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Preface

This book covers key areas of Medicine and Medical Research. The contributions by the authors include Health worker, pharmacist, medical errors, clinical information, Healthcare Safety Environment, Honey, Recurrent apthous stomatitis (RAS), ulceration, erythema, therapeutic benefits, recurrent apthous ulcer, Douching, Hausa-Fulani, vaginal, various vaginal pathology for effective control, Bacterial vaginosis, Nugent score, clue cells, Amsel criteria, whiff's test, Tertiary Health Facility, Antenatal Clinic Attendees, Atypical flutter, left atrial flutter, mitral valve surgery, surgical maze, Mitral Isthmus Flutter, Post Mitral Valve Repair, Prevalence, bacterial vaginosis, women, abakaliki, transmission of HIV and herpes simplex virus (HSV), Plant extract, Saussurea costus, anti-cancer, apoptosis, Anticancer Bioactives, cytotoxic activity, anti-apoptotic proteins, Knowledge, practices, perception, fear, stigma, contagious, PWE (People with Epilepsy), CORPS (Community Own Resource Persons), epilambanein, Stigmatization of Epilepsy, Ketamine, opioid epidemic, opioid misuse, detoxification, chronic pain, g ketamine assisted opioid detoxification, Oral submucos firbrosis, turmeric, kenacort, precancerous condition, jaggery and turmeric application, Mitochondria, mitochondrial dysfunction, sexual constitution, Maternal mortality, pattern, Tertiary Care Hospital, pregnancy and child birth related issues, Anemia, proper antenatal care, Airway volume, obstructive sleep apnea, Uvulopalatopharyngoplasty, three-dimensional computed tomography, velopharyngeal and glossopharyngeal airways, cardiovascular disease (CVD), Airway volume, velopharyngeal and glossopharyngeal, Anatomical balance theory, craniofacial bony structures, Type 2 diabetes mellitus, GLP-1, oral antidiabetic medications, metabolic relationships, Antidiabetic Medications, dipeptidyl peptidase- IV (DPP-IV), Schwannoma, vagus, presaccral, S-100, hypointense on T1, heterogeneously hyperintense etc. This book contains various materials suitable for students, researchers and academicians in the field of Medicine and Medical Research.

Chapter 1
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The Healthcare Safety Environment: A Study on Healthcare Workers and Predictors of Medical Errors

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ABSTRACT

The healthcare environment has always been overwhelmed by medical errors, worldwide. The aim of this search is to identify correlates associated with of the healthcare workers' (HCW) perception toward medical errors prevention within their health facilities' environment. Distinguished health services now immensely depend both upon adherence to highest performance standards and the need to embrace advanced, and often sophisticated, technologies which provide better diagnostic and therapeutic opportunities to the patient's and the provider's best interest. In this research, healthcare providers from a variety of health facilities in Cairo, Egypt, as a model, would be surveyed. The work load (WL), burnout (BO), and leader-member exchange (LMX) quality and their influences upon the HCWs' perception and attitude toward medical errors (ATEs) would be studied. Findings of this research were as follows: Among 5,725 health professionals surveyed, 2,260 (39.5%) returned valid responses. The studied population's mean age was 33.4 years ±7.76; male-female ratio was 1.26:1. Nursing predominate other occupations, e.g., 35.4% vs. 21.6% physicians. Both LMX and ATEs scores were significantly higher in male workers [t(df=2258)=0.106, p<0.05; t(df=2258)=1.22, p<0.05, respectively]. The LMX and ATEs scores varied by occupation [F(df=4, 2,255)=2.48, p=0.045]; physicians scored higher than technicians, nurse, and pharmacists, [F(df=4, 2,255)=6.65, p=0.02]. Respondents' LMX score was increasing by age [F(df=3, 2,237)=3.52, p=0.016]. Burnout score decreased by decreasing age [F(df=3, 2,237)=3.37, p=0.042]. Both LMX and ATEs were significantly correlated (r= 0.16, p=0.015); WL directly correlated with BO (r= 0.351, p<0.001) but indirectly correlated with ATEs (r= –0.161, p<0.016). Otherwise, ATEs and BO were indirectly correlated (r=-0.473, p<0.001). Significantly, BO was predictor for the changes in ATEs (β= –0.032, p<0.001). Likewise, work experience was a predictor for BO (β = –0.122, p=0.008). In conclusion, work stressors impact ATEs of HCWs, including those under a financial target pressure. Given their favorable ATEs and LMX attitude, older health workers can play a role in combating medical errors risk in the healthcare institutions arena. Future research on the pattern and determinants of medical errors in the Cairo health institutions, probably utilizing a hybrid methodological approach, such as sampling the medical records for detailed clinical information, reviewing morbidity and mortality reports.

Keywords: Health worker; pharmacist; medical errors; clinical information.

1. INTRODUCTION

Enthusiastic health systems strive to achieve quality and "perfection" in performance throughout the healthcare providing process to assure a maximum leverage upon the patient's safety and health outcomes. Distinguished health services now immensely depend both upon adherence to highest performance standards and the need to embrace advanced, and often sophisticated, technologies

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which provide better diagnostic and therapeutic opportunities to the patient's and the provider's best interest. Organization-wide deployment of continuous improvement principles engages frontline staff and embeds a scalable methodology for growing and coordinating improvement activities. The key challenge is to understand how to lead the implementation of continuous quality improvement tenets successfully and sustainably across large-scale and increasingly complex healthcare organizations to deliver the benefits it can provide [1]. In such a highly challenging atmosphere, a system's management approach is inevitable in order to assure well adjusted and smooth flow of the healthcare process, with minimal flaws, uncertainty, and errors potential. Although errors are an unavoidable trait of mankind, (e.g., "to err in human") [2] planners for interventional human services, including healthcare, work diligently to furnish an appropriate environment for the most favorable outcome, safety, and minimal unwanted events. Medical errors involve mistakes medical professionals make in patient testing, care, or treatment [3]. Medical errors are a serious public health problem and a leading cause of death in the United States. It is a difficult problem as it is challenging to uncover a consistent cause of errors and, even if found, to provide a consistent viable solution that minimizes the chances of a recurrent event [4,5]. Categories of medical errors include failures of planned actions, mistakes of execution and use of wrong plans to achieve outcomes. Specifically incorporated are wrong or inaccurate diagnosis or wrong site, wrong surgery, wrong procedure, and incomplete treatment of illness or injury. Also encountered are hospital acquired infections (HAIs), blood transfusion reaction and anesthesia errors. Incriminated causes of medical errors include modest experience, poor communication either between patients and healthcare workers or between healthcare workers and unclear lines of authority, emergency care inadequate staffing, and complexity of the procedure [6]. To reduce the incidence of errors, health care providers must identify their causes, devise solutions and measure the success of improvement efforts. Moreover, accurate measurements of the incidence of error, based on clear and consistent definitions, are essential prerequisites for effective action [7,8].

Regardless the quality and resources devoted to assure perfection, many of the negative patient safety events are still related to systems and how people operate within them. This attitude likely leads to continued suffering of patients so that every year, thousands of patients die because of medical errors. Such errors can occur anywhere in the health care system, whether public hospitals, private practices, nursing homes, patients' homes, and pharmacies.

To help reduce the incidence of medical errors and hence improve patient safety, we need to identify error-*prone* initiation *factor*s and to develop approach for prevention. For instance, BO has been considered a major cause of medical errors [9]. The negative outcome of such risk for individuals and organizations is overwhelming. If burnout is a proximal cause of medical error, efforts by individuals and healthcare organizations to overcome stressful work conditions, particularly burnout, may help decrease the occurrence of medical errors [10]. Other factors impacting medical errors include workload work experience, load tolerance, continuous professional training, the organization's reward policy, and the organization's leadership philosophy; how far it is internalized within the mindset of the health workers community. Scholars working on these risk areas often prioritize a risk of interest over other risks. For instance, one critique of the burnout literature is that it has largely ignored the study of how the leadership processes affects burnout. For this reason, correlates of impaired patient safety and medical errors, such as burnout, LMX quality, and workload need to be addressed integrally yet in a more balanced way.

In essence, LMX involves an approach that conceptualizes leadership from the perspective of relationship and implies that leaders develop different relationships with their followers [11]. When the leader has a strong LMX relationship with the subordinates in terms of mutual trust, respect, and obligation, a positive effect on employees' attitudes toward patients, the organization and the perception toward the organization are high, and expectedly, medical errors may well be prevented from happening. Especially in health care, supporting high quality LMX relationships may indirectly influence organizational and personal outcomes by reducing BO [12]. Since healthcare managers often tend to discriminately treat their subordinates, the influence of LMX quality on the overall work performance either subjectively and objectively varies.

Workload refers to the amount of work or number of work units assigned to an individual to perform or complete over a given period [13]. Occupationally, WL has been linked to fatigue, anxiety, and overall

impaired physiological, mental, and physical performance. In most healthcare settings WL can be a primary risk for BO, which predisposes to medical errors [9]. The "WL- BO - medical errors" series is well observed in most healthcare stingers that overloaded nurses are liable to emotional stress, cynicism, anger and ultimately BO. Especially the heavy WL of nurses has been considered as a major problem for healthcare systems across the world. For instance, in Europe, heavy WL adversely affected patient safety to the degree that patients may die after common surgery when they are cared for by a heavier workload nurse. The issue is that the burden of care for nurses increased in recent years as a result of an increase in patient numbers and population aging, in the presence of an inadequate nurse staffing and mandatory overtime work to treat chronic understaffing. Moreover, nursing overload negatively affects job satisfaction which contributes to high nurse turnover and the nursing shortage. Physicians, too, are subject to workload, especially in such highly competitive healthcare climate and where national economic difficulty forces a cut on budget and reluctance to invest in human resources. In which case over working physicians render patient safety in jeopardy. For instance, it was found that nearly half of surveyed physicians by Johns Hopkins investigators believe that excessive WL can affect the safety of their patients and consider as the cause of a large number of medical errors [14].

On the other hand, BO refers to a state of emotional, physical and mental exhaustion results from excessive and prolonged occupational stress, such as work overload [15]. Burnout is considered a serious public health problem due to its increasing rate of incidence and negative impact on the entire healthcare system, for its has been largely connected with work performance, job satisfaction, quality of life (QoL), and psychological health. Particularly nursing is a stressful occupation because of their work characteristics that requires mutual interactions to maintain services, and due to work overload and on the job conflicts. These stressors lead to professional BO. Physicians, too, are prone to BO that is directly connected with increased medical errors and impaired patient care [10]. In Shanafelt, et al., 2009 [9] work to evaluate the relationship between BO and perceived major medical errors among, the majority of medical errors American surgeons have reported were strongly related to the degree of BO and mental QoL they have been suffering. Another source of BO which has received attention in recent years involves self efficacy, a social learning concept the reinforcement of which prevents BO [16]. A self efficacious employee is in a better position to keep and perform calm in stressful conditions, and therefore is less likely to fall into BO as easy. Ultimately, a healthcare place that is disturbed by workload and burnout and incapacitated by poor control on medical errors risk is prone to a multitude of devastating complications on the patient's part, in terms of worsened morbidity, mortality, and disability likelihood, and on the organization's stability.

In the presence of terrifying figures, e.g., 98,000 deaths in 1999 due to mistakes in hospitals, [2] many of which could have been prevented, or 180,000 of Medicare deaths in 2010, [17] the credibility of the whole healthcare system is questionable. The cost to hospitals and community is unbearable. Medical errors, adverse effects, and mistakes committed during care providing can be prevented or reduced [18] and hospitals undoubtedly need to seek all possible opportunities to reduce and avoid the threat of medical errors. Definitely, error reduction is a difficult mission due to the sophisticated nature of today's healthcare organizations, however understanding key factors contributing to medical errors and developing approach for prevention bring us closer to a healthcare environment with minimal error potential and maximum patient safety opportunity.

In Egypt, the fabric of the public healthcare system is rather unique in terms of types, ownership, access and delivery of service. All people have the right for a free healthcare service, regardless their social, employment or citizenship status. The MOHP is currently the major provider of primary, preventive, and curative care in Egypt (with around 5,000 health facilities and more than 80,000 beds spread nationwide). Specifically, with respect to inpatient services, the MOHP is the largest institutional provider of inpatient care services in the country. It has about 1,048 inpatient facilities, accounting for more than 80,000 beds. Comparatively, the private sector has 2,024 inpatient facilities, with a total of about 22,647 beds, accounting for approximately 16 percent of the total inpatient bed capacity in Egypt [19]. Overall, the Egyptian health care system faces multiple challenges in improving and ensuring the health and wellbeing of the Egyptian people, especially the low social class populations. The system faces not only the burden of combating illnesses associated with poverty and lack of education, but it must also respond to emerging diseases and illnesses associated with modern, urban lifestyle. To this end, quality, comprehensive coverage, long waiting lists, and the

limited availability of updated technologies at a significant proportion of governmentally sponsored public health organizations are issues concerning both the health officials and the people of Egypt. The relatively limited resources the governmental healthcare environment suffers, and the emerging access to global communications which raises the people's expectations for a better and advanced health care, urge a considerable number of middle-class and most high-class populations to seek healthcare at the private healthcare market. After the declaration of an open economic policy in 1974, the private health sector began to grow. Between 1975 and 1990, the total number of private beds rose significantly. Private care facilities in Egypt range from hospitals that are large, modern, and sophisticated to smaller hospitals, day care centers, and polyclinics. This private sector provision of services includes everything from private doctor practices, practice groups, high-tech diagnostic and therapeutic centers and laboratories, as well as private hospitals of all sizes and levels. The Egyptian National Health Care Provider Survey [20] showed that 89 percent of the physicians with private clinics had multiple jobs. Seventy-three percent of the physicians had two jobs (i.e., they had another job outside their private clinic), 14 percent had three jobs, and 2 percent had four jobs. Commonly, those governmentally employed physicians who cannot afford to open their own private clinics opt to work in more than one private facility in addition to their governmental jobs to make up for the income deficit and to achieve some social and prestigious standard of living many doctors are traditionally striving. Likewise, other healthcare professionals, including nurses, pharmacists, health technicians, and allied health care providers, all consider working with private employers upon part-time contractual agreement and many opt to work full-time with these organizations to assure better earning, often at the expense of their family - and leisure times. Such healthcare professionals could be practicing under rather stressful work conditions on daily basis, and this paves the way for work loaded, exhaustion, and probably burnout, a situation that may well be reflected upon their physical and mental well being and eventually work performance. This work has been built on the hypothesis that health workers attitude to medical errors is an intermediary healthcare outcome that may be affected by a set of factors, including LMX quality, WL, and BO, particularly the private care health professional in Cairo, Egypt who are at risk of assuming long working hours and stressful work conditions. Understanding this relationship may well be a step toward the preventing risk of medical errors among health workers and hence alleviating their harmful impact on the entire healthcare outcome including patient safety.

2. METHODS

2.1 The Study Design

A multiphase project joining a group of interested researchers and healthcare workers from health organizations in several Arabic districts, including Cairo-Egypt, Saudi Arabia western province, and central Saudi Arabian province was established to study some determinants and outcomes associate with patient safety, performance indicators, and quality assurance of the healthcare environment in these districts. Medical errors, a major component of the risk profile of patient safety, would be among the studied outcomes.

2.2 Sampling Framework and Criteria Used for Selecting Sampling

In this work, healthcare providers from selected private health organizations in Cairo, Egypt were surveyed during the period between March 2014 and June 2014; their WL, BO, LMX quality and the influences of these determinants upon the healthcare workers' ATEs would be analyzed. One-hundred and fifteen health organizations fulfilling official medical, municipal and commercial registration requirements in greater Cairo district were surveyed. Inclusion criteria also included health organizations with a minimum of 95 professional health workers, including medical staff, nursing, technicians, and allied health services staff. Participating hospitals and facilities were randomly selected in a stratified fashion to represent the approximate proportion of licensed private healthcare organizations working in northern, southern, eastern, and western Cairo (29=25.2%, 26=22.6%, 32=27.8%, 28=24.3% hospitals/healthcare facilities, respectively). Further, healthcare employees were proportionately randomly selected from each organization's departments/ sections/units.

2.3 Data Collection Instruments and Procedures

A validated predesigned questionnaire to screen the healthcare staff of the selected institutions was utilized. A study sample from the participating healthcare organizations mounting up to 9,340 subjects was reached and invited to respond to the questionnaire, 5,725 (61.3%) of whom returned the self-administered questionnaire. Every effort was done in order to deliver the questionnaire by hand and obtain consent of the participants throughout the study period to assure effective communication and hence maximum response rate of the targeted population. In order to be included in the analysis, only returned questionnaires reporting valid answers on ≥80% of the items would be considered. The questionnaire included a total of eighty-eight items. (The term item may be used throughout this work to describe every single "question" asked to identify to what extent it "measures the same point of interest". Should an item be manipulated, e.g., for a statistical analysis purpose, the term may be referred to as "variable"). Included also are items if the answer to the preceding question was "yes". The questionnaire items cover the following domains: a) demographic and background information (10 questions), b) hospital/health organization information (5 questions), c) work system information (25 questions), d) patient safety climate and culture in the organization (37 questions), perceived performance on unit effectiveness and satisfaction with care provided (6 questions), e) quality of working life (5 questions). The relatively large number of questions was carefully decided and set to assure maximum validity and comprehensiveness of the questionnaire. For instance, the work system domain contained questions on vital work processing and flow, such as communication openness, communication accuracy, communication timelines; time pressures affecting patient safety, workload, coordination mechanisms, workplace design, equipment design, and access to supplies. The personal and demographic domain addresses items related to age, gender, socio-economic status, education, professional information, including occupation, previous years of experience, and years of experience in the current hospital work area of the respondent. Importantly, too, the subscale about factors affecting ATE included questions addressing vital information regarding the LMX, WL, and BO. Likewise, the quality of working life scale included clear questions about fatigue, tension, and also job satisfaction. Specifically, input variables of this research's interest that would potentially influence the study outcome were based on definitions drawn from evidence-based resources. For instance, LMX quality was addressed based on definition by Deluga, 1998, [11] WL as defined by Jex, 1998, [13] and BO, as defined by Maslach, et al. 2001 [15] (see before). On the other hand, both ATEs and medical errors would be dealt with as described by Farger, 2012 [21].

Generally, a five-point Likert scale could be used for stratifying such categorical variables. The Likert format uniformly provides options ranging from 1 to 5. The response selection ranges between "strongly agree" and "strongly disagree"; whether or not a "strongly disagree" response would be given maximum score five or least score one depends on the nature of the question. For instance, in questions addressing inquiries the agreement to which is in favor of a positive workers' ATEs (an outcome of this work's interest), "strongly agree" scores five and "strongly disagree" scores one; and vise versa. The questionnaire takes 35-45 minutes to complete. All required official permissions were obtained; arrangements with the participating organizations done prior to conducting the survey. In preparation for the study, a pilot administration was conducted to assess the questionnaire's test-retest reliability. Thirty health-worker colleagues were given the questionnaire to respond to (response-a). The same questionnaire was re-administered by the same group one week later (response-b). A panel of juries consists of experts in research, healthcare quality, preventive medicine, medical directing, and chief nursing, was selected to judge the responses. Test-retest reliability was calculated to assess the temporal stability of the questionnaire items, using appropriate correlation techniques. An acceptable – to - strong reliability evidence for the questionnaire's items was found: reliability alphas 0.78 for the selected determinants, 0.83 for lifestyle, 0.76 for chronic diseases, and 0.91 for screening tests scales. Onsite, participants were informed about the aim of the study prior to the completion of questionnaire. A verbal consent from each participant was considered a personal permission to participate in the study. Otherwise, it was made clear that participation was voluntary, and that any participant could opt to withdraw any time during the study. We have also stressed the anonymity and confidentiality of any collected information, and that only generic outcome data might be disseminated in scientific settings.

2.4 Data Management and Analysis

Data were entered to a Microsoft program with adequate backups; open-ended questions coded, and observations made ready for statistical analysis. First, descriptive statistics, including frequency data, would be displayed. Parametric techniques, e.g., *t*-test of two independent samples, could be used comparing mean differences, considering normal distribution of the continuous data. Testing the differences between three groups or more in their observed levels of a continuous data, considering normality assumption, one-way ANOVA test would be used. Correlation techniques, whether Pearson's or Spearman's depending on normality distribution, to compare the strength of correlation between any two continuous variables of interest could also be used, where appropriate. Multiple linear regression models, e.g., to predict the change in the workers' ATE as a result of a unit change in the predictor variables (e.g., work experience, occupation, LMX, workload, burnout), could be constructed. The SPSS software for Microsoft- version-20 was used for statistical analysis. All tests were at level of significance α=0.05; results with *p*-values <0.05 were considered "statistically significant."

3. RESULTS

In the study, 2,260 returned questionnaires with fulfilling response validity criteria out of 5,725 responses (39.5%) were entered in the analysis. (In the display of data, either term such as "respondents", "participants", "health professionals", or "health providers", might be used interchangeably, study individuals, would be used to describe the individuals who were included in the analysis).

Table 1a describes selected demographic and professional data of the study participating group. Age-wise, younger age group (20 - <30 years) constitutes almost one-third of the participants (32.3%, n=730). The majority (46.0%, n=1040) belong to the next age group (30 - <40). The number of the study individuals then decreased by decreasing age: 340 (15%) were 40 - <50 years old, 130 (5.8%) were 50 or above (Table 1a). Male workers slightly dominate the study population (55.31%, n = 125), constituting a male – to female ratio of 1.26:1.

Table 1a. Distribution of the study group by demographic characteristics

Characteristic		n	%
Age (y)*	20 to <30	730	32.3
	30 to <40	1040	46.0
	40 to <50	340	15.0
	≥50	130	5.8
	Missing	20	0.9
	Total	2260	100.0
Gender	Male	1250	55.3
	Female	990	43.8
	Total	2260	100.0
Primary work area	Medical ward	50	2.2
	Surgical ward	140	6.2
	Intensive care unit (ICU) (any type)	260	11.5
	Oncology	110	4.9
	Hematology	70	3.1
	Emergency department	40	1.8
	Anesthesiology	50	2.2
	Laboratory	360	15.9
	Pharmacy	230	10.2
	Radiology	180	8.0
	Other unit/ward	630	27.9
	No specific unit	120	5.3
	Missing	20	0.9
	Total	2260	100.0

** Mean age = 33.4 (±7.76SD)*

Table 1b. Distribution of the study group by professional characteristic

Characteristic		n	%
Occupation	Physician	510	22.6
	Nurse	800	35.4
	Pharmacist	160	7.1
	Technician	580	25.7
	Other	190	8.4
	Missing	20	0.9
	Total	2260	100.0
Work experience duration *	Less than 5 years	460	20.4
	5 to 10 years	980	43.4
	11 to 15 years	380	16.8
	16 to 20 years	190	8.4
	More than 20 years	200	8.8
	Missing	50	2.2
	Total	2260	100.0
Years of experience in the current organization **	Less than1 year	280	12.4
	1 to 5 years	1690	74.8
	6 to 10 years	270	11.9
	Missing	20	0.9
	Total	2260	100.0

** Mean work experience 9.5y (±6.98SD)*
*** Mean Years of experience in the current hospital = 3.1 (±1.2SD)*

The participants work at 12 primary work areas (Table 1a), highest of which was "other unit/ward" category (63 = 27.9%), followed by laboratory (36 = 15.9%), ICU, all types (26 = 11.5%), and pharmacy (23 = 10.2%). The remaining areas recorded low numbers of participants, e.g., surgery (14 = 6.2%), until the emergency department which recorded least participating frequency (4 =1.8%) (Table 1a).

According to the study design, five healthcare occupations were reported: "physician", "nurse", pharmacist", "technician" "other" (Table 1b). Nurses constituted the greatest frequency of participation (800 = 35.4%), followed by technician (580 = 25.7%), physicians (510 = 21.6%), and least were pharmacist (160 = 7.1 %) and "other" occupation category (190 = 8.4%).

Among the five categories endorsed of years of experience, those who have 5-10 years of experience outnumber all other experience groups, accounting 980 (43.4%) subjects, next were participants with less –than 5 year experience (460 = 20.4%), followed by those with 11-15 experience years (380 = 16.8%) (Table 1b). The least common work experience durations were >20y and 16-20y; including 200 (8.8%) and 19 (8.4%) participants, respectively. As in Table 1b, too, 1,690 (74.8%) of the respondents dominantly had been employed with their current employers for 1 to 5 years, 280 (12.4%) spent less than 1 year, and lastly 270 (11.9%) were those who had spent 6 - 10 years.

Table 2 shows the distribution of the participants' scores of the LMX, WL, BO, and ATEs scales by gender analyzed. The mean scores both for LMX (male 3.64±0.96, female 3.52±0.84) and ATEs (male 3.524±0.56, female 3.445±0.40, respectively) shows a statistically significant difference between male and female workers [t(df=2258)=0.106, p<0.05and t(df=2258)=1.22, p<0.05]. Both the participants' WL and BO mean scores did not significantly vary in the two gender groups.

In the one-way ANOVA testing (Tables 4a) to measure the influence of age upon the difference in the study population scores of the main study scales, first, LMX showed a significant different in the mean scores between age groups [F(df=3, 2237)=3.52, p=0.016] (Table 3a). [Further, respondents aged 20-<30 scored significantly lower compared to those who age ≥50 (post hoc LSD test, mean difference = –0.769, p=0.005), and those aged 30-<40 had a significantly lower LMX score than the ≥50 peers (mean difference = – 0.75, p=0.005)]. Unexpectedly, the respondents' BO mean score significantly decreased by decreasing age [F(df=3, 2237)=3.372, p=0.042], where those aged 30-<40 scored

significantly higher compared to those who age 40-<50 (post hoc LSD test: mean difference = 0.331, *p*=0.031). Otherwise, both WL and ATEs mean scores were not influenced by age (Table 4a).

Table 2. Difference in the mean scores of the study variables of interest among the participants two gender groups

Variable	Gender	n	Mean	SD	Test statistic	*p*-value
LMX	Male	1250	3.64	0.963	t(df=2258)= 0.106	<0.05
	Female	1010	3.52	0.838		
WL	Male	1250	3.05	0.914	t(df=2258)= 0.112	>0.05
	Female	1010	3.07	0.893		
BO	Male	1250	2.30	0.788	t(df=2258)= 0.699	>0.05
	Female	1010	2.23	0.767		
ATEs	Male	1250	3.52	0.559	t(df=2258)= 1.22	<0.05
	Female	1010	3.44	0.405		

Table 3. Difference in the mean scores of the study variables of interest among the study occupation groups (physician nurse, technician, pharmacist, other)

Variable	Test statistic	*p*-value
LMX	*F*(df = 4, 2255) = 2.48	0.045
WL	*F*(df = 4, 2255) = 3.13	0.043
BO	*F*(df = 4, 2255) = 2.65	0.020
ATEs	*F*(df = 4, 2255) = 2.75	0.041

Table 3a. Difference in the mean scores of the study variables of interest among the study's age groups

Variable	Test statistic	*p*-value
LMX	*F*(df = 3, 2237) = 3.521	0.061
WL	*F*(df = 3, 2237) = 2.350	0.094
BO	*F*(df = 3, 2237) = 3.372	0.042
ATEs	*F*(df = 3, 2237) = 2.960	0.117

Table 3b. Difference in the mean scores of the study variables of interest among the study's occupation groups

Variable	Test statistic	*p*-value
LMX	*F*(df = 3, 2237) = 2.408	0.045
WL	*F*(df = 3, 2237) = 3.139	0.033
BO	*F*(df = 3, 2237) = 1.325	0.098
ATEs	*F*(df = 3, 2237) = 3.758	0.014

Another set of 4 ANOVA tests has been calculated to analyze the difference in the mean scores of each of the principal study scales among different occupation groups (Table 3b). The LMX mean scores were significantly different [*F*(df=4, 2255)=2.408, *p*=0.045]. *[Further, post-hoc test for LMX score differences within occupation groups showed that physicians had a significantly higher mean LMX scores than all occupations (technicians, nurse, pharmacists), except "other" occupation (mean score differences: physician – technician=0.53, p=0.02, physician – nurse=0.55, p=0.01, physician – pharmacist= 0.64, p=0.012)].* The WL mean scores varied between occupation groups [*F*(df=4, 2255)=3.139, *p*=0.033]. *[Within group post hoc test showed that "other" occupation was significantly higher than "technicians" (mean score difference = 0.47, p=0.0034). The WL score differences within the remaining occupation groups were not statistically significant].* The difference for the ATEs mean scores between occupation groups was also significant [*F*(df=4, 2255)=3.758, *p*=0.014]. *[Within group post hoc test showed that "other" occupation was significantly higher than "technicians" (mean score difference = 0.47, p=0.0034)].* The score differences for the WL scale between the occupation groups were not statistically significant (Table 3b).

In Table 4, a correlation analysis between the study scales, both LMX and ATEs had a rather weak, yet significant, mean scores correlation (r= 0.162, p=0.015). No significant correlations between LMX and both WL and BO have been reported. On the other hand, WL and BO mean scores moderately positively correlation (r= 0.351, p<0.001). Significantly too, WL and ATEs weakly inversely correlated (r= –0.161, p<0.016). The ATEs mean score and BO's were inversely moderately correlated, too (r= –0.473, p<0.001).

Tables 5a and 5b exhibit data of the two linear regression analysis attempts. The first regression (Table 6a) shows how WL could predict the variability in the ATEs as a result of BO change. For each unit score increase in BO, ATEs score decreases by 0.032 score unit (β = –0.032 p<0.001) (Table 5a).

Table 4. Correlations analyses of scores of the scales of interest

		LMX	WL	BO	ATE
LMX	Pearson correlation	1	0.003	-0.123	0.162
	Sig. (2-tailed)		0.963	0.064	0.015
	n	2260	2260	2260	2260
WL	Pearson correlation	0.003	1	0.351	-0.161
	Sig. (2-tailed)	0.963		<0.001	0.016
	n	2260	2260	2260	2260
BO	Pearson correlation	-0.123	0.351	1	-0.473
	Sig. (2-tailed)	0.064	<0.001		<0.001
	n	2260	2260	2260	2260
ATE	Pearson correlation	0.162	–0.161	–0.473	1
	Sig. (2-tailed)	0.015	0.016	<0.001	
	N	2260	2260	2260	2260

Table 5a. Predicting the change in ATEs against the change in BO: a bivariate linear regression analysis

Coefficients [a]							
Model	**Un-standardized coefficients**		**Standardized coefficients**	***t***	**Sig.**	**95% CI for β**	
	β	**Std. Error**	**Beta**			**Lower**	**Upper**
Constant	4.175	0.090		46.245	<.001	3.997	4.353
BO mean	–0.302	0.038	–0.473	–8.031	<.001	-0.376	–0.228

a. Dependent variable: ATEs mean

In the second regression model (Table 5b) to predict the variation in BO, as an intermediary dependent variable, due to changes in a selected group of predictors, including gender, work experience, occupation, LMX, and WL was calculated. (Only WL and work experience were significantly entered to the model). Each unit increase in WL score significantly yields ATEs decreases by 0.302 unit score in BO (β = 0.302, p<0.001). Significantly, too, a unit increase in work experience score leads to a decrease in BO score by 0.112 units (β = –0.122, p=0.008).

Table 5b. Predicting the change in BO against the change in WL and work experience: a multiple linear regression analysis

Coefficients [a]								
Model		**Un-standardized coefficients**		**Standardized coefficients**	***t***	**Sig.**	**95% CI for β**	
		β	**Std. Error**	**Beta**			**Lower**	**Upper**
1	Constant	1.348	0.172		7.830	<0.001	1.009	1.687
	WL mean	0.302	0.054	0.351	5.607	<0.001	0.196	0.408
2	Constant	1.567	0.188		8.320	<0.001	1.196	1.938
	WL mean	0.318	0.054	0.369	5.948	<0.001	0.213	0.424
	Experience mean	–0.112	0.042	–0.167	–2.689	0.008	–0.194	–0.030

a. Dependent variable: BO mean

4. DISCUSSION

4.1 The Private Health Care General Environment in Egypt

Early in the design of this work, there was keenness to admit to the study the largest sample possible of Cairo health professionals. The private healthcare field in Cairo sector covers a considerable portion of the health demand of the Egyptian society. Further, this sector enjoys a wide veriety of healthcare expertise with profession-related risks to exercise analyzing the healthcare ATEs patterns in the target population. First the study sampling frame contained 9,340 affiliates who were diligently reached to assure as large sample size as possible and to cover up for any low questionnaire response rates to predict (61.3% return rate and 39.5% valid response rate). Not uncommonly, lower figures, (e.g., 25.5%) of a response rate, e.g. to Web-based surveys or a slightly higher rate (31.5%) surface-mail surveys have been reported. [22] In the first phase of the analysis, we meant to thoroughly describe the study scales (LMX, WL, BO, ATEs); their distributions by socio-demographic/ professional status, including occupation and work experience. This helped identify the weight and significance of each of these factors in exploring the medical errors impression trends of the studied population. For instance, younger professionals (20-30 years old) tended to report lower LMX scores than older-age counterparts, and vise versa. Since the majority (46.0%) of respondents aged 30 to <40, age should be given an utmost consideration in interpreting the organization's medical errors profile.

4.2 Why Young Age People Are Less Resistant to Burnout

The 30 to <40 year old respondents reported a significantly higher BO score compared to older groups. Apparently, older professionals seem to tolerate BO more frequently than younger colleagues. To start with, the pattern of the effect of age, e.g., on LMX could easily be understood, since age has been perceived as human trait that promotes the individual's ability to make wiser decisions and more effective leadership performance. Therefore, we can easily accept the finding that the health professionals' age is pro better LMX quality. In contrast, it sounds little uncommon that younger individuals report a higher subjectivity, and hence less tolerability to BO while on the job. As such, we may argue that BO itself is not an "all or none" issue. Burnout in the healthcare arena has many causes to think about other than age [23]. For instance, several job traits come into play in determining the level of BO in healthcare institutions, such as role conflicts and role overload. Absence of a clear guideline for the tasks and duties assigned to health workers makes them uncertain about the limits of the task and therefore they become liable to put-off more easily. In which case, even junior practitioners may be at risk of BO in a shorter time interval as compared to doing the same task under better job conditions. The organization's characteristics also have an important role in the BO challenge, for linking the organization's reward-punishment policy to work performance does guard against BO overload. Personal traits other than age also have a role in the higher incidents of BO, such as the health worker's self-efficacy [24] and the amount of social support workers receive from the surroundings. Thereby, age here should be handled carefully while planning for a medical errors improvement. For instance, tasks that need more communication and leadership experience, or tasks of planning and policy making nature may be assigned to older age professionals who have higher communication and LMX skills, until all staff has been able to live up to the expected level of LMX and BO tolerance standard, all in parallel with diminishing the effect of the factors that lead to a BO tendency among staff.

Of note, too, is that BO was not significantly related to our study group occupations. Instead, it was only significantly related to WL; the latter varied by occupations, (e.g., "other" specialties tend for higher WL levels than technicians). In fact, BO has been a matter of focus in medical errors research. The issue encompasses emotional, physical and mental exhaustion as a result of excessive work stresses, especially work overload [15]. Neglecting the BO challenge leads to devastating drawbacks on the whole healthcare system. Otherwise, BO not uncommonly varies between different healthcare occupations, elsewhere. For instance, 50% of physicians believe that among the contributing factors to medical errors is fatigue, as a form of BO [25]. Likewise, medical errors reported by surgeons were significantly linked to their degree of BO and their mental QoL [14]. Nursing also experiences a

voluminous work overload and has to perform an endless number of duties and may eventually end up with BO [26].

4.3 Work Experience Influence upon Burnout

Work experience, as well as self efficacy on the job has been given the due care in the analysis. More than 43% of our participants had 5 -10 years of relevant work experience. Also, 74.8 % of the respondents had 1- 5 years of experience with their current employers. Work experience scoring enabled us to predict the variation in BO tendency secondary to the change in the work experience standard. In a study by Perry et al. [27] on voluntary medical male circumcision (VMMC) services in Kenya, South Africa, Tanzania, and Zimbabwe, a multivariate analysis for predictors of work fatigue/BO had been undertaken. The average work experience for Kenyan providers was 31 months compared to South Africa (10 months), Tanzania (15 months), and Zimbabwe (11 months). In comparison to our health professionals, except for a considerable proportion of the Kenyans (67%), less number of VMMC providers started to experience work fatigue/burnout around the end of the work durations (33% South African, 17% Zimbabwean, and 15% Tanzanian providers). In their regression analysis, Perry et al. [27] first report an increase both in age and duration of work which was associated with an increased likelihood of experiencing work fatigue/BO. However, higher career duration total at VMMCs decreased the likelihood of experiencing BO. Evidently, the same trend of a decreasing variability in BO by work experience in the VMMC survey has been shared by our study.

4.4 How is Health Workers' Ates Influenced by the Study's Medical Errors Correlates

In the analysis of our ATEs domain by occupation, physicians attained a significantly higher ATEs score than nurses. As a matter of fact, both professions have always been obsessed with medical errors committed at the worksite and down the healthcare road. Both professions are held accountable for their patients' safety and remotely unlikely that an average physician or nurse would mean to intentionally inflict harm upon their patients. In a study by Valiee, et al. [28] conducted a study to evaluate nurses' perception about nursing error who had at least one year of work experience in critical care units in Tehran and Kurdistan, the participants reported that nursing errors were deemed unavoidable. Work pressure, caring blindly, and lack of coordination were among the condemned reasons. The nurses supported the recommendations given to alleviate their concern about errors, not to impact patients' wellbeing. Shanafelt, et al. [9] also indicate that when American surgeons were surveyed utilizing a validated depression screening instrument and standardized assessments of BO and QoL, they showed a strong desire that medical errors be diminished on the job. The surgeons blamed burnout and their mental QoL for medical errors, some of which may have been as fatal.

4.5 Medical Errors as an Ultimate Consequence of Work Stresses

Medical errors, whether those related to failure of planned actions or mistakes of execution and the use of wrong plans to achieve outcomes, alongside with the risks of medical malpractice, all involve a multitude of underlying causes and triggering factors. Among these factors are heavy WL and communication problems in the health organization. Under such climate, medical errors are prone to be encountered and their occurrence could be on the rise, unless otherwise mitigated by effective measures. To that end, medical errors largely jeopardize both patient safety and the health organizations stability. In a given health facility, unless the service environment was designed to the best outcome of patients' wellbeing, at least patients should not be harmed by the care they are given at the healthcare facility ("primum non nocere" = "first, do no harm" principle) [29]. Especially in BO among healthcare workers' research and in the realm of healthcare profession, seemingly there is a moving of the focus from just "errors" to the broader favorable outcome of health service, which is patient safety [30]. An adequate understanding of medical errors plays a pivotal role in achieving the improved patient safety goal.

Three main levels of inputs were studied in this work: organization level, as expressed by LMX quality, job level, as expressed by WL, and individual level, as expressed by BO; each has been analyzed as a hypothesized predictor for the health professionals' ATEs. The philosophy of selecting healthcare staff attitude toward medial errors as an outcome is envisioned in a sense that ATEs

stands as an important intermediary step toward the prevention and control of medical errors [31]. In the literature, too, researches such as that by Frager [21] tended to utilize ATEs to measure and provide recommendations for medical errors prevention. Actually, utilizing ATE as an outcome in error research provides a vehicle for understanding opportunities to improve patient care safety. Understanding the circumstances related to errors is the starting point to work on the prevention of medical errors; furnishing a healthy environment for quality care and improved outcomes of the provided service. On the health institution's part, establishing a culture where a shift from punitive - to non-punitive approach, e.g., adopting "root cause analysis" (RCA) technique to depict the reason of errors so not to repeat, and "forward mode and effect analysis" (FMEA) to forecast potential reasons for errors, so prevent initially, enhances the success potential of the institution's health maintenance and improvement mission.

4.6 Rationale of Addressing the Selected Study Variables

The score trends of the principal study's input scales (LXM, WL; BO) reflect the prevalence status of these factors among the studied population sample. The mean LMX score of the study group was 3.59±9.1; the higher the age, the higher the health professionals' LMX. Gellert, [32] too, studied the influence of age upon the workers' perception of LMX in physically and mentally demanding case working settings. In agreement with our age-dependent LMX findings, Gilbert [32] reported that older employees tend to have a better exchange of relationship with their supervisors; and that mediated the relationship between age and job satisfaction. Since age positively impacts LMX, older health workers in Egypt can have an important role in transmitting a sound leadership experience to coworkers, e.g., being proactive in leadership promotion education and training activities. Incorporating leadership and communication skills in continuous medical education activities on a periodic basis where the attending staff are incentivized by credit hours and further rewarded by opening to them attractive career promotion opportunities, all are creative ideas to invest the currently preferable LMX result in this healthcare population.

The mean WL score of the study population was borderline moderate (3.06 ± 0.903). Workload is another universal cause of medical errors in most health occupations and in most healthcare settings, worldwide [9]. Fortunately, the WL standard within our study boundary was only revolving around a moderate level, an encouraging situation so that with some more effort to improve the workload domain in the studied institutions WL stressor could be suppressed and a workplace with the least medical errors burden could be brought about. Workload among health professionals can vary by the type of healthcare occupation, particularly in the presence of shortage of staff, patient overflow, and shortage of logistics to satisfy the required volume of service. In our study, the mean score of WL reported by healthcare occupations ranged between 2.87 and 3.34. The highest score (3.34 ± 0.75) was reported by "other" occupation, followed by "physician" (3.23 ±0.78), followed by "nurse" (3.03 ± 0.86), "pharmacist" (3.02±1.02), and least (2.87±1.04) to encounter were health technicians.

Among the work stressors analyzed, too, BO has achieved the lowest mean score (2.27±0.78) in the main two work stressors (WL and BO) analyzed. There has been a traditional critique about using BO as a parameter for assessing the healthcare work environment and the often inherent stressors, meanwhile ignore studying the possible counter-regulatory effect of the LMX processes on the level of BO of healthcare workers [33]. Nonetheless, we can argue here this criticism claiming that our addressing the most recognizable factors affecting medical errors, particularly LMX could treat for the deficiency in the BO inquiry. Although LMX in our population shows a favorably negative, yet insignificant correlation with BO, LMX could exercise its effect on reducing the tendency for medical errors through other pathways, e.g., its direct effect on staff's ATEs. The ATEs scores are already high, especially in older workers, and also high between different health professions. More tangible effect of LMX on BO and subsequently a bolstered patient safety could be accomplished through supporting a distinguished LMX interaction throughout the health care process at the studied institutions. Thomas and Lankau [12] also supports that providing a quality leadership within the health organization's workers community may indirectly influence organizational and personal outcomes, e.g., reducing BO rate. Reduced BO burden minimizes the health workers' absenteeism due to tiredness from overwork. It also increases their job satisfaction, and ultimately promotes their loyalty to their affiliated organizations. Moreover, to the best interest of the participating institutions' outcomes, ATEs was

inversely correlated with BO level, meaning the higher ATEs the lower would be the BO tendency of staff. Until the role of LMX has been mobilized, together with other supportive approaches to minimize the BO among Cairo care providers and necessary logistics have been furnished, there is a good opportunity to work on BO through utilizing the remarkably useful relationship between ATEs and BO among the surveyed professionals.

4.7 Predictability Potential of the Selected Study Variables

Near the end of the analysis, it was useful to examine, e.g., how BO could be used to predict the probability of the change in ATEs of the study sample members. The regression model indicates that BO makes health workers unlikely to control the occurrence of medical errors on the job. On the other hand, WL was predictor for the probability of an increase in the degree of BO (β = 0.318 units, p<0.05). Van Bogaert, et al. [34] studied the relationships between nurse practice environment, WL, BO, job outcomes and nurse-reported quality of care in psychiatric hospital staff in Belgium. They found that an improved data collection model could explain 50% of the variation in job outcomes. Thereby, WL itself was a predictor for the job outcomes and enabled the model to significantly explain the variation in these variables.

In conclusion, medical errors occurrence has been s a health service “chronic syndrome”, health organizations are often barely immune to. Several types and risks for medical errors are quite preventable; yet, healthcare providers often continue to fall into the trap of error due to a multitude of reasons, many of which could have been avoided early in the care process. The price of medical errors in terms of jeopardized patient safety, lost opportunities of a better health outcome, and the cost and liability on the part of the health organization and on the overall public health system is devastating. Many hospitals and care providers have started seeking to reduce medical errors within their boundaries particularly through maintaining and improving the quality of care, especially after the era of quality and patient safety awareness, the presence of which have become basic licensing requirements at the international and local levels, and that most health organizations have to assure in order to maintain their licensed status. Interestingly, the levels of three major factors of a notable influence on the frequency of medical errors among our health professionals support the probability of rather reduced medical error load within the studied organizations. For instance, BO frequency is on the low side, and both LMX and ATEs are more than moderate level. Another finding in support of this BO situation is that it has not been related to the health workers’ occupation. Moreover**,** in the presence of such relatively low burnout trend, the health professionals may well be entitled for a relatively high level of ATEs, which is a mediator for a reduced frequency of medical errors. Alarmingly, the age profile of our participants indicates a dominance of middle age (46.0% aged 30 to <40), as the tendency for BO in this age group has been higher than older ages. However, the professionals’ age sustains a desirable LMX pattern; the latter is a strong mediator both toward burnout and ultimately medical errors frequency. A tendency for a desirable level of ATEs on the job, especially physicians is predominant. Having such positive ATEs provides a convenient opportunity to a lowered medical errors workplace. Just less than half (43%) of our staff are among those who have 5 -10 years of relevant work experience. The latter has been associated with reduced errors, e.g., experienced practitioners can make better decision about patients’ health, and handle the increasing demand for patient safety. A future medical error improvement plan, e.g., retrieving findings of the study’s regression analyses, needs to consider those factors predisposing medical errors, especially BO and WL. It is quite feasible to utilize our relatively low BO level in improving the medical errors strategy, in collaboration with other supportive measures, such as thrusting the organization’s staff communication, and an employee reward system for commitment to quality recommendations and immediate reporting of error incidents. Prevention of medical errors should go simultaneously with any measures contemplated to treat the prospected medical errors situation. Accordingly a systematized plan to prevent errors using database from this research could be established. The issue is that medical errors prevention, by far, implies many personal, cognitive, and behavior considerations health workers have to recognize in order to improve their attitude to prevention of medical errors. Specifically better leadership management and wise supervision help reduce medical errors and adverse events by those health professionals striving providing better care to their patients. All healthcare staff members are in need for continuous training in patient care focusing on medical error prevention and patient safety incidents reporting. Nursing overload should be handled with an utmost

care in order to reduce the possibility of nursing errors, e.g., investing in preparing quality nursing cadres and offering generous incentives and career promotion opportunities for distinguished calibers. Among the methods to alleviate the consequences of medical errors incidents is medical malpractice insurance. Physicians and some other health professions in Egypt are encouraged to sustain an appropriate insurance to pay off any claims and settle lawsuits brought by harmed patients. However, malpractice insurance is not the radical solution for medical errors. It may pay a portion of the cost of harm and disabilities incurred as a result of the providers' malpractice, yet, it does not restore lost lives or restore trust in the health system which could not guard its affiliates against errors and did not provide an adequate climate for an error-protected environment.

5. CONCLUSION

Medical errors occurrence has been s a health service "chronic syndrome", health organizations are often barely immune to. Several types and risks for medical errors are quite preventable; yet, healthcare providers often continue to fall into the trap of error due to a multitude of reasons, many of which could have been avoided early in the care process. The price of medical errors in terms of jeopardized patient safety, lost opportunities of a better health outcome, and the cost and liability on the part of the health organization and on the overall public health system is devastating. Many hospitals and care providers have started seeking to reduce medical errors within their boundaries particularly through maintaining and improving the quality of care, especially after the era of quality and patient safety awareness, the presence of which have become basic licensing requirements at the international and local levels, and that most health organizations have to assure in order to maintain their licensed status. Interestingly, the levels of three major factors of a notable influence on the frequency of medical errors among our health professionals support the probability of rather reduced medical error load within the studied organizations. For instance, BO frequency is on the low side, and both LMX and ATEs are more than moderate level. Another finding in support of this BO situation is that it has not been related to the health workers' occupation. Moreover**,** in the presence of such relatively low BO trend, the health professionals may well be entitled for a relatively high level of ATEs, which is a mediator for a reduced frequency of medical errors. Alarmingly, the age profile of our participants indicates a dominance of middle age (46.0% aged 30 to <40), as the tendency for BO in this age group has been higher than older ages. However, the professionals' age sustains a desirable LMX pattern; the latter is a strong mediator both toward BO and ultimately medical errors frequency. A tendency for a desirable level of ATEs on the job, especially physicians is predominant. Having such positive ATEs provides a convenient opportunity to a lowered medical errors workplace. Just less than half (43%) of our staff are among those who have 5 -10 years of relevant work experience. The latter has been associated with reduced errors, e.g., experienced practitioners can make better decision about patients' health, and handle the increasing demand for patient safety. A future medical error improvement plan, e.g., retrieving findings of the study's regression analyses, needs to consider those factors predisposing medical errors, especially BO and WL. It is quite feasible to utilize our relatively low BO level in improving the medical errors strategy, in collaboration with other supportive measures, such as thrusting the organization's staff communication, and an employee reward system for commitment to quality recommendations and immediate reporting of error incidents. Prevention of medical errors should go simultaneously with any measures contemplated to treat the prospected medical errors situation. Accordingly a systematized plan to prevent errors using database from this research could be established. The issue is that medical errors prevention, by far, implies many personal, cognitive, and behavior considerations health workers have to recognize in order to improve their attitude to prevention of medical errors. Specifically better leadership management and wise supervision help reduce medical errors and adverse events by those health professionals striving providing better care to their patients. All healthcare staff members are in need for continuous training in patient care focusing on medical error prevention and patient safety incidents reporting. Nursing overload should be handled with an utmost care in order to reduce the possibility of nursing errors, e.g., investing in preparing quality nursing cadres and offering generous incentives and career promotion opportunities for distinguished calibers. Among the methods to alleviate the consequences of medical errors incidents is medical malpractice insurance. Physicians and some other health professions in Egypt are encouraged to sustain an appropriate insurance to pay off any claims and settle lawsuits brought by harmed patients. However, malpractice insurance is not the radical solution for medical errors. It may pay a portion of the cost of harm and disabilities incurred as a result of the

providers' malpractice, yet, it does not restore lost lives or restore trust in the health system which could not guard its affiliates against errors and did not provide an adequate climate for an error-protected environment.

A continuing research on the pattern and determinants of medical errors in the Cairo health institutions, probably utilizing a hybrid methodological approach, such as sampling the medical records for detailed clinical information, reviewing morbidity and mortality reports, and interviewing stakeholders, including administrative and technical staff, is suggested. It would also be interesting to consider conducting a qualitative research project where patients, and probably health practitioners in parallel, are interviewed, their opinions, wants, needs and demands for a safe care environment are identified.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 2
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Describing Honey: Nature's Wonder of Healing

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ABSTRACT

Aim: Aim of this present study is to quantify, evaluate and assess the therapeutic benefits of honey on the treatment of recurrent apthous ulcer.
Materials and Methods: Sixty patients with minor oral ulcers were attended by Dept. of Oral Medicine and Radiology for treatment for oral ulcers. A double blinded clinical trial was carried out by topical application of honey or placebo therapy and the reduction in size and duration of complete elimination of ulcerations were noted.
Results: The ulcers had reduced in size considerably after 3 days of treatment by honey dressings, some with complete remission after another 3 days. Successful use of honey as a topical medication for decreasing healing time and pain remission in recurrent apthous stomatitis was proven in our study. However, only a weak conclusion can be drawn due to several limitations and further studies are warranted to prove its efficacy for therapeutic benefit on a boarder scale.

Keywords: Honey; Recurrent apthous stomatitis (RAS); ulceration; erythema.

1. INTRODUCTION

Natural products have been used for several years and for several purposes, honey being one of them. "Honey" which is a nectar collected from many plants and processed by honey bees, is used by human beings as a source of energy and nutrition. Honey has been used by humans since ancient times, nearly 5500 years ago [1,2]. It seems to dispense considerable benefits in wound care (chronic and infected), burns dressings, ulcers and for various systemic diseases. Utility of topical application of honey includes antifungal, anti inflammatory and antibacterial properties [3,4]. It causes faster elimination of bacterial infection, accelerates wound healing and stimulates tissue regeneration, hence restricting the use of antibiotics. Honey's acidity, osmolarity and antioxidant peculiarity have significant role in eradicating microbial infection while due to its high viscosity, a moist wound condition is maintained accomplishing its healing properties [3]. Evidence indicates that honey can exert several health-beneficial effects including antioxidant, [5] anti-inflammatory, [6] antibacterial, [7] antidiabetic, [8] respiratory, gastrointestinal, [9] cardiovascular, and nervous system [10] protective effects.

Oral mucosal wounds/mouth ulcers are sores or open lesions in the mouth which are caused by various disorders. Among them, recurrent apthous ulceration or recurrent apthous stomatitis is the most prevailing oral mucosal disease. It is signalized by periodic appreance of a painful small round or oval crater form ulceration on the mucosa of cheeks, lips, vestibule, soft palate, tongue, floor of mouth and pharynx. These ulcers have bright red circulatory inflammatory zone around the ulceration with a pseudo membrane that ranges from gray to yellow in color. These ulcers are painful and hinder mastication. Both sexes are almost equally affected with a slight higher prevalence in females. Regardless of much clinical & research attention, the clarity of etiology of RAS remains poorly understood and resulted in treatment that are largely multifactorial [3,4].

Nowadays, there is a rapidly increasing interest and research into natural health remedies and supplements with proliferations of publications now being offered on the diversity of is therapeutic

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properties. Henceforth, the aim of present study was to assess and evaluate the prophylactic use of honey as a natural wound healer.

2. METHODOLOGY

A total of 60 patients within a period of 6months with minor oral ulcers (2-6 mm) were enrolled in this study. The diagnosis was based on history, clinical features & exclusion of other similarly appearing diseases. Demographic parameters included age, gender, size of ulcer and erythema. Informed consent was obtained from all patients after thorough explanation of the efficacy of drug in the treatment of ulcers and they were instructed to spread a thin layer of medicament which was provided to them by our dept, using sterile cotton, three times daily, as a sole remedy without using any other medications. The diagnostician and the patients were blinded for the drug trial to avoid any biased results. No food, drinks, smoking, gum chewing was permitted for 30 mins after application.

Two groups of patients were examined: (Tables 1, 2, Graph 1).

Group 1: 30 patients (13 male, 17 females) were treated by honey application on their ulcers three times a day for three consecutive days.

Group 2: 30 patients (12 males, 18 females) were treated by a placebo formula three times a day for three consecutive days.

Both groups of patients were called for follow up re-examination after treatment of three days to check the response of honey and placebo therapy. The size of ulceration, character of lesions, erythema were evaluated using sterile divider and scale by a single examiner.

3. RESULTS

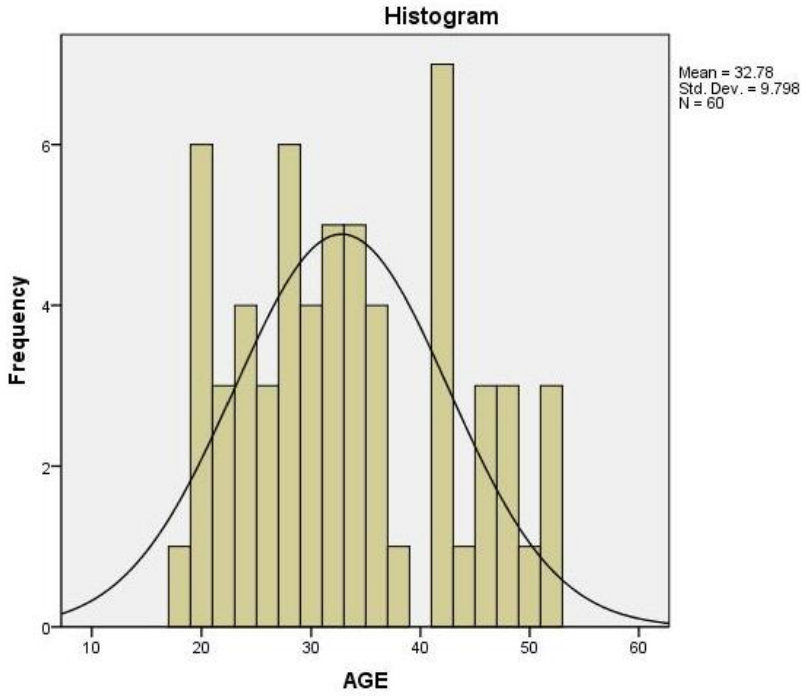
No systemic adverse reactions were noted in any case. Reduction in pain (through VAS scale) and erythema were scored as "Reduced", "Completely Healed" and "No Change". In this study, patients under group 1 showed marked reduction in size of ulcer, reduced pain as well as erythema within 3days of time interval whereas group 2 patients had reduced symptoms within 7-10 days of time interval with some of them representing no change at all. The difference in the effect of honey and placebo therapy on the healing time was significant (Table 3, Graph 2, Figs. 1, 2). T test was done for comparison of pain scores between two groups and *p*- value for honey treated group compared to placebo group was significant in ulcer size, pain and erythema.

Table 1. Gender wise division of subjects in two groups

Group			Frequency	Percent	Valid percent	Cumulative percent
Honey	Valid	Male	13	43.3	43.3	43.3
		Female	17	56.7	56.7	100.0
		Total	30	100.0	100.0	
Placebo	Valid	Male	12	40.0	40.0	40.0
		Female	18	60.0	60.0	100.0
		Total	30	100.0	100.0	

Table 2. Mean age of subjects

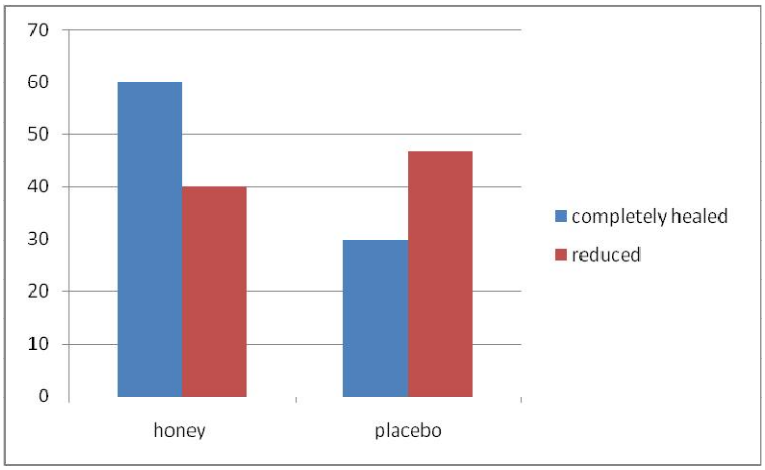
N	Valid	60
	Missing	0
Mean		32.78
Std. deviation		9.798



Graph 1. Mean age of subjects

Table 3. Patient response in two groups

Group			Frequency	Percent	Valid percent	Cumulative percent
Honey	Valid	Completely healed	18	60.0	60.0	60.0
		Reduced	12	40.0	40.0	100.0
		Total	30	100.0	100.0	
Placebo	Valid	Completely healed	7	23.3	23.3	23.3
		No change	9	30.0	30.0	53.3
		Reduced	14	46.7	46.7	100.0
		Total	30	100.0	100.0	



Graph 2. Patient response in two groups

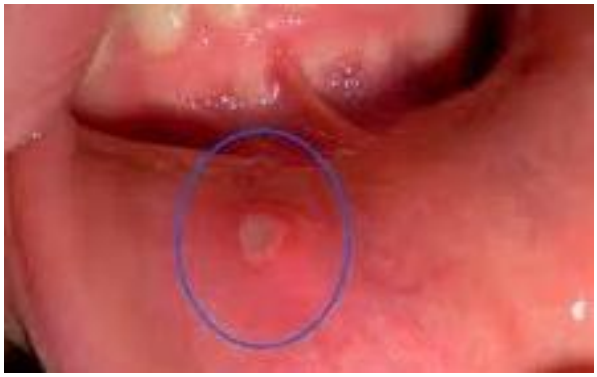


Fig. 1. Unhealed ulcer

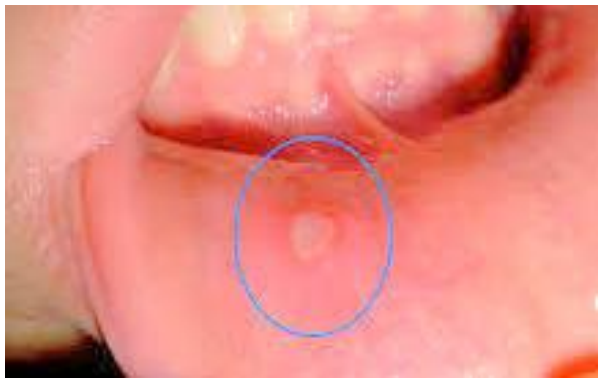


Fig. 2. Reduced erythema

4. DISCUSSION

Honey is a super saturated nectar collected by bees from a wide variety of plants [3]. Honey is an ancient remedy for the treatment of infected wounds, which has recently been 'rediscovered' by the medical professionals, particularly where conventional modern therapeutic agents fail. The first written reference to honey, Sumerian tablet writing, dating back to 2100-2000 BC mentions honey use as a drug and an ointment. The immuno-modulatory property of honey is relevant to wound repair too. In addition, honey is hygroscopic, which means that it can draw moisture out of the environment and dehydrate bacteria, and its high sugar content and low level pH also prevent the microbes from growing [4].

The natural anti-oxidants and flavonoids exhibit a wide range of biological effects including antibacterial, anti-inflammatory, anti-allergic, anti-thrombotic and vasodilatory action [11]. The properties of moisture retention, antimicrobial and angio-genetic activity, and granulation tissue formation and epithelialisation have also been confirmed by in-vitro and animal studies [12-14]. Honey also contains other bioactive constituents such as organic acids, ascorbic acid, trace elements, vitamins, amino acids, proteins and a total of approximately 200 components [15].

Honey is easy to apply, painless and comfortable, harmless to tissues, creates a moist healing environment, is antibacterial and stimulates healing and epithelialisation. Its antibacterial activity is due to the high osmolarity created by its sugar content, some is due to hydrogen peroxide released by exudate, and some is due to photochemical that come from the nectar of plants [16]. Hydrogen peroxide is the major contributor to the antimicrobial activity of honey, and the different concentrations of this compound in different honeys result in their varying antimicrobial effects [17]. In addition, its physical properties provide ideal moist conditions for healing and a stimulating effect on the growth of

wound repair tissues. Unlike other antiseptics, honey has no harmful effects on tissues because slow enzymatic production of hydrogen peroxide is one thousandth hydrogen peroxide 3% [18].

Though honey has a valued place in traditional medicine for centuries and its therapeutic potential of uncontaminated, pure honey is grossly underutilized. It is widely available and although the mechanism of action of several of its properties remains obscure, the time has now come for conventional medicine to lift the blinds off this traditional remedy and give it its due recognition, as we did in our study [19].

Recurrent aphthous stomatitis (RAS) is the most common form of recurrent oral ulceration, reportedly affecting up to 20% of the population. A more typical pattern of recurrent oral ulceration will be characterized by periods of ulceration with remissions between bouts of ulceration. The diagnosis and management of the patient with recurrent apthous stomatitis requires a systematic approach based on the adequate history, clinical examination, investigations as appropriate, institution of management and, finally, review to allow for any necessary modifications of that management. So a thorough history, clinical evaluation and investigations were carried out in this study prior to implementating treatment [20].

It is a chronic inflammatory, ulcerative condition of the oral mucosa and the higher incidence of RAS in people with a higher socio-economic status is observed. Due to unclear etiopathogenesis of the disease, the treatment is mainly symptomatic, not very effective and does not prevent their recurrences [21]. Since the etiology and pathogenesis of RAS remain unclear, there is currently no consensus regarding a definitive curative therapy. The commonly accepted treatment strategy is to lessen the pain and duration of lesions [22]. Topical corticosteroids, antibiotics, and analgesics are highly recommended for patients with RAS [23]. However, longer treatment and frequent exposure to these medications may cause fungal infection and drug resistance, which may further lead to more severe adverse effects or even life-threatening complications [24].

The plethora of treatment used for the treatment of oral ulcerations is testament to the lack of any single effective treatment [20]. So here honey is tried as a topical medication for its natural and beneficial properties [25].

Natural herbal medications are on the rise for its negligible side effects as Ghalayani et al. [26] reported a significant difference in the mean healing time between 8.6±0.99 days of placebo treatment and 5.3±0.81 days of treatment with *Punica granatum* extract ($P>0.001$). In yet another study, it was found that Tian-zhu aerosol oral rinse remarkably reduced the duration of the ulcer compared with chlorhexidine rinse ($P>0.05$) [27].

In a double blinded randomized clinical trial of a total of 75 eligible adult participants conducted in 2011, it was suggested that Oral mucositis can be successfully treated by a combination of honey and coffee as an alternative medicine, though the impact may be due to the synergistic effect of these two substances. This study is in collaboration with our study suggesting honey as a novel topical drug and an antioxidant [28].

In a meta-analysis done, it was showed that all the studies reported a statistically significant reduction of the ulcer size, shortening ulcer duration and remission of pain using visual analog scale without severe complications in patients receiving herbal medicine therapy compared with the controls. In the study, the author focused on the efficacy and safety of the topical application of natural herbal medicines for the treatment of RAS. A total of 1,515 subjects in 13 clinical trials were analyzed in the present meta-analysis. Thus, there is some evidence to suggest that topical herbal medicine therapy is an effective and safe alternative option to current Western medicine-based treatments for RAS [29].

In patients with constant and aggressive outbreaks (major aphthae), pain is intense and topical treatment is unable to afford symptoms relief. Systemic therapy is indicated in such situations, in the form of corticosteroids (prednisone) or thalidomide, among other drugs, but its severe adverse reactions are well documented. In view of the above facts, natural topical products like honey which is readily available, can be promoted with almost no side effects [30]. The glycosylated proteins present

in honey can induce TNF-a secretion by macrophages, and this cytokine is known to induce the mechanism of wound repairing. Furthermore, the ability of honey to reduce 'reactive intermediates' release may well limit the tissue damage by activated macrophages during wound healing of ulcers. Thus, the immune-modulatory property of honey is relevant to wound repair and healing of ulcer [31]. In a case control study conducted on 56 patients in Iran, Salivary malondialdehyde MDA level was significantly higher ($p<0.001$) and total antioxidant capacity (TAC) level was significantly lower ($p<0.042$) in RAS as compared with the control group indicating the alteration of oxidant/antioxidant status in recurrent aphthous stomatitis. It seems that the increase of oxidative stress may lead to imbalance of oxidant / antioxidant status which is crucial in inflammatory mechanism of RAS. Since honey is known to have antioxidant property, it appears useful in cases of recurrent apthous stomatitis, so was used in the present study [32]. Honey is also used in radiation induced mucositis which is more severe and grave compared to recurrent apthous stomatitis, and has proved to be successful in reducing the size of ulcerative areas by its use for a two-week period. The research subjects who were studied by use of the honey as a mouthwash felt calm during the research and expressed that using honey had led to the relief of pain and had soothing effect on the ulcerative sites in the oral cavity [33]. Thus honey is a safe, satisfying and effective healing agent and also in its natural form, it is extremely cost-effective.

5. CONCLUSION

Herbal medications such as honey are not only natural, safe, soothing but cost effective too and the most ancient of wound treatments, it is taking its place in modern age wound care. Successful use of honey as a topical medication for decreasing healing time and pain remission in recurrent apthous stomatitis was proven in our study. However, only a weak conclusion can be drawn due to several limitations and further studies are warranted to prove its efficacy for therapeutic benefit on a boarder scale.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 3
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Studies on Douching Practices among Hausa-Fulani Pregnant Women with and without Bacterial Vaginosis in Zaria, Northwest Nigeria

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DOI: 10.9734/bpi/ctmamr/v10

ABSTRACT

Douching, or the process of applying liquid or semi-liquid, powdery or herbal substances into the vagina for various reasons may have been an age-old practice in *many* cultures
Objective: The objective of this study was to examine patterns of douching practices and their association to vaginal infection among Hausa-Fulani pregnant women in Zaria, Northwest Nigeria.
Study Design: This health facility-based study was a descriptive cross-sectional investigation, with laboratory analysis for bacterial vaginosis and other vaginal flora.
Results: Of 220 participants, 85.5% consented to regular douching practices. Commonly identified methods of douching were using hand to insert plain water (80.0%), insertion of toilet soap (55.0%), using warm water plus disinfectant/salt/ black soap (18.6%) and using a jet or stream of water (8.6%). Frequent douching was associated with douching during bathing (69.5%), after passing urine (34.1%), after sexual intercourse (16.4%), before sexual intercourse (5.9%) and at any other times (6.8%). Pregnant women who douche using fingers to insert plain water were over 1½ times more likely to have bacterial vaginosis (χ^2=1.30, P-value=0.25, OR=1.67, 95% CI: 0.69, 4.09) and those who douche after sexual intercourse were about 3½ times more likely to develop Bacterial vaginosis (χ^2=8.88, P-value=0.003, OR=3.42, 95% CI: 1.47, 7.93). Douching during bathing and after sexual intercourse were more prevalent among subjects aged Bacterial vaginosis positive women aged 30-34 years (100.0%) and those aged 35-39 years (75.0%) respectively.
Conclusions: The practice of douching was common among the Hausa-Fulani ethnic group in Nigeria. the Hausa-Fulani ethnic group is just one out of hundreds of ethnic groups in Nigeria and findings in this study do not necessarily apply to douching practices or vaginal infection in other ethnic groups or other geo-political locations of the country. Further studies are desirable to confirm douching practices and various vaginal pathology for effective control, education, and management of female genital tract. A prospective study to look at the effect of douching on pregnancy outcome is desirable in the future.

Keywords: Douching; Hausa-Fulani; vaginal.

1. INTRODUCTION

Douching, or the process of applying liquid or semi-liquid, powdery or herbal substances into the vagina for various reasons may have been an age-old practice in many cultures [1]. Douching probably originated from the time when the vagina was regarded as unclean and women menstrual period was erroneously viewed as a filthy process. Like any other orifice of the body such as the eyes, ears, mouth, nose or rectum, the vagina also possesses natural systems to keep itself healthy. Just like the eyes secrete tears from lacrimal glands, the ears produce wax, the nostrils produce mucous and the mouth produces saliva, the vaginal produces its own organic secretions that are expected to kept it clean. It also has its own acid pH of 3 produced by glycogen degradation to lactic acid in

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vaginal epithelial cells functioning as a defense mechanism against invading pathogens. The surfaces of vaginal walls, which are usually moist, are easily separable due to lubrication by about 1 ml of basal vaginal fluid (BVF). Sources of BVF which collect in the vagina are from the peritoneal fluid, fluid from the entire uterine system which includes the follicles, the fallopian tubes, the uterus itself, the cervix and the vaginal secretion. Other sources of vaginal BVF are the Bartholin's and Skene's gland. It is uncommon for women to douche daily; sporadic douching is more common [2-4].

The epidemiology of douching, women's behavioral pattern to it, substances used and reasons behind douching as well as its health implication have been studied extensively, mostly outside of Africa [5,6]. Surveys have shown that douching practice was common in the United States [7,8] Martino, et al. stated that [9] douching prevalence varies according to race, and age cohort and Diclemente argued that increased age [10], lower socioeconomic status, and older partners are probable relevant risk factors for douching among African American adolescents. Major critical reviews of douching data suggest that current evidence is strong enough to conclude that douching can be harmful and is associated with pelvic inflammatory disease (PID), ectopic pregnancy, and other morbid conditions [11-14].

One study in Turkey found that many women who practiced vaginal douching believed in its health benefits [15]. Another study reported age, religion, place of residence, and poverty influence behavioral attitudes towards douching and that most women who practiced douching alleged it to be a routine conduct [16]. The belief of some adult women in the southeastern United States is that douching is necessary for good hygiene [8]. Numerous research studies confirm that women of African American ancestry douche more than others [17-19].

Bacterial vaginosis and vaginal douching are both reported to be more common in African-American and Caribbean than white women [20]. Surveys of adolescents and adults who douche reveal that women are more likely to douche if they have a history of one or more sexually transmitted diseases (STDs), including HIV and herpes simplex type 2 infection [21-24]. While various studies associate douching with various sexually and non-sexually transmitted infections of the genital tract such as Bacterial vaginosis [25], Chlamydia trachomatis [26], pelvic inflammatory disease [27] and to gynecological pathologies such as endometriosis and upper genital tract infection [28], a study actually documented a beneficial effect of douching in prevention against cervical cancer [29] and another contested any association between douching and pelvic inflammatory disease [30].

Apart from infertility and fibroid, vaginal indications such as abnormal odor, either accompanied by discharge or not, seem to be cause of gynaecological visits or referral [31]. Earlier publications declared that the occasionally unpleasant odor from normal vaginal fluid [32] probably emanates from presence of infection with Bacterial vaginosis [20], though in some cases neither bacterial vaginosis nor any other invading pathogens are confirmed in symptomatic women [33,34]. Invariably, the practice of douching has been associated with vaginal odor [35].

Although one study opined that vaginal douching and vaginal substance use is a common practice in sub-Saharan Africa [36], douching practices among various ethnic groups in West Africa have not been widely studied hence scanty data on douching is available in this geographical location. Therefore, the main objective of this study was to explore vaginal douching practices among a cohort of pregnant women in Nigeria. Information gather can help as in planning a more robust study on douching and its perceived association with personal hygiene, genital infection and other gynecological and obstetric pathologies not only in pregnancy but prior to or after a woman has delivered.

2. METHODS

Regular Antenatal Clinic (ANC) holds once weekly at Ahmadu Bello University Teaching Hospital, Shika area of Zaria City, Kaduna State of Nigeria. Between 40 and 60 women were usually seen at ANC where pregnancies were not only monitored for any present or impending anomaly but the pregnant women were also screened for high blood pressure, proteinuria and infections such as HIV. In the 3-month period of April and June of 2008, a total of 244 pregnant women consulting for the first

time were seen at this ANC and, in addition to the regular clinical examinations, were also screened for bacterial vaginosis. In addition, questionnaires were served to these pregnant women to assess douching practices among this specific ethnic group.

The study was cross-sectional and descriptive. Pregnant woman who presented at the ANC for the first time, were admitted into the study. The tripartite study included (i) clinical examination (ii) laboratory investigation for Bacterial vaginosis and (iii) questionnaire administration to all eligible patients.

The study population was pregnant women who agreed to participate in the study and who were residing in Zaria City in Kaduna State, one of the five divisions of the Northwest geo-political zone in Nigeria. The Hausa-Fulani ethnic group, who dominate most parts of these states, are mostly traders, herdsmen and farmers, though some work in government offices as administrators.

Each consecutive pregnant woman who were attending their first ANC into the study was recruited into the study. Inclusion criteria were then (i) attending ANC for the first time (ii) resides in Zaria and not just visiting (iii) black indigenous Nigerian (iv) willing to participate in the study. The exclusion criteria were (i) those with severe systemic diseases such as diabetes mellitus, high blood pressure demonstrated kidney diseases (ii) presence of uterine anomaly and placenta abruption and cervical incompetence (iii) multiple pregnancy (iv) antibiotic consumption two weeks prior to study.

Baseline demographic information and outline of reproductive, gynaecological and medical history of each patient was recorded. The questionnaire focused on douching practices and any adverse event in current pregnancy.

At first point of contact at the ANC, the study subjects were briefed about the study, instructed on the importance of coming early to ANC, briefed about the study and encouraged to participate. It was clearly explained to them that they would still enjoy full benefit of ANC should they decide not to participate in the study. The advantages of participating in the study was also clearly explained to them. Only 4 (1.8%) women of the 224 women who were initially recruited decided not to participate the main reason being that their husband was away and had had not been informed.

2.1 Privacy and Confidentiality

Confidentiality was maintained by interviewing, performing clinical examination and collecting specimen for laboratory analysis from each pregnant woman in a private, well-lit and cool consulting room.

2.2 Questionnaire Study on Douching

The structured questionnaire was initially pre-tested and adjusted to fit the respondents. The questionnaire was at first constructed in English language and later translated into Hausa language and back into English language. Mostly female health workers, especially trained for the purpose, explained the study to the pregnant women, screened each one of them for eligibility, obtained informed consent and recorded responses of the pregnant women on the questionnaire served. Douching practices, especially method and time of douching were extracted from the pregnant women.

2.3 Collection of Specimen

To collect vaginal swabs, a sterile, un-greased speculum was inserted into the vaginal orifice and opened to expose the vaginal canal and the cervix. The pH of vaginal secretion was measured using a pH strip. Thereafter, a dry, sterile cotton on stick was used to collect secretion or substances from the posterior fornix of the vagina. This wet smear of vaginal secretion from the posterior fornix was

then mixed with normal saline and examined under an electric microscope with magnification power of x400. This was to identify (i) clue cells and (ii) bacteria morphocytes. Each sample was then processed per Amsel and Nugent criteria and cultured for the offending organisms. Ancillary investigations included screening for anemia and for Human Immuno-Deficient Virus (HIV).

2.4 Diagnosis

Classical Amsel's criteria and Nugent score were used to ascertain diagnostic confirmation of Bacterial vaginosis. Using Amsel's criteria, a minimum of three out of four criteria should be present for diagnosis of to be confirmed. These 4 criteria are (i) thin, white homogenous discharge, (ii) clue cells on microscopy, (iii) pH of vaginal fluid greater than 4.5, and (iv) release of fishy odor on adding alkali (10% potassium hydroxide) or positive whiff test.

Nugent score, the main diagnostic test, approximates comparative quantity of bacteria morphotypes, apportioning scores of between 0 and 10. While a score >6 is confirmatory of Bacterial vaginosis, score <4 indicates normal vaginal flora and score of 4-6 is intermediate.

2.5 Data Management

Data collected was entered into a laptop computer and coded accordingly. Data analysis was conducted using SPSS computer software version 15.0. Frequency tables were constructed for variables that were categorical. Cross-tabulations, Student's t-test and χ^2 (Chi-square) analysis were employed where appropriate. Significance was considered at a p-value of <0.05. The resulting data were illustrated as Tables and figure.

2.6 Ethical Considerations

Ethical considerations involved taking verbal informed consents from the pregnant women enlisted in the study. Data confidentiality and patient anonymity and privacy were ensured by coding and by not using the name of any participant in the study. The Ethical Committee of ABUTH Zaria approved the study protocol.

3. RESULTS

Approximately 34% of the 220 pregnant women who were seen at the Antenatal Clinic of the tertiary health facility where this study was carried out were aged 25-29 years. Surprisingly, the proportion of those aged =40 years was higher (14.6%) than those aged 35-39 years (12.3%) (Table 1). Most of the subjects (32.7%) had secondary education, however, about 32% did not have any formal education while only 13.6% attained postsecondary education. Almost 40% of them were housewives with no other occupation and 35.9% were engaged in a polygamous matrimony. Only 3.1% of the pregnant women reported for ANC in the first trimester of pregnancy, indicating that early medical check-up is rare until the second trimester (81.3%) among the ethnic group of study.

Laboratory analysis of vaginal smear indicated that 32 (14.5%) of the pregnant women were positive for Bacterial vaginosis and HIV screening showed only 4.5% were positive. The highest proportion of pregnant women with pH of =4.5 was among those aged 25-29 years. This age group also had the highest proportion of women with Clue cells present (40.6%), positive Whiff test (45.1%), vaginal discharge (30.2%) and positive Bacterial vaginosis (50.0%). The highest proportions of pregnant women with Clue cells present (46.9%), positive Whiff test (34.5%), vaginal discharge (42.9%), Bacterial vaginosis (34.4%) and HIV positivity (60.0%) were found among those with no formal education (Table 2). The Table also illustrates morbidity patterns of study subjects by various educational levels, indicating that the highest proportions of pregnant women with Bacterial vaginosis (34.4%), HIV positivity (60.0%) vaginal discharge (42.9%) and positive Clue cells (46.9%) were among the group with no formal education.

Table 1. Socio-demographic characteristics of respondents

Variable	Item	Frequency	%
Age group	20-24	57	25.9
	25-29	74	33.6
	30-34	30	13.6
	35-39	27	12.3
	≥40	32	14.6
Formal Education	Non	70	31.8
	Elementary	48	21.8
	Secondary	72	32.7
	Post-secondary	30	13.6
Occupation	Trader	41	18.6
	Government worker	17	7.7
	Artisan	59	26.8
	Student	16	7.3
	Housewife	87	39.6
Matrimony	Monogamous	141	64.1
	Polygamous	79	35.9
Number of children	0	42	19.1
	1	36	16.4
	02-Apr	84	38.2
	≥5	58	26.4
Trimester	First	11	3.1
	Second	172	81.3
	Third	37	15.6

Using fingers of the hand to insert plain water was the most common method of douching (153, 80.0%) followed by insertion of toilet soap (121, 55.0%). Using a jet or stream of water was the least prominent method (19, 8.6%) of douching (Table 3). Pregnant women who used fingers to insert plain water for douching (25,78.1%) were 1.67 times more likely to be positive for bacterial vaginosis than those who use other methods of douching. The most prevalent time of douching was during bathing (153, 69.5%) followed by after urinating (75, 34.1%). Those who douched after sexual intercourse were about 3½ times more likely to have bacterial vaginosis than those douched at other times (χ^2=3.42, P-value=0.25, OR=1.67, 95% CI:0.69, 4.09). Incidentally, those who douched before sex were only 1.8 times more likely to have bacterial vaginosis when compared to those who douched during bathing, after urinating or at other times.

Table 2. Clinical findings by age group (years) and by educational status

Variable	Item	Characteristics of vaginal fluid (pH of vaginal fluid)		Clue cell		Whiff test		Vaginal discharge on examination		Bacterial Vaginosis		HIV status	
		≤4.5	>4.5	Present	Absent	Positive	Negative	Yes	No	Positive	Negative	Positive	Negative
		Freq. (%)	Freq.(%)	Freq. (%)	Freq. (%)	Freq. (%)	Freq. (%)	Freq. (%)	Freq. (%)	Freq. (%)	Freq. (%)	Freq. (%)	Freq. (%)
All pregnant women		15 (6.8)	205 (93.2)	64 (29.1)	156 (70.9)	113 (51.4)	107 (48.6)	63 (28.6)	157 (71.4)	32 (14.5)	188 (85.5)	10 (4.5)	210 (95.5)
Age group (years)	20-24	3 (20.0)	54 26.3)	12 (18.8)	45 (28.8)	29 (25.7)	28 (26.2)	17 (30.2)	40 (25.5)	8 (25.0)	49 (26.1)	3 (30.0)	54 (25.7)
	25-29	5 (33.3)	69 (33.7)	26 (40.6)	48 (30.8)	51 (45.1)	23 (21.5)	17 (30.2)	57 (36.3)	16 (50.0)	58 (30.9)	2 (20.0)	72 (34.3)
	30-34	3 (20.0)	27 (13.2)	9 (14.1)	21 (13.5)	9 (8.0)	21 (19.6	10 (15.9)	20 (12.7)	4 (12.5)	26 (13.8)	3 (30.0)	27 (12.9)
	35-39	1 (6.7)	26 (12.7)	10 (15.6)	17 (10.9)	14 (12.4)	13 (12.1)	15 (23.8)	12 (7.6)	4 (12.5)	23 (12.8)	2 (20.0)	25 (11.9)
	≥40	3 (20.0)	29 (14.1)	7 (10.9)	25 (16.0)	10 (8.8)	22 (20.6)	4 (6.3)	28 (17.8)	0 (0.0)	32 (17.0)	0 (0.0)	32 (15.2)
Educational status	No formal education	3 (20.0)	67 (32.7)	30 (46.9)	40 (25.6)	39 (34.5)	31 (29.0)	27 (42.9)	43 (27.4)	11 (34.4)	59 (31.4)	6 (60.0)	64 (30.5)
	Elementary	5 (33.3)	43 (21.0)	22 (34.4)	26 (16.7)	29 (25.7)	19 (17.8)	20 (31.7)	28 (17.8)	9 (28.1)	39 (20.7)	2 (20.0)	46 (21.9)
	Secondary	3 (20.0)	69 (33.7)	9 (14.1)	63 (40.4)	34 (30.0)	38 (35.5)	11 (17.5)	61 (38.9)	9 (28.1)	63 (33.5)	1 (10.0)	71 (33.8)
	Post-secondary	4 (26.7)	26 (12.7)	3 (4.7)	27 (17.3)	11 (9.7)	19 (17.8)	5 (7.9)	25 (15.9)	3 (9.4)	27 (14.4)	1 (10.0)	29 (13.8)

Douching practices in terms of method (Fig. 1) and timeperiod (Fig. 2) and in terms of presence or absence of Bacterial vaginosis were assessed per age group and educational status, as shown in Table 4. Overall, using fingers to insert plain water was more common in age 25-29 (90.5%) and in those with no formal education. However, insertion of toilet soap was practiced more in younger age group of 20-24 (71.9%) and among those with elementary educational status (60.4%). Douching before or after sexual intercourse was observed more in age group 35-39 years (14.8% and 29.6% respectively) and in those with secondary (6.9%) and elementary (22.9%) educational levels respectively. Among those with bacterial vaginosis, using finger to insert plain water was most prevalent in age group 25-29 (93.8%) and among those with no formal education (100.0%) while insertion of toilet soap was found more in age group 30-34 years (75.0%) and among those with post-secondary education (66.7%). All women aged 30-34 years (100.0%) and 9 (81.8%) of those with formal education reported douching during bathing. Douching after sexual intercourse was noted more in pregnant women aged 3539 (75.0%) and among those with primary (44.4%) and secondary (44.4%) education respectively.

Table 3. Prevalence, method, and time of douching by presence or absence of bacterial vaginosis

Variable	Item	Total (%)!	Bacterial vaginosis positive (n=32, 14.5%)		Bacterial vaginosis negative (n=188, 85.5%)		χ^2 (P-value)	OR (95% CI)
			Freq.	%	Freq.	%		
Douching practices	Yes	188 (85.5)	26	81.3	162	86.2	0.53 (0.47)	0.70 (0.26, 1.85)
	No	32 (14.5)	6	18.7	26	13.8		
Method of douching	Using fingers to insert plain water	153 (80.0)	25	78.1	128	68.1	1.30 (0.25)	1.67 (0.69, 4.09)
	Insertion of toilet soap	121 (55.0)	13	40.6	108	57.4	3.13 (0.08)	0.51 (0.23, 1.09)
	Using warm water and disinfectant/salt/black soap	41 (18.6)	5	15.6	36	19.1	0.22 (0.64)	0.78 (0.28, 2.17)
	Using a jet or stream of water	19 (8.6)	2	6.3	17	9	0.03 (0.86)	0.67 (0.15, 3.05)
Time of douching	During bathing	153 (69.5)	21	65.6	132	70.2	0.27 (6.0)	0.81 (0.37, 1.79)
	After urinating	75 (34.1)	7	21.9	68	36.2	2.49 (0.11)	0.49 (0.20, 1.20)
	After sexual intercourse	36 (16.4)	11	34.4	25	13.3	8.88 (0.003)	3.42 (1.47, 7.93)
	Before sexual intercourse	13 (5.9)	3	9.4	10	5.3	0.24 (0.62)*	1.84 (0.48, 7.09)
	At other times	15 (6.8)	2	6.3	13	6.9	0.06 (0.81)	0.90 (0.19, 4.18)

!=Multiple responses; *Fisher's exact test

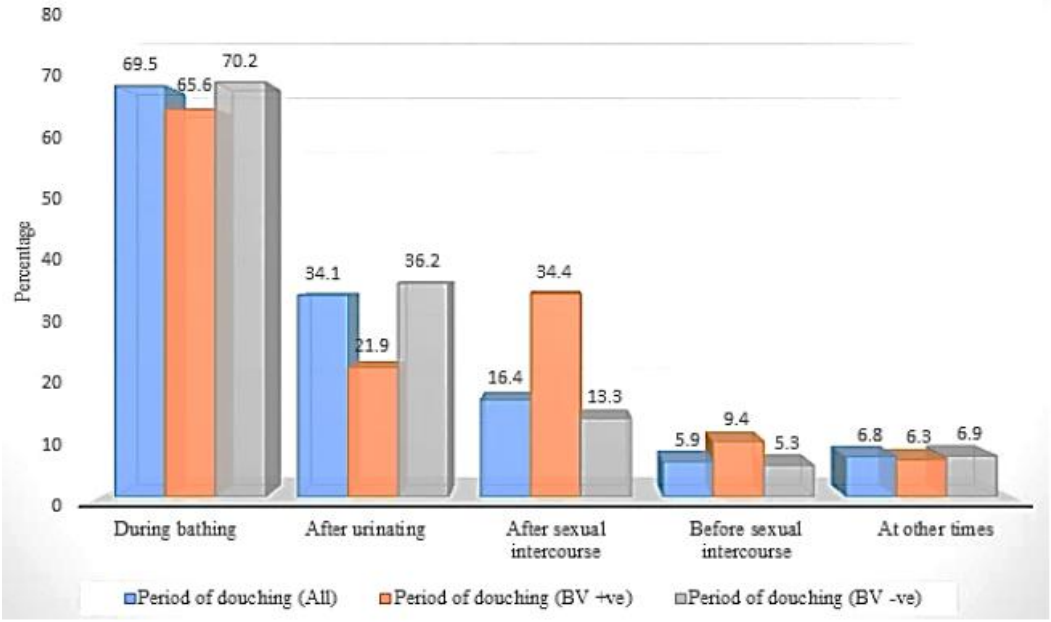


Fig. 1. Period of douching among Bacterial vaginosis positive and negative pregnant women

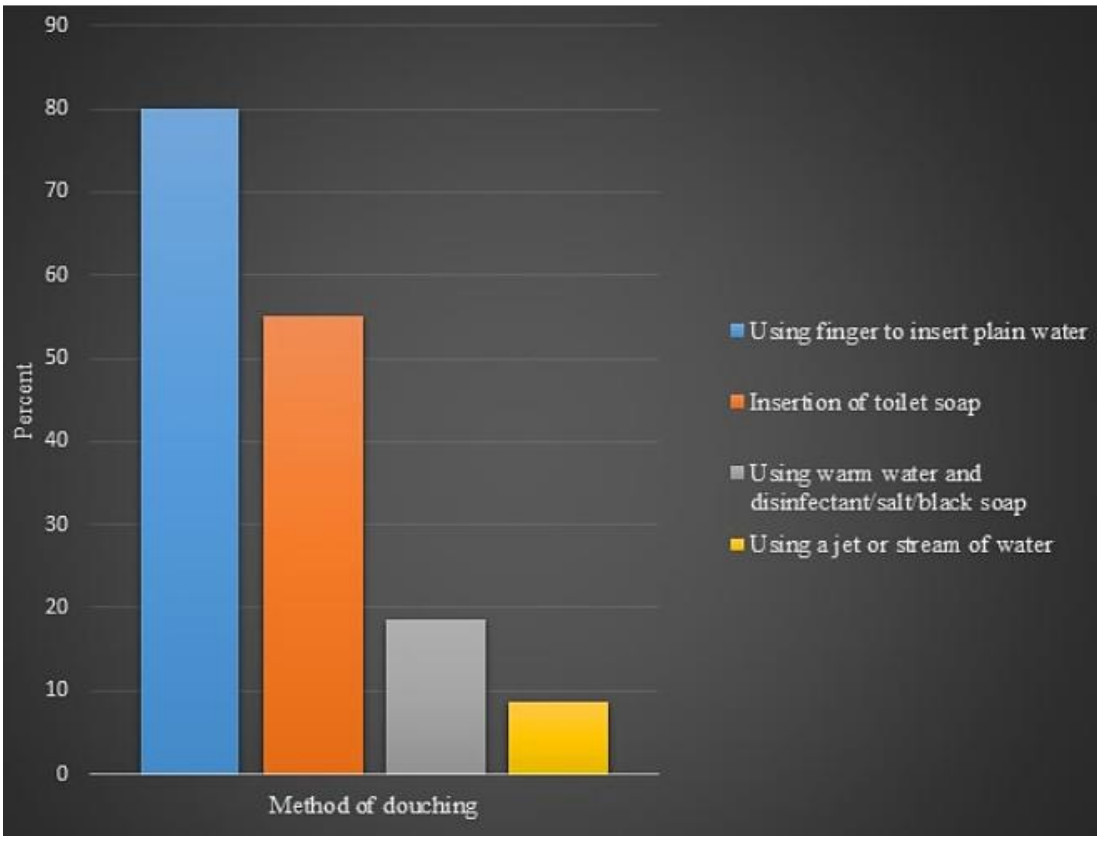


Fig. 2. Percent distribution of douching methods among pregnant women in Zaria

However, among those without Bacterial vaginosis, using finger to insert plain water for douching was also very prominent among those aged 25-29 (89.7%) and those without formal education (83.1%). Douching during bathing was found more among those in the age group of 35-39 (75.0%) and those with no formal education (89.8%).

Fig. 3 shows morbidity pattern by age groupings depicting Bacterial vaginosis to be most prevalent among pregnant women aged 25-29 years but HIV positivity to be highest in age groups 20-24 (30.0%) and age group 30-34 (30.0%) respectively.

4. DISCUSSION

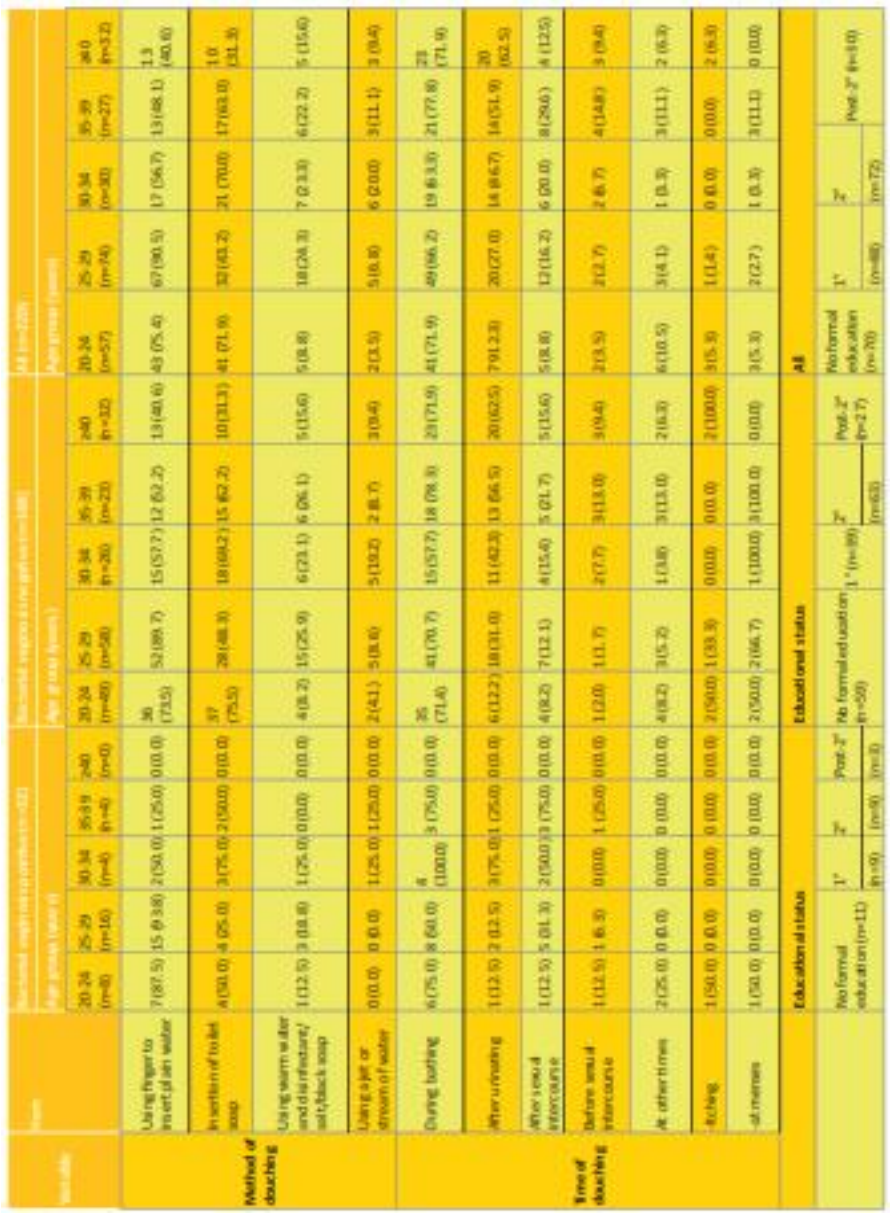
It may seem quite natural for women in reproductive age group to douche either regularly or periodically. Whether douching is necessary or not or whether it is a tradition handed down from the past is still contentious. Since the vagina produces secretions at various times and since menstrual flow exit through the vagina, it might seem customary for women to douche. Douching may have started early in life while mothers bathe their newborn females. At such instances, especially in sub-Saharan Africa, powdery substances are often administered over parts of the baby's body including the female genitalia.

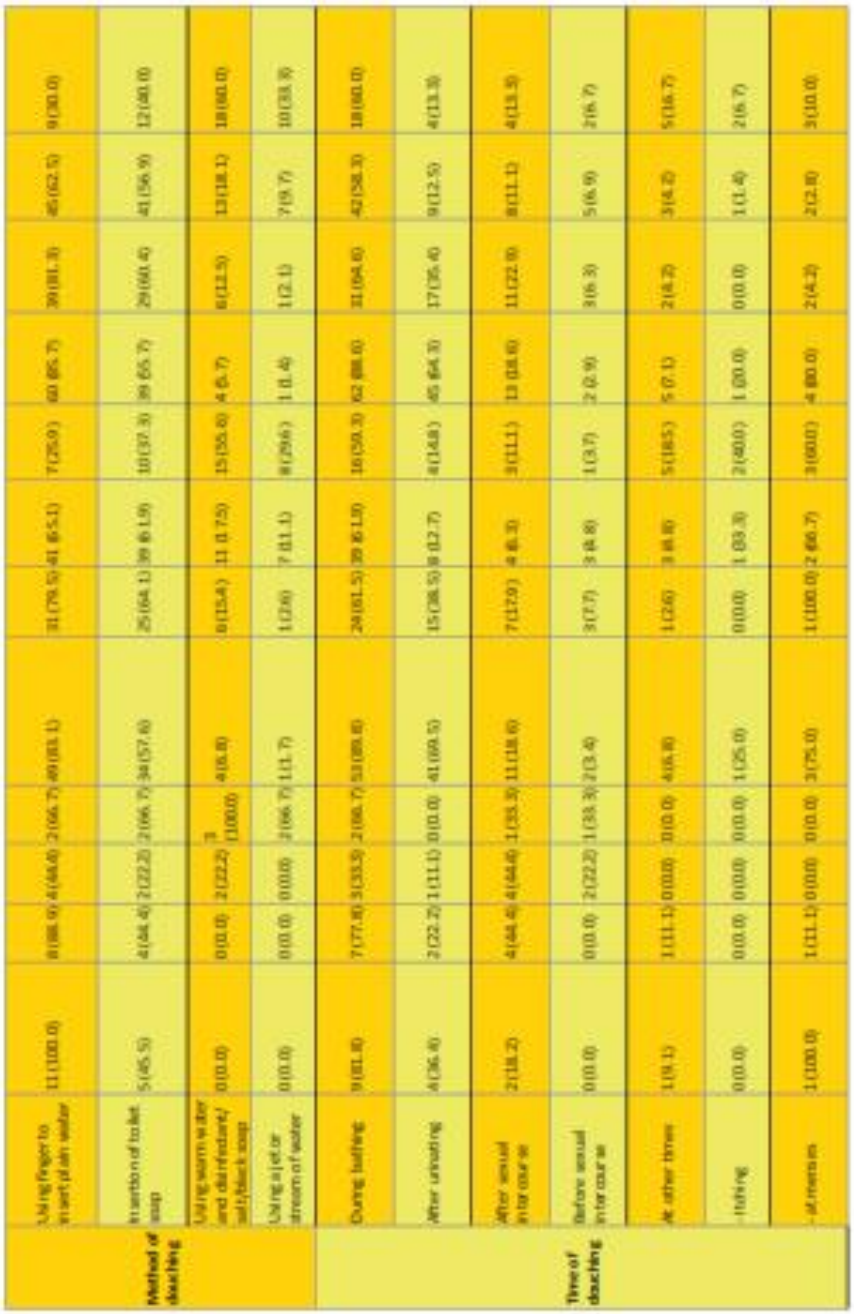
The exact age when douching starts is not yet established. Foch et al. reported an average age of 17 years among women who douche in USA which was confirmed by [37]. However, it seems douching probably commences at the time of debutant into sexual activity and continues till older age but for different reasons. One of the possible reasons for douching in younger age may be to wash out semen after sexual intercourse to prevent getting pregnant if condom is not used. This study however

observed that douching was more prevalent in younger pregnant women than in those in their 30's or 40's. It is supposed that pregnancy may be the outcome of a douching failed to prevent it.

One of the main findings of this study high prevalence of douching among the Hausa-Fulani ethnic group of Northern Nigeria. The 85.5% prevalence of douching found in this study was higher than the 70% reported in Egypt [38], the 58.5% reported among African American women in child-bearing age [39], close to the 90% documented in a South African study [40] but lower than the 97% reported in Cote d'Ivoire (La Ruche et al. 1999). Vaginal douching appears to be common especially among Black women of African descent in general, by a third of women in the USA [41,42] as well as in a large proportion of women in Turkey [15].

Table 4. Douching practices by age group and educational status among bacterial vaginosis positive and negative pregnant women





Using fingers to insert plain water was found to be the most common method of douching among as reported by 80% of women in this study. Very few other studies reported douching with plain water. Incidentally, bacterial vaginosis was found more among those aged 25-29 years with no formal education, who used fingers to insert plain water than among any other sub-group. Education is one of the various factors which impact women's douching behaviors, the others being social and cultural influences (Cottrell). It is possible that using fingers to insert plain water for douching may have a causal relationship with vaginal infections, such as Bacterial vaginosis observed in the patients. Plain water is not always free from pathogens, neither are hands. This may be the most important singular iatrogenic factor by which pathogens are introduced to the vagina, especially in early age groups between 20-29 years when douching was found to be more common. Using fingers to insert plain

water may not be associated with 'dry sex', a process of drying and tightening the vagina in preparation for sexual intercourse as reported by Bekinska, et al. but to personal hygiene of the vagina or when there is an indication of a sexually transmitted infection [43]. Selfmedication is rampant in Nigeria [44] and douching may be the first step in home-management of an impending serious vaginal infection prior to clinic attendance. Douching was observed to be less common before than after sexual intercourse, a finding in line with the suggestion that frequent douching is significantly more associated with douching after sex [45]. This may lend credence to the notion of douching for cleanliness.

It is surprising that method of douching also differed by educational status. Douching was observed more among women with no formal education or among those with only primary education. This agrees with other studies that douching is more prevalent among females of low educational [22,46] and socioeconomic status [10]. However, our result of douching being more prominent in younger age groups was in contrast with what Declemente et al. reported [10].

Dettol (chloroxylenol, 4 mg/100 ml) used by 18.6% of pregnant women in this study was the most common disinfectant for douching, especially among those between the ages of 25 to 39. This is much lower than the 67% of women who use Dettol as disinfectant in another study [40]. There may be an economic and/or educational factor to the use of Dettol as 60% of those with post-secondary education applied this disinfectant during douching in contrast to only 15% of those without formal education.

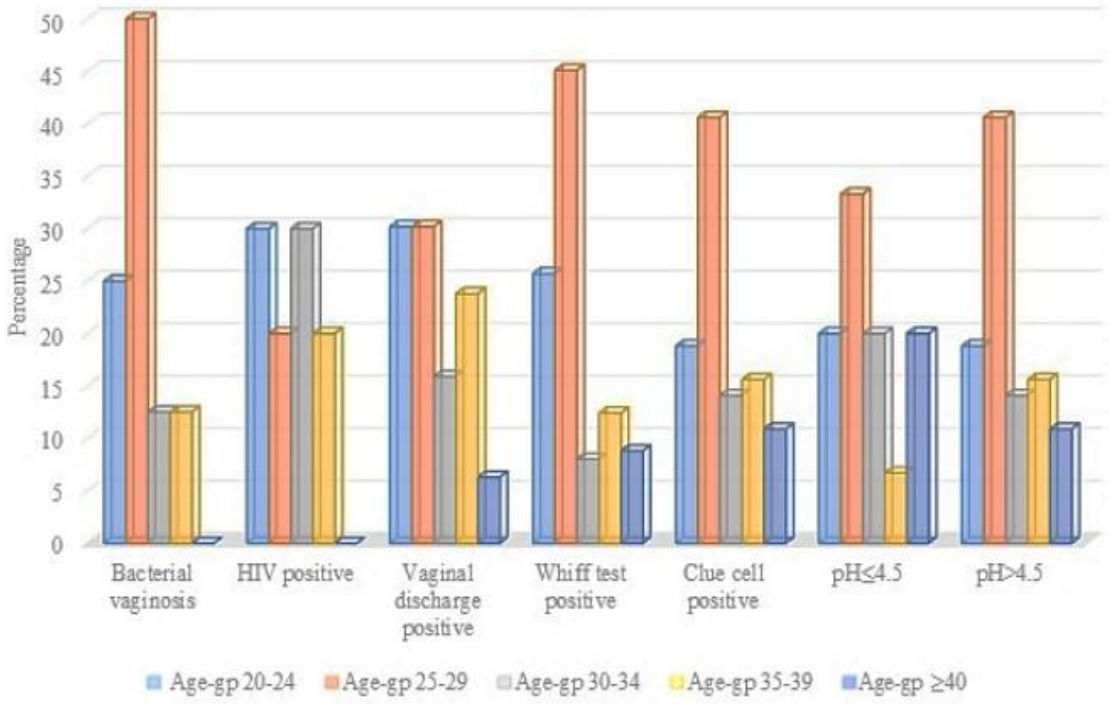


Fig. 3. Morbidity pattern of study subjects by age group (years)

Bacterial vaginosis was observed in 14.5% of pregnant women in this study, more in ages 20-29 than in other age groups and more among those with low than those with high educations status. Many studies have associated douching with various adverse reproductive and gynecologic consequences such as bacterial vaginosis, preterm birth, low-birth-weight infants, pelvic inflammatory disease, chlamydial infection, tubal pregnancy, higher rates of HIV transmission, and cervical cancer. The deleterious effects of douching cannot be over-emphasized.

5. CONCLUSION

Douching was a common practice among the Hausa-Fulani ethnic group in Nigeria amongst whom the most practice of douching was inserting plain water with fingers followed by insertion of toilet soap and using warm water and disinfectant such as Dettol. Douching seemed to be more associated with personal hygiene than cleaning after menstrual flow. A high proportion of women in this study douche during bathing and after micturition. A sizable proportion also douche after and not before sexual intercourse. Bacterial vaginosis was found more among those who used fingers to insert plain water for douching, especially those aged 25-29 years and those with no formal education. An aggressive health education, using the print and electronic media, should give health education to women, especially those in early reproductive age group, on the risks associated with douching such as pelvic inflammatory disease (PID), endometriosis, ascending and upper genital tract infection, infertility and ectopic pregnancy, among others. A multi-center study on anthropological factors associated with douching is encouraged. Survey of the true prevalence of douching in various cohorts of African women associated with gynecological, obstetric, and other pathological indices should be embarked upon for the health of women and their offspring.

6. STUDY LIMITATIONS

This study has some limitations. First, only pregnant women were studied and, in this part of the country, these usually come for their first ANC at later stages of pregnancy. Therefore, conclusions based on our findings are limited to this cohort of women and may not be generalizable to other women who are not pregnant. Secondly, the sample size is small and stratification makes it even smaller. Younger women aged less than 20 were excluded from this study and it is a well-known fact that age of debutant into sexual activity is much younger than 20 in this location. Therefore, douching practices of young girls are not represented in this study. Thirdly, this study focused mainly on douching practice and information on concomitant vaginal infections is not well focused upon. However, Bacterial vaginosis in relation to douching practices appears to be undocumented in Nigeria, thus the attention given to it in this paper. This study did not identify risk factors for acquiring bacterial vaginosis or any other diseases observed through laboratory investigations. A prospective study to look at the effect of douching on pregnancy outcome is desirable in the future. Patients were observed at health facility setting, which mitigates against the socio-cultural, anthropological, and genealogical background to douching practices. Finally, the Hausa-Fulani ethnic group is just one out of hundreds of ethnic groups in Nigeria and findings in this study do not necessarily apply to douching practices or vaginal infection in other ethnic groups or other geo-political locations of the country.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 4
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Diagnosis of Bacterial Vaginosis a Common Vaginal Infection among First-Time Antenatal Clinic Attendees: Evidence from a Tertiary Health Facility in North-West Nigeria

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ABSTRACT

Context: Bacterial vaginosis has been prospectively linked to various adverse reproductive and pregnancy related events.
Objective: To determine the prevalence of bacteria vaginosis among first time antenatal clinic attendees in Northwest Nigeria.
Study Design, Setting and Subjects: A Cross sectional study conducted at the Antenatal clinic of the Ahmadu Bello University Teaching Hospital, Zaria, north-west Nigeria. A total of 228 consecutive booking clients were enrolled from April to June, 2008.
Main Outcome Measures: Presence of at least three of the following: (i) thin, white homogenous discharge, (ii) clue cells on microscopy, (iii) pH of vaginal fluid >4.5 (iv) release of fishy odor on adding alkali (10% potassium hydroxide) or positive whiff test (Amsel's criteria) and (v) relative proportion of bacteria morphotypes on gram staining (The Nugent score).
Results: In all 220 (96.5%) clients concluded the study and were analyzed. Prevalence of bacteria vaginosis (BV) was 14.6% using the Nugent score. Prevalence of asymptomatic BV was 9.6%. Eleven (34.4%) of the clients with BV were symptomatic while 21 (65.6%) were asymptomatic. There was no significant difference in the prevalence of BV between symptomatic and asymptomatic women. Presence of clue cells on microscopy (χ^2=10.5, p=0.001), absence of yeast cells (χ^2=4.120, p=0.042) and isolation of *Gardnerrella vaginalis* (χ^2=36.480, p=0.000) were significantly associated with BV. BV was more prevalent in the second trimester (81.3%) among parous women with low education, low economic status, in polygamous marriages, who had not used hormonal contraceptives or who were HIV positive. Amsel criteria method had a low sensitivity of 37.5% and a specificity of 70.7%, a positive predictive value of 17.9% and negative predictive value of 86.9%.
Conclusion: The overall prevalence of Bacterial vaginosis in pregnant women attending antenatal care for booking at ABUTH, Zaria is high and thus should be considered as an important condition in pregnant women by clinicians. It is necessary to screen for BV in high risk women with previous untoward events like low birth weight and preterm delivery especially in the second trimester. A larger prospective study should be carried out to better demonstrate risk factors and possible adverse effects of Bacterial vaginosis.

Keywords: Bacterial vaginosis; Nugent score; clue cells; Amsel criteria; whiff's test; Northwest-Nigeria.

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1. INTRODUCTION

The impact of many infectious diseases is complicated by pregnancy [1]. These infections are usually of considerable concern to clinicians because of the potential threats to the lives of the pregnant woman and that of her fetus [2]. Evidence suggests a boosted innate response [3,4,5], which may represent a compensatory immune mechanism to protect the pregnant mother and the fetus and which may imply decreased susceptibility to initial infection [6]. Feto-maternal wellbeing in pregnancy and pregnancy outcome are certainly of great worry to the obstetrician and gynecologist. Bacterial vaginosis has the potential of causing late miscarriages, preterm births, preterm premature rupture of membranes, pelvic inflammatory disease, increased risk of human immune-deficiency virus infection, post-partum, postabortal and post-operative endometritis. Detection, treatment and prevention of bacterial vaginosis may help improve perinatal and maternal morbidity and mortality. Bacteria vaginosis (BV) is the commonest cause of abnormal vaginal discharge in women of childbearing age and the most common infectious condition in women [7-9]. Many cases of BV remain asymptomatic or present with only malodorous vaginal discharge with no inflammatory complaints [10]; thus BV is therefore referred to as “vaginosis” and not “vaginitis” [11,12]. BV is particularly common in black women, especially in sub-Saharan Africa [13-16]. It is usually detected in 10-40% of women worldwide and is most prevalent among women who have multiple sexual partners; those of low income status and lower levels of education; among women who smoke cigarettes; in African-American women; and in women who do not use hormonal contraception [17]. Certain intra-vaginal practices have been linked with risk of acquiring Bacterial vaginosis. Intra-vaginal practices are common among sexually active women and have been described in several sub-Saharan African countries and parts of Asia and the United States of America [15]. These practices include vaginal wiping, vaginal washing (the most studied risk factor for BV) and inserting substances into the vagina. Vaginal washing may be by douching with a stream or jet of water or by using fingers to insert water, soap or variety of other substances into the vagina [15]. Women report using intra-vaginal practices for the purpose of genital hygiene, treatment of sexually transmitted diseases and to enhance sexual pleasure for male partners (dry sex). Although most women engage in these practices for perceived benefits, a number of studies have demonstrated potential harmful association with Human Immunodeficiency Virus 1 (HIV-1) infections, Sexually Transmitted Diseases (STDs), BV and other obstetric and gynecologic conditions such as Pelvic Inflammatory Disease (PIDs), decreased fertility and ectopic pregnancy [15]. Bacterial vaginosis has the potential of causing late miscarriages, preterm births, preterm premature rupture of membranes, pelvic inflammatory disease, increased risk of HIV infection, post-partum, post-abortal and post-operative endometritis [16]. Detection, treatment and prevention of bacterial vaginosis may help improve perinatal and maternal morbidity and mortality.

2. MATERIALS AND METHODS

The study was conducted between April and June 2008 at the Antenatal Clinic of Ahmadu Bello University Teaching Hospital, Shika, Zaria in Kaduna, one of the states in Northwest Nigeria. It was a cross sectional study combining the use of administered questionnaires with clinical examination and laboratory results. The interviewers completed the standardized baseline questionnaires containing information about patient demographics, reproductive profile, gynecologic history, previous adverse events during pregnancy and risk factors for BV. Laboratory analysis of samples taken from the study population was also carried out.

Kaduna State in Northwest Nigeria has a population of approximately 1.5 million (National Population Commission. Nigeria National Population Census 2005) and Zaria, one of the major cities in the State, has some prominent Federal institutions of higher learning and research. The Ahmadu Bello University Teaching Hospital (ABUTH) serves as a tertiary/ referral health facility for Zaria and its environs. Zaria occupies a portion of the high plains of Northern Nigeria, 652.6 m above sea level and some 950 km from the coast. It is located at 11°31 N, 7°42 E.

The Hausa-Fulani ethnic group constitutes more than 70% the population, and are mainly peasant farmers, predominantly of the Islamic faith. Other ethnic groups that live and work in Zaria include the

Yorubas from the Southwest and the Igbos from the Southeast of the country. The study population were consenting pregnant women seeking antenatal care (first time attendees) at the ABUTH.

All consenting pregnant women encountered at the antenatal booking clinic of the health facility who met the inclusion criteria were recruited. The inclusion criteria were absence of systemic diseases such as diabetes mellitus, hypertension, renal disease; no prior history of placental abruption, uterine anomaly, incompetent cervix, twin pregnancy or prior antibiotic use in preceding two weeks before the study.

The antenatal booking clinic at the facility was held once a week with an average of forty women seen per week. The women were initially counselled to secure their consent for collection of various biological samples. All consecutive consenting pregnant women attending the antenatal clinic for the first time were recruited for the study until the desired sample size was attained.

Questionnaires were structured and pre-tested. Doctor and nurse interviewers specifically trained for the study screened each woman for study eligibility, explained the purpose and practice of the study and obtained informed consent. The same interviewers completed the standardized baseline questionnaires which contained information about patient demographics, reproductive profile, gynaecologic history, previous adverse effect in pregnancy and risk factors for BV.

The questionnaire was administered in a confidential location within the antenatal clinic by mostly female interviewers. Vaginal swabs for BV assessment were collected from clients using swab sticks which were immediately transported to the laboratory. At first, a clean, unlubricated speculum was introduced into the vagina and vaginal pH measured using pH strips. A sterile cotton swab was used to obtain material from the posterior vaginal fornix for vaginal smear. Wet smear of vaginal secretion from posterior fornix was diluted with normal saline, examined microscopically (x400) for identification of clue cells and bacteria morphocytes. These vaginal secretions (on wet smears) were also subjected to studies using Amsel and Nugent criteria. Each sample was also cultured for the offending organisms. The remainder of the prenatal care and BV screening was under the direction of the principal investigator.

The protocol and consent was approved by the ethical committee of ABUTH Zaria. Diagnoses were by Amsel criteria and Nugent score. By Amsel's criteria, at least three of the four criteria should be present for diagnosis of Bacterial vaginosis to be confirmed. The criteria included (i) thin, white homogenous discharge, (ii) clue cells on microscopy, (iii) pH of vaginal fluid greater than 4.5, and (iv) release of fishy odor on adding alkali (10% potassium hydroxide) or positive Whiff's test. The Nugent score estimated relative proportion of bacteria morphotypes to give a score between 0 and 10. Less than 4 was regarded as normal, 4-6 as intermediate, greater than 6 as indicative of the presence of Bacterial vaginosis. The Nugent score was the main diagnostic test.

Reference group in each case referred to pregnant women who were confirmed to have Bacterial vaginosis. Data was analyzed using SPSS computer software version 15.0. Significance was considered at a p-value of <0.05. Data was presented in tables and figures.

3. RESULTS

A total of 228 clients were initially recruited for the study but 220 (96.5%) of them completed it. Of these 220 clients, 32 (14.5%) were confirmed to have Bacterial vaginosis (BV) based on Nugent score (Table 1).

There was no significant statistical difference (t=-0.85, df=49.9, P-value=0.20) in the mean (± SD) age (in years) of clients with (25.5 ± 4.7) and without (26.3 ± 6.0) BV. The infection was more prevalent (16, 50.0%) among those aged 25-29 years who were 2.2 times more likely to present with BV than any other age group (χ^2=4.49, P-value=0.03, OR=2.24, CI: 1.05, 4.79). Bacterial vaginosis was more prevalent among women who had only Koranic education (10, 31.3%), among occupational housewives (12, 37.5%) and among those with a parity of 2-4 (13, 40.6%).

Table 1. Socio-demographic characteristics of respondents,*Fisher's exact test

Variable	Item	Bacterial vaginosis		χ^2 (P-value)	OR (95% CI)	Total
		Positive	Negative			
		Freq. (%)	Freq. (%)			
Age (years)	20-24	8 (25.0)	48 (25.5)	0.04 (0.95)	0.97 (0.41, 2.31)	57 (25.9)
	25-29	16 (50.0)	58 (30.9)	4.49 (0.03)	2.24 (1.05, 4.79)	74 (33.6)
	30-34	4 (12.5)	26 (13.8)	0.006 (0.94)*	0.89 (0.29, 2.75)	30 (13.6)
	35-39	4 (12.5)	24 (12.8)	0.06 (0.81)*	0.98 (0.31, 3.03)	27 (12.3)
	≥40	0 (0.0)	32 (17.0)	5.08 (0.02)*	Undefined	32 (14.6)
Educational status	None	1 (3.1)	1 (0.5)	0.18 (0.67)*	6.03 (0.37, 98.98)	2 (1.0)
	Koranic	10 (31.3)	58 (30.9)	0.002 (0.96)	1.02 (0.45, 2.29)	68 (30.9)
	Primary	9 (28.1)	39 (20.7)	0.87 (0.35)	1.50 (0.64, 3.49)	48 (21.8)
	Secondary	9 (28.1)	63 (33.5)	0.36 (0.55)	0.78 (0.34, 1.78)	72 (32.7)
	Tertiary	3 (9.4)	27 (14.4)	0.23 (0.63)*	0.62 (0.18, 2.17)	30 (13.6)
Occupational status	Housewife	12 (37.5)	75 (39.9)	0.07 (0.80)	0.90 (0.42, 1.96)	87 (39.5)
	Business/trading	7 (21.9)	34 (18.1)	0.26 (0.61)	1.27 (0.51, 3.17)	41 (18.6)
	Civil servant	1 (3.1)	16 (8.5)	0.49 (0.49)*	0.35 (0.04, 2.71)	17 (7.7)
	Artisan	7 (21.9)	52 (27.7)	0.47 (0.49)	0.73 (0.30, 1.80)	59 (26.8)
	Student	5 (15.6)	11 (5.9)	3.87 (0.04)	2.30 (0.96, 9.24)	16 (7.3)
Occupation of partner	Civil servant	12 (37.5)	69 (36.7)	0.01 (0.93)	1.03 (0.48, 2.24)	81 (36.8)
	Farmer	1 (3.1)	12 (6.4)	0.10 (0.75)*	0.47 (0.06, 3.77)	13 (5.9)
	Business man	3 (9.4)	42 (22.3)	2.08 (0.15)*	0.36 (0.10, 1.24)	45 (20.5)
	Petty trader	9 (28.1)	14 (7.4)	12.49 (0.0004)	4.86 (1.89, 12.49)	23 (10.4)
	Artisan	2 (6.3)	25 (13.3)	0.69 (0.41)*	0.43 (0.10, 1.93)	27 (12.3)
Parity	0	3 (9.4)	39 (20.7)	1.61 (0.20)*	0.40 (0.11, 1.37)	42 (19.1)
	1	6 (18.8)	30 (16.0)	0.16 (0.69)	1.22 (0.46, 3.21)	36 (16.4)
	2-4	13 (40.6)	71 (37.8)	0.09 (0.76)	1.13 (0.52, 2.42)	84 (38.2)
	5 and more	10 (31.3)	48 (25.5)	0.46 (0.50)	1.33 (0.59, 3.00)	58 (26.4)
Type of marriage	Monogamous	16 (50.0)	125 (66.5)	3.23 (0.07)	0.5 (0.24, 1.07)	141 (64.1)
	Polygamous	16 (50.0)	63 (33.5)			79 (35.9)
Total		32 (14.5)	188 (85.5)	-	-	220 (100.0)

Table 2. Prevalence of bacterial vaginosis by gestational age of index pregnancy, history of vaginal discharge and risk factors, *Fisher's exact test

Variable		Item	Bacterial vaginosis		Total (%)	χ^2 (P-value)	OR (95% CI)
			Positive	Negative			
			Freq. (%)	Freq. (%)			
Gestational Age (weeks)		1-13	1 (3.1)	10 (5.3)	11 (5.0)	0.01 (0.93)*	0.57 (0.07, 4.65)
		14-26	26 (81.3)	146 (77.7)	172 (78.2)	0.21 (0.65)	1.25 (0.48, 3.23)
		27-39	5 (15.6)	32 (17.0)	37 (16.8)	0.04 (0.85)	0.90 (0.32, 2.52)
		40 and over	-	-	-	-	-
History of vaginal discharge		Yes	11 (34.4)	74 (39.4)	85 (38.6)	-	-
		-Whitish discharge	11 (100.0)	45 (60.8)	56 (65.9)	-	-
		-Thick	6 (54.5)	51 (68.9)	57 (67.1)	-	-
		-Itching	4 (36.4)	21 (28.4)	25 (29.4)	-	-
		-Rashes	3 (27.3)	17 (23.0)	20 (23.5)	-	-
		-Remarkable Odor	2 (18.2)	10 (13.5)	12 (14.1)	-	-
Risk factors	Douching	Yes	26 (81.3)	162 (86.2)	188 (85.5)	0.53 (0.47)	0.70 (0.26, 1.85)
		No	6 (18.8)	26 (13.8)	32 (14.5)		
	Hormonal contraceptive use	Yes	3 (9.4)	35 (18.6)	38 (17.3)	1.05 (0.31)*	0.42 (0.13, 1.57)
		No	29 (90.6)	153 (81.4)	182 (82.7)		
	HIV status	Positive	3 (9.4)	7 (3.7)	10 (4.5)	0.88 (0.35)	2.63 (0.64, 10.76)
		Negative	29 (90.6)	178 (94.7)	207 (94.1)		
		Unknown	-	3 (1.6)	3 (1.4)	-	-
Total			32 (14.5)	188 (85.5)	220 (100.0)	-	-

Only 1 (3.1%) of the 32 BV positive clients presented for ANC in the first trimester (1-13 weeks) compared to 26 (81.3%) who presented in the second trimester (14-26 weeks) and 5 (15.6%) in the third trimester of pregnancy (Table 2). Though 11 (34.4%) of the BV positive and 74 (39.4%) of BV negative clients respectively complained of vaginal discharge, the overall prevalence of asymptomatic Bacterial vaginosis was 9.6% (21/220). Bacterial vaginosis was observed more among pregnant women who practiced douching (26, 81.3%) but not among those who used hormonal contraceptives (3, 9.4%) or those who were HIV positive (3, 9.4%). Of the 32 BV positive clients, 11 (34.4%) gave a history of vaginal discharge. All (11, 100.0%) had whitish discharge which was thick in 6 (54.5%) women. The discharge was associated with itching in 4 (36.4%) women, with rashes in 3 (27.3%) women and with remarkable odour only in 2 (18.2%) women (Fig. 1).

Whiff's test was positive for 14 (43.8%) BV positive and 99 (52.7%) BV negative clients with no significant statistical difference between these two groups (Table 3).

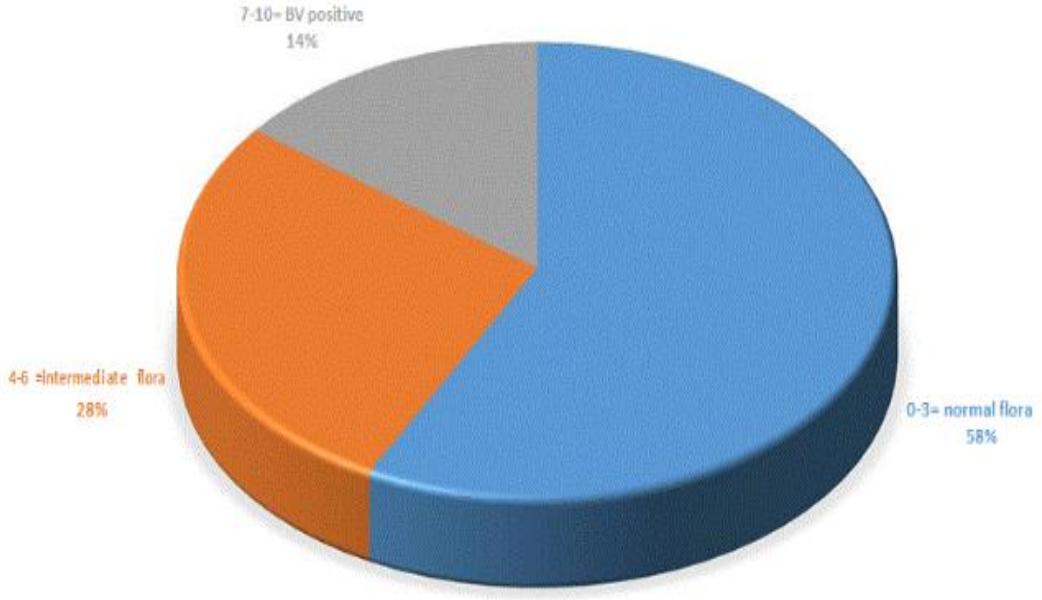

Fig. 1. Nugent score for all clients

Pregnant women who were BV positive were about 1½ times more likely to present with thin, non-offensive homogenous discharge than those who were BV negative (χ^2=0.78, P-value=0.38, OR=1.44, 95% CI: 0.64, 3.28). In addition, BV positive clients were about twice more likely to present with typical thin, non-offensive homogenous vaginal discharge that BV negative clients (χ^2=1.63, P-value=0.20, OR=2.06, 95% CI: 0.68, 6.23). Using Amsel's clinical criteria, 25% had BV with a combination of i) positive Whiff's test with 10% KOH, (ii) presence of clue cells on microscopy, (iii) vaginal pH>4.5 and (iv) thin, homogenous non-offensive discharge, thereby confirming the diagnosis of bacteria vaginosis The mean (± SD) pH of clients with bacterial vaginosis was 4.7 (0.5) while that of clients without BV was 4.8 (0.5) without any notable difference among the two. However, BV positive clients were 3.3 times more likely to have pH<4.5 than BV negative patients (χ^2=4.57, P-value=0.03, OR=3.30, 95% CI: 1.05, 10.38) (Table 3). There was also a noteworthy variance in the proportion of BV positive clients among whom Clue cells were observed at microscopy (17, 53.1%) compared to those who were BV negative (47, 25%). Furthermore, patients who were BV positive were about 3½ times more likely to be Clue cell positive than those who were BV negative (χ^2=10.49, P-value=0.001, OR=3.40, 95% CI: 1.58, 7.33).

Of the 32 clients who were diagnosed to be BV positive using Nugent score, only 12 (37.5%) were confirmed to be BV-positive while 20 (62.5%) were confirmed to be BV-negative using Amsel's

criteria. Using the Nugent score as the gold standard, Amsel criteria has a sensitivity of 37.5%, specificity of 70.7%, positive predictive value (PPV) of 17.9% and a negative predictive value (NPV) of 86.9% (Table 4).

Overall, 358 organisms were isolated from all the clients including 55 (15.4%) from the BV positive patients and 303 (84.6%) from BV negative patients (Table 5). Clients who were positive for BV were over four times more likely to have *Gardnerella vaginalis* co-infection (χ^2=16.79, P-value=0.00004, OR=4.17, 95% CI: 2.03, 8.57) and were 1.15 times more likely to have Pus cells (χ^2=0.23, P-value=0.63, OR=1.15, 95% CI: 0.64, 2.08) compared to those who were BV negative. Fig. 2 shows the relative preponderance of these organisms.

Table 3. Prevalence of BV by components of the amsel criteria

Variable	Item	Bacterial vaginosis		χ^2 (P-value)	OR (95% CI)
		Positive	Negative		
		Freq. (%)	Freq. (%)		
Characteristics	pH≤4.5	5 (15.6)	10 (5.3)	4.57 (0.03)	3.30 (1.05, 10.38)
	pH>4.5	27 (84.4)	178 (94.7)		
Clue cells	Present	17 (53.1)	47 (25.0)	10.49 (0.001)	3.40 (1.58, 7.33)
	Absent	15 (46.9)	141 (75.0)		
Whiff test	Positive	14 (43.8)	99 (52.7)	0.87 (0.35)	0.70(0.32, 1.49)
	Negative	18 (56.3)	89 (47.3)		
Vaginal discharge on examination	Yes	18 (56.3)	45 (23.9)		
	-Typical (thin, non-offensive, homogeneous)	8 (44.4)	17 (37.8)	1.63 (0.20)	2.06 (0.68, 6.23)
	-Atypical	8 (44.4)	28 (62.2)		
	No	14 (43.7)	143 (76.1)	-	-

**result of vaginal discharge on examination was not available for 2 BV +ve women and were excluded*

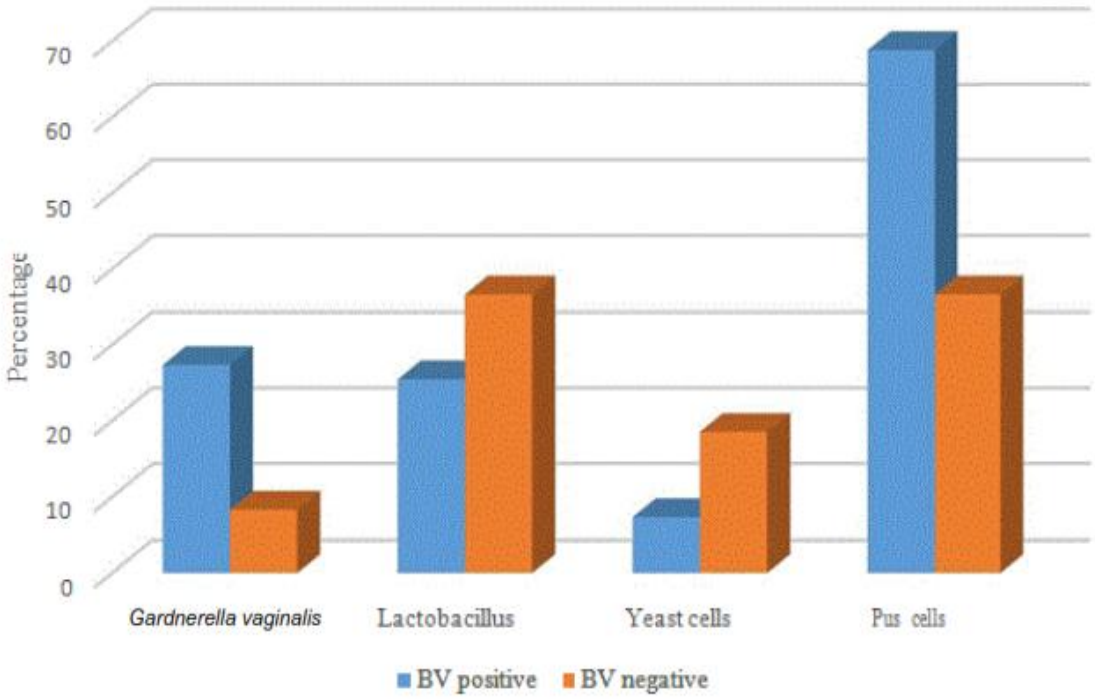

Fig. 2. Relative preponderance of organisms in co-infectivity among bacterial vaginosis positive and negative clients

Table 4. BV status by method of diagnosis

	Nugent BV positive	Nugent BV negative	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
	Freq. (%)	Freq. (%)				
Amsel positive BV	12 (37.5)	55 (29.3)	37.50%	70.00%	17.90%	86.90%
Amsel negative BV	20 (62.5)	133 (70.7)				

4. DISCUSSION

Bacterial Vaginosis (BV), the commonest lower female genital tract condition, causes a lot of problems in and outside of pregnancy [7-9]. In pregnancy, it causes late miscarriages, preterm labor, premature rupture of membranes, post-partum endometritis, low birth weight etc. [18-23]. All these impact on perinatal and maternal morbidity and mortality. BV is thus a significant infection in pregnancy.

The prevalence of Bacterial vaginosis among this study population was 14.6% and this falls within 10-40% of the prevalence reported among women in the literature worldwide [18]. This is quite significant considering the complications of Bacterial vaginosis such as miscarriages, premature rupture of membranes and preterm birth, especially in an environment in sub-Saharan Africa, where facilities for neonatal resuscitation and intensive care are inadequate. Using polymerase chain reaction (PCR) method combined with Nugent score, Anukam et al. [8] also reported similar prevalence of BV (14.6%) among non-pregnant women in the southern part of Nigeria. This possibly indicates that pregnancy may not necessarily influence presence or absence of bacterial vaginosis. Certain key findings in this study need further discussion. The first is that Bacterial vaginosis was found to be most prevalent in certain socio-demographic spectrum.

Table 5. Organisms seen on microscopy according to presence of BV, NB: Some organisms were mixed on a single microscopy

Organism	BV positive (n=32)	BV negative (n=188)	Total	χ^2	OR (95% CI)
	Freq. (%)	Freq. (%)			
Gardnerella vaginalis	15 (46.9)	25 (13.3)	40 (11.2)	16.97 (0.00004)	4.17 (2.03, 8.57)
Lactobacillus	14 (43.8)	111 (59.0)	125 (34.9)	2.56 (0.11)	0.59 (0.31, 1.13)
Yeast cells	4 (12.5)	56 (29.8)	60 (16.8)	3.43 (0.06)	0.35 (0.12, 1.00)
Pus cells	22 (68.8)	111 (59.0)	133 (37.1)	0.23 (0.63)	1.15 (0.64, 2.08)
Total number of organisms	55 (15.4)	303 (84.6)	358 (100.0)	-	-

It is interesting to note that this condition was not observed in pregnant women aged 40 years and above but was seen more in the age group of 25-29 years in consonance with what Turovskiy et al. [24] reported. Taking age group of 20-25 years into consideration, 75% of pregnant women consulting for ANC in this tertiary hospital would have been positive for Bacterial vaginosis. Early sexual debut and possible multiple sexual partners due to frequent divorce, are observed in Northern Nigeria and may be risk factors for BV as reported in some studies [25-28].

Bacteria vaginosis was not prevalent among clients with tertiary education but was more commonly found among those with little or no education. This finding agrees with what Harville et al. [18] reported that poor education and income are linked with higher risk of BV.

Bacteria vaginosis was rarer (9.4%) among women in their first pregnancy that among those who had had one child or more. In fact, the proportion of women with Bacterial vaginosis increased as parity increased till 4 after which the proportion declined. Also, more pregnant women in polygamous (16/79, 20.4%) than in monogamous (16/141, 11.3%) marriage presented with Bacterial vaginosis. One of the

possible reasons for these situations is exposure to either multiple sexual partners and/or exposure to several heterosexual activities which are identified risk factor for BV [29].

Another key finding was that Bacterial vaginosis was more prevalent in the second and third trimester. The mean gestational age at which pregnant women in this study booked for ANC was 21 weeks. Trabet and Misra [30] reported increased likelihood of BV among women who have greater frequency of intercourse during the first trimester of pregnancy. On the other hand, after an 8 week follow-up from 14 weeks of gestation, Krauss-Silva et al. [31] observed a reversal to BV negative in about 40% of pregnant women who were initially positive. Presence or absence of BV at any stage of pregnancy seems to be a controversial issue. For example, a study reported that certain cytokines produced in mid-pregnancy were protective against BV, while others, such as IL6-174 G>C polymorphism increased the risk of developing BV [32]. The diagnosis of BV in the second trimester has been linked with high risk of preterm delivery and premature rupture of the membrane [33] though there may be other causes of preterm delivery and premature rupture of the membrane.

Incidentally, the proportion of pregnant women who practiced douching among the BV positive and BV negative clients was not significantly different, though among the 188 pregnant women who claimed they practiced douching, 26 (13.8%) were BV positive in contrast to the 6 (18.8%) of the 32 who claimed to not practice douching. Douching is a strong risk factor for BV, preterm birth, low-birth-weight infants, pelvic inflammatory disease, chlamydial infection, tubal pregnancy, higher rates of HIV transmission, and cervical cancer [34,35].

One interesting finding was that BV positive patients were over 2½ times more likely to be HIV positive than BV negative patients. The prevalence of HIV among this cohort of pregnant women studied was 4.5% which agrees with the national HIV prevalence of 4.4% reported from the National Sentinel Survey [36]. This is in line with the documented suggestion that BV enhances heterosexual HIV transmission [37,38].

The finding of typical vaginal discharge – thin, non-offensive and homogenous and non-offensive – in about 56% of the BV positive women correlates with the 58% reported by Srinivasan et al. [39]. The use of gram stained smear has been found to have higher sensitivity and specificity [40-42]. Using the Nugent score, only 14.6% had Bacterial vaginosis compared to 25% who had a combination of at least 3 of the 4 composite Amsel clinical criteria. Only 12 (37.5%) of the clients were BV positive by both Nugent score and Amsel clinical criteria [43]. Although Amsel criteria method is convenient and relatively inexpensive it is not always reliable. In this study, it had a low sensitivity compared to Nugent score. Kurki et al. [44] claimed that determination of vaginal pH is deficient in specificity since elevated vaginal pH may also depend on a variety of other conditions of the female lower genital tract.

Final major findings were the isolations of various organisms such as *Gardnerella vaginalis* more in BV positive (15/32, 46.9%) pregnant women similar to the 44.4% reported in The Gambia [13]. This was not surprising as this organism has been implicated as the causative agent of Bacterial vaginosis. For example, some authors [45,46] proposed *Gardnerella vaginalis* biofilms as hazardous in BV pathogenesis and symptomatology. *Lactobacillus* spp., recorded less among BV positive pregnant women, was found to be one of most prevalent natural microbiota of the lower genital tract in women; others being *L. crispatus*, *L. jensenii* and *L. iners* [47-49], together creating a critical frontline guard against possible invading pathogens. Circulating hormones were thought to modulate the delicate symbiotic balance between Lactobacilli and the vaginal tract. Our finding of decreased concentration of Lactobacilli spp. was in accord with the explanation that BV, a poly microbial syndrome, resulted in milieu of decreased *Lactobacilli* spp. concentration and in increased pathogenic bacteria, including *Gardnerella vaginalis* [50,51].

These findings have some clinical and practical implications. The high prevalence of BV among first time antenatal attendees (and by extension pregnant women in this study) reflects the potential negative effects BV can have on pregnancy and its outcome such as miscarriages, preterm births and increased risk of infection to the pregnant woman among other negative effects. The fact that most of the women in this study first presented for ANC relatively late (in their

second trimester) when the damage may have been done is worrisome. Our study will therefore recommend early commencement of ANC and screening for BV at least in high risk women. Women in child-bearing age should have access to health education on prevention of BV at school, or through radio and television. The clinical impact of the right measures taken as a result of the findings from this study will be a reduction of perinatal and maternal morbidity and mortality related to BV.

5. CONCLUSION

The overall prevalence of Bacterial vaginosis in pregnant women attending antenatal care for booking at ABUTH, Zaria is high and thus should be considered as an important condition in pregnant women by clinicians. The presence of clue cells on microscopy and isolation of *Gardnerella vaginalis* were significant indicators of presence of Bacterial vaginosis. There was no significant difference in mean pH of BV positive and BV negative patients and douching and other intra-vaginal practices were common practices among women in the study population. Screening for bacterial vaginosis preferably using the Nugent score, at least in high risk pregnant women may be worthwhile even if they are asymptomatic especially in the second trimester. A larger prospective study should be carried out to better demonstrate risk factors and possible adverse effects of Bacterial vaginosis.

6. STUDY LIMITATIONS

This study had some limitations that need explanation. Firstly, this was a facility-based study on a small non-representative sample of pregnant women. Therefore, extrapolating our findings to the general population may not be possible.

Secondly, first-time ANC clients typically presented during the later stages of pregnancy at ABUTH. Screening at earlier gestation would have been preferred. The study was unable to establish the temporality of BV infections, past pregnancy outcomes and risk factors for BV acquisition.

Thirdly, a prospective study to look at the effect of BV on pregnancy outcome was hampered by ethical issues and lack of consensus on whether to treat BV in pregnancy or not and the effect of either action. Equally of concern was the fact that follows up was difficult because patients/clients who enrolled at ABUTH ANC may not deliver in the hospital. Deliveries at home and elsewhere were still prevalent in Northern Nigeria when this study was carried out. These and other risk factors such as use of IUCD, previous STI, frequency of coitus and cigarette smoking could not be linked with presence of Bacterial vaginosis.

Lastly, the study was carried out in only one out of six geo-political zones and several ecological locations such as the Atlantic Ocean coastline in the south and the mountainous regions on the eastern flank of the country. As such, conclusions based on findings may lack external validity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 5
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A Case Report on Catheter Ablation of Mitral Isthmus Flutter Post Mitral Valve Repair and Surgical Maze

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ABSTRACT

The present study describe a case of LAF developed after a surgical Maze procedure that demonstrates the importance of a systematic approach to mapping and ablating atypical atrial flutter to prevent a recurrence of symptomatic arrhythmia. A typical atrial flutter is a regular arrhythmia characterized by a non-cavotricuspid isthmus dependent macro-reentry. In patients with previous cardiac surgery procedures, and in particular, after a surgical maze, there are many different potential LA reentry circuits that involve various pathways. Both activation mapping and entrainment mapping were performed to identify the critical isthmus of the circuit and to terminate the arrhythmia effectively. Catheter-based mapping and ablation of atypical atrial flutter are feasible and effective, although technically challenging. The combination of activation mapping using a non-fluoroscopic 3D mapping system and entrainment mapping is crucial to achieving satisfying acute success and long-term outcomes.

Keywords: Atypical flutter; left atrial flutter; mitral valve surgery; surgical maze.

1. INTRODUCTION

A typical atrial flutter is a regular arrhythmia characterized by a non-cavotricuspid isthmus dependent macro-reentry. Clinical electrophysiology has made the traditional classification of rapid atrial rhythms into flutter and tachycardia of little clinical use. Electrophysiological studies have defined multiple mechanisms of tachycardia, both reentrant and focal, with varying ECG morphologies and rates, authenticated by the results of catheter ablation of the focal triggers or critical isthmuses of re-entry circuits [1,2]. Considering that atypical atrial flutter reentry circuit may involve various locations in both atria, activation mapping of the flutter circuit is important to confirm that catheter ablation is being performed at an isthmus involved in the circuit. Left atrial flutter (LAF) most commonly involves reentry around a scar from prior cardiovascular surgery, catheter ablation, or congenital heart disease. Macroreentrant atrial tachycardia is a common complication following surgery for congenital heart disease (CHD), and is often highly symptomatic with potentially significant hamodynamic consequences. Medical management is often unsuccessful, requiring the use of invasive procedures [3,4]. One aspect that derives from the widespread diffusion of ablation procedures for atrial fibrillation, both percutaneous and surgical, is the onset of LAF linked to the presence of a gap at the pulmonary vein (PV) ostia or antra. Early detailed intraoperative mapping studies of sustained AF suggested that it was the result of several simultaneous wave fronts moving across the atrial surface in a seemingly random fashion [5,6].

2. CASE REPORT

A 72-year-old man presented at our Centre for an elective atrial flutter ablation procedure. Previous cardiac history includes myxomatous mitral valve, severe prolapse with a flail of the posterior mitral

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leaflet with severe mitral valve regurgitation requiring mitral valve repair at age 69. He also had a history of paroxysmal atrial fibrillation. At the time of his valve surgery, the left atrial appendage was removed, and a simplified Cox-Maze III procedure (PV encircling and connecting lines) was also performed. Three years after surgery, he developed symptomatic rapid atrial flutter. The patient complained of mild swelling of his ankles and worsening shortness of breath on exertion. He received oral anticoagulation therapy, and a rate control treatment with metoprolol was started waiting for catheter ablation. He was scheduled for atrial flutter ablation after a transesophageal echocardiogram. The procedure was performed in fasting state and under conscious sedation. The patient was brought to the electrophysiology lab in atrial flutter. 12-lead ECG showed flutter waves morphology positive in V1, flat in lead I, and negative in lead II, III, aVF (Fig. 1). Venous access was obtained twice through the right femoral vein. A 6 Fr decapolar deflectable catheter was advanced into the right atrium and placed into the coronary sinus (CS). The CS activation sequence was distal to proximal, compatible with an atypical LAF, and the cycle length was 260 msec (Fig. 2). Transseptal access to the left atrium (LA) was then established from the right femoral vein under fluoroscopic guidance using contrast injection to visualize the fossa ovalis and the left atrial cavity. Systemic anticoagulation with heparin was given to maintain an activated clotting time >300 seconds. A 3.5 mm tip ThermoCool Surround Flow bi-directional F-J curve catheter (Biosense Webster Inc., Diamond Bar, CA, USA) was placed into the LA.

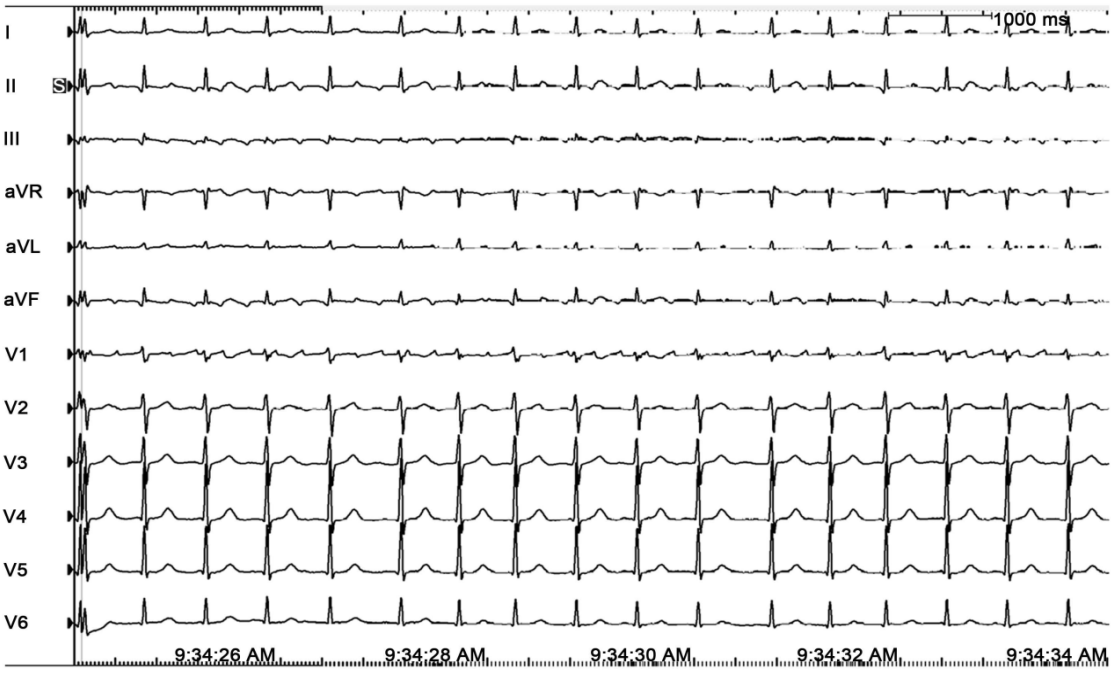

Fig. 1. 12-lead ECG shows atrial flutter with flutter waves morphology positive in V1, flat in lead I, and negative in lead II, III, aVF

The ablation catheter was sequentially moved into the LA to map the PV ostia and antra. All the PV antra, the posterior wall, and the roof were silent. Interestingly, a dissociated potential was recorded on the posterior wall (Fig. 3). We found LA potentials only in the mitral isthmus region. The entrainment of the tachycardia was performed from the mitral isthmus region pacing at 230 msec. The difference of the post-pacing interval (PPI) and the tachycardia cycle length (TCL) after the entrainment pacing indicates the proximity of the entrainment catheter to the reentry circuit, and the PPI–TCL at the mitral isthmus was 10 msec with concealed entrainment (Fig. 4). This was indicative that the mitral isthmus was part of the critical zone of the reentry circuit. A mitral isthmus line from the left inferior PV to the mitral isthmus was performed. During ablation, the atrial flutter terminated (Fig. 5), and the ablation line was completed from the left inferior PV to the mitral valve annulus. After

ablation, we demonstrated a failure to capture at maximum output (20 V @ 3 msec) on the line, and significant conduction delay was recorded while pacing the LA across the line. Repeated atrial stimulations, on and off isoproterenol infusion up to 4 mcg/min, with programmed atrial stimulation and burst down to the atrial refractory period demonstrated no inducible atrial arrhythmia. Post-procedure, the patient recovered well with no complications. His dyspnea on exertion is resolved, and he remains in stable sinus rhythm after nine months of follow-up.

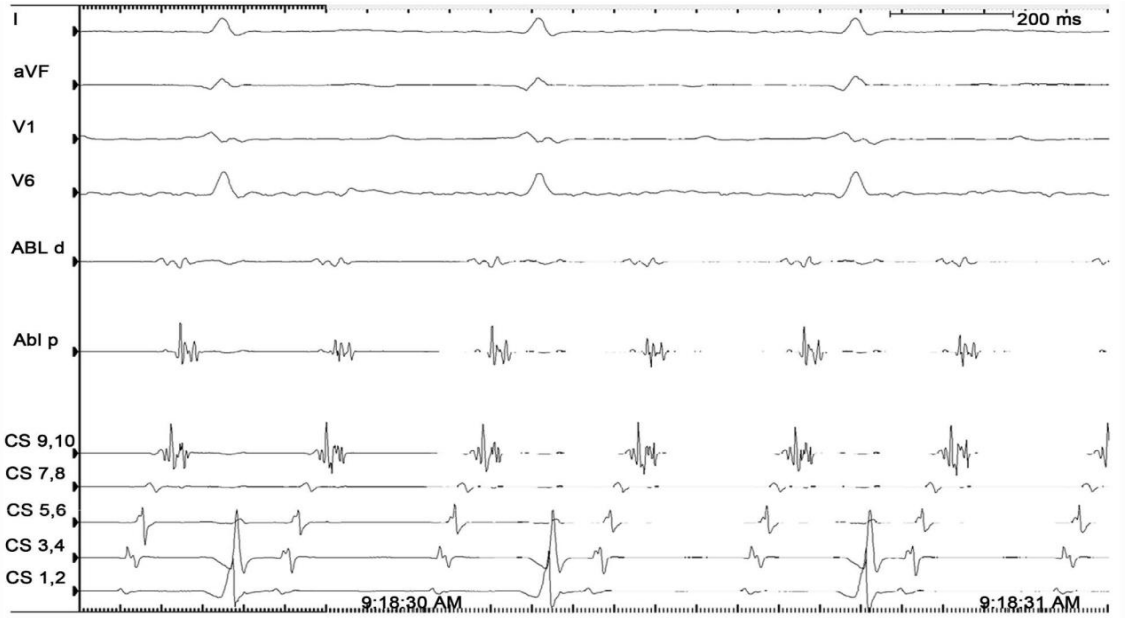

Fig. 2. Coronary sinus (CS) activation sequence is distal (CS 1-2) to proximal (CS 9-10), compatible with an atypical LAF. The cycle length of the flutter is 260 msec

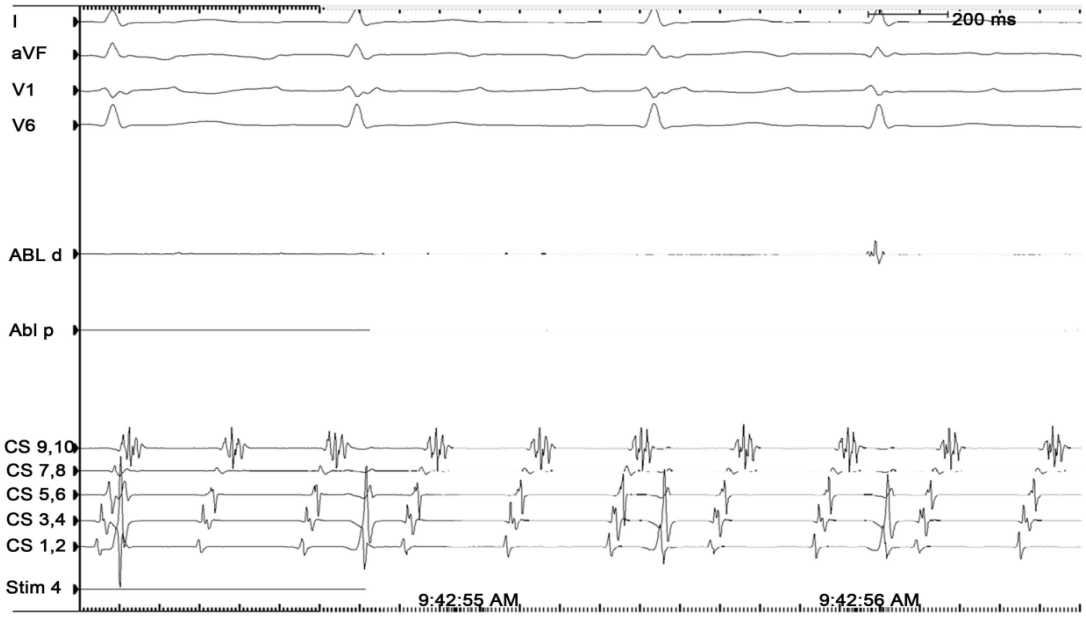

Fig. 3. A dissociated potential is recorded from the ablation catheter (ABL d) on the posterior wall of the left atrium

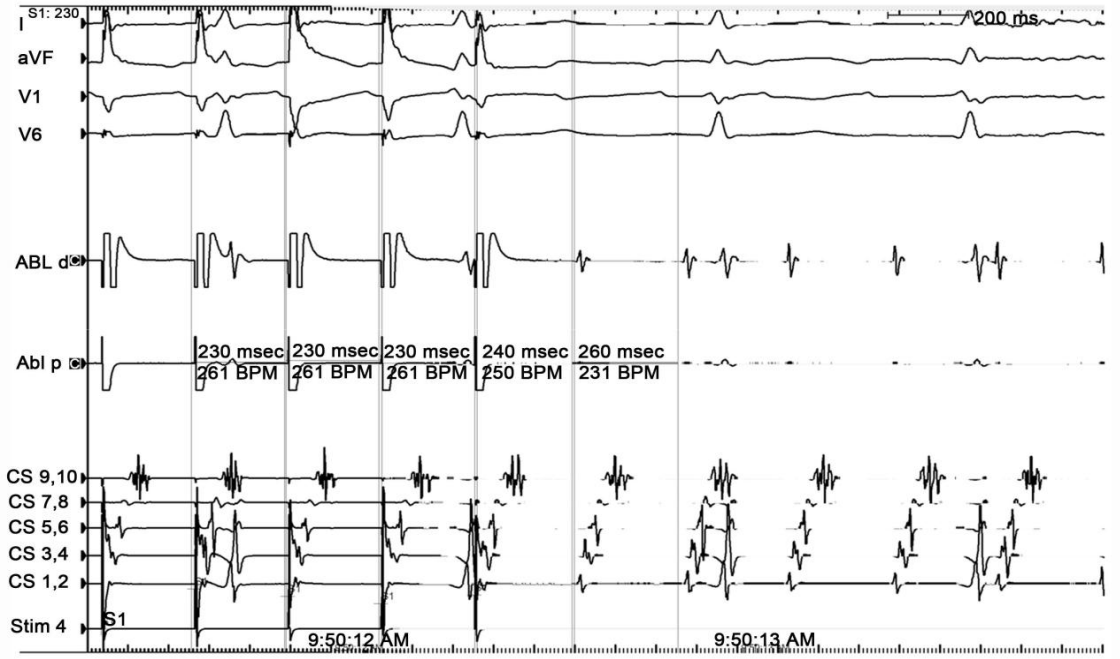

Fig. 4. Entrainment of the tachycardia (CL 260 msec) is performed from the mitral isthmus region pacing at 230 msec. The difference of the post-pacing interval (PPI) and the tachycardia cycle length (TCL) after the entrainment pacing indicates the proximity of the entrainment catheter to the reentry circuit, and the PPI–TCL at the mitral isthmus is 10 msec with concealed entrainment

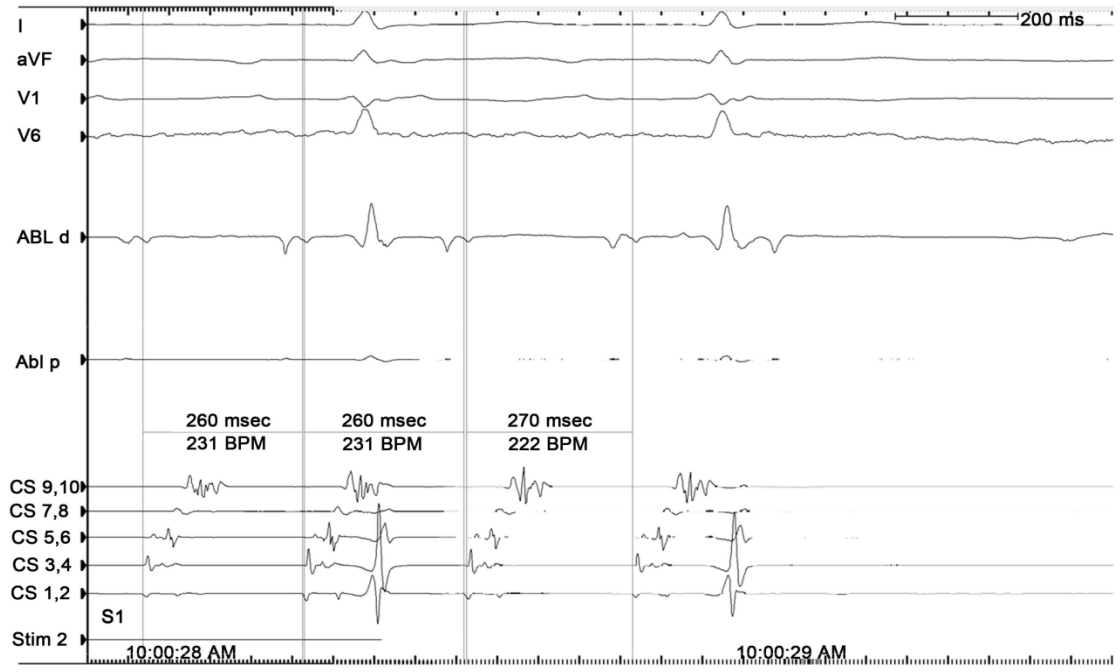

Fig. 5. During ablation on the mitral isthmus, the atrial flutter prolonged (270 msec) and then terminated

3. DISCUSSION

Cardiac surgeons were the pioneers of curative ablation of atrial fibrillation (AF). Since the ’80s, when Cox and colleagues introduced the LA isolation procedure to restore regular rhythm and containing AF to the LA in dogs, several techniques have been developed. The “cut-and-sew” surgeries performed under direct visualization (Cox-Maze I to III procedures) for AF have a success rate ranging from 75 - 95% at preventing recurrent arrhythmia [7,8,9,10,11]. The atrial incisions of the procedure are designed to block potential macroreentrant pathways and narrow the atrial tissue to block propagation of microreentrant wavelets. Moreover, focal activation from PVs is blocked by the isolation of the left atrial posterior wall, including all of the PVs.

The most common arrhythmias following a Cox-Maze III procedure include LAF from reentry around the surgical incisions, AF from PV reconnection, and focal atrial tachycardia. Scar-related atypical LAF is challenging to manage medically and frequently recur after electrical cardioversion [12,13,14]. Ishii et al. reported that an atrial arrhythmia of some form occurred in 43% of patients after the Maze procedure and that most of these arrhythmias were AF [15]. Wazni et al. found in their cohort of 23 patients with atrial arrhythmias after surgical maze that 48% had atypical atrial flutter, with more patients having LAF than a right atrial flutter, implying that the surgery itself creates a substrate that can lead to another arrhythmia. They also found that AF recurrence was secondary to the recovery of conduction around the lines encircling the PVs [16]. Bai et al. found that in patients with a prior history of cardiac surgery or AF ablation, the open-irrigated-tip catheter was superior to the 8-mm solid-tip catheter for radiofrequency ablation of scar-related atypical LAF. Patients ablated with the open-irrigated-tip catheter had higher acute success rate and more favorable long-term outcomes with more patients maintaining sinus rhythm without antiarrhythmic drugs therapy [17]. Recently, Coffey and colleagues published a multicenter retrospective review of 91 patients with atypical atrial flutter. The authors found that patients with a history of a surgical Cox-Maze procedure had the highest long-term success rate, at 88% over 16 ± 12 months [18].

In addition to the activation map performed with a non-fluoroscopic 3D mapping system, successful use of entrainment mapping was crucial to identify the isthmus and to terminate the arrhythmia effectively. Indeed, there are many different potential LA reentry circuits that involve various pathways, particularly after a surgical maze procedure (*i.e.,* PV, septum, mitral valve annulus). Entrainment maneuver refers to the concept that pacing at a slightly shorter cycle length than a reentrant tachycardia will accelerate the tachycardia without interrupting. Concealed entrainment accelerates the tachycardia without fusion on the ECG or intracardiac recordings. If concealed entrainment occurs and the PPI is equal to the TCL, the pacing electrode is likely at an isthmus of slow conduction within the reentry circuit. In atypical LAF, confirming that the ablation catheter is within the reentry circuit by demonstrating concealed entrainment and a PPI–TCL of ≤20 msec is important for the success of the procedure [19,20]. The most significant limitation of entrainment mapping in patients with atypical flutter is the frequent conversion of the clinical arrhythmia into a different circuit, morphology, or AF deterioration during pacing.

4. CONCLUSION

Atypical atrial flutter is a challenging arrhythmia that can occur in the presence of an atrial scar, often related to either previous cardiac surgery or catheter ablation of AF. Catheter-based mapping and ablation of atypical atrial flutter are feasible and effective, although technically challenging. The combination of activation mapping using a non-fluoroscopic 3D mapping system and entrainment mapping is crucial to achieving satisfying acute success and long-term outcomes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 6
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Critical Study on Prevalence of Bacterial Vaginosis among Antenatal Patients at Federal Teaching Hospital Abakaliki, South East Nigeria

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ABSTRACT

Bacterial vaginosis (BV) is the most common cause of vaginal discharge in women of child bearing age. About half of these women are asymptomatic. Adverse outcomes are consistently associated with bacterial vaginosis in pregnancy and in the puerperium. This study, which was done to look at the prevalence, involved recruiting 230 participants, and screening them for BV using the Amsel's criteria. It is hoped that identification and treatment of these women will help prevent some of the sequela associated with BV. Restoration of the vaginal microflora by treating identified cases of BV will also help reduce the transmission of HIV and herpes simplex virus (HSV) as BV propagates their replication and vaginal shedding. Two hundred and thirty women in the age range 16 - 40 years were screened for BV. All the participants douched, soap and water being predominantly used. 55 women (23.9%) had BV based on Amsel's criteria. Women with only one sexual consort had the highest incidence of BV (51.9%). Raising the awareness of bacterial vaginosis among our pregnant women and their health care providers will be an important first step in preventing BV and promoting its diagnosis and treatment. It will also be an important first step in preventing the sequela of BV in pregnancy and in the puerperium.

Keywords: Prevalence; bacterial vaginosis; women; abakaliki; Nigeria.

1. INTRODUCTION

Bacterial vaginosis is the most common cause of vaginal discharge in women of childbearing age [1] [2-4]. In some populations its prevalence is greater than 50 percent [1], and almost half of the affected women are asymptomatic [2,4]. With normal physiological vaginal discharge increasing during pregnancy [5], distinguishing between a normal physiological discharge and that caused by bacterial vaginosis is of significant interest and benefit to the mother, yet to be born baby and the Obstetrician. Observational studies have consistently shown that an association exists between bacterial vaginosis and adverse pregnancy outcome [2]. These include preterm delivery [2,6,7], preterm premature rupture of membranes, postpartum endometritis [8]. Inflammation of the vagina, or vaginitis, is caused by various infectious and non-infectious factors [9]. The most common causes of infectious vaginitis are bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and trichomonal vaginitis (TV) [10,11]. Bacterial vaginosis carries a five to seven fold increased risk for late miscarriage and preterm labour [5] and hence prematurity. Prematurity remains the leading cause of perinatal and neo-natal morbidity and mortality [12]. Prematurity as a result of bacterial vaginosis infection can be prevented if pregnant women infected with bacterial vaginosis are identified and treated. Long standing or untreated bacterial vaginosis may lead to more serious sequelae, such as endometritis, and salpingitis [13].

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Bacterial vaginosis have been recognised as a state of diminished resistance to colonization. It also renders women particularly vulnerable to the acquisition of *Trichomonas vaginalis, Neisseria gonorrhoeae*, *Chlamydia Trachomatis*, Herpes Simplex Virus 2 (HSV-2) and Human Immunodeficiency Virus-1 (HIV-1) [4,14-18]. It has been documented that bacterial vaginosis propagates viral replication and vaginal shedding of the HIV-1 and HSV-2 viruses, thereby further enhancing the spread of these sexually transmitted diseases [4]. The prevalence rate of BV as determined by Gram stain analysis of vaginal fluid ranges from 29% in the United States [19] to >50% in Ugandan villages [20,21].

Bacterial vaginosis (BV) was previously called non-specific vaginitis because bacteria are the main etiologic agent, and an associated inflammatory response is lacking. Studies have shown that a diagnosis of BV based on symptoms alone is often inaccurate compared to diagnosis based on laboratory criteria [22,23]. In addition, basing diagnosis solely on symptoms will miss those women who would have laboratory-confirmed diagnosis of the infection but are asymptomatic [24].

Amsel's criteria have been used for making the diagnosis of BV for many years. It combines both laboratory criteria and symptoms. The four diagnostic criteria are: a vaginal fluid pH > 4.5; >20% of epithelial cells are "clue" cells; milky homogenous adherent vaginal discharge; and a positive "whiff" test, which is an amine or "fishy" odour noted after the addition of 10% potassium hydroxide. The presence of three out of the four criteria is recommended by Amsel for diagnosis [25-27].

Risk factors for bacterial vaginosis include douching [23], race [14,15] no condom use [23], new male partner, and smoking [25].

Though sexual activity can increase the risk of developing bacterial vaginosis, BV can occur in virgins [28]. BV is thought not to be sexually transmitted.

Bacterial vaginosis also contributes significantly to increased incidence of foetal wastage as it has been implicated as a cause of premature rupture of membranes, chorioamnionitis and spontaneous abortions. Untreated bacterial vaginosis has been shown to cause postpartum endometritis [8] and salpingitis [13], conditions that can invariably alter the Obstetric carrier of the patient.

2. METHODOLOGY

This was a prospective study in which the sample size was calculated based on the known prevalence from a similar study done elsewhere using the formula by Daniel WW [29]. 230 consenting pregnant women, who did not meet the exclusion criteria, were recruited over a period of 13 weeks at the antenatal clinic of the Federal Teaching hospital Abakaliki, Ebonyi state to look at the prevalence of BV.

Ethical clearance was obtained from the ethics committee of the hospital.

The criteria for exclusion included: refusal to give consent, recent history of vaginal bleeding (within the last 48 hours), intercourse within the last 24 hours, gestational age greater than 42 weeks, unsure date, threatened abortion, diabetes Mellitus and premature rupture of membranes.

The purpose of the study was explained to the client at the antenatal clinic hall by residents and house officers trained for the study, and her informed consent obtained before being enlisted in the study. The client had the right to opt out of the study anytime within the study period. Through a structured questionnaire, the medical history was obtained from the clients. These included the age, educational status, history of smoking, douching and the substance used, number of lifetime sexual partners and history of previous premature birth.

After counselling and signing of consents, coded structured questionnaires were administered to the client. Thereafter, clients were taken to the examination room and in the presence of a chaperon, they are put in a lithotomy position and a sterile disposable speculum Cusco's speculum passed. A sterile

swab-stick with an identification mark, which tallied with that on the questionnaire was then used to collect a sample of vaginal fluid from the posterior fornix.

During the specimen collection, presence or absence of the characteristic thin gray adherent vaginal discharge was noted and filled out in the same questionnaire. The swab was smeared onto a grease-less glass slide which was allowed to air dry and on a pH paper. The pH is recorded. The glass slide had been marked with a diamond pencil with a number that corresponds with the number on the corresponding questionnaire. The swab-stick is then put in a test tube that contains about one millilitre of 10% potassium hydroxide. This was wafted to the nose for the characteristic fishy odour "whiff test". This was also noted on the questionnaire. The smeared glass slide was then sent to the laboratory at the end of the sample collection for staining and examination for clue cells, by a laboratory scientist or the chief researcher. This was immediately noted on the questionnaire.

The information on the questionnaire was matched and cross checked. This was fed into a personal computer and subsequently analyzed using the EPI, info statistical software package version 3.3.2 (2005) of the Centre for Disease Control.

The sensitivities and specificities of the various signs and symptoms of BV were calculated using the standard formula.

3. RESULTS

Two hundred and thirty women who booked for antenatal care, consented and did not meet the exclusion criteria were enrolled into the study. None of them opted out of the study. The sociodemographic characteristics of the study population are as shown in Table 1. The participants' ages ranged from 16 - 40 years with a mean age of 29.2 ± 4.6 years. Women who were nulliparous were 75 (32.6%), multiparae were 139 (60.4%) while grand multiparous women were 16 (7.0%). Three (1.3%) of the women had no formal education, 21 (9.1%) had primary, 79 (34.4%) had secondary while 127 (55.2%) had tertiary education. All the participants douched, those that used soap and water formed the bulk-155 (67.4%). One hundred and thirty (56.5%) participants have had only one sexual partner, while 88 (38.3%) have had more than one and 12 (5.2%) have had more than three sexual partners in their lifetime. 109 (47.8%) of the total participants reported having any symptom, those presenting with vaginal discharge (81% - 74.3%) accounting for the bulk.

Fifty five women (23.9%) of the studied population had BV. Of the 55 that had BV, 32 (58.2%) reported having any symptoms. 19 (59.4%) had whitish discharge that stuck to the vaginal wall alone, 12 (37.5%) had both whitish discharge and fishy odour after sexual intercourse, while only 1 (3.1%) had fishy odour alone. Considering the diagnostic criteria, vaginal pH of more than 4.5 was present in most (52) [94.5%] of the studied population while 3 (5.5%) had PH less than <4.5. The whiff test was positive in 51 (92.7%) participants who had BV. Clue cells were present in 32 (58.2%), while white vaginal discharge was present in 48 (87.3%) of the BV positive Cases. This is summarized in Table 2, Table 3.

When the diagnostic criteria were compared between the BV positive group and the BV negative group; the whiff test gave the highest number of true positives (51/55) while the presence of clue cells gave the least number of false negatives. Table 4 lists the sensitivity and specificity of the different diagnostic criteria.

Table 1. Sociodemographic characteristics

Variable			Level of significance (%)			
Age	**BV + ve**	**%**	**BV − ve**	**%**	**BV + ve**	**BV – ve**
15-24	10	18.2	27	15.4	13.4 - 44.1	17.2 - 30.7
25-34	38	69.1	124	70.9	17.2 - 30.7	69.3 - 82.8
35-44	7	12.7	24	13.7	9.6 - 41.1	58.9 - 90.4
Total	55		175			

Chi square-0.25, df-2, p value-0.88; This is not significant as p is not less than 0.05.

Variable					Level of significance (%)		
Edu. status	**BV + ve**	**%**	**BV – ve**	**%**	**total**	**BV + ve**	**BV – ve**
None	0	0.0	3	1.7	3	0.0 - 6.5	0.4 - 4.9
Primary	7	12.7	14	8.0	21	5.3 - 24.5	4.4 - 13.1
Secondary	26	47.3	53	30.3	69	33.7 - 61.2	23.6 - 37.7
Tertiary	22	40.0	105	60.0	127	27.0 - 54.1	52.3 - 67.3
Total	55		175		230		

Chi square-0.51, df-3, p value-0.03; This is significant as p is less than 0.05

Table 2. Incidence of BV

	Frequency	%
BV + ve	55	23.9
BV – ve	175	76.1

Table 3. Diagnostic criteria

		BV + ve	BV − ve	Total
Ph	<4.5	3	58	61
	>4.5	52	117	169
Whiff test	Present	51	30	81
	Absent	4	145	149
Clue cells	Present	32	3	35
	Absent	23	172	195
White sticky discharge	Present	48	61	109
	Absent	7	114	121

Diagnosis of BV is based on any three positive out of the four diagnostic criteria

Table 4. Sensitivity and specificity of the diagnostic criteria

	Sensitivity (%)	Specificity (%)
pH	31	95
whiff test	62	97
clue cells	91	88
white discharge	44	94

Presence of clue cells gave the highest sensitivity, while the whiff test is the most specific

The presence of clue cells has the highest sensitivity (fewest false negatives) and the whiff test the highest specificity (fewest false positives).

4. DISCUSSION

Various prevalences for BV have been published, this being primarily dictated by the study group, and to a lesser extent the biosocial characteristics of the participants. Out of the two hundred and thirty participants, fifty five met three out of the four Amsel's Criteria required for the diagnosis of BV. This gave an incidence of 23.9%. This incidence is not consistent with that of Adinma *et al.* in Nnewi, south east Nigeria 17% [30] and Adeoye *et al.* 10.5% [31] but is consistent with incidences of 25% [32] and 23% [33] obtained by Adekunle and Cecil Klufio respectively. Various figures have been recorded from other regions; India 27.5% [34], 31.6% [35], Helsinki Finland 10.4% [36], USA 16% [37], Kenya 37% [38], South Africa recorded the highest incidence of 52% [39]. The Canadian Study summed up the incidences in most of the groups as they quoted a range of 6.0% to 32% [36,40,41] in different studies.

The biosocial characteristics of BV positive participants showed that the highest incidence was in the age range of 25 - 34 (69.1%) followed by those in the age range of 15 - 24 (18.2%). This is not consistent with that of Adinma *et al.* (Nigeria) which was 17.0% for 16 - 20 years [30] and Larsson

(Sweden) 18 - 25 years [42]. Age though was not statistically significant (Chi square = 0.25, df = 2, p = 0.88), and therefore, no age group has a greater tendency of BV acquisition.

When the mother's level of education and the incidence of BV was subjected to statistical analysis, it was found to be significant (Chi square-0.51, df-3, P-0.04). When the educational level of the mother was matched against douching; there was a steady increase in the number that douched as the educational level increased. Also the number increased as the wash agent included other things aside water. Considering that the educational level of the mother helps determine the social class [43] and that all the study participants douched; it could be surmised that aside water, it is likely that those in the higher social classes and thus more educated, used some form of soap and vaginal foams; are likely to have bath with antiseptic liquids, perfumed bubble baths, uses vaginal deodorants and washes underwear with strong detergents [44]. These upset the balance of the naturally occurring bacterial flora and increase the risk of developing BV [45]. Zhang et al. [39] found that BV was more in women who douched once or more times per week when compared to those that douched less frequently or not at all. Schwebke [24] collaborated this when he discovered that douching in the previous week was positively associated with BV. When different douching products (vinegar, povidone-iodine, physiological saline) were evaluated, Oderdonk et al. [34,35] found that all, to varying degrees led to a reduction in the total bacteria count.

5. CONCLUSION

This study demonstrates that bacterial vaginosis is common among pregnant women in Ebonyi State, Nigeria commoner than was previously documented [30,31].

Studies have shown that awareness of BV is low among pregnant women and physicians alike [45]. Raising the awareness of bacterial vaginosis among our pregnant women and their health care providers will be an important first step in preventing BV and promoting its diagnosis and treatment. It will also be an important first step in preventing the sequela of BV in pregnancy and in the puerperium.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Saussurea costus: A Source of Anticancer Bioactives

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ABSTRACT

Cancer is the second leading cause of death globally and is responsible for an estimated 9.6 million deaths in 2018. Plant-derived products or extracts are used in folk/traditional medicine to treat several ailments or disorders. The anticancer activity of *Saussurea costus* and its mode of intervention in human cancer cells of breast, colon, and liver were investigated. The bio-actives of *S. costus* leaves extensively extracted in five solvents of different polarity were investigated for cytotoxic activity against breast (MCF-7), liver (HepG2), and colon (HCT116) cancer cell lines. Secondary metabolites extracted in hexane, methanol, ethyl acetate, and chloroform solvents had the highest cytotoxicity and thus the greatest anticancer effect on all the cancer cell lines tested, while as that of butanol was comparatively less active. Further investigations revealed that the extract arrested the cells in the G1 phase of cell cycle and induced apoptosis. The elevated expression of pro-apoptotic proteins and decreased expression of anti-apoptotic proteins confirmed that the intrinsic (mitochondrial) pathway was involved in mediating the apoptosis of cancer cells upon treatment with *S. costus* extract. These results suggest that the *S. costus* extract is the potential source of the secondary metabolites that could be used as anti-cancer agent to treat diverse cancers of breast, colon, and liver. However, further evaluations, active compound isolations, in vitro and in vivo evaluations are recommended for future research on these active ingredients.

Keywords: Plant extract; Saussurea costus; anti-cancer; apoptosis.

1. INTRODUCTION

Cancer is the second leading cause of death globally and is responsible for an estimated 9.6 million deaths in 2018 [1]. The reasons are complex but reflect both aging and growth of the population, as well as changes in the prevalence and distribution of the main risk factors for cancer, several of which are associated with socioeconomic development [2-4]. The most common cancers are lung, breast, liver, colorectal, prostate, skin, and stomach. Hepatocellular carcinoma (HCC) is a leading cause of death in people with cirrhosis [5]. Chronic liver disease and cirrhosis by viral hepatitis remain the important risk factors for the development of HCC [6]. Recently, the incidence of HCC has been rising worldwide, particularly in highly industrialized countries such as the United States or Japan [7-11]. Significant progress has been made in diagnosis and treatment using multidisciplinary approaches of surgical, non-surgical [12] and systematic chemotherapy [13]. Breast cancer, the most common cancer among women, is one of the leading causes of morbidity and mortality for women worldwide [14]. Colorectal cancer is a multifactorial disease, of which several risk factors have been identified involving genetic and environmental factors, lifestyle, and gut microbiota. Though emerging chemotherapeutic agents inhibiting the cellular pathways involved in the cellular proliferation have revolutionized the treatment of various cancers, the universal drug resistance development demands the generation of new therapeutic agents [15].

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Plants are known to have a long history in cancer treatment [16] and medical practices. *Saussurea costus* (Costaceae family), habitant of the sub-alpine region of Jammu and Kashmir, Himachal Pradesh, and Uttarakhand is used to treat various ailments, *viz*, ulcer, headache, rheumatism, cough and cold, throat infection, etc., while in Korea it is frequently used in Korean traditional prescriptions for inflammatory diseases [17]. Its anticancer [18-20] activity, including other activities of anti-inflammatory [21,22], and antioxidant [23,24] has gained the attention of the scientific community to explore the underlying mechanisms of aforesaid activities. All these beneficial uses of a taxonomically related *S. costus*, commonly known as kuth in Kashmiri, have not been investigated particularly in the cancer cell lines taken in this study.

Apoptosis, a major form of programmed cell death, plays a vital role in regulating tissue development and maintenance of the homeostasis in eukaryotes [25-27]. One of the hallmarks of cancer is the deregulation of apoptosis [27]. Therefore, an effective mode of intervention for chemo-preventive and chemotherapeutic agents in many types of cancers could be the upsurge of apoptosis. In this study, we explored the possibility that whether *S. costus* could function as a chemotherapeutic agent in human cancers.

2. METHODOLOGY

2.1 Crude Extracts Preparation

100 g of dry leaves of *S. costus* obtained from an herbal store (Khamis Mushayt, Saudi Arabia) were washed with distilled water and ground by a grinder with 500 ml of 80% aqueous ethanol. The leaves were occasionally stirred for seven days at room temperature (18–24°C). The extract was filtered using filter paper and dried under reduced pressure using a rotary evaporator at 37°C. The crude extract weighing 10 g was reconstituted in 400 ml of distilled water and further extracted with different polarity solvents, *viz*, methanol, hexane, chloroform, ethyl acetate, and butanol, using liquid-liquid extraction method. The separated solvent phase was re-evaporated using a rotary evaporator. 0.01 g aliquots of each crude extract were stored as stock solutions at -20°C for bioactivity assays.

2.2 Cell Culture, Cytotoxicity Assessment, Cell Cycle Distribution and Apoptosis Assessment

The cells of the human hepatocellular carcinoma cell line (HepG2), colorectal adenocarcinoma cell line (HCT116), and breast adenocarcinoma cell line (MCF-7) obtained from the American Type Culture Collection (ATCC) were maintained in RPMI-1640 supplemented with 100 µg/ml penicillin and heat-inactivated fetal bovine serum (10% v/v) in a humidified 5% (v/v) CO_2 atmosphere at 37°C [28]. The cytotoxicity of different compounds was tested against human tumor cells using the Sulphorhodamine B assay (SRB) as described [29]. Western blotting was performed as described [30].

3. RESULTS

3.1 Cytotoxic Activity of *S. costus* Leaf Extracts

Taking the advantage of SRB assay, the viability (%) of the cancer cells was used as an indicator to determine the cell toxicity towards the treatment with different concentrations of extract. The results demonstrate that the viability of cells decreased in a dose-dependent manner (Fig. 1), potentially killing all cells at 100 µg/ml. The cytotoxic activity of methanol, hexane, chloroform, and ethyl acetate was very strong (IC_{50}; 0.5 – 2.5 µg/ml), almost similar to that of a prominent anticancer drug, doxorubicin (IC_{50} <1 µg/ml). The methanolic extract exhibited comparatively less cytotoxic effect (IC_{50}; 25 to 32.2 µg/ml) against all the cell lines tested (Table 1). IC_{50} values of the extracts, except methanol, were being within the closer range to that of doxorubicin.

3.2 *S. costus* Leaf Extract Induces Cell Cycle Arrest

In order to determine whether the inhibition of cellular growth was due to the arrest of the cell division at a specific phase of the cell cycle. The cell lines were individually treated with the extracts at their

IC_{50} concentrations, and the cell cycle profile was determined by PI staining followed by DNA flow cytometry analysis. As shown in Table 2 the significant population of all the tumor cell lines, except HepG2, was found in the G_1 phase of the cell cycle after their treatment with either methanol or hexane or ethyl acetate, compared to the DMSO-treated control. Rather than increasing the HepG2 cellular population in the G_1 phase, there was a significant increase in the population of cells in the G_2/M phase upon their treatment with ethyl acetate compared to the control. Chloroform displayed varying effects on the cell lines tested. Compared to control, chloroform treatment significantly increased the MCF-7 and HepG2 cell population in G_1 and G_2/M phase, respectively. While as, compared to control a significant population of HCT116 cells was observed in G_1 and G_2/M phases of the cell cycle upon their treatment with chloroform. Butanol, on the other hand, exhibited markedly increased cell population of all cell lines in both G_1 and G_2/M phases. The results demonstrate that all the extracts actually arrested all the types of cancer cells in the G_1 and G_2/M phases of the cell cycle, though the percentage of cells showed a variation.

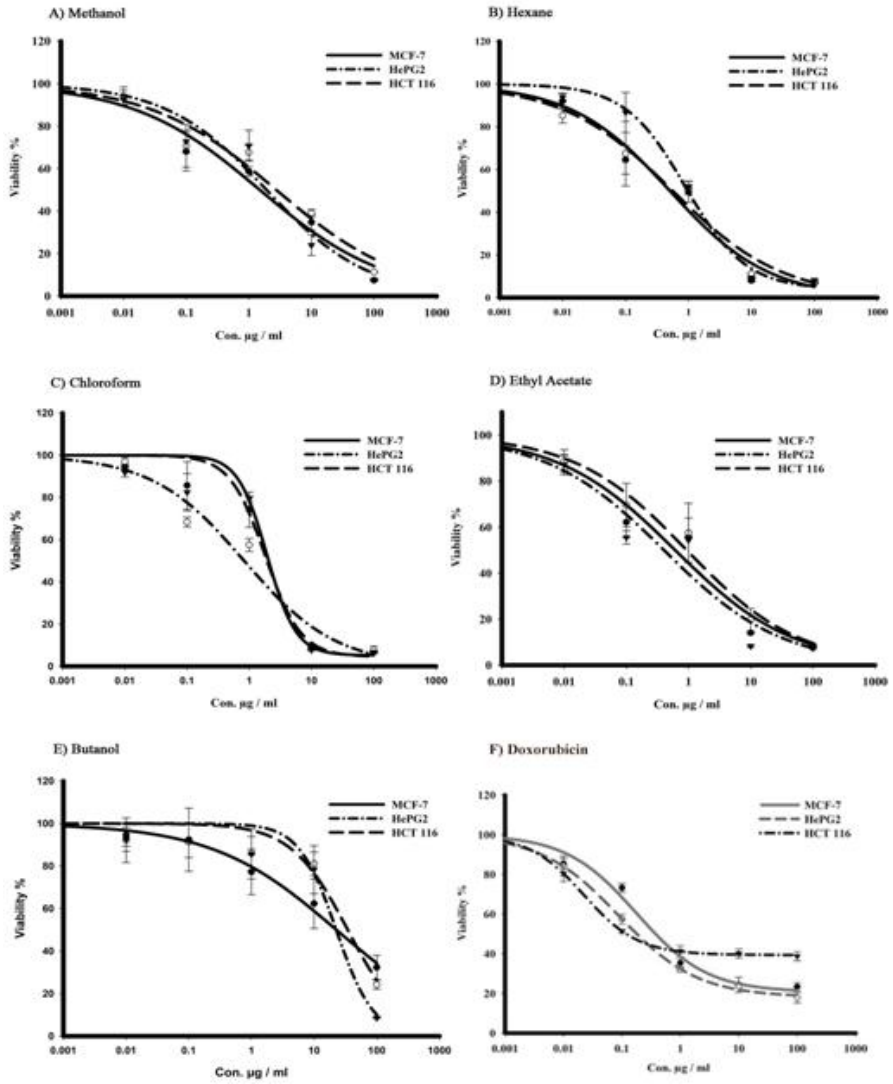

Fig. 1. IC_{50} values of the *Saussurea costus* extracts against cancer cell lines

The graph plotted between the percentage of viable cells of the cell lines MCF-7, HepG2, and HCT116 treated against the various concentrations (µg/ml) of extracts, viz, methanol (A), hexane (B), chloroform (C), ethyl acetate (D), butanol (E), and the drug doxorubicin (F).

Source: Biomed Res Int. 2020 Jul 12;2020:1608942. DOI: 10.1155/2020/1608942

Table 1. The IC_{50} (µg/ml) of different extracts of *Saussurea costus* against different tumor cell lines

Extract	MCF-7	HepG2	HCT116
Methanol	1.3 ± 0.2	2.5 ± 0.6	1.7 ± 0.3
Hexane	0.54 ± 0.04	0.5 ± 0.09	0.99 ± 0.09
Chloroform	1.8 ± 0.3	0.8 ± 0.01	2.1 ± 0.18
Ethyl Acetate	0.67 ± 0.05	1.2 ± 0.2	0.4 ± 0.05
Butanol	25.5 ± 2.8	33.2 ± 2.5	24.9 ± 3.07
Water	250.31 ± 10.9	380.4 ± 163.7	204.3 ± 65.12
Doxorubicin	0.45 ± 0.0516	0.6 ± 0.022	0.42±0.103

Results were expressed as mean ± SD for three different independent replicates.
Source: Biomed Res Int. 2020 Jul 12;2020:1608942. DOI: 10.1155/2020/1608942

Table 2. Cell cycle phase distribution (%) of the tumor cell lines after their treatment with different solvent extracts of *Saussurea costus*

Tumor Cell	Extracts	Cell Phases		
MCF-7		G_1	S	G_2/M
	Cell Control	50.32 ± 0.98	23.21 ± 0.6	26.47 ± 0.9
	Methanol	63.42 ± 1.5	5.87 ± 0.5	30.71 ± 1.01
	Hexane	68.92 ± 0.9	13.45 ± 1.1	17.63 ± 0.52
	Chloroform	64.25 ± 0.5	11.43 ± 0.7	24.32 ± 0.6
	Ethyl Acetate	67.82 ± 0.7	12.65 ± 1.1	19.53 ± 0.8
	Butanol	54.71 ± 1.1	12.08 ± 0.5	33.21 ± 0.7
HePG2	Cell Control	56.44 ± 1.1	24.58 ± 1.1	18.98 ± 2.1
	Methanol	66.87 ± 1.5	13.76 ± 1.16	19.37 ± 1.1
	Hexane	69.11 ± 2.2	12.12 ± 1	18.77 ± 1.3
	Chloroform	58.25 ± 0.4	18.31 ± 1.4	23.44 ± 1
	Ethyl Acetate	56.74 ± 0.8	20.9 ± 1	22.36 ± 1.6
	Butanol	61.39 ± 1.7	16.3 ± 1.7	22.31 ± 0.9
HCT116	Cell Control	48.54 ± 2.3	23.13 ± 1.4	28.33 ± 1.1
	Methanol	59.35 ± 1.3	6.91 ± 1	33.74 ± 1.7
	Hexane	68.2 ± 2.7	16.13 ± 1.3	15.66 ± 1.6
	Chloroform	56.8 ± 1.3	11.1 ± 1.4	32.1 ± 0.8
	Ethyl Acetate	66.87 ± 1.8	20.48 ± 0.8	12.65 ± 1.5
	Butanol	51.87 ± 2.5	16.61 ± 1.7	31.52 ± 1.4

Results were expressed as mean ± SD for three independent replicates. Source: Biomed Res Int. 2020 Jul 12;2020:1608942. DOI: 10.1155/2020/1608942

3.3 *S. costus* Extracts Induces Apoptosis in Cancer Cells

To further investigate the extract-induced inhibitory effect, cells treated with various extracts were analyzed under a fluorescent microscope for nuclear morphological changes (apoptosis or necrosis) after AO/EB staining.

As shown in Fig. 2A, B, and C (sub-panels a-f), the extracts of *S. costus* induced morphological changes, DNA fragmentation, nuclear shrinking, etc. which are characteristics of various stages of apoptosis, *viz*, early or late phase apoptosis. The major cellular populations of all tumor cell lines tested against all types of extracts were in the early apoptotic stage (sub-panel g). However, a marked percentage of HCT116 cells were in the necrotic stage of apoptosis when treated individually with chloroform, ethyl acetate, and butanol.

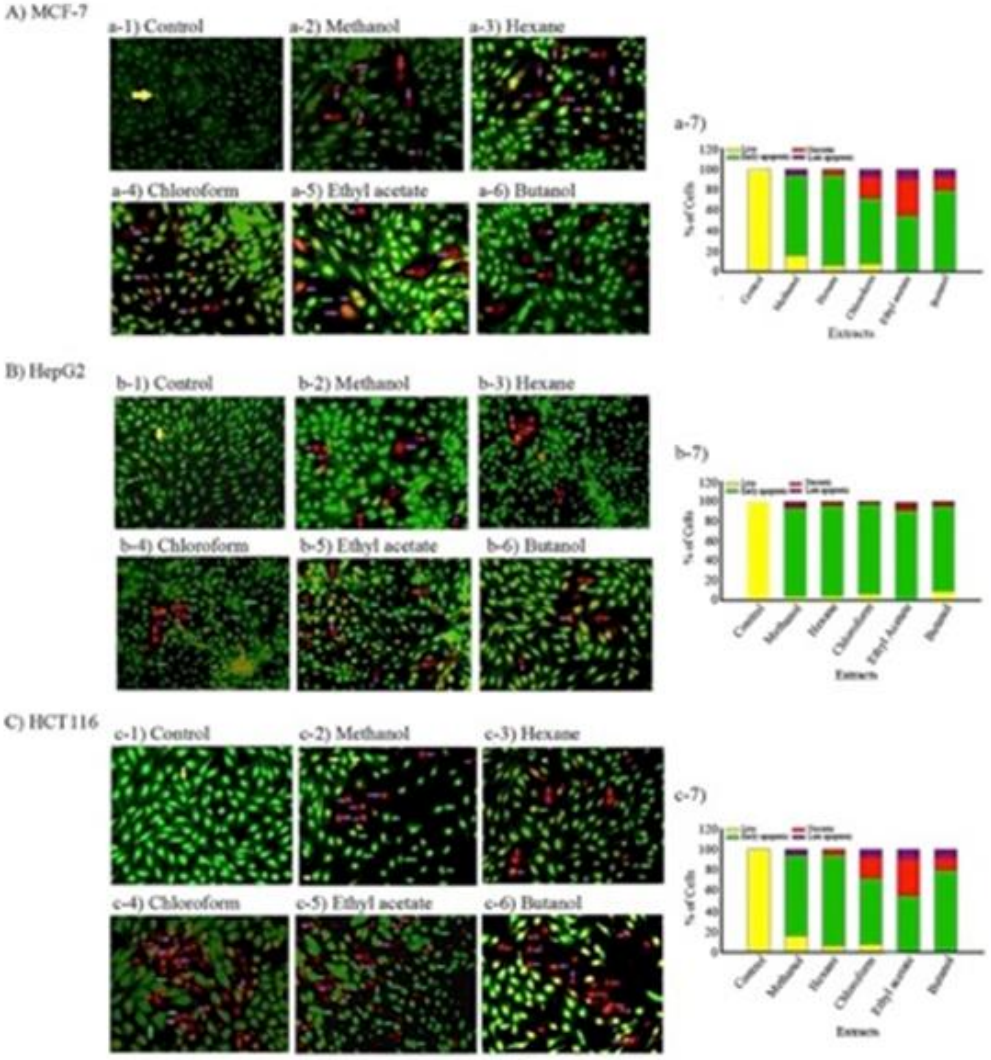

Fig. 2. The representative fluorescent images of the cancer cell lines treated with a plant extract

Bar graphs show the percentage of cells of a cell line in different stages of apoptosis after extract treatment. Arrow: Yellow; live cell, green; early apoptotic, red; necrotic, purple; late apoptotic. Source: Biomed Res Int. 2020 Jul 12;2020:1608942. DOI: 10.1155/2020/1608942

3.4 Apoptosis of Cancer Cells Occurs Through Caspase-3

The disturbance of apoptotic molecular signaling pathways is involved in carcinogenesis. In apoptosis, the activation of the executioner caspases-3 and -7 is regulated both by extrinsic (death ligand) and intrinsic (mitochondrial) pathways.

The BCL2 family of proteins, including both anti-apoptotic (Bcl-2) and pro-apoptotic (Bax) regulators, is the hallmark of apoptosis regulation. To investigate the molecular mechanism, we tested the hexane extract (having the least IC_{50}) for all the four cancer cell lines. Treatment of *S. costus* extract decreased the levels of Bcl-2 in all cancer cells, while the level of Bax expression is highly induced (Fig. 3). The caspase family activation being one of the earliest known steps in the apoptosis, the cancer cells exposed to *S. costus* extract exhibited the robust activation of caspase-3 (Fig. 3).

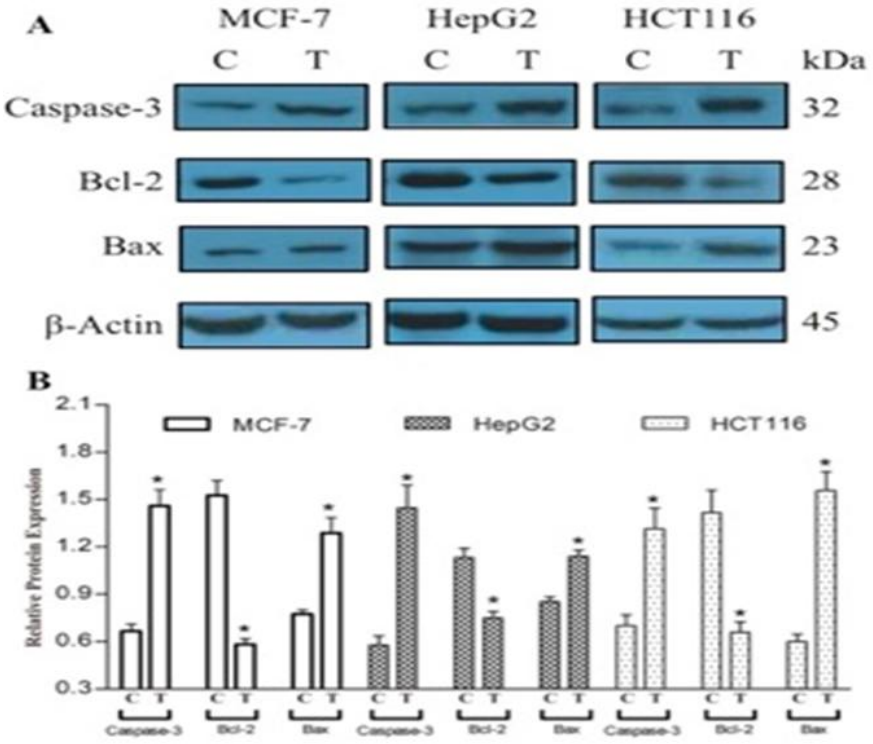

Fig. 3. Expression profile of Bcl-2, Bax, and caspase-3.(A) Western blot analysis of Bcl-2, Bax, and caspase-3 proteins in the cell lysates of cancer cells after hexane extract treatment (B) Relative protein expression level of Bcl-2, Bax, and caspase-3 in control (C) and extract-treated (T) cells

**: P < 0.05. Source: Biomed Res Int. 2020 Jul 12;2020:1608942. doi: 10.1155/2020/1608942*

4. DISCUSSION

Natural products extracted in their crude form or as purified compounds, have been used as bases for discovering new drugs. Plant extracts have been investigated *in-vitro* for antibacterial as well as anticancer activity [31]. Herbal medicine treatment provides some advantages over the use of single purified chemicals [32]. In this study, the cytotoxicity properties of various *S. costus* extracts obtained from its dried leaves have been investigated for their biological efficacy against cancer cells. We used several solvents (methanol, hexane, ethyl acetate, butanol, and chloroform) to extract all compounds of varying polarity. The four solvent extracts methanol, hexane, chloroform, and ethyl acetate showed strong cytotoxic activity against all the three cancerous cell lines tested (IC_{50} < 2.6 µg/ml). Cell cycle analysis showed that the extracts basically arrested all types of cancer cells in the G_1 or G_2/M phase of the cell cycle, though the percentage of cells showed a variation with respect to a particular cell line (Table 2). Studies show that anticancer molecules cause cell cycle arrest and subsequently induce cell death by apoptosis [33]. Certain anticancer agents are shown to cause DNA damage, which results in stagnation of cells in the G_1 or G_2/M phase before inducing apoptosis. The present results of cell cycle arrest suggest the association of apoptosis-inducing property of the extracts in the tested cancer cells. The observed physiological changes of condensed/fragmented and highly fluorescent nuclei (Fig. 2) in extract-treated cancer cells suggested features of apoptosis. A crude extract of IC_{50} less than 50 µg/ml considered to have anti-tumor properties [34], suggests, that the *S. costus* extracts exhibit strong anti-cancer effects.

The expression profile of Bax, Bcl-2, and Caspase-3 post hexane-extract treatment indicated that the expression of Bax increased and of Bcl-2 decreased (Fig. 3), suggesting a critical role of the *S. costus* extract in the apoptosis of cancer cells. Increased expression of Bax causes subsequent release of

cytochrome C and other pro-apoptotic molecules, thus activating downstream caspases and ultimately caspase-3 [35]. Increased expression of caspase-3 was observed in the extract-treated cells (Fig. 3), which is in agreement with the apoptosis of human gastric cancer by *S. costus* extract [36].

These results demonstrate that the mitochondrial-mediated caspase activation pathway is involved in extract-mediated apoptosis of human cancer cell lines of breast, liver, and colon. More investigation is required to determine the role of other regulators/signals involved in the cytotoxic and apoptotic effect of the *S. costus* extracts.

5. CONCLUSION

These findings suggest that *S. costus* extracts contain bioactives that might act potential therapeutic agents for the treatment of breast, liver, and colon cancers. However, further evaluations, active compound isolations, *in vitro* and *in vivo* evaluations are recommended for future research on these active ingredients.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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Chapter 8
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Factors Underlying Stigmatization of Epilepsy: Case Study of Abasuba and Ameru Communities, Kenya

Tiberry D. O. Nyakwana[1*], Jemimah A. Simbauni[2] and James O. Jowi[3]

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ABSTRACT

Aims: To determine factors underlying stigmatization of People with Epilepsy (PWE) among Abasuba and Ameru communities, Kenya.
Study Design: Cross sectional design was used in this study.
Place of the Study: The study was carried out in Abothuguchi, Miriegameru and Nkuene divisions in Meru Central Sub-counrty and Central, Gwasi and Mbita divisions of Suba Sub-county in Kenya.
Methodology: It was a descriptive cross-sectional study. A modified participatory rapid appraisal technique was used which involved the use of questionnaires, interview schedules and focused group discussions. Household heads, medical personnel, members of community-based organizations, patients, parents, administrators, teachers, faith healers and herbalists were interviewed.
Results: The study results reveal a significant statistical relationship between negative attitude and fear epilepsy (χ^2 = 43.69354, df=1, p<0.05). The fear of epilepsy is dependent on knowledge about it (χ^2 = 7.41663, df=1, p=0.00646). Occupation was not found to influence fear except among the Meru Central District female respondents (χ^2 = 6.19763, df=2, p=0.04510). However, there was no significant relationship between fear of epilepsy and the level of education (χ^2 = 0.15773, df=2, p=0.092436). The belief that epilepsy results from a curse or witchcraft is transferable and contagious was deeply entrenched in the culture of the two communities and that the society views them with resentment resulting into isolation and social stigma.
Conclusion: The pattern of traditional belief systems about epilepsy is similar in the community and it is considered a contagious disease highly associated with witchcraft. Stigma and discrimination of PWE is aptly evident in both study areas cultural beliefs notwithstanding. This has the effect of shaping the expression of stigma and social isolation of PWE. Lack of information is responsible for people's fear of epilepsy and perception is a driver of negative attitude towards people with epilepsy in the community. The study recommends the provision of effective treatment and a comprehensive community sensitization program with target specific IEC materials to counter the negative beliefs.

Keywords: Knowledge; practices; perception; fear; stigma; contagious; PWE (People with Epilepsy); CORPS (Community Own Resource Persons).

1. INTRODUCTION

The term 'epilepsy' is derived from a Greek word "epilambanein" which means 'attack', 'grab', 'capture' or 'seize' [1]. It is a ubiquitous disorder known to mankind since antiquity with no racial, religious, class or geographical boundaries. It is associated with stigma, neuro-psychiatric comorbidity and high economic costs [2].

Epilepsy is one of the most common neurologic conditions, with an incidence of approximately 50 new cases per year per 100,000 population [3,4]. Epilepsy is a chronic condition of the brain characterized

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by enduring predisposition to generate two or more spontaneous seizures occurring more than 24 hours apart that resulting from excessive discharge of cerebral neurons [5]. These discharges are unprovoked or provoked by an identified cause [6]. The abnormal, excessive electrical discharges are non-specific responses to an insult to the brain causing sudden and transitory phenomena of motor, sensory, autonomic or psychic nature in different measures [7]. These phenomena may result in a diversity of clinical forms of epileptic seizures [8]. Without proper treatment, epilepsy has neurobiological, cognitive, psychological and social consequences with significant morbidity and mortality. However substantial disparities exist in its outcomes. Its care has propensity to be fragmented and poorly coordinated in countries where it exists. Healthcare system factors associated with its improved outcomes are modifiable.

Epilepsy is a significant public health problem representing 0.5% of the global burden of disease [9]. Globally about 70 million people have epilepsy worldwide, 10 million of whom live in Sub-Saharan Africa [10]. It affects another additional 500 million people are affected as family members and caregivers of patients [11]. In 2010, WHO's Global Burden of Disease ranked epilepsy as the second most burdensome neurologic disorder worldwide in terms of disability-adjusted life years [12]. In Kenya it is one of the 12 common diseases identified by local names at family level with a prevalence rate of 18 per 1000 [13,14]. The pooled lifetime prevalence of epilepsy is 7.60 per 1,000 persons and a median lifetime prevalence of 7.06 per 1,000 persons in high income countries but in middle- and low-income countries, it is 49.00 per 1,000 persons [15].

In Africa, though very few extensive population-based studies have been conducted, the prevalence rate of epilepsy has been seen to vary with individual reviews and country by country ranging from 0.7 to 1.5% with bimodal distribution peaks at 20-29 and 40-49 years [16]. In Tanzania, random cluster sample survey in Ulunga District reported a prevalence of 10.2 per 1000 [17]. A door-to-door survey in Zambia found an unadjusted incidence of 14.5 per 1000 [18]. A meta-analysis found a prevalence rate of 9.39 per 1000 individuals with a median of 14.2 (interquartile range of 8.0-33.2 per 1000 in Sub-Saharan Africa [19]. Generally, in Africa, the prevalence rate of epilepsy is 11.29 per 1000 population [20].

In rural Kenya and other African countries, about 69% of the seizures begin in childhood. Its prevalence in these countries is about 20 cases in every 1,000 people and about 77 in every 100, 000 new cases are diagnosed every year. These estimates are two to three times higher than in developed countries. In a cross-sectional survey carried out in Kilifi County in Kenya, a prevalence of 3.5 per 1000 inhabitants at risk with 3.8 for males and 3.3 for females in [21]. An earlier survey carried out in Nakuru County (Kenya) found a prevalence rate of 18 per 1000 of the population [22]. It is a well-known condition among the top 12 common diseases usually identified by families, often using local names [23].

The history of epilepsy has been riddled with prejudices and counter-prejudices. The Hammurabi code dated 1780 BC dictated that a person with epilepsy could not marry or testify in a court of law and the purchase contract of a slave was considered void if they suffered a seizure in the first three months. In China, PWE are generally withdrawn from the society, denied employment feel isolated and are either overprotected or neglected [24]. Most people would object to their children marrying or even playing with a person with epilepsy.

In some communities, epilepsy is equated with a mental disorder or insanity. Until recently, compulsory sterilization of PWE was legally permitted in some states in the US. Those who experience seizure in public places faced the risk of being arrested for disorderly conduct or mistaken for being under the influence of alcohol or illicit drugs [25]. In many parts of Sub–Saharan Africa, epilepsy continues to be associated with witchcraft [26,27,28].

Patients with Epilepsy are more vulnerable to often disabling psychosocial difficulties. The neurological deficit they may have come to bear on their families who may therefore be hesitant to disclose the illness to neighbors as such information may engender fear and social rejection. Consequently, these patients suffer segregation and exclusion in the society with subsequent stigma.

Many of them do not seek biomedical treatment and many of those who are on prescribed treatment do not adhere to it thus contributing to the wide treatment gap [29].

Quality healthcare for people with epilepsy maximizes patients' welfare, maintains standards and increase the likelihood of favourable health outcomes of individuals, households, families and communities. Aspects attributed to quality healthcare for people with epilepsy encompass effectiveness, efficiency, accessibility, evidence-based, timeliness and limited wastage. Effective healthcare delivery should therefore adhere to the evidence base that results in improved outcomes for them based on their expressed needs. Efficiency in health care for people with epilepsy should limit waste and maximize resource utilization within the context of healthcare provision.

2. MATERIALS AND METHODS

The study was carried out in Gwasi, Central and Mbita wards, Suba Sub-County and in Nkuene, Mirigamieru West and Abothuguchi Central Sub-Counties of Meru County. The study areas were purposively selected due to contrasting epilepsy care interventions. These varied epilepsy care interventions were noted by the investigators during their involvement in epilepsy care programmes in the two study areas run by Kenya Association for the Welfare of People with Epilepsy (KAWE).

2.1 Study Population

The study population comprised two hundred and twenty-five (225) household-heads, twelve (12) focus discussions and 30 key informants. Only household heads present at the time of the visit and had been residing in the area for at least five years and consented to take part in the study were recruited and interviewed. It excluded non-household heads, household heads that had not lived in the area for at least five years and those who did not consent. Informed consent was obtained in writing from study subjects prior to the interviews and confidentiality was assured.

2.2 Study Design

This was a cross-sectional descriptive study which involved the use of a 30-item structured questionnaire, Focus Group Discussion guidelines and key interview guides for household heads, focused groups and key informants respectively.

2.3 Investigation Process

The authority to carry out the study was obtained from Kenyatta University Research and Ethical Committee. Once in the field, further clearance was obtained from respective Deputy County Commissioners, County Executive Committee Members and Chief Officers of Health. Multistage sampling was adopted for selection of the locations and households. Three wards in each of the two sub-counties were selected randomly using random numbers. The first sample in each ward was determined arbitrarily considering the infrastructure and proximity to urban centres. Systematic random sampling method was used where the heads of every seventh household present at the time of the visit was interviewed face-to-face. The interval of every seventh household was considered appropriate following the fear and stigma attached to epilepsy as revealed from the pilot study where respondents considered it an abomination never to be called by name or only discussed in strict confidence. The key informants were served with self-administered questionnaires.

2.4 Sample Size Determination

The sample size was determined by the formula as used by Fisher et al. (1998)

$$n = \frac{Z^2pqD}{d^2}$$

Z = standard normal deviation usually 1.96 at 95% confidence interval
n = is the desired sample size

p = proportion of population estimated to have working knowledge on epilepsy = 0.07
q = 1 – p (1 – 0.2) = 0.93
d = degree of accuracy = 0.05
D = design effect =2

$$n = \frac{1.96 \times 1.96 \times 0.07 \times 0.93 \times 2 = 200}{0.05 \times 0.05}$$

A sample size of 119 was selected from Suba sub-county and 106 from Meru Central Sub-County were interviewed based on the prevailing environmental and geographical factors.

2.5 Data Collection

Data collection was carried out using semi-structured questionnaires, key informant interviews and focus group discussion guides which were designed to evaluate the awareness, knowledge, perception, attitude and practices of household heads. Face- to- face interviews were held with the household heads who met the inclusion criteria between 8am and 6pm. Focus Group Discussions (FGDs) were held with youth, women groups, parents, patients and C.B.O. members using FGD guidelines. Key Informant interviews were held with government officials, teachers, religious leaders, traditional healers and health workers to supplement the interviews held with the household heads. A total of twelve (12) focus group discussions were held and thirty (30) questionnaires were administered to various key informants.

3. RESULTS

A total of 225 study household heads were recruited and interviewed. Their mean age was 45.5years, mode of 42 years, a standard deviation of 14.63 years and range of 80 (18-98) years. They comprised 140 male respondents comprising of 62.2% and 85 female respondents comprising of 37.8%. For Suba Sub-County, 80.7% (n=96) were males compared to 41.5% (n=44) males for Meru Central Sub-County. They were distributed as follows: Mbita, 16.4% (n=37), Gwasi, 19.6% (n=44), Central, 16.9% (38), Nkuene, 14.7% (n=33), Mirigamieru, 15.6% (n=35) and Abothuguchi, 16.9% (n=38). By occupation, the distribution was: self-employed - 51.6 % (n=114); Salaried employees - 25.8% (n=57); unemployed - 22.6% (n=50). Upto 54.3% of Suba Sub-County respondents had education level of secondary and post secondary level as compared to 35.2% for Meru Central in the same category.

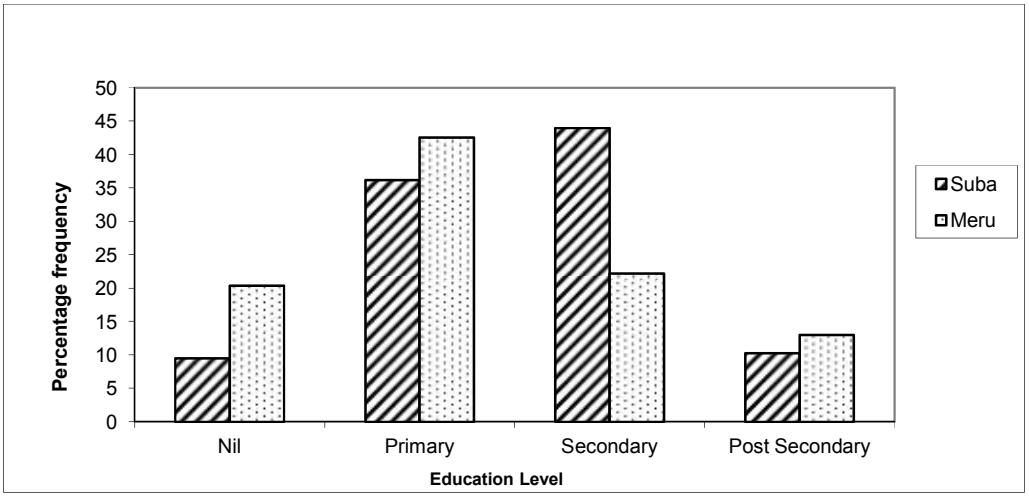

Fig. 1. Distribution of study participants by level of education

There was 100% awareness about epilepsy with respondents mentioning schools, CORPS and newspaper as their major sources of information. However, level of knowledge on epilepsy for Suba

Sub-County respondents was 34.2% compared to 11.3% for Meru Central Sub-County respondents. Consequently 56.9% of respondents of Suba Sub-County had a working knowledge of the causes of epilepsy compared to 37.3% for Meru Central Sub-County.

Upto 51.3% of Suba Sub-County respondents feared epilepsy as compared to 46.2% of Meru Central Sub-County respondents. In overall, 62.4% of those who feared epilepsy did not know what causes it ($p<0.05$).

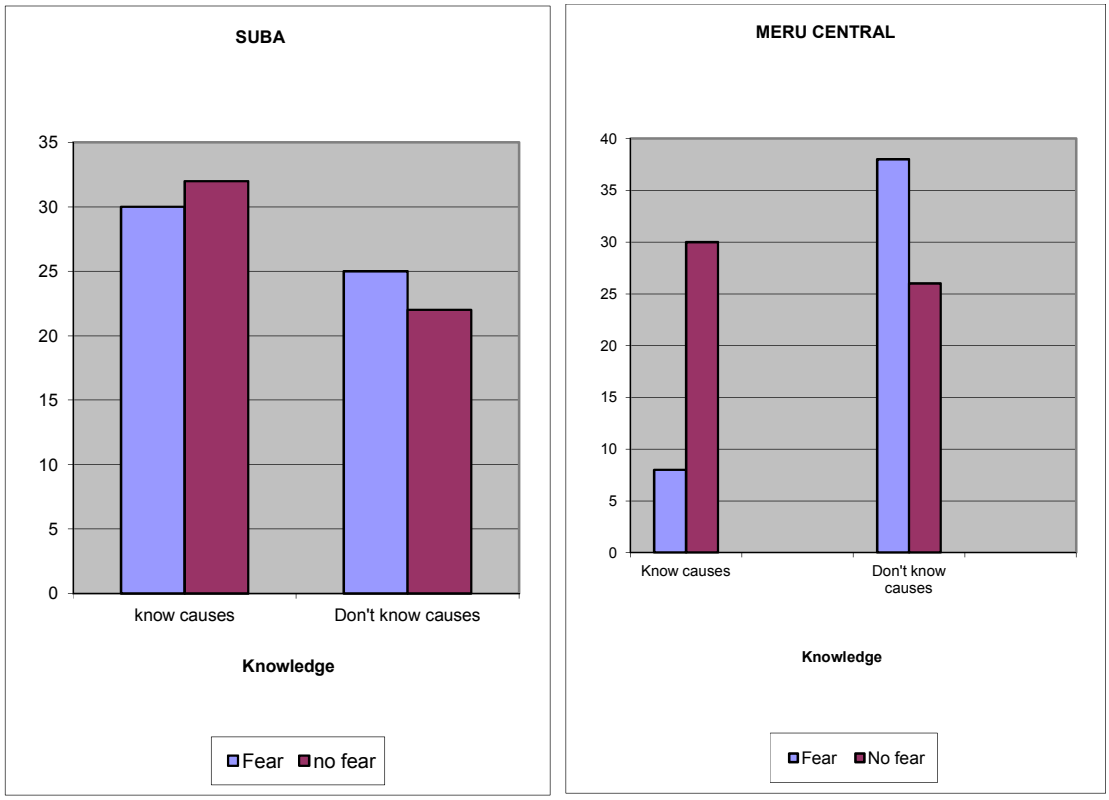

Fig. 2. Association between fear and knowledge on causes of epilepsy by Sub-Counties
(χ^2 = 0.24685, df=1, p=0.6193); (χ^2 = 14.14238, df=1, p=0.00017)

The relationship between fear and knowledge about epilepsy in both study areas was found to be statistically significant (χ^2= 7.41663, df=1, p=0.00646).

Table 1. Education level and fear of epilepsy

	No education	Primary	Secondary	Total
Fear	11 (13.3%)	33 (39.8%)	39 (47.0%)	83 (49.1%)
No fear	11 (12.8%)	32 (37.2%)	43 (50.0%)	86 (50.9%)

(χ^2 = 0.15773, df=2, p=0.092436)

For all the respondents in both sub-counties, there was no significant relationship between level of education and fear of epilepsy (χ^2 = 0.1573, df=2, p=0.92436). Upto 51.1% of all respondents had negative attitude toward PWE but 87.3% of those who fear epilepsy had negative attitude towards PWE while 83.5% of those who did not fear epilepsy had good attitude (χ^2=112.6283, df=1, p<0.05).

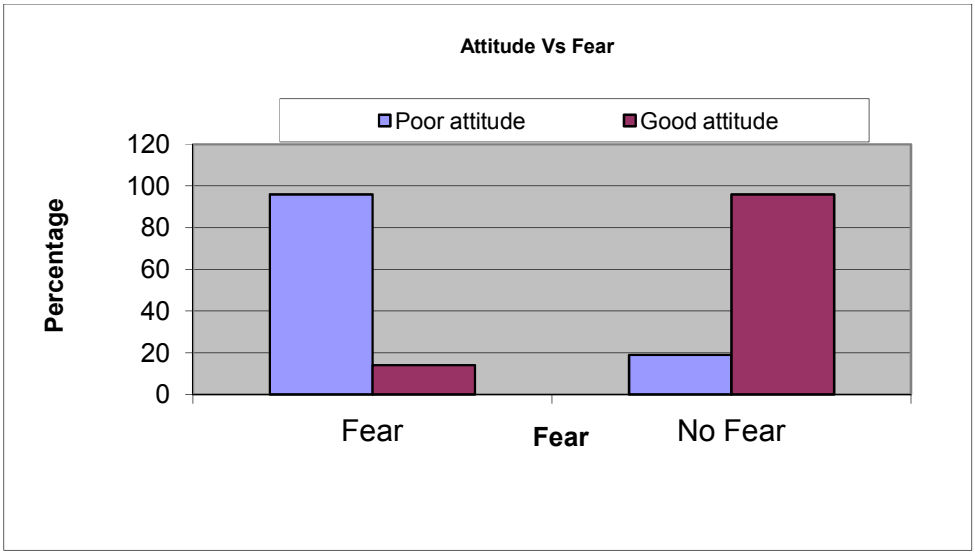

Fig. 3. Overall association between fear and attitude towards PWE

In Suba Sub-County, 59.6% of those with negative attitude and 57.4% of those with positive attitude had poor perception of behaviour of people with epilepsy (p=0.81062). In Meru Central Sub-County, 56.9% of those with poor attitude had poor perception while those with positive attitude had good perception of their behaviour (χ^2 = 4.14216, df=1, p=0.04183).

3.1 Belief Models in the Study Areas from Focus Group Discussions

3.1.1 Belief models in Meru Central Sub-County

In Meru Central Sub-county the respondents believed that epilepsy is an incurable disease and that most attacks occur in public places or schools. It is also associated with accidents, birth problems, head injuries, childhood diseases, and "worm in the head". Epilepsy is also believed to be hereditary and/or transmitted through witchcraft, bodily contact (contagious) and crossing (jumping over) faeces, urine or foam of the victims during or after the attack. It is also thought that staying for a long time with a person living with epilepsy, especially in marriage, could make one get the disease. This belief transcended formal education as exemplified in a statement by an education officer who stated "epilepsy is transferable and if you stay in close contact with them, you develop the disease". Other causes remain mysterious as summarized in a statement by an elderly woman who stated "*twaugaa ni nkoma yabageraga mwankine* "(we used to say that it is the devil that used to throw them into fires).

People with epilepsy are feared in Meru Central sub-County community. Members of the community illustrated this in the following statements: "During school days, I wouldn't like to come anywhere near these people as they used to be violent especially after the attacks". "Staying with these people is a big problem not unless there is no otherwise (like a family member)". Another participant stated that "the first time we saw one, we ran away including the teacher". An elderly woman who gave a story about a lady in her village retorted "she got pregnant soon after getting married. She was not on medication. She got a severe attack in her advanced pregnancy out of which she died together with the unborn baby".

Epilepsy has no cure but treatment is known to ameliorate the condition especially if started early. Some of the known modes of treatment include prayers, formal hospital medication and herbal medicines which they described as "highly effective" especially when administered through the nose. Others include crude methods like "burning a crow to ashes" and then sniffing the ashes to kill the worm and chasing away the spirits using drum beats. This is exemplified in this statement from an elderly woman who said *"uni ndi mwana, twonete mtu akigwatwa ni kibaba na mukuru umwe aratwira*

tujukie mtu mukebe jututheri tujuure tukiringaaga gituma gikiinge kenda okiira" (when I was a child, we once witnessed a person with epilepsy having a fit and an old man told us to take an empty tin and bang it while we make a lot of noise in order to make him recover from the attack). Another one stated "I once took a patient to the herbalist who made a lot of cuts on the body as he thought the patient had wrong blood". "Where I work, they are prayed for by the Imams" said one respondent from Isiolo, Muslim community in Kenya.

Some modes of treatment were found to amplify ostracism and stigma. A female participant said "there was a student who used to take herbal medicine for the illness whom we used to avoid as she used to smell like the herbs". "Sometimes medication is taken for long and properly supervised," I always make follow-up to ensure they take their drugs regularly." This was told to an audience by a care-giver.

3.1.2 Belief models in Suba Sub-County

In Suba Sub-County, epilepsy is a mysterious condition that carries a serious social burden due to stigma and low esteem. Many a times relatives hide the victims from the neighbours for fear of ridicule and shame to the extent that household heads who had cases in their families found it difficult to discuss the condition. It is considered an abomination associated with witchcraft where victims are restricted from socially interacting with the rest of the society. Herbalists and other traditional healers were found to hold the information in extreme secrecy and the methods they use are so mysterious that the society thinks epilepsy has no cure.

4. DISCUSSION

Suba and Meru Central sub-Counties are culturally and geographically distinct areas in Kenya. The two sub-counties share limited medical infrastructure, communal living situations and modest education standards especially among the female population. Life in these areas is constrained by high unemployment rate, low income and extended family relationships.

Based on their demographic profile with the mean age was 45.5 years, mode of 42 years, a standard deviation of 14.63 years and range of 80 (18-98) years, the study respondents were considered mature enough to answer questions appropriately. They were evenly distributed in the six wards in order to check on selection bias.

The high level of awareness (100%) and relatively low level of knowledge (26%) about epilepsy in the two districts is a strong pointer to the prevailing "wide knowledge gap". This compares favorably with findings from a study in Nigeria which found out that only 26% of respondents had good knowledge of epilepsy [30]. Respondents with some knowledge on epilepsy mentioned basic clinical signs which included falling down, stiffening, jerking and soiling of clothes. They associate epilepsy with trauma, infections or genetics and their major sources of information was the media, relatives and personal experiences.

The relatively higher level of knowledge about epilepsy among Suba Sub-County respondents at 34% could be attributed to their relatively higher education level. The low level of knowledge on epilepsy in Meru Central (11%) where there had been an established community intervention programme was a powerful pointer to the ineffective and inappropriate public education and promotion strategies used by the concerned agency. The lack of knowledge about the care centres in both sub-counties points to the poor healthcare infrastructure of epilepsy in Kenya. This concurs with WHO standards which observed that very few countries have national plans for epilepsy reinforcing the prevailing wide "treatment gap" [31]. This scenario had direct bearing on the level of care for PWE and a fertile ground for the dissemination of old-age retrogressive beliefs and superstitions which foster fear and discrimination of people with epilepsy. In some communities, the disease is considered an abomination; never to be called by name.

The statistical relationship between level of education and fear of epilepsy, which was insignificant, was considered responsible for the widespread poor response among respondents especially during

emergency situations like an attack. Running away from the victims during the attack was commonly reported across the respondents in both subcounties. This represented the maximum expression of fear of the condition among respondents regardless of their education level. This implies dearth of information on non-communicable diseases, particularly epilepsy, in the educational curricula in Kenya.

In both study areas, epilepsy is portrayed as a mysterious disease whose causality lies in the supernatural world. These belief models are informed by negative cultural heritage passed over generations and therefore entrenched in the culture and traditions of the two communities. It is associated with witchcraft, magical powers and evil spirit where and people fear even to walk in company of PWE. Stigma is due in part to a lack of understanding by people they see every day. Some of these people mistakenly believe that epilepsy is a form of mental illness, a curse or that seizures are something to fear. There is a belief that attributes it to supernatural rather than medical causes; this belief system is aggravated by lack of adequate facilities in the developing world as noted by authors in [32].

In Meru Central Sub-County, epilepsy causality had ancestral beliefs attributing it to a curse or witchcraft [33]. These authors stated that "though epilepsy is so widespread, it is widely misunderstood leading to stigmatization and the risk of social and legal penalties". In Suba sub-County, fear of epilepsy was also found to be driven by myths, supernatural beliefs and superstition surrounding its origin and mode of transmission which are considered mysterious. Stigma, psychiatric comorbidities, social or physical limitations and the possibility of unexpected death are personal and communal attributes to people with epilepsy [34].

Majority of the respondents in both sub-counties showed good practice towards epileptic patients under attack, with Meru Central Sub-county respondents having a higher percentage (80.2%) compared to Suba (79.1%). They ensured safety of the patients during attack and did not run away from the victims. This finding compares favourably with the observation [30] that although a lot of misconceptions existed about epilepsy in the study population (e.g. epilepsy is transmissible by contact and that epileptics must be isolated or avoided) several respondents would share a room, eat with or employ persons with epilepsy [35]. In the face of suffering, men are socialized to have unintentional language of distress. Testosterone, a hormone synthesized and secreted by interstitial (Leydig) cells in the testes, is a principal mediator in producing accelerated linear growth in puberty, virilization, masculinity, personality and behaviour traits in men [36]. It is the Long-term potential (LTP) that is modulated by the testosterone resulting in favourable fear responses in men in the face of adversity [37]. They exhibit courage and remain stoical under a disciplined exterior, a feat that is important in facilitating appropriate response and taking up quick action in emergency situations. This in essence translates to good practice. Consequently, male respondents had higher percentage of good practice towards people with epilepsy during an attack. Though they are known to be caring and sympathetic to the sick and needy, women were found to remain indifferent and resort to prayers instead of securing safety measures for the victims. The frightening and devastating nature of the attack can make people resort to any measures including offering prayers [38].

Majority of Meru Central sub-County respondents (53.3%) had better perception of behaviour of PWE as compared to Suba respondents (41.4%). Despite the higher education level of the Suba sub-county respondents, 58.6% of them had poor perception of behaviour of PWE as compared to 46.7% Meru Central sub-County respondents.

Unlike in Suba Sub-County, 54.3% of Meru Central sub-County respondents did not fear epilepsy. Among those respondents who expressed fear of epilepsy, 54.2% were those with poor perception about behaviour of PWE (Table 1). However, like Suba, the relationship between poor perception and fear was not significant (Suba: χ^2 = 0.28234, df=1, p=0.59517, Meru Central χ^2 = 1.99836, df=1, p=0.15747). This finding is at variance with earlier research that reported a significant association between literacy and the type of feeling exhibited towards epileptics by the participants where those who had higher level of literacy expressed positive attitude and vice versa in Northern Nigeria.

5. CONCLUSIONS

The study revealed that:

I. The pattern of traditional belief systems about epilepsy was similar in both study areas. This pattern shaped the expression of stigma and social isolation as a social burden associated with epilepsy in both sub-Counties.
II. Social stigma and negative attitude towards people with epilepsy was a common phenomenon. There was overwhelming belief that epilepsy is caused by witchcraft and associated with ancestral curse and was thought to be contagious, thus transmissible from one person to another.
III. Epilepsy was also thought to be an incurable disease when treated with modern medicine. This mindset amplified social discrimination and drove families with people with epilepsy to seek healing from traditional practitioners.
IV. Traditional practitioners, as care-givers, were found to play a major role in epilepsy case management as they are often visited before the patient is brought to hospital. This is a major cause of delay in obtaining appropriate care and treatment.
V. The gap between formal education and fear of epilepsy indicates dearth of information about epilepsy in the educational and training curricula. Therefore, chronic non-communicable diseases like asthma, epilepsy, diabetes and hypertension could be demystified by increasing the bulk and accuracy of information about them in the school training curricula.
VI. The superstitions and belief models compounded by a low knowledge level and information on epilepsy was responsible for the systematic discrimination of people with epilepsy both in employment and social life.
VII. People living with epilepsy were recommended for low-paying jobs under the convenient label of “low risk jobs”. This subjects them to perpetual poverty giving them an identity of stigmatized and down-trodden personality thus contributing to the negative attitudes towards them.

6. RECOMMENDATIONS

Results from this study indicate that a number of problematic and stigmatizing ideas about epilepsy are still prevalent in Suba and Meru Central Sub-counties. Lack of accurate information and the enduring negative beliefs obtained provides a foundation for the misunderstanding of problems facing people with epilepsy. These problems are compounded by poor healthcare infrastructure with limited or inadequate intervention measures.

Perspectives of quality services for people with epilepsy should be accessible, acceptable, equitable and patient centered thus taking into account aspirations and preferences including community cultural heritage. It should be as appropriate for clinical care as for management services that support service delivery. Skills used by healthcare providers to promptly respond to the healthcare needs of the population and keeping the facilities within a reasonable geographical distance enhances accessibility and amounts to equitability. These aspects should be kept safe with minimal risk and harm to service users and should not vary with personal characteristics. Therefore, the dimensions of quality of care for people with include efficiency, continuity, safety, availability of amenities, technical competence, access to services, effectiveness and interpersonal relations, each considered in the light of specific programs and the local context.

In order to address them the study recommends:

- Development of national quality framework and implementation of practice management guidelines, improving treatments, developing, implementing, and assessing performance metrics
- Improving communications between the care team and patients; and evaluation and accreditation of epilepsy care centres are equally central in this arrangement.

- Well-designed comprehensive community sensitization programme accompanied by audience specific Information, Education and Communication (IEC) materials to disseminate accurate information on epilepsy.
- Review of school and teacher training curricula in order to achieve a reasonable level of technical information to reverse the misunderstanding
- Fostering well designed controlled research to establish tool kits that can be used to monitor attitude and perception of the public on PWE on a continuous basis
- The provision of accessible and affordable integrated health care of PWE to improve their health outcomes

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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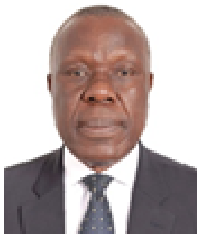

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Chapter 9
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Update on Ketamine Infusion Therapy for Sustained Opioid Cessation for Chronic Pain and for Depression

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ABSTRACT

Objectives: Opioid misuse and opioid use disorders (OUD) continue to challenge America's veteran population with estimates of chronic opioid use for noncancer pain at over 28%. The primary outcome of the original study utilizing ketamine assisted opioid detoxification is long-term opioid cessation, while secondary outcomes are assessments of opioid withdrawal, pain reduction, and ketamine side effects. This update also reviews all composite results to date.
Design: Preliminary observational study involving a comprehensive review of a prospectively collected database.
Setting: Veterans Affairs Medical Center in Nashville, Tennessee; update from private practice clinic in Franklin, TN.
Subjects: 41 veterans with chronic noncancer pain and on chronic opioids who received ketamine assisted opioid detoxification; update includes results from 114 patients.
Methods: The authors conducted a review of a real time data collection of forty-one patients who met inclusion criteria. The authors reviewed data collected over a 28-month period (April 2016-July 2018). Following detoxification and the initial ketamine infusion series, the patients were monitored at regular intervals for up to 12 months post-infusion; this monitoring period extended through October 2018 to ensure all patients had at least 3 months of monitoring data.
Results: Most veterans remained opioid free long after treatment: 83%, 75%, and 58% at one, three, and six months respectively (p=0.0001). Seventy-six percent of patients reported opioid withdrawal as either none or mild severity. Median pain reductions at one and three months were 50% and 40%, respectively. The incidence of troubling ketamine side effects was low.
Conclusion: Overall, this update provides new evidence that the utilization of a standardized ketamine infusion protocol coupled with a rapid opioid detoxification is very effective, results in a high rate of prolonged opioid reduction, decreases chronic pain, minimizes opioid withdrawal using strictly non-opioid analgesics, and decreases depressive symptoms. Future studies could consider a randomized, controlled trial, although patient and clinician blinding may be challenging. Ketamine assisted opioid detoxification appears to be a safe and effective tool to target opioid misuse and has the potential to decrease opioid consumption, overdose related deaths, and chronic pain.

Keywords: Ketamine; opioid epidemic; opioid misuse; detoxification; chronic pain.

1. INTRODUCTION

The United States is currently battling an ongoing opioid crisis [1]. Opioid use for non-cancer chronic pain, opioid diversion, opioid abuse, and unintentional deaths due to opioid overdose have all increased exponentially over the past 20 years [2,3,4]. Most recent opioid statistics are staggering with an estimated 11.4 million people misusing opioids and an estimated 130 people dying daily (over 48,000 annually) from opioid overdoses [5]. Opioids have the potential for misuse and addiction. Worldwide, use of opioid analgesics doubled between 2001 and 2003 and 2011–2013 [6,7].

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Opioid misuse and opioid use disorders (OUD) continue to challenge America's veteran population with estimates of chronic opioid use for noncancer pain at over 28% [8,9]. To combat the opioid crisis, the U.S. Department of Veterans Affairs (VA) established the Opioid Stewardship Initiative in 2012, and the Department of Health and Human Services declared opioid abuse as a public health emergency in 2017. More than 700,000 individuals died from a drug overdose between 1999 and 2017 in the U.S. [10]. Drug overdose fatalities exceeded 70,000 in 2017 and two-thirds (68%) was attributable to opioids [7,11].

In spite of a high prevalence of OUD in veterans, long-term opioid treatment has been shown to be ineffective for many chronic pain patients and may in fact increase dysfunction and opioid related adverse effects including death [12,13,14]. In addition, chronic pain patients frequently struggle with a triad of intertwined co-existing disorders including both anxiety and depression [8,15]. Chronic musculoskeletal pain patients have higher depression than individuals without pain in a general population study [16,17]. Ketamine, with its profound anti-hyperalgesic properties [18], may uniquely benefit all of these codependent conditions. Since most substance abuse treatment centers treat OUD with medication assisted treatment [19], long term opioid cessation is infrequent [20,21,22]. There is a need to assist with national metrics related to opioid misuse/abuse and to provide an effective, alternative treatment for chronic pain. Given ketamine's anti-hyperalgesic properties, this study sought to establish a novel ketamine assisted opioid detoxification program aimed at safely decreasing both opioid use while simultaneously addressing chronic pain.

2. METHODS

Study data was prospectively collected and managed using Research Electronic Data Capture, a secure, web-based application designed to support data capture for research studies [23]. After obtaining approval from the VA Tennessee Valley Healthcare System Institutional Review Board, the authors reviewed data collected from 41 patients over a 28-month period (April 2016-July 2018) who had met inclusion criteria and had undergone rapid opioid detoxification coupled with ketamine infusion therapy. Following detoxification and the initial ketamine infusion series, the patients were monitored at regular intervals for up to 12 months post-infusion; this monitoring period extended through October 2018 to ensure all patients had at least 3 months of monitoring data. The primary outcome was opioid cessation, while secondary outcomes were assessments of opioid withdrawal, pain reduction, and ketamine side effects.

2.1 Patient Selection

During the 28-month enrollment period, 169 patients with chronic pain lasting at least 6 months were referred to us for consideration for ketamine infusion therapy for reduction in chronic pain and possible opioid detoxification. Patients were referred primarily from the VA Pain Clinic, but also from a variety of other specialties including neurology, psychiatry/addiction medicine, rheumatology, and primary care. Chronic noncancer pain syndromes could include chronic regional pain syndrome (CRPS), amputation pain, low back pain, neuropathy, head and neck pain, central pain syndrome, radiculopathy, post-surgical pain, and fibromyalgia. From this referral population, 102 patients were excluded from participation in the program either due to specific exclusion criteria or patient declination following a comprehensive medical evaluation including review of radiographic, laboratory, and psychological evaluations, as well as a discussion of the risks, benefits, and alternatives. Exclusion criteria for the program included significant mental illness involving psychosis, symptomatic coronary artery disease, severe heart failure or valvular disease, severe hepatic or renal disease, elevated intracranial pressure, Body Mass Index (BMI) > 40, and poorly controlled diabetes mellitus (A1c > 9%). Of the remaining 67 patients who qualified and received ketamine infusion therapy, 22 patients who were not on chronic opioids were also excluded from this study. Finally, the analysis defined "intent to treat" as patients who received at least 3 out of the 4 initial ketamine infusions and who were using pure opioid agonists or buprenorphine. In total, 41 opioid-tolerant patients were included for analysis who had underwent rapid opioid detoxification coupled with ketamine infusion therapy.

2.2 Protocol

Initially, to ensure patient safety, all patients were hospitalized for four consecutive days with daily ketamine infusions. In an effort to adhere to Centers for Medicare and Medicaid Services InterQual (Copyright © 2014 McKesson Corporation and/or one of its subsidiaries) criteria for inpatient admission, the need for hospitalization was later based on their level of opioid tolerance and medical co-morbidities. Patients taking greater than 35 morphine milligram equivalents daily (MMED) were admitted for the four day rapid opioid detoxification daily infusion program; those patients on low dose opioids (< 35 MMED) underwent rapid opioid detoxification with a series of four infusions over a ten week period with possible hospitalization for the first 2-3 days depending on patient co-morbidities and social support at home. Patients could return quarterly for "booster" infusions if needed during the monitoring phase.

Ketamine infusions were administered over four hours with progressive dosing each day as tolerated. Based on a literature review and Borsook's concept that treatment for chronic pain requires higher dosing than that for depression [24], ketamine infusions were started at 50-90 mg/hour and progressed daily as tolerated to 50-125 mg/hour on subsequent days depending on patient age and comorbidities. In addition, patients were pretreated with oral diazepam, as well as anti-emetics including ondansetron, dexamethasone, and/or scopolamine. If necessary, additional parenteral sedation including intermittent midazolam or propofol infusion was titrated for agitation or self-reported troubling dreams to a goal of 0 to -2 on the Richmond Agitation Sedation Scale [25]. All potential ketamine induced side effects experienced during each of the infusions were recorded. At the AMG Ketamine and Wellness Center, the chronic pain protocol aims for 4-5 infusions over a two-week period; dosing begins with an average of 1mg/kg/hour for four hours and is increased as tolerated by 0.1 mg/kg/hour for each infusion. The depression protocol aims for 6 infusions over a two-week period; dosing begins with an average of 1mg/kg/hour for one hour and is increased as tolerated by 0.2 mg/kg/hour for each infusion.

2.3 Management of Opioids and Withdrawal

Extended release opioids were discontinued 72 hours prior to admission and all immediate release opioids were discontinued on the first infusion day; patients who discontinued extended release opioids were allowed to increase their immediate release opioids by 50% during the 72 hours prior to the first infusion if necessary for analgesia. No opioid antagonists were given to precipitate withdrawal. Opioid withdrawal was assessed using the Clinical Opioid Withdrawal Scale (COWS) [26] and was aggressively managed using anti-emetics, anti-diarrheals, anxiolytics (usually oral diazepam as needed for up to one week), oral clonidine for up to two weeks as tolerated, and oral muscle relaxants as needed. After each ketamine infusion, chronic pain was treated with only non-opioid medications including acetaminophen, anti-inflammatories, muscle relaxants, anticonvulsants (gabapentin or pregabalin), antidepressants, and topical analgesics.

2.4 Monitoring Phase

Following detoxification and the initial ketamine infusion series, the patients were monitored at regular intervals including one week, one month, three months, six months, and twelve months post-infusion. Patients were monitored at follow up either during clinic or telephone encounters. Data collection included current analgesics, pain level assessment, opioid withdrawal side effects, and ketamine side effects. All analgesics and their doses were recorded; this included documentation of opioid utilization. Regular chart reviews and occasional random urine drug screens (UDS) were performed during the monitoring phase to reaffirm a patient's report. Pain level assessment utilized a percent pain reduction compared to baseline.

2.5 Statistical Analysis

Sample size was based on our primary outcome of long-term opioid cessation. With a desired effect of at least 50% sustained opioid cessation at 6 months, as well as an alpha of 0.05 and a beta of 0.20, a sample size of 19 patients was required. Continuous variables that were parametric were represented by the mean $\pm$ SD (Standard Deviation), while non-parametric results were represented

by median (IQR-Interquartile range) values. T-test was used for all continuous data. Fisher's exact analysis was used to evaluate categorical data. All analyses were performed using Excel (Microsoft Office 2013) or GraphPad 2018 Software systems.

3. RESULTS

3.1 Demographics

The demographics of the 41 patients are summarized in Table 1. Mean patient age was 53.8 years. Males comprised 85% of the study population. Most patients, 54%, were ASA (American Society of Anesthesiologists) III status. The mean BMI was 29.6 kg/m^2. The three most common pain conditions were low back pain (68%), nonspecific (non-CRPS) extremity pain (39%), and head and neck pain (39%). Mean pain intensity score was 6.5/10 with a mean duration of 15 years. Mean MMED was 117.9 mg. Veterans also had a high incidence of comorbid mental health conditions including depression (80%), anxiety (73%), insomnia (66%), and post-traumatic stress disorder (PTSD) (54%). Poly-pharmacy was common with 85% of patients prescribed antidepressants and 68% prescribed anticonvulsants (most commonly gabapentin or pregabalin); while all study patients were taking chronic opioids prior to treatment, types of opioids varied as follows: 85% prescribed short-acting opioids, 59% long-acting opioids, and 5% buprenorphine as well as various combinations.

Table 1. Baseline characteristics

Demographics, n= 41	
Age in years, mean (SD)	53.8 (11.9)
Male, N (%)	35 (85)
ASA II/III, N (%)	19 (46)/ 22(54)
BMI mean (SD)	29.6 (4.8)
Baseline MMED, mean (SD)	117.9 (96.6)
Tobacco Use, N (%)	15 (37)
Average Pain Score, mean (SD)	6.5 (1.5)
Duration of pain in years, mean (SD)	15 (10.2)
Pain Diagnoses, N (%)	
Low Back Pain	28 (68)
Extremity Pain (non-CRPS)	16 (39)
Head and Neck	16 (39)
Global Body Pain/Fibromyalgia	7 (17)
CRPS	4 (10)
Chronic Post-Surgical Pain	4 (10)
Neuropathy	4 (10)
Spinal Cord Injury	3 (7)
Amputation Pain	2 (5)
Mental Health Comorbidities, N (%)	
Depression	33 (80)
Anxiety	30 (73)
PTSD	22 (54)
Medications, N (%)	
Antidepressants	35 (85)
Opioid-Short Acting	35 (85)
Gabapentin/Pregabalin	28 (68)
Opioid-Long Acting	24 (59)
NSAIDs	21 (51)
Muscle Relaxant	19 (46)
Topical Medications	17 (41)
Other Analgesics	14 (34)
Buprenorphine	2 (5)

SD, standard deviation: n, total number of subjects; N (%), number (percentage) of subjects in the specific category; ASA, American Society of Anesthesiologists classification; BMI, body mass index; MMED, morphine milligram equivalents daily; PTSD, post-traumatic stress disorder; CRPS, chronic regionalpain syndrome; NSAIDs, non-steroidal anti-inflammatory drugs

3.2 Ketamine Dosing and Protocols

The majority of patients (93%) undergoing rapid opioid detoxification were treated as inpatients with most receiving four consecutive days of ketamine infusions (Table 2); the remaining patients were treated primarily as outpatients with four infusions over a ten-week period. All patients completed four-hour infusions for each session with progressive mean dosages of 77.5 mg/hr on day one, 92.0 mg/hr on day two, 107.5 mg/hr on day three, and 118.0 mg/hr on day four. Overall mean ketamine dosing was 1.08 mg/kg/hr. Nineteen patients (46%) elected to return for booster infusions within the first year, most frequently presenting three to four months after their first ketamine infusion. Twelve (63%) received only one booster, while three (16%) received four booster infusions during the first year.

Table 2. Ketamine protocol/dosing

Protocol (n=41)	**N (%)**
4 consecutive days	35 (85)
4 days over 10 weeks	4 (10)
Status (n=41)	**N (%)**
Inpatient	38 (93)
Outpatient	3 (7)
Dosage [a]	**Mean (SD)**
1st Infusion	311 (50)
2nd Infusion	368 (52)
3rd Infusion	430 (54)
4th Infusion	472 (75)
Overall (mg/kg/hr)	1.08 (0.26)
Booster Administered [b] (n = 41)	**N (%)**
3-4 months	10 (24)
5-8 months	7 (17)
9-12 months	2 (4)
Number of Boosters [c] (n = 19)	**N (%)**
1	12 (63)
2	4 (21)
3 or more	3 (16)

a. total dosage of 4 hr infusion in milligrams
b. time in months to first booster infusion after initial series
c. number of booster infusions within 1st year after initial series
n, total number of subjects; N (%), number (percentage) of subjects in the specific category; SD, standard deviation

3.3 Opioid Cessation-Primary Outcome

Most patients remained opioid free long-term after their rapid opioid detoxification: 83%, 75%, 58%, and 50% at one, three, six, and twelve months respectively (p=0.0002) (Table 3). Of the patients who reinitiated opioids following their opioid detoxification, their mean opioid consumption was reduced from 117.9 at baseline to 55.5 and 74.3 at six and twelve months (p=0.0001) representing a 53% and 37% reduction in opioid consumption respectively. In addition, the distribution of MMED doses shifted dramatically from baseline to 6 months: patients consuming less than 50 MMED increased from 29% to 86%, while patients consuming over 100 MMED decreased from 44% to 6% (Fig. 1). At the AMG Ketamine and Wellness Center, patients have experienced an average opioid reduction of 83% MMED at one month.

We also examined specific variables at six months following detoxification, which could correlate with long term opioid cessation. There was no difference in age, gender, ASA, or BMI. In addition, greater ketamine dosing did not correlate to a higher degree of success. However, patients taking lower baseline opioids (87 MMED) enjoyed greater abstinence than those patients taking higher doses (162.5 MMED) (p=0.04); in addition, those patients who reported a greater reduction in pain had a higher likelihood of remaining off opioids (p=0.03).

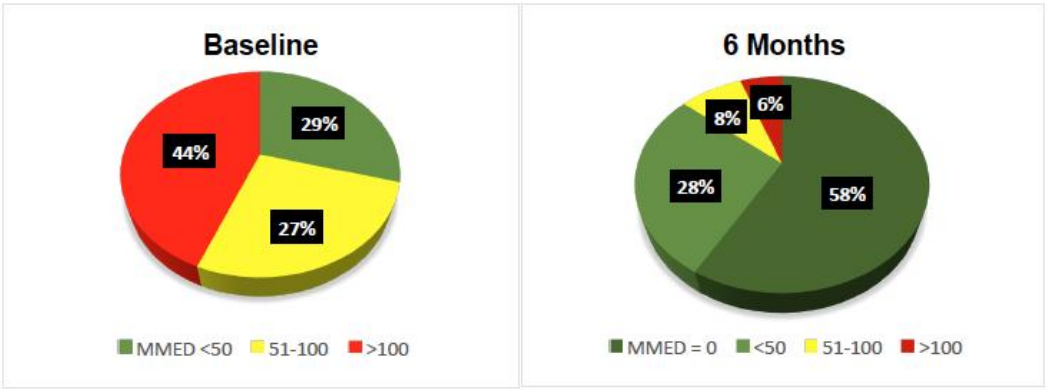

Fig. 1. Change in opioid use at baseline vs 6 months following ketamine assisted opioid detoxification. MMED (morphine milligram equivalents daily)

Table 3. Opioid cessation/reduction

	Baseline	1 week	1 month	3 month	6 month	12 month
Opioid Cessation N (%)						
	(n=41)	(n=41)	(n=41)	(n=40)	(n=36)	(n=22)
On opioids	41 (100)	6 (15)	7 (17)	10 (25)	15 (42)	11 (50)
Off opioids	0 (0)	35 (85)*	34 (83)*	30 (75)*	21 (58)*	11 (50)+
Opioid Reduction [a]						
	n = 41	n = 6	n = 7	n = 10	n = 15	n = 11
Mean MMED	117.9	38.8*	48.2*	56.45*	55.5*	74.3*
<50 MMED, N (%)	12 (29)	4 (67)	3 (43)	5 (50)	10 (67)	6 (55)
50-100 MMED, N (%)	11 (27)	2 (33)	4 (57)	4 (40)	3 (20)	4 (36)
>100 MMED, N (%)	18 (44)	0 (0)	0 (0)	1 (10)	2 (13)	1 (9)

Correlations with Opioid Cessation at 6 months			
	Off Opioids (n=21)	**On Opioids (n=15)**	**P value**
Age, average (SD)	55.6 (10.9)	52.6 (12)	0.44
Male Gender, N (%)	20 (95)	11(73)	0.14
ASA III, N (%)	13 (62)	5 (33)	0.18
BMI, average (SD)	30 (5)	27.7 (3.6)	0.12
Depression, N (%)	16 (76)	14 (93)	0.37
Anxiety, N (%)	16 (76)	11 (73)	1.0
PTSD, N (%)	13 (62)	7 (47)	0.50
Baseline Pain level, mean (SD)	6.2 (1.6)	6.9 (1.4)	0.16
Baseline MMED, mean (SD)	87 (71.5)	162.5 (116.9)	0.04
Ketamine, mg/kg/hr, mean (SD)	1.01 (0.2)	1.23 (0.3)	0.02
% Pain Reduction, mean (SD)	45.8 (34)	19.4 (32)	0.03

a. patients reinitiated on opioid therapy
**p-value ≤ 0.0001*
+p-value = 0.0002
n, total number of subjects; N(%), number (percentage) of subjects in the specific category; MMED, morphine milligram equivalents daily; SD, standard deviation; PTSD, post-traumatic stress disorder; ASA, American Society of Anesthesiologists classification; BMI, body mass index

3.4 Opioid Withdrawal

The severity of opioid withdrawal was low during the initial series of ketamine infusions using this rapid opioid detoxification protocol; 76% of veterans described none to mild symptoms based on the

COWS (Table 4). One week after detoxification, diarrhea was the most common symptom with 30% of patients reporting this complaint, followed by agitation in 24% of patients, and insomnia in 22% of patients. Diarrhea lingered in some patients with 19% still reporting this symptom at one month, 13% at three months, and 7% at six months. One month following the initiation of ketamine infusions, 97% of veterans reported none to mild symptoms. Other withdrawal symptoms were very infrequent (< 3%) at three months.

Table 4. Side effects

Opioid Withdrawal Symptoms N (%)								
	Diarrhea	**Agitation**	**Insomnia**	**Body Aches**	**Nausea**	**Leg Jerking**	**Diaphoresis**	**Yawning**
Days 2-4 (n=41)	6 (15)	10 (24)	6 (15)	7 (17)	4 (10)	6 (15)	3 (7)	4 (10)
1 Week (n=37)	11 (30)	9 (24)	8 (22)	7 (19)	4 (11)	3 (8)	1 (3)	0 (0)
1 Month (n=36)	7 (19)	3 (8)	4 (11)	1 (3)	2 (6)	1 (3)	1 (3)	1 (3)

Opioid Withdrawal Severity N (%) [a]				
	None	**Mild**	**Moderate**	**Severe**
Days 2-4 (n=41)	11 (27)	20 (49)	9 (22)	1 (2)
1 Week (n=37)	17 (46)	14 (38)	6 (16)	0 (0)
1 Month (n=36)	25 (69)	10 (28)	1 (3)	0 (0)
3 Month (n=32)	27 (84)	4 (13)	1 (3)	0 (0)

Ketamine Side Effects N (%)								
	Halluci-nations (None/Mild)	**Polyuria**[b]	**Urinary Retention**	**Halluci-nations (Disturbing)**	**Hypoven-tilation**[c]	**Nausea and/or Vomiting**	**Hypo-tension**[d]	**Headache**
Days 1-4 (n=41)	33 (80)	15 (37)	14 (34)	8 (20)	7 (17)	3 (7)	2 (5)	2 (5)

3.5 Pain Reduction

Following the rapid opioid detoxification utilizing ketamine infusions, most patients reported substantial long-term pain reduction. Median pain reduction at both one week and one month was 50% (IQR=25-65) and at three months was 40% (IQR=10-60). However, at six months, the mean reduction in pain diminished to 30%, representing a gradual decrease in effect with time. Currently, at the AMG Ketamine and Wellness Center, patients have experienced a 53% reduction in pain one month after initiation of ketamine therapy.

3.6 Ketamine Side Effects

Mild hallucinations were common, occurring in up to 80% of patients, while troubling hallucinations that were reported at least once during their 4-day series, occurred in 20% (Table 4). Hypoventilation occurred in 17% of patients, all of whom had obstructive sleep apnea and was effectively treated with application of continuous positive airway pressure, usually at their home settings. Other uncommon side effects included hypotension, hypertension, headache, and nausea, all of which were easily managed. In the monitoring phase, ketamine related side effects were uncommon and fully resolved within three months post-infusion.

In our primary veteran study, urinary complaints were frequent during the infusions with 37% of patients having polyuria (defined as urine output greater than twice the intravenous fluids), and 34% experienced urinary retention. There were no cases of interstitial cystitis. Ketamine-induced Diabetes Insipidus (DI) was diagnosed by either polyuria (two times the intravenous fluids administered or

greater than 4 ml/kg/hr of urine output or by supportive laboratory findings (urine: low Na^+, low specific gravity, low osmolality or serum: high Na^+ and/or high osmolality). Prior to either vasopressin or desmopressin, 81% of our veteran patients had probable DI based on either polyuria (58%) or supportive lab work (58%). 64% required in/out urinary catheterization. After the institution of prophylactic vasopressin infusions, the rate of DI fell to less than 5%.

In terms of depression, patients at the AMG Ketamine and Wellness Center have experienced a 53% average reduction in their depression scores (Quick Inventory Depressive Symptomatology) at one and three months post-ketamine treatment; 67% of patients with treatment resistant depression have responded positively to the therapy with depression scores in the “none -mild” category one month post-ketamine treatment.

4. DISCUSSION

This comprehensive retrospective observational study using prospectively collected and audited data provides new evidence that moderate dose ketamine infusions may contribute towards successful opioid detoxification, while simultaneously decreasing chronic pain and opioid withdrawal. These beneficial effects from ketamine-assisted opioid detoxification may be preferable compared to existing techniques including opioid maintenance therapy (OMT) and opioid tapering techniques. While opioid maintenance therapy using methadone or buprenorphine may control opioid administration in patients with opioid use disorders, such treatment does not adequately address the incumbent risks including cardiac arrhythmias, respiratory depression, constipation, opioid diversion, opioid induced hyperalgesia (OIH), tolerance, addiction, physical dependence, death, as well as risks to the fetus in case of parturients on chronic opioids (i.e. intrauterine fetal demise and neonatal abstinence syndrome) [18,27,28,29,30]. Interestingly, Uebelacher reported that a majority of patients actually prefer to be "opioid free", rather than choosing OMT, for a variety of reasons, including the goals of minimizing clinic visits, side effects, and complications.

Our success rate of complete opioid cessation of 83%, 75%, and 58% at one, three, and six months respectively, as well as a dramatic overall reduction in opioid consumption, compares favorably to current techniques at opioid cessation /detoxification. With opioid tapering techniques, high dropout rates (32-100%) and high recidivism (45-90%) are common, especially when depression, high MMED, and increased levels of pain coexist [31,32,33] such as in our patient population. During an opioid wean, difficulty managing pain, poor coping skills, unrealistic expectations, significant withdrawal symptoms, poor social support, ongoing stressors, pseudo-addiction or addiction, medicolegal issues including diversion and aggravating mental health conditions, and illicit opioid use may contribute to the challenge with opioid tapers [33,34]. Diaper describes a “mountain of detoxification” with current approaches that is simply too high to climb for many patients, resulting in either disinterest in entering or completing a detoxification program [21]. In addition, our results exceed the latest Consensus Guidelines from the American Society of Regional Anesthesia and Chronic Pain, the American Academy of Pain Medicine, and the American Society of Anesthesiologists on what constitutes a "positive treatment response" involving ketamine infusions for chronic pain, namely a 20% reduction in opioid consumption for at least six weeks [35]. Our finding that a greater ketamine dose was not associated with a higher likelihood of long-term opioid cessation suggests that lower dosing protocols may be equally effective. Since the original publication, 74 chronic pain patients who were on prescription opioids have undergone ketamine infusion therapy; their average morphine equivalents were 88 mg at baseline. At one and three months following the initiation of ketamine infusion therapy, there was a 94% and 86% reduction in opioid consumption respectively.

Our protocol using ketamine-assisted rapid opioid detoxification was initiated without prior knowledge of previous programs using ketamine for detoxification, but rather on ketamine’s unique ability to abolish OIH after both acute and chronic opioid administration [18,27]. However after protocol initiation, we did identify other studies which support ketamine assisted detoxification. Jovaisa employed ketamine 0.5 mg/kg/hr in a randomized controlled trial with 58 patients undergoing precipitated opioid withdrawal and noted “better control of withdrawal symptoms” in the ketamine infusion group [36]. Quinlan briefly describes a small cohort of chronic pain patients who were treated with chronic opioids using subanesthetic ketamine infusions for five days to assist with opioid

withdrawal in patients with chronic pain, opioid tolerance, and OIH; 27% of patients were opioid free at six months and most patients felt "better" for at least the first month after the program [37]. Strickler successfully employed a low-dose ketamine infusion for seven days to assist in a rapid opioid taper in a patient on over 400 MMED [38].

While 25% and 42% of our study patients reinitiated opioids at three and six months respectively, their dose was substantially less than pre-infusion doses, reducing the risk of dose-related opioid side effects. Mean opioid dose of those patients who restarted by six months was 55.5 MMED, representing a 53% decrease in opioid consumption at six months. In addition, patients consuming greater than 100 MMED, a well-recognized dose associated with significant increased risk of respiratory depression, decreased from 44% at baseline to 9% at six months. Ketamine appears to "reset" opioid receptors via N-methyl-D-aspartate (NMDA) receptor antagonism allowing far less dosing needs in the chronic pain patient (i.e. decreased opioid tolerance).

Weiss reported that the major deterrent to opioid cessation in chronic users is the fear of opioid withdrawal; Mattick states that opioid withdrawal can be "immiserating, like a week of bad influenza" [22,39]. With our protocol, 76% of patients had minimal (none to mild) opioid withdrawal during their rapid opioid detoxification. Most symptoms including agitation, insomnia, irritability, nausea, and diarrhea were effectively treated with both symptom directed treatment and clonidine administration as tolerated. Interestingly, other reports demonstrate ketamine's ability to minimize opioid withdrawal partly by decreasing NMDA activation and central nervous system hyperactivity [38,40]. Most opioid detoxification programs employ an opioid taper over months to minimize opioid withdrawal yet may unnecessarily prolong the opioid cessation process contributing to patient dissatisfaction and may still be associated with some opioid withdrawal [21]. The Food and Drug Administration, however, has recently published a Safety Announcement having received reports of serious harm when opioid medicines are discontinued rapidly including serious withdrawal symptoms, uncontrolled pain, and suicide [41]. While we recognize this concern of rapid opioid detoxification without a proper treatment plan or monitoring, we believe ketamine assisted opioid detoxification addresses these concerns by minimizing withdrawal, decreasing chronic pain, and decreasing depression and suicidality [42]. While ultra-rapid opioid detoxification programs using aggressive naloxone administration under general anesthesia to precipitate opioid withdrawal have been associated with significant risks [39], our antagonist-free protocol is well tolerated without serious side effects in our series. Unexpectedly, persistent diarrhea beyond six months was reported in 7% of patients, but was effectively treated with anti-diarrheals, generally diminished with time, and was still associated with high patient satisfaction for the program.

In addition, the fear of worsening pain is also a major deterrent to opioid cessation for patients with chronic pain syndromes [21,22]. Our ketamine infusion protocol simultaneously assists in opioid withdrawal while treating the patient's chronic pain condition. Rather than increasing pain levels as opioids were discontinued, most patients reported a prolonged reduction in their pain, with a median pain reduction of 50% at one week and one month and 40% at 3 months. As noted, the large standard deviation in our pain reduction data indicates a large variance between individual patient responses, suggesting that some patients or pain conditions respond better than others to ketamine infusions; for example, patients who prefer to remain on opioids may respond differently to this infusion therapy compared to those who truly want to discontinue opioids. The primary mechanism in decreasing pain is ketamine's antagonism of NMDA pain receptors within the central nervous system [43] and reversal of the opioid induced hyperalgesia. [30]. Other studies demonstrate ketamine's effect on decreasing inflammation [44], excitatory amino acids [45], and depression [42,46] as well as modulation of the descending inhibitory pain pathway, all of which contribute to a reduction in chronic pain.

Finally, the above benefits of opioid cessation and decreased chronic pain outweighed the risks involved with ketamine administration. While the greatest fear with moderate dose ketamine infusion therapy is troubling hallucinations, 20% of patients reported this concern at any time during their four-day infusion; troubling hallucinations were successfully treated with either increasing sedation or changing from intermittent benzodiazepine use to a low dose propofol infusion. Our current protocol at the AMG Ketamine and Wellness Center utilizes low-dose propofol sedation for *all* patients with only a rare incidence of troubling dissociative hallucinations. Two (5%) patients reported post-infusion

nightmares yet both patients remained highly satisfied and in fact returned for subsequent booster infusions. In addition, elderly patients in particular reported transient post-infusion confusion (<5%), decreased memory (<5%), and/or falls (<5%) during the first three months, emphasizing the need for careful observation of older patients in the post-infusion period. We have since modified our protocol in patients over 55 years of age by diminishing ketamine dosing, administering only low dose propofol infusions for sedation, and adding cognitive/ memory impairment testing to better monitor cognitive status following ketamine administration.

Interstitial cystitis has been seen in patients who have chronically exposed to high dose ketamine. While interstitial cystitis was not seen in our patients, we did see significant urinary morbidity prior to the prevention of ketamine-induced diabetes insipidus with vasopressin or desmopressin. At the VA Medical Center, the rate of DI fell from 81% to less than 5% with the use of prophylaxis with vasopressin or vasopressin. At the AMG Ketamine and Wellness Center, oral desmopressin 50 mcg is now administered due to low cost, improved patient safety, and ease of administration. Bladder scans are routinely performed after two hours of ketamine infusion. Currently, our practice has experienced a polyuria rate of 14%, but there have been no cases of distended bladders, urinary retention, urinary incontinence, urinary catheterization, nor any known side effects or complications from the use of single, low dose desmopressin.

In spite of the significant results, there are certain limitations to this study. Most importantly, this preliminary study is an observational database study involving 41 consecutive patients with real time data collection at a single institution. Therefore, results may have been impacted by a number of variables, which have not been controlled, including patient and provider bias. In addition, ketamine dosing was not dosed by weight but rather by a pre-established protocol based on clinical judgment and a literature review, with flexibility for titration based on age and /or possible side effects. This study did not require scheduled UDS during the monitoring phase nor was the Controlled Substance Monitoring Database checked for opioid prescriptions. However, investigators did utilize random, unannounced UDS as well as regular chart reviews at each monitoring point with verification of no opioid prescription in the electronic medical record. Finally, we recognize the possible economic challenge in applying this government-based model in a private or academic practice. We agree with Bahji who recently discussed the high value of hospitalization in detoxification in order to adequately manage these complex patients with multiple co-morbidities, to minimize withdrawal symptoms with the use of pharmacologic assistance (e.g. ketamine, clonidine, benzodiazepines), and to provide adequate multidisciplinary support including mental health and chronic pain specialists [47]. Compared to long-term opioids and the need for long-term care, regular close follow-up, costs of complications and side effects [28], and decreased productivity, we believe that this ketamine-assisted opioid detoxification with short-term hospitalization is cost-effective.

5. CONCLUSION

Overall, this update provides new evidence that the utilization of a standardized ketamine infusion protocol coupled with a rapid opioid detoxification is very effective, results in a high rate of prolonged opioid reduction, decreases chronic pain, minimizes opioid withdrawal using strictly non-opioid analgesics, and decreases depressive symptoms. Future studies could consider a randomized, controlled trial, although patient and clinician blinding may be challenging. In addition, studies could focus on a dose response curve, identifying the lowest possible dose that is still effective in opioid cessation, as well as determining which patient populations most benefit from ketamine-assisted detoxification. Finally, additional research could focus on the feasibility of application in a strictly outpatient setting to increase external validity and facilitate widespread adoption.

A high prevalence of chronic opioid misuse persists despite evidence that chronic opioid use is frequently ineffective for chronic pain and may increase dysfunction and opioid related adverse effects including death. We demonstrate a significant rate of long-term opioid cessation following ketamine infusion therapy with minimal withdrawal symptoms while simultaneously decreasing chronic pain. Ketamine assisted opioid detoxification could contribute to the fight against the U.S. opioid crisis.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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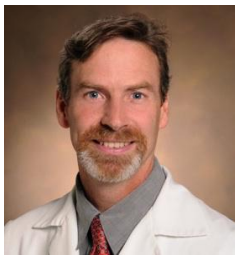

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Chapter 10
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Oral Sub Mucous Fibrosis: Comparison of Different Non Surgical Modalities

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ABSTRACT

Background: Oral submucous fibrosis (OSMF) is a chronic, complex, irreversible, highly potent pre-cancerous condition characterized by juxta-epithelial inflammatory reaction and progressive fibrosis of the sub mucosal tissues (lamina propria and deeper connective tissues). The condition is linked to oral cancers and is associated with areca nut chewing, the main component of betel quid. Various treatment modalities were compared the efficacy of intraoral administration of vitamin B combined with lycopene with intralesional triamcinolone acetonide combined with hyaluronidase and oral vitamin B complex with lycopene.
Objectives: To compare and evaluate the easy, safe and non invasive procedure for treatment of oral submucous fibrosis.
Materials and Methods: Total of 30 patients with clinically diagnosed OSMF patients. Each group consisted of ten patients with an age ranging from 30 -75years. A thorough case history along with a detailed clinical examination was performed for all individuals. The interincisal mouth opening was recorded at initial visit. Each group of patients received different modalities of theraphy for three weeks and mouth opening was measured after three weeks modalities that were to be compared. Group A Patients were advised use mixture of Turmeric and Jaggery Application twice a day all over the oral mucosa. Group B patients were advised to use Topical Kenacort Application twice a day and Group C patients were advised to do Physiotherapy exercise twice daily. The patients were recalled after a period of three weeks and their inter-incisal mouth opening was recorded. The statistical calculation was performed using SPSS version 19.
Results: 30-45 years - It was found that kenacort application suited the best treatment modality for this age group as it showed a very good prognosis in the inter incisal mouth opening. 46-75 years - It was found that jaggery and turmeric application suited the best treatment modality for this age group as it showed a very good prognosis in the interincisal mouth opening.
Conclusion: In our study group jaggery and turmeric application found to be most easy effective and safe modality of treatment for OSMF patients.

Keywords: Oral submucos firbrosis; turmeric; kenacort; precancerous condition.

1. INTRODUCTION

Oral sub mucous fibrosis (OSMF) is a high risk potentially malignant disorder characterised by changes in the connective tissue fibers of the lamina propria and deeper parts resulting into stiffness of the mucosa along with restricted mouth opening [1]. Submucous Fibrosis was first described by Schwartz in a series of Indian women [2] but it was Joshi in 1953 who first coined the term Oral Submucos fibrosis [3]. OSMF is mainly reported from India, but has also been diagnosed in Malaysia, Srilanka, Nepal, South Vietnam, and Thailand [4]. The potential precancerous nature of oral submucous fibrosis was first mentioned by Paymaster in 1956, with description of development of slow-growing squamous cell carcinoma in one third of the cases with OSMF [5]. OSMF is also

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characterized by reduced movement and depapillation of the tongue, blanching and leathery texture of the oral mucosa, progressive reduction of mouth opening, and shrunken uvula [6-9].

The strongest risk factor for OSMF is betel quid chewing containing areca nut. This condition predominantly affects women with f:m ratio of 3:1 between the ages of 45–54 years [10]. The potentially malignant nature of this condition has been well documented. A malignant transformation rate of 4.5% to 7.6% was found [11].

In initial phase of disease, mucosa feels leathery with palpable fibrotic bands. In advanced stage the oral mucosa loses its resiliency and becomes blanched and stiff. Other features of disease include xerostomia, recurrent ulceration and pigmentation of oral mucosa, dryness of mouth, burning sensation, decreased mouth opening and tongue protrusion [12]. In severe stage; hypomobility of soft palate & tongue, xerostomia, loss of gustatory sensations, fibrosis of pharyngeal & esophageal mucosa, hearing impairment, sunken cheeks, muscular dystrophy, hoarseness of voice, nasal twang and significant functional morbidity is noticed [13-15].

There are various treatments for OSMF including conservative therapy and surgical modalities. This chapter aims towards evaluating, best non-surgical method for the treatment for OSMF patients who do not prefer invasive surgical methods.

2. MATERIALS AND METHODS

The present study was conducted in the Department of oral medicine and Radiology, school of Dental Sciences, Karad, India. The ethical committee clearance was obtained before commencing the study. A written informed consent was obtained from the patients before conducting the study.

2.1 The Study Population

A total of 30 patients with clinically diagnosed OSMF were with the age group of 30 – 75years were recruited for the study.

2.1.1 Inclusion criterion

(1) Patients clinically diagnosed with oral sub mucous fibrosis showing reduced mouth opening.
(2) Patients who reported after 3 weeks for follow up.

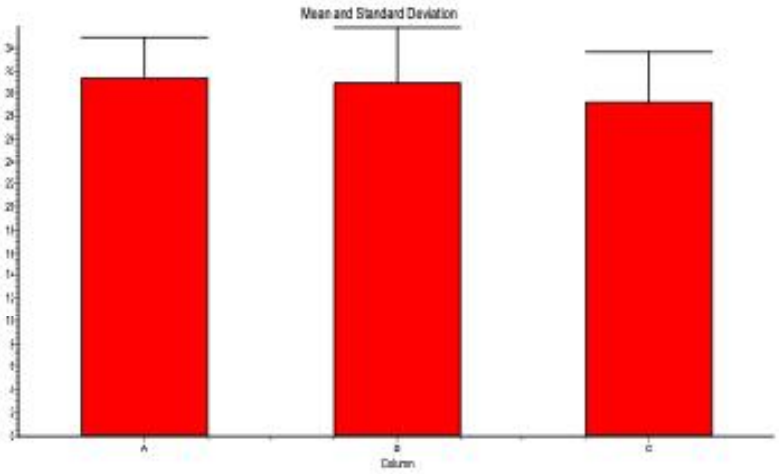
2.1.2 Exclusion criteria

(1) Patients who are systemically compromised (severe cardiovascular, hepatic, immunologic, renal, hematologic, or other organ disorders).

Through clinical examination was done using gloves and mouth mirror and vernier caliper was used to measure the inter-incisal mouth opening at initial visit. All 30 subjects were divided in to three groups consisting of ten in each group. Group A patients were advised application of Turmeric and Jaggery mixture twice a day all over the oral mucosa. Group B patients were advised to use Topical Kenacort Application twice a day and Group C patients were advised for Physiotherapy exercise like use of ice cream stick to open the mouth and ballooning twice daily. The patients were recalled after a period of three weeks and their inter-incisal mouth opening was recorded. The statistical analysis was performed using SPSS version 19.

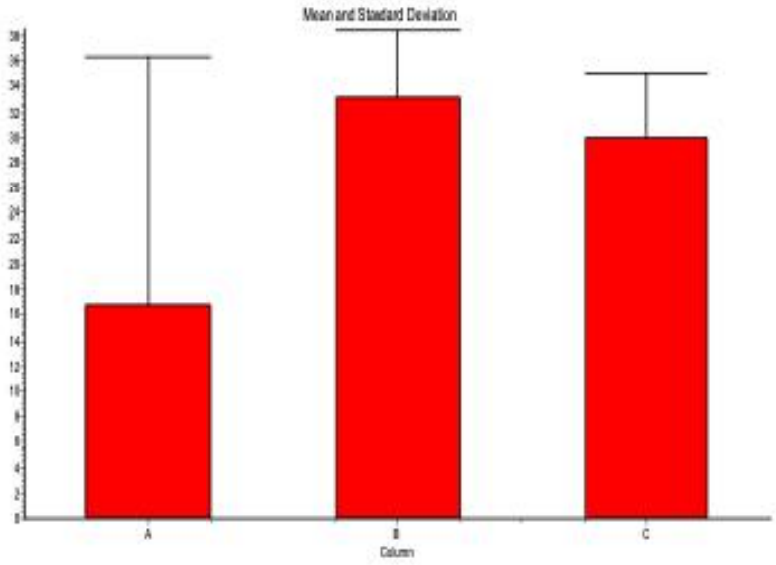
3. RESULTS

Out of 30 patients, 27 were males and 3 were females. When the different non -surgical treatment modalities where compared, it was found that turmeric and jaggery application was the best treatment when compared to kenacort application and physiotherapy but with no statistical siginificance. (p=0.5314) [Graph 1].



Graph 1. Relation between the 3 non-surgical method

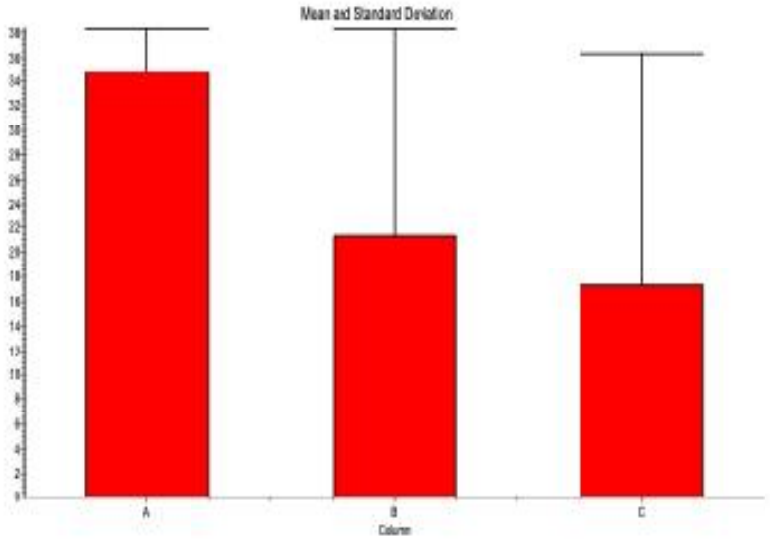
The three non surgical treatment modalities were compared within the age group of 30- 45 years. It was found that kenacort application was suited as the best treatment modality for this age group as it showed a very good prognosis in the inter incisal mouth opening. The data collected revealed that it was statsticaly significant. (p= 0.1838) [Graph 2].



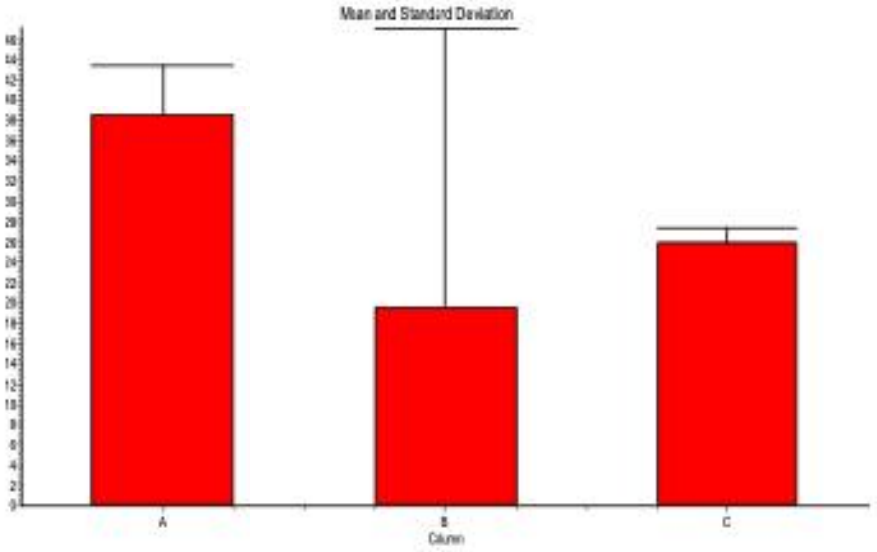
Graph 2. Relation between the 3 non-surgical method for the age group of 30-45 years

The relation between the 3 non- surgical method for the age group of 46-50 years found that jaggery application was suited as the best treatment modality for this age group as it showed a very good prognosis in the inter-incisal mouth opening. The data collected revealed that it was significant graphically (p=0.1353) [Graph 3].

The relation between the 3 non- surgical method for the age group of 61-75 years found that jaggery application was suited as the best treatment modality for this age group as it showed a very good prognosis in the inter- incisal mouth opening .The data collected revealed that it was significant graphically. (p=0.5588)[Graph 4].



Graph 3. Relation between the 3 non-surgical method for the age group of 46-50 years



Graph 4. Relation between the non-surgical method for the age group of 61-75 years

4. DISCUSSION

Areca nut chewing is one of the most important factors responsible for OSMF. Relationship between Consumption of areca nut in variable forms and its relation to OSMF has been established by many studies [16-19]. Areca nut plays a role in OSMF by generating free radicals as well as by causing immunosuppression. It affects patients with all ages but more common in second and third decades with male predominance which was also observed in our study. n our study group out of 30 patients, 27 were males and 3 were females. The results of our study was in consistent with Lai et al study who reported 96.67% male preponderance [20]. All the patients included in this study were using areca nut

in variable forms. This was also reported by Canniff et al in the study on pathogenesis and management of oral submucous fibrosis [12].

The treatment of OSMF includes topical or intra lesional corticosteroids because of their anti-inflammatory and immunosuppressant properties. Iron and antioxidants also helps in treating OSMF. Physiotheraphy exercises like forceful mouth opening exercises by using ice cream sticks and ultrasound also helps in reducing symptoms of OSMF. Kakar et al reported that patients treated with hyluronidase showed quicker improvement in symptoms but a combination of dexamethasone gave better and long term results [21]. Hyaluronidase degrades the fibrous matrix promoting lysis of fibrinous coagulum and activating specific plasmatic mechanism. Relief of symptoms and stiffness in oral cavity occurs through softening and diminishing fibrous tissue.

In a series of cases studied by Khanna JN, Andararade NN in 1995, revealed improvement in clinical symptoms and mouth opening after triamcinolone injection while advanced stages treated surgically gave encouraging results in mouth opening [22].

Administration of turmeric powder offers protection against benzopyrene induced increase in micronuclei in circulating lymphocytes and it is an excellent scavenger of free radical in vitro. Turmeric oil & turmeric oleoresin both act synergistically in vivo to offer protection against DNA damage [23].

Muscle stretching exercises includes forceful mouth opening with the help of sticks, ballooning of mouth, hot water gargling. This is thought to put pressure on fibrous bands. Forceful mouth opening have been tried with mouth gag & acrylic surgical screw.

In our study we compared three different non surgical modalities safety, effectiveness. As per our knowledge till now none of the studies have compared combination of turmeric and jaggery application with topical kenacort and physiotheraphy mouth opening exercise. In our study group it was observed that out of all three modalities turmeric with jaggery application found to be best non invasive, safe therapy for OSMF patients [24].

5. CONCLUSION

OSMF is a crippling disease of oral cavity, having multifactorial etiology with arecanut chewing the most elicited one. Oral submucous fibrosis is one of the most poorly understood and unsatisfactorily treated oral diseases. Though variety of treatments available for OSMF the most effective easy one which is affordable by the patient is important when treating the patient. In our patients application of turmeric with jaggery was found to be most effective safe modality in OSMF patients.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Chapter 11
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Mitochondrial Function in the Formation of Sexual Constitution of Men

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DOI: 10.9734/bpi/ctmamr/v10

ABSTRACT

The article provides a brief overview of mitochondrial functions and factors leading to the development of mitochondrial dysfunction. The sexual constitution of a person is formed in the prenatal period and early ontogenesis. Reasoned data suggest the influence of mitochondrial function on the formation of sexual constitution.

Keywords: Mitochondria; mitochondrial dysfunction; sexual constitution.

1. BACKGROUND

Energy exchange in cells is carried out at the expense of universal cellular organelles — mitochondria. Mitochondria are organelles of energy supply, in which the cell's metabolic processes also occur.

Their number in the cell is from 50 to 1000 or more. It is distinguished from other cellular organelles of mitochondria by the presence of its own well-studied mitochondrial DNA, which determines the ability of mitochondria to autoproduce.

In specialized cells, mitochondria are concentrated in those areas where there is the greatest need for energy. For example, in muscle cells, large numbers of mitochondria are concentrated along the working elements-contractile fibrils. In cells whose functions are associated with particularly high energy consumption, mitochondria form multiple contacts, combining into a network, or clusters (for example, cardiomyocytes and skeletal muscle simplasts). In the cell, mitochondria perform the function of respiration. It was found that some components of the respiratory chain (coenzyme Q, cytochrome oxidase), along with the transfer of electrons along the chain, also carry out the transfer of protons from the mitochondrial matrix to the intermembrane space, resulting in a proton gradient. During the reverse flow of protons into the mitochondrial matrix, the energy released in the respiratory chain is utilized by phosphorylation of adenosine diphosphate (ADP) into adenosine triphosphate (ATP) and other macroergic phosphates, and a reserve of energy for biological oxidation is created. In addition to electron transport and oxidative phosphorylation, mitochondria provide another process involving redox reactions - b-oxidation of fatty acids. Free fatty acids are transformed into acetyl-CoA and then form esters with carnitine. Carnitine-acetyl-CoA is transferred through the mitochondrial membrane, acetyl-CoA is released and participates in b-oxidation [1]. Also in the mitochondria there is a regulation of the intracellular distribution of calcium, the formation of steroids, the regulation of apoptosis. In mitochondria, the pathways of metabolism of proteins, fats and carbohydrates are integrated, the main energy processes are carried out. In this regard, changes in mitochondria can cause a complex chain of pathological processes at the level of the cell and the whole organism as a whole [2]. Also, mitochondria play an important role in such processes as aging and cell death, the development of certain diseases and pathological processes, and the physiological adaptation of the body to endurance exercises [3].

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There is no doubt that violations of the bioenergetic systems of the cell play a primary role in the implementation of the damaging effects of various factors. Mitochondrial dysfunction leads to insufficient energy supply of cells, disruption of many other important metabolic processes, further development of cellular damage, up to cell death [4,5]. Therefore, the energy state of the body as a whole depends on the state of the mitochondria.

Currently, the effect of impaired ATP synthesis in mitochondria on the functional activity of cells and tissues has been studied to the greatest extent. It was found that when the ATP content in the cell decreases by 10-20%, the activity of all energy — dependent processes decreases by 70-80%. The effects of insufficient ATP can include suppression of anabolic processes, disruption of ion pumps and, consequently, ion homeostasis in the cell, and inhibition of cytoskeletal functions [6].

Inhibition of energy formation processes in mitochondria is accompanied by a weakening of b-oxidation of lipids, which results in a violation of lipid homeostasis in the cell and the accumulation of acyl-CoA-thioesters, acyl-carnitines, ceramides and triglycerides, which have potential toxicity for the cell. An exception is the mitochondria of the nervous tissue, the biochemical feature of which is the lack of the ability to carry out b-oxidation of lipids [7].

Mitochondria have an exceptional role both in maintaining the life of the cell, and a high destructive potential. Violation of any of the functions of mitochondria-energy, thanatogenic, or activation of their production of free radicals can cause the development of functional and morphological disorders in various tissues and organs [8].

2. GENERAL DISCUSSION

Many environmental factors and medications are a significant cause of pathological changes in the mitochondria. These factors include the action of alkylating agents (for example, nitrosamines from the environment), hydroxyl radicals, high doses of ultraviolet and ionizing radiation, medications (briostatin, azidothymidine), and other chemical agents (alloxan, cyanides, carbon monoxide, etc.). The cause of mitochondrial damage may also be the lack of some microelements, such as selenium [9]. In many cases, the sensitivity of mitochondrial DNA to environmental damage is several times higher than that of the nuclear genome.

It is known that taking into account the numerous metabolic functions of the microflora, the violation of its colonization resistance is considered as a trigger factor for the development of a number of different diseases. Thus, studies conducted in recent years demonstrate a significant association of dysbiotic disorders of the intestinal microflora with the pathogenesis of non-alcoholic fatty liver disease (NAFLD), which occurs due to damage to the mitochondria. The presented data demonstrate the significance of dysbiotic changes in the development of mitochondrial dysfunction, the formation of steatosis in NAFLD with its subsequent transformation into steatohepatitis and progression to fibrosis and cirrhosis [10].

A number of publications report an increasing amount of evidence that impaired mitochondrial function can have a significant impact on mood and psychotic disorders.

Recently, data from a wide range of research studies involving animals and humans have supported the hypothesis that impaired mitochondrial functions can disrupt neural plasticity pathways and reduce cellular elasticity, which potentially contributes to the development or progress of mood disorders, such as severe depression or bipolar disorder, as well as other mental diseases such as schizophrenia or autism [11].

A link was found between mitochondrial dysfunction and Alzheimer's disease. The results obtained are consistent with previous work that established that the accumulation of beta-amyloids in neurons (distinctive feature of Alzheimer's disease) is directly involved in mitochondrial dysfunction [12].

Experimentally on animals, it was found that prenatal stress of a female mouse during early maturation leads to the birth of cubs with higher levels of androstenedione and lower levels of testosterone than in normal mice.

This may mean that males have less masculinization. In addition, although the mice were born with a normal weight, their growth began to slow down in the future. Their body weight was 10-20 % lower than that of control mice [13]. Researchers have identified several sets of genes related to the structure and function of mitochondria, which are responsible for energy production. Indeed, mice born of stressed mothers have dramatically reduced mitochondrial function in the hypothalamus compared to normal mice.

It is known that the sexual constitution is formed under the influence of hereditary factors and conditions of development in the prenatal period and early ontogenesis.

Consequently, factors (psychoemotional overload, burdening the body with xenobiotics, intestinal microflora dysbiosis) affecting the prenatal period and early ontogenesis can lead to mitochondrial dysfunction in the central nervous system (CNS), in particular – in the hypothalamus.

Wherein various molecular, biochemical and cellular disorders occur, leading to the development of hypothalamus dysfunction. As a result, there is an imbalance of hormones ⇔ violation of blood flow in the body, organ or part of it ⇔ metabolic disorders ⇔ etc., there is a "vicious circle".

Hence, mitochondrial dysfunction in the hypothalamus will contribute to the formation of weak and weakened versions of the sexual constitution. This will be manifested by early detection of vascular endothelial dysfunction.

One of the first studies that established a link between low birth weight (intrauterine development delay) and early vascular endothelial dysfunction was the work of Leeson C. P. et al. [14,15].

Thus, we assume that the mitochondrial function of the CNS affects the formation of the sexual constitution of a person. Mitochondrial dysfunction is a standard pathological phenomenon that develops under the influence of one or more often a combination of pathological factors. These are psychosomatic overloads; burdening the body with xenobiotics, intestinal microflora dysbiosis and injuries, including various radiations.

Mitochondrial dysfunction in the CNS leads to a violation of energy exchange in the cells of the hypothalamus. The integration and regulatory functions of the hypothalamus are disrupted – these are vegetative, metabolic, endocrine and trophic functions, and the immunological reactivity of the body.

One can achieve the formation of the desired sexual constitution by eliminating or minimizing the impact of harmful factors on the body of a pregnant woman and child after birth (2-3 years).

Each type of sexual constitution of a person has its own energy balance. The energy balance of the body affects the body's resistance to pathological factors.

"If the energy balance of the body is below average, the body will not be able to resist painful aggressions and will become hopelessly ill," wrote the Russian doctor and philosopher A. S. Zalmanov more than 50 years ago [16].

The energy balance of the body is determined by the mitochondrial function of the cell. Energy-material exchange is a set of mechanisms that determine the processes of vital activity at the cellular level [17].

The sexual constitution of a man, being a particular form of the general human constitution, is formed under the influence of hereditary factors and conditions of development in the prenatal period and early ontogenesis. That is, what is inherited from the mother and father (nuclear DNA), as well as the conditions of development after birth and early development (2-3 years). This means that

mitochondrial DNA (mtDNA), the replication of which is more intensive than nuclear, therefore, there is a rapid accumulation of mutations caused by pathogens [18].

Thus, male children acquire the mother's mtDNA sequence, since it is transmitted exclusively through the egg [19]. In the prenatal period and early ontogenesis, the proportion of damaged mtDNA will increase when pathogenic factors are applied. Therefore, the violation of energy-material exchange of cells will affect the overall energy balance of the body. This is how the general human constitution is formed.

Energy-material exchange formed at the level of merged cells (egg and spermatazoid) will determine the cytoenergetic status of the body. This status of maintaining energy homeostasis will then be regulated by the hypothalamus, which provides the relationship between the nervous and humoral systems of regulation. The hypothalamus controls the activity of the human endocrine system due to the fact that its neurons are able to secrete neuroendocrine transmitters that stimulate or inhibit the production of hormones by the pituitary gland. In other words, the hypothalamus, the mass of which does not exceed 5% of the brain, is the center of regulation of endocrine functions and maintenance of homeostasis of the whole organism.

Even V. D. Dilman (1986) [20] pointed out the leading role of the hypothalamus in the systematic development of metabolic dysfunction, leading to obesity, diabetes, cardiovascular diseases, oncological diseases, and aging. According to the theory of hyperadaptosis formed by Dilman V. D., the sensitivity of hypothalamus receptors to signals coming from body tissues (leptin, insulin, etc.) gradually systematically decreases with age. In order to trigger its "response" one need more and more of this or that hormone — more insulin, more leptin. It develops insulin and leptin resistance, metabolic diseases that lead to aging and death.

It is known that mitochondrial dysfunction of hypothalamus cells will affect the function of the pituitary gland with the subsequent development of an energy-deficient state, the so-called "energy-deficient diathesis". Energy-deficient diathesis is a hidden form of relative individual insufficiency of the cytoenergetic status of the organism [21].

Today, it is known that most diseases are accompanied by a failure in the operation of the intracellular mitochondrial quality control machine. Failure occurs, for example, when psycho-emotional overload (stress) [22], the impact and burden of the body with xenobiotics, toxins, exposure to ionizing radiation, intestinal microflora dysbiosis, as well as aging of the body, as a result, good and bad mitochondria begin to coexist in the cell. When the proportion of bad ones exceeds a certain threshold, there is a "phenotypic manifestation" of the disease, which until now had an invisible, latent character [23].

According to the literature data, there are morphological, biochemical criteria and clinical manifestations of mitochondrial insufficiency [24]. One of the methods for diagnosing violations of cellular energy exchange is to determine the level of lactate and pyruvate in the blood. This condition is characterized by increased levels of lactic and pyruvic acids. The balance of lactic and pyruvic acids is determined by the content of acyl-CoA inhibitor of the pyruvate dehydrogenase complex in mitochondria [25]. There is an accumulation of lactate and H+ in the cytosol, which leads to a decrease in pH inside the cell and to impaired function. Intermediate metabolites of free fatty acids hinder the transfer of macroergic phosphates through their membrane, contributing to further activation of glycolysis. As the oxygen deficit increases, acidosis occurs, which causes functional changes and a violation of the integrity of the membranes [26,27]. To diagnose energy exchange disorders, it is advisable to evaluate the activity of succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) enzymes. The main function of LDH is to catalyze the oxidation of lactic acid to pyruvate. Almost all cells in the human body contain the LDH enzyme.

The maximum concentration is observed in skeletal (9,000 u/g) and cardiac (25,000 u/g) muscles, as well as in the kidneys (15,000 u/g), lungs (9500 u/g)and liver (9,000 u/g).

If the cell is damaged, the enzyme is actively released into the bloodstream. Blood cells also contain LDH, but their content is insignificant. That is why even with a slight destruction of the tissues of any of the organs, there is a sharp increase in the level of LDH in the blood serum, which is used for the diagnosis of various diseases. This fact allows us to attribute the blood test for LDH to highly sensitive, but low-specific criteria.

According to literature data, mitochondrial dysfunction of liver cells, endothelium and vascular intima can form multiple "vicious" circles with components of "classical" mechanisms of atherogenesis, creating favorable conditions for their implementation. The main targets of mitochondrial dysfunction factors are the exchange of lipoproteins in the liver and the processes of atherogenesis in blood vessels, starting with endothelial dysfunction and ending with erosion and rupture of the atherosclerotic plaque [8,28,29].

One of the clinical manifestations of mitochondrial dysfunction is endothelial artery dysfunction. It should be noted that to see the manifestations of abnormalities of the mitochondrial respiratory chain, the amount of mutant mtDNA must exceed the threshold level. The percentage of altered DNA usually differs both within the family and in the body's systems [30].

The sexual constitution of a man, which was laid down genetically from his parents and formed phenotypically from the conditions of development in the early period, will be characterized by a certain energy balance. The energy balance of the body is a somatobiological background that determines the resistance to pathological factors.

Thus, mitochondrial function affects the formation of the male sexual constitution and determines endothelial function.

Today, we can confidently say that the period of pregnancy and the first months of life is the most important in a person's life. All available data show that it is during this period that the foundations of not only physical, but also mental health are laid. And the influence of this initial period of life is so great that it does not disappear until very old age, shaping the fate of a person.

German neuroscientist Peter Shpork clearly and aptly stated: "in old age, our health is sometimes much more affected by the diet of our mother during pregnancy than food at the current moment of life." [31].

It's hard to believe, but the facts are telling.

Our cells have a memory - they remember not only what you usually eat for breakfast, but also what your mother and grandmother ate during pregnancy. Our cells remember well whether you do sports and how often you drink alcohol. Cell memory stores your encounters with viruses and how much you were loved as a child. Cellular memory decides whether you will be prone to obesity and depression. And this amazing feature of our cells helps to understand the science of epigenetics [32].

3. CONCLUSION

Mitochondrial function is transmitted to children through the maternal line. It is already known how important physiological structures are set up in the womb and in the first years of life. The basic mitochondrial function of cells will determine the overall and sexual constitution. The sexual constitution of future children and grandchildren can be formed if it is here that reasonable preventive measures are taken for pregnant and young parents, and in the long term it is possible to change the health of an entire nation for the better.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Chapter 12
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Discussion on a 5 Year Retrospective Study of Pattern of Maternal Mortality in a Tertiary Care Hospital in South India

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ABSTRACT

Introduction: Maternal mortality remains one of the most daunting public health problems in India. For every minute one mother is dying due to pregnancy and child birth related issues. A woman in developing country has 30 to 40 times greater risk of dying due to pregnancy and child birth than her counterpart in developed country. Even today 20% of global maternal deaths occur in India. The total maternal deaths in India are around 63,000 a year, approximating one death every minute.
Materials and Methods: A retrospective analysis of 78 cases of maternal mortality over a period of 5 years from January 2007 to January 2012 in tertiary care hospital were analyzed with special emphasis on parity, cause of death, time interval from admission.
Results: Hemorrhage was the leading cause of maternal death accounting for 26.92% followed by sepsis 23.08%. Pre-eclampsia contributed to 20.51% of maternal death. Anemia was responsible for 17.95% deaths. The age group in which most (74.36%) maternal deaths occurred was 21-30 years group. When the parity of the women was compared, it was seen that most maternal deaths was in multi-para accounting for more than half the maternal deaths (56.41%).Most of the women (62.5%) died within 24 hours of admission followed by many women dying in the next 24-48 hours being 12.5%.
Conclusion: The maternal mortality rate at referral hospitals in India is very high. Accurate estimation of maternal mortality depends mainly on a sound vital registration system and proper reporting of maternal death. Accurate estimation of maternal mortality depends mainly on a sound vital registration system and proper reporting of maternal death. Solutions of the issues comprises of 3Ds: Delay in diagnosis, immediate treatment and decision to transfer, delay in transport for reaching to proper hospital and delayed therapy. Most of the deaths in our study have been avoided, if they had registered and received proper antenatal care, early diagnosis, timely intervention and early referral with well equipped transport facilities.

Keywords: Maternal mortality; pattern; Tertiary Care Hospital.

1. INTRODUCTION

Maternal mortality is one of the most important burning issues in our country. For every minute one mother is dying due to pregnancy and child birth related issues. A woman in developing country has 30 to 40 times greater risk of dying due to pregnancy and child birth than her counterpart in developed country [1]. Estimates of the global burden of disease for 1990 also showed that India contributed 25% to disability-adjusted life-years lost due to maternal conditions alone [2,3]. In the developing world, “A pregnant woman has her one foot in the grave” as stated by Gwyneth Lewis in’ Beyond the number’. Maternal mortality is defined as the death of any woman when pregnant or within 42 completed days following termination of pregnancy, irrespective of duration or site of pregnancy but not from accidental or incidental death. Maternal mortality ratio is defined internationally as the maternal mortality rate per one lakh live births. Maternal mortality remains one of the most daunting

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public health problems in India. Even today 20% of global maternal deaths occur in India [4]. Globally 1% of deaths occur in developed world while 99% occur in developing countries [5,6]. The total maternal deaths in India are around 63,000 a year, approximating one death every minute. Therefore, reducing maternal mortality ratio faster is a fundamental national and international concern [7,8,9,10,11]. It is being estimated that half of all death from pregnancy could be prevented with better prenatal care, quality of care and life style habits. One of the six health related millennium development goals set by WHO is to reduce the maternal mortality ratio [12]. Most of the deaths occur within one week and it is 100 times likely to occur on the first day after birth of the child. In India current MMR is 212 per lakh live births (census of India sample registration system 2011). Institutional mortality rates are 2-10 times higher as compared with field surveys because most of the seriously ill patients are referred to the nearest tertiary care centers. Maternal mortality is the tip of iceberg, there is a large base of the severe acute maternal morbidity, the identification and analysis of which will tell the story of true complications. It can be reduced by adequate antenatal care and appropriate interventions at the right time.

2. MATERIALS AND METHODS

A retrospective analysis of 78 cases of maternal mortality over a period of 5 years from January 2007 to January 2012 in Katuri Hospital, Guntur(A.P.) were analyzed with special emphasis on parity, cause of death, time interval from admission . Ethical Clearance has been obtained from the institute ethical committee.

3. RESULTS

There were 78 maternal deaths during the period from January 2007 to January 2012 in the hospital. Hemorrhage was the leading cause of maternal death accounting for 26.92% followed by sepsis 23.08%. Preeclampsia contributed to 20.51% of maternal death. Anemia was responsible for 17.95% deaths (Table 1). The age group in which most (74.36%) maternal deaths occurred was 21-30 years group. This was followed by <20 years (15.38%) and >30 years age groups (10.26%) (Table 2). When the parity of the women was compared, it was seen that most maternal deaths was in multi-para accounting for more than half the maternal deaths (56.41%). (Table 3) The women who came to our hospital, most of them were referred. 35.90% were delivered in our hospital (Table 4). Most of the women (62.5%) died within 24 hours of admission followed by many women dying in the next 24-48 hours being 12.5%. Few women died after 48 hours accounting for 24.99%.

Table 1. Causes of maternal death (n=78)

Cause of death	No (%)
Direct causes	
Hemorrhage	21(26.92%)
Severe pre-eclampsia	16(20.51%)
Septicemia	18(23.08%)
Abortion	5(6.42%)
Indirect causes	
Anaemia	14(17.95%)
Acute renal failure	1(1.28%)
Cardiac failure	1(1.28%)
Malaria	2(2.56%)

Table 2. Age distribution of maternal deaths

Age group	No (%)
< 20 yrs	12(15.38%)
21-30 yrs	58(74.36%)
>31 yrs	8(10.26%)
Total	**78(100)**

Table 3. Parity distribution of maternal deaths

Parity	No (%)
Primi	28(35.90%)
Multipara(2-4)	44(56.41%)
Grand multipara(> 4)	06(7.69%)
Total	**78(100)**

Table 4. Place of delivery

Place of delivery	No (%)
Medical college	28(35.90%)
District/Govt hospital	12(15.38%)
Primary health centre	11(14.10%)
Private nursing home	14(17.95%)
Home delivery	7(8.98%)
Undelivered	6(7.69%)
Total	**78(100)**

4. DISCUSSION

Maternal mortality is the death of a woman in relation to pregnancy. According to WHO “A maternal death is defined as the death of a woman while pregnant or within 42 days of termination of pregnancy, irrespective of the duration and site of pregnancy, from any cause related or aggravated by pregnancy or its management”. The present study revealed that 23.07% maternal death was due to indirect obstetrical causes, 76.93% due to direct cause. Other studies have shown variations in direct obstetrical death from. 68.7% in a study by Kulkarni et al. [13] and 60% by Salhan et al. [14]. Direct obstetric deaths accounted for 76.93% of all deaths in our study that included hemorrhage 26.92%, severe pre-eclampsia 20.51%, sepsis 23.08%, abortion 6.42%. Hemorrhage especially during post partum is sudden, unpredictable and more dangerous when woman has pre-existing anemia. Globally 25% of all maternal deaths are due to hemorrhage. Other studies show variation between 9.72% and 27.5% [6,15]. In our study the rate of deaths due to hemorrhage was 26.92%. This is due to lack of proper antenatal care, poor nutritional status, home deliveries and late referrals. Sepsis which is a direct consequence of poor hygiene during delivery, account for 15% of maternal deaths globally. In our study it was 23.08%. Globally, indirect cause of maternal deaths account for 20% of all maternal deaths, particularly from anemia, malaria, HIV, etc. Other studies show their range between 17.2% and 40%. In our study it was 23.07% and included deaths due to anemia 17.95%, ARF 1.28%, cardiac failure 1.28% and malaria deaths 2.56%. This was similar to in a study by Chhabra et al. [16] in which the main indirect cause of death was anemia (13.9%). Our study showed that 74.36% of women died between the age group 21 and 30 years, as highest number of women belong to this age group. Similarly, multigravidas contribute 56.41% of maternal deaths. Admission death interval of our study revealed that 62.5% of women died within 24 hours of admission, probably due to poor general condition of women at the time of admission and late referrals.

5. CONCLUSION

The maternal mortality rate at referral hospitals in India is very high. Accurate estimation of maternal mortality depends mainly on a sound vital registration system and proper reporting of maternal death. Solutions of the issues comprises of 3Ds: Delay in diagnosis, immediate treatment and decision to transfer, delay in transport for reaching to proper hospital and delayed therapy. The classical triad of maternal mortality causes in our study remained hemorrhage, eclampsia and sepsis. Most of the deaths in our study have been avoided, if they had registered and received proper antenatal care, early diagnosis, timely intervention and early referral with well equipped transport facilities.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 13
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Does Uvulopalatopharyngoplasty Change the Airway Volume?

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ABSTRACT

Obstructive sleep apnea (OSA) syndrome increases the risk of developing cardiac diseases and hypertension. Therefore, effective treatment of patients with OSA is critical. The aim of this study was to investigate the changes in velopharyngeal and glossopharyngeal airway morphology and volume after uvulopalatopharyngoplasty (UPPP) in three adult OSA patients who had bilateral large tonsils using three-dimensional computed tomography.

Case Report: All three patients (one male and two females) who presented with a history of heavy snoring and excessive daytime sleepiness were examined with overnight nocturnal polysomnography, which indicated moderate-to-severe OSA. Because all patients had large tonsils, UPPP was expected to enlarge the pharyngeal airway. Polysomnography and three-dimensional computed tomography (3D CT) scanning were performed and compared, both before and 3 months after UPPP.

Results: Unexpectedly, although the morphology of the glossopharyngeal airway clearly changed after UPPP, the volume changes in the velopharyngeal and glossopharyngeal airways were negligible. In conclusion, in our patients, the morphology of the velopharyngeal and glossopharyngeal airway changed after UPPP; however, the volume did not change. Further analysis of 3D CT images could contribute to our understanding of changes in morphology and air volume in the upper airway that are caused by UPPP.

Keywords: Airway volume; obstructive sleep apnea; Uvulopalatopharyngoplasty; three-dimensional computed tomography.

1. INTRODUCTION

Obstructive sleep apnea (OSA) syndrome increases the risk of developing cardiac diseases and hypertension [1]. OSA is highly prevalent, estimated to affect 34% of men and 17% of women in the general population [2] and 40% to 60% of patients with cardiovascular disease (CVD) [3,4]. Therefore, effective treatment of patients with OSA is critical. Recent imaging studies have described pharyngeal morphology in patients with OSA. In particular, the assessment of upper airway volume has received much attention from specialists in OSA as a challenging approach to understanding the pathogenesis of OSA [5,6,7]. The occlusion of the upper airway in OSA mainly affects the velopharyngeal and glossopharyngeal portions [8]. Uvulopalatopharyngoplasty (UPPP) is a surgical procedure that is used to enlarge the pharyngeal space. To the best of our knowledge, there is no report that has used three-dimensional computed tomography (3D CT) images to quantify the change in air volume in the velopharyngeal and glossopharyngeal airway from pre- to post-UPPP in adult OSA patients.

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In this report, we present velopharyngeal and glossopharyngeal morphology and volume before and after UPPP, assessed by 3D CT in three adult OSA patients who had bilateral large tonsils.

2. CASE PRESENTATION

All three patients (one male and two females) who presented with a history of heavy snoring and excessive daytime sleepiness, indicated by a high Epworth Sleepiness Scale (ESS) score, were referred to our department by a medical practitioner. They requested surgical treatment to enlarge their pharyngeal airway. Before surgical treatment, all three patients underwent a complete otorhinolaryngological physical examination that was conducted by a researcher. Body mass index (BMI), tonsil size, and palate grade were recorded for analysis. BMI was calculated as weight (kg)/height $(m)^2$. Tonsil size and palate grade were evaluated according to the scale proposed by Friedman et al. [9]. All patients were examined with overnight nocturnal polysomnography (PSG) in our sleep laboratory in the standard manner, and each had an apnea hypopnea index (AHI) >20 events/hour, indicating moderate-to-severe OSA. A hypopnoea is currently defined as being at least a 30% reduction in airflow combined with a 3% arterial oxygen desaturation levels, or an arousal from sleep [10,11].

Because the patients had bilateral large tonsils, the UPPP surgical procedure to enlarge the pharyngeal airway was performed by a sleep surgeon under general anesthesia, according to the originally described technique [11]. The large tonsils were removed bilaterally and the tonsil's weights were measured. All patients tolerated the surgery without complications.

BMI and ESS score were evaluated both before and 3 months after UPPP. PSG and 3D CT scanning were also performed at both time points. Postoperative PSG revealed moderate-to-great improvement in the severity of OSA (Table 1).

2.1 3D CT

3D CT was performed under the same condition before and after UPPP. 3D CT was performed using a 64-row multidetector CT scanner (Brilliance 64®, Phillips, Cleveland, OH, USA) under routine neck examination conditions at the end of inspiration while the patients were holding their breath. Thin axial sections of 0.9 mm thickness at 0.45 mm intervals were obtained to reconstruct multiplanar reformation images and 3D CT images. Multiplanar reformation and 3D CT image reconstructions were performed at an image workstation (Ziostation2, Ziosoft, Tokyo, Japan), which enabled us to visualize and objectively quantify the dimensions and volume of the airway. Axial-view multiplanar reformation images were used for this analysis. The 3D CT images of the velopharyngeal and glossopharyngeal airway were constructed from images of areas in which the CT number was −400 Hounsfield units or less.

Axial and sagittal images of the glossopharynx before (Fig. 1(a)) and after (Fig. 1(b)) UPPP in patient 1 were shown in Fig. 1. The maximal diameter of the glossopharynx in the transverse dimension and the minimal diameter in the anteroposterior dimension were recorded for analysis.

In patient 1, the transverse diameter of the glossopharynx was enlarged from 6.4 mm preoperatively to 25.2 mm postoperatively, whereas the anteroposterior diameter was reduced from 27.8 mm to 17.6 mm. In like manner, in patients 2 and 3, the transverse diameter was enlarged, whereas the anteroposterior diameter was reduced postoperatively. To provide a better understanding of these morphological changes, the anterior-view 3D CT images of the velopharyngeal and glossopharyngeal airway in patient 1 before (Fig. 2(a)) and after (Fig. 2(b)) UPPP were shown in Fig. 2. In addition, 3D composite images from before and after UPPP in patient 1 were also generated at an image workstation (ParaView®, Kitware, Clifton Park, NY, USA). 3D images were adjusted for their opacities and displayed by superimposition in Fig. 3.

Table 1. Physiologic and polysomnographic variables before and after uvulopalatopharyngoplasty

Patient	Age/sex	BMI (kg/m^2)		Tonsils				ESS		AHI(events/h)		CT90(%)		LSAT(%)	
				TS	TW		PG								
		Pre-	Post-		R	L		Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-
1	36F	34.9	31.0	3	7.6	6.9	IV	16	7	112.1	3.8	45.1	0.2	61	84
2	28 M	26.4	25.8	3	9.4	4.4	II	15	14	47.0	16.2	3.5	6.2	77	78
3	30 F	20.0	19.9	3	10.7	10.7	IV	13	5	22.9	0.4	0	0	94	95

AHI = apnea hypopnea index; BMI = body mass index; CT90 = percentage of time with oxygen saturation below 90%; ESS = Epworth Sleepiness Scale; F = female; M = male; pre- = preoperatively; post- = postoperatively; PG = palate grade; R = right; TS = tonsil size; TW = tonsil weight

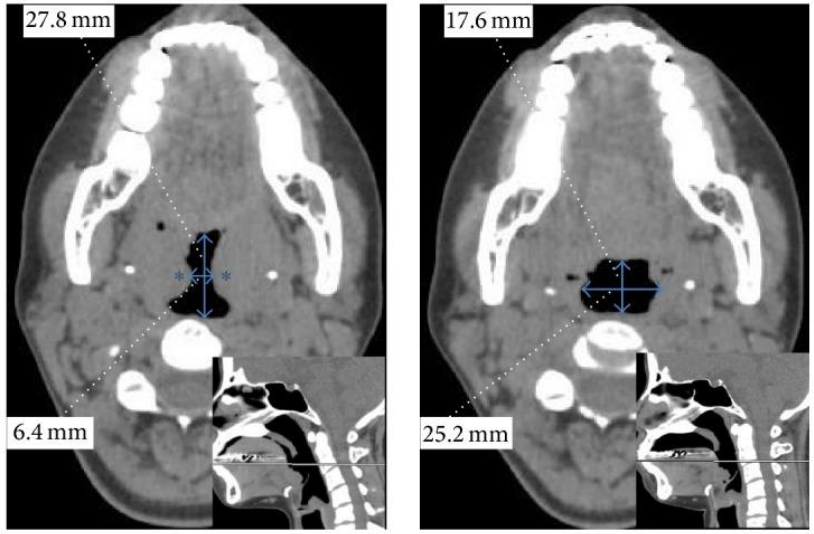

Fig. 1. Axial-view computed tomography images from patient 1. (a) The narrowing of glossopharynx (blue double-headed arrows) evident before uvulopalatopharyngoplasty (UPPP) was caused by the impression of large tonsils (asterisk). (b) The narrowing was enlarged transversely; however, the glossopharynx became narrower in the anteroposterior direction after UPPP (indicated by the number). The lines on midsagittal view indicate the level of the axial-view

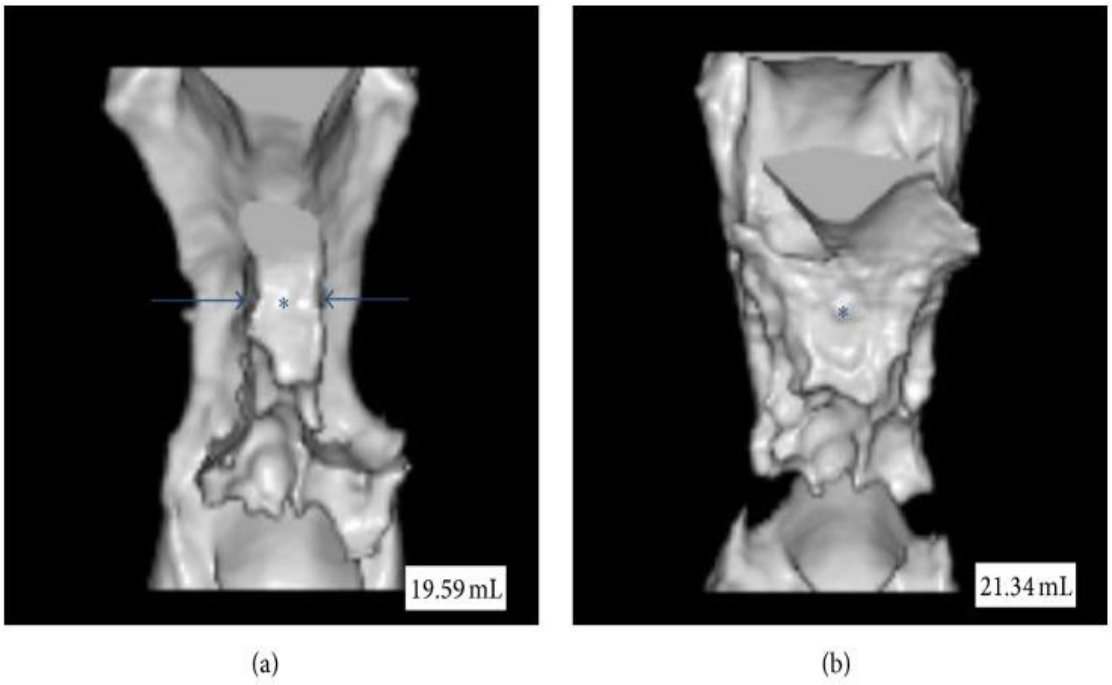

Fig. 2. Anterior-view three-dimensional computed tomography images from patient 1. (a) Bilateral large tonsils impressed on the glossopharyngeal airway from both sides (blue arrows) before uvulopalatopharyngoplasty (UPPP). The gap between two arrows shows the airway (asterisk). (b) The airway was enlarged after UPPP. Nevertheless, the change in the volume of the airway (indicated by the number) was negligible

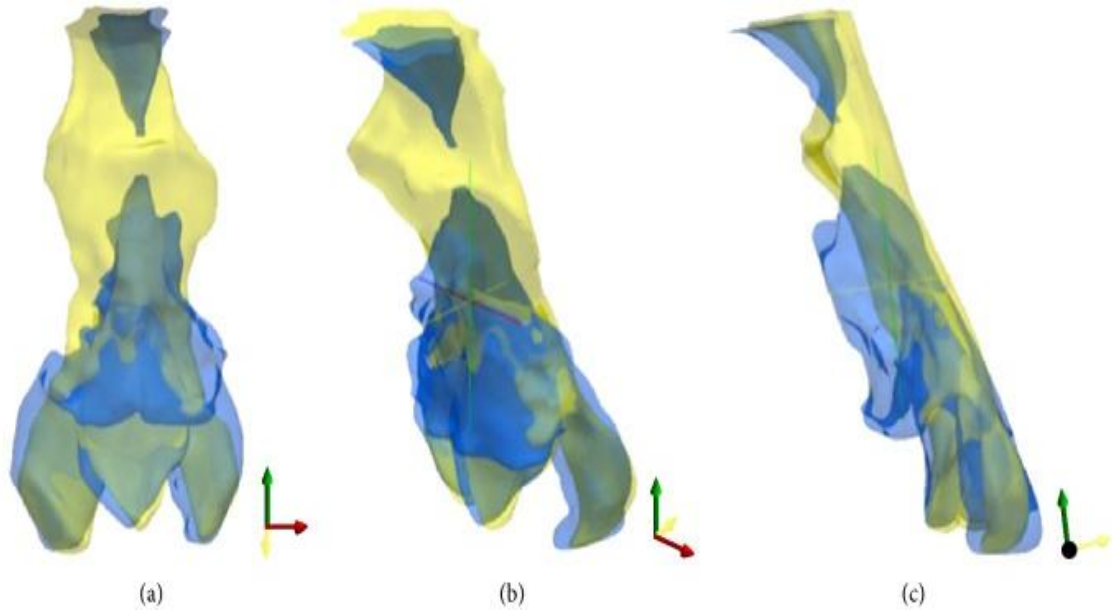

Fig. 3. 3D composite images before UPPP (blue) and after UPPP (yellow) from patient 1. Anterior view (a), left oblique view (b), and left lateral view (c). The 3D composite images were superimposed to visualize these morphological changes clearly

The following limits were adopted when measuring the velopharyngeal and glossopharyngeal airway volume: The superior margin was the posterior nasal spine, and the inferior and anterior margins were the hyoid bone in the midsagittal view. The dimensions of the glossopharynx and the volume of the velopharyngeal and glossopharyngeal airway before and after UPPP in each of the three patients were summarized in Table 2. In all patients, the transverse diameter of the glossopharynx increased after UPPP, whereas the anteroposterior diameter decreased. Thus, morphological changes were observed after UPPP; however, the change in total air volume was negligible (Table 2).

Table 2. Anatomical measures from 3D CT before and after uvulopalatopharyngoplasty

Patient		3D CT			
		Diameter (mm)		Air volume (mL)	
		Pre-	Post-	Pre -	Post-
1	T	6.4	25.2	19.59	21.34
	A-P	27.8	17.6		
2	T	13.2	19.3	8.78	9.03
	A-P	14.8	12.1		
3	T	12.8	19.0	10.39	11.53
	A-P	16.8	12.6		

3D CT = three-dimension computed tomography; A-P = anterior-posterior; OSA = obstructive sleep apnea; pre- = preoperatively; post- = postoperatively; T = transverse

3. DISCUSSION

Since Fujita et al. [11] first described UPPP as a surgical procedure for OSA in 1981, it has remained the main surgical approach for the treatment of patients with OSA. UPPP is designed to enlarge the airway lumen at the level of the velopharyngeal area and decrease the collapsibility of the pharyngeal walls. UPPP enlarges the upper airway by removing the redundant excessive distal palatal tissue while preserving the function of the proximal palatal musculature. However, the mechanism by which UPPP improves OSA is still controversial. Moreover, the success rate of this surgery, which is based mainly on the measurement of respiratory parameters, was only around 50% in a long-term follow-up study [12].

Patients with more severe OSA tend to have larger tonsils and a smaller airway volume [13]. OSA is considered to be caused by an imbalance in the pharyngeal airway size that is determined by the anatomical balance between the large volume of contents (i.e., tonsils and surrounding soft tissues structures) and the small volume of the container, which is constrained by craniofacial bony structures. When this anatomical balance theory [14] is applied to UPPP, the residual air space should be smaller when the contents are larger. The aim of UPPP should be to reduce the content volume, which should result in an increase in the volume of the pharyngeal airway.

In this report, for all three patients, bilateral large tonsils were removed as a part of UPPP. For example, in patient 1, the weight of the bilateral large tonsils which were removed during UPPP was 14.5 g (7.6 g and 6.9 g for the right and left side, resp.). It was expected that the air volume of the velopharyngeal and glossopharyngeal spaces would increase by the equivalent amount (i.e., 14.5 g/14.5 mL) after UPPP.

Our results showed obvious morphological changes after UPPP; however, changes in velopharyngeal and glossopharyngeal air volume were negligible—from 19.59 mL to 21.34 mL. As shown in Fig. 1, the transverse diameter of the glossopharynx was enlarged from 6.4 mm to 25.2 mm; however, the anteroposterior diameter narrowed from 27.8 mm to 17.6 mm because the tongue base was displaced from anterior to posterior after UPPP. It was supposed that, before UPPP, the bilateral large tonsils maintain the tongue base in a forward position. However, after bilateral large tonsils were removed, the tongue base moved backwards, thus, reducing the AP diameter of the airway. These morphological changes could underlie the lack of change in air volume in the velopharyngeal and glossopharyngeal airway after UPPP.

To the best of our knowledge, there is no report that has used 3D CT images to quantify the change in air volume in the velopharyngeal and glossopharyngeal airway from pre- to post-UPPP in adult OSA patients with large tonsils. Recently, Chiffer et al. [5] reported volumetric magnetic resonance imaging analysis before and after transoral robotic surgery for OSA patients. They reported total airway volume increased postoperatively, whereas the volumes of the soft palate, tongue, and total and retropalatal lateral pharyngeal walls decreased. Cossellu et al. [6] also reported morphological and volume changes in the upper airway using 3D CT during oral appliance in patients with OSA. They showed an improvement of the total upper air volume in nine out of ten patients. To our knowledge, no reports discussed volume changes before and after UPPP.

Schwab et al. [15] already described that the airway caliber increased substantially after UPPP, with a large increase in the lateral airway dimension and a decrease in the thickness of the lateral pharyngeal walls. Langin et al. [16] also performed CT in patients with OSA before and after UPPP and demonstrated an increased width of the fauces after surgery. Nevertheless, these studies evaluated morphological changes but did not quantify volume change after UPPP.

The patients reported here indicate that the mechanism by which UPPP improves OSA cannot fully be explained using the anatomical balance theory.

In three cases, there was marked improvement in OSA symptoms as well as AHI and ESS scores despite negligible changes in the airway volume after UPPP. We believe that this improvement could be attributed to three factors. First, muscle tone might have increased after UPPP as a result of scar formation on the palatopharyngeal wound, which could have contributed to reduced collapsibility of the pharyngeal walls during sleep. Second, the morphological change, that is, enlargement of the restricted area, might have altered pharyngeal air circulation and swirl flow, which would be expected to normalize pharyngeal air flow during sleep [17]. Finally, this postoperative alteration in anatomy could have led to an equalization of the airway lumen diameter. We believe this equalization implies a decrease in airflow velocity and postoperative diminution in the collapsibility of the pharyngeal air lumen during sleep according to Bernoulli's principle [18]. However, these aeromechanics theories require further studies with more patients using computational fluid dynamics.

In this report, we presented small number of patients. However, our investigation showed that UPPP did not always increase air volume in the velopharyngeal and glossopharyngeal airway even after bilateral large tonsils were removed. This potentially valuable report suggests an alternative working mechanism of UPPP.

4. CONCLUSION

In conclusion, in our patients, the morphology of the velopharyngeal and glossopharyngeal airway changed after UPPP; however, the volume did not change. Further analysis of 3D CT images could contribute to our understanding of changes in morphology and air volume in the upper airway that are caused by UPPP.

ADDITIONAL POINTS

This work was done in the Department of Otorhinolaryngology, Second Hospital, Fujita Health University School of Medicine, Nagoya, Aichi, Japan.

CONSENT

Written informed consent and human subjects' understanding were obtained from all participants.

ETHICAL APPROVAL

This study was conducted in accordance with Declaration of Helsinki (1964).

DISCLOSURE

One case from this series was presented as a poster presentation at the 15th Korea-Japan Joint Meeting of Otorhinolaryngology-Head and Neck Surgery, Seoul, Korea, April 3–5, 2015.

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COMPETING INTERESTS

The authors declare that they have no competing interests regarding the publication of this paper.

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Chapter 14
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Letter to the Editor: Streamlined Upper Airway Collapsibility Measurement for Uvulopalatopharyngoplasty (UPPP): Perspectives

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ABSTRACT

As a surgical approach for treating adults with obstructive sleep apnea (OSA), dilating a small airway might be an important treatment component of therapy, but it might not be primary surgical goal. Surgical goal must be to stabilize the upper airway against dynamic collapse that threatens its patency in patients with OSA. For this reason, evaluation of pharyngeal collapsibility (critical pressure: Pcrit) for individual patients with OSA, especially streamlined Pcrit measurements before and after surgery would be valuable.

Keywords: Airway volume; obstructive sleep apnea; Pcrit; Uvulopalatopharyngoplasty.

Obstructive sleep apnea (OSA) syndrome is a highly prevalent disease, with an estimated prevalence of approximately 4% in men and 2% in women [1]. It is associated with significant morbidity and mortality that increases with age and its prevalence peaks at approximately 55 years of age in men [2]. Therefore, effective treatment of patients with OSA is critical. Unfortunately, treatments that bypass the airway, or simply open the pharyngeal airway are either morbid or unsuccessful, respectively. Uvulopalatopharyngoplasty (UPPP), since Fujita et al. [3] first described as a surgical procedure in 1981, has been developed as a surgical approach for treating adults with OSA, with the aim of opening (dilating) the pharyngeal airway. It still remains the most common surgical procedure performed to treat adults with OSA, with an overall success rate of approximately 40% in unselected patients [4,5]. Therefore, OSA surgeons do not favor UPPP as a treatment of choice for all patients with OSA. UPPP could be effective, in appropriately selected patients, such as those with hypertrophic large tonsils, webbing of the posterior pillars, elongated and thickened uvulas, redundant pharyngeal folds and a normal tongue with a retro-displaced soft palate ("favorable" anatomic structures). OSA is characterized by upper airway collapse and/or occlusion during sleep, which mainly affects the middle pharyngeal area especially velopharyngeal and glossopharyngeal portions. Patients with OSA tend to have a more narrow middle pharyngeal space, smaller middle pharyngeal airway volume [6], that is characterized with the anatomical imbalance between the large volume of upper airway contents (i.e., tonsils and surrounding soft-tissues) and small volume of container (i.e., craniofacial bony structures) (Anatomical balance theory) [7]. This means if patients have a large volume of soft tissue content and/or small container volume, the residual pharyngeal air space might be result in crowed and stuffing, which may cause airway occlusion during sleep. In applying this theory to sleep surgery, OSA surgeons often try to reduce the soft tissue contents and/or dilate the bony container surrounding the pharyngeal airway, which should increase airway volume and enlarge the pharyngeal airway. UPPP is designed to resect large hypertrophic tonsils, removing the redundant excessive distal palatal tissue. It will dilate the airway lumen at the level of velopharyngeal area, which is expected to increase upper airway volume. It is not clear, however, why UPPP does not always increase upper airway volume as the OSA surgeon anticipates.

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Recently, the changes in velopharyngeal and glossopharyngeal airway morphology and volume after UPPP were examined in adult patients with OSA and bilateral large tonsils by three-dimensional computed tomography [8]. In this paper, morphology of the glossopharyngeal airway was compared before and after UPPP. In their three cases, patients' apnea-hypopnea indices and daytime sleepiness had improved dramatically after UPPP, but interestingly enough, they found that the glossopharyngeal airway clearly dilated after UPPP, although the volume changes in the velopharyngeal and glossopharyngeal airways were negligible.

Just imagine two 250ml coca-cola or pepsi bottles, one is made of plastic bottle and the other is made of aluminum. Consider their characteristics: is the crushability (collapsibility) of those two bottles equal? As we imagined, even if the size of two empty coca-cola or pepsi containers is the same, the plastic bottle is crushable and aluminum can might be quite stiff. Even a plastic bottle of 500ml would be easy to be dent, whereas an aluminum can of similar or even smaller size would be hard to crush or collapse. Thus, the ultimate size of the airway impacts less on the success of surgery than the change in its mechanical properties (stiffness or collapsibility).

It is worth recalling that OSA is characterized upper airway collapse and/or occlusion during respiration and sleep, which is not static but dynamic phenomenon. Treating OSA is complicated, of course. Dilating of the upper airway might be necessary yet insufficient to open the upper airway. To be sure, dilating a small airway might be an important treatment component of therapy, but it might not be the main goal of therapy. Even of the airway becomes wide and dilated post-operatively if it is still soft and collapsible (and easy to dent), the airway will dynamically collapse and/or occlude very easily during sleep. In a similar vein, just imagine a rubber band, when stretched (dilated), it will become stiff or even rigid and less collapsible. It is possible that when we dilate (stretch) patient's pharyngeal airway with a UPPP procedure, it could become less collapsible. But anatomical factors (i.e., small airway) may not be the only reason a patient has OSA. Instead, a dynamic phenomenon (i.e., respiration and sleep), airway characteristics (i.e., not easy to dent), airway stiffness (collapsibility) must be also considered in evaluating the airway and the potential effects of surgery. It is likely that airway dilation (with a concomitant increase in volume) is not our primary surgical goal. Our goal must be to stabilize the upper airway (i.e., make it uncrushable: not easy to dent) against dynamic collapse that threatens its patency in patients with OSA.

Whereas investigators have identified both anatomic and neuromuscular control factors that increase pharyngeal collapsibility during sleep in patients with OSA [9], a physiologic basis for measuring pharyngeal collapsibility (critical pressure: Pcrit) would be useful to evaluate in patients with OSA; the collapsibility of individuals with varying levels of pharyngeal airway obstruction during sleep could be examined before surgery; and the relationship changes in pharyngeal collapsibility and changes in the severity of OSA before and after UPPP could be elucidated [10]. Evaluation of Pcrit for individual patients with OSA before surgery, might help predict success in treating a patient pre-operatively. Despite the potential utility of Pcrit measurement, Pcrit cannot be easily measured, especially in the outpatient clinic or in the hospital ward. Such measurements should be facilitated in the outpatient clinic or in the hospital ward for individual OSA patients before and after surgery, as if it were simply a blood pressure measurement.

Recently, a simple, novel non-invasive streamlined approach for measuring Pcrit was published [11]. Additional research to extend this approach to Pcrit measurements in prospective UPPP patients is warranted.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 15
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Detailed Study on the Mechanism of Metabolic Influences on the Endogenous GLP-1 by Oral Antidiabetic Medications in Type 2 Diabetes Mellitus

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is now the commonest type of diabetes mellitus in most economically developed nations. Incretin-based therapy is now a prevalent treatment option for patients with type 2 diabetes mellitus (T2DM). It has been associated with considerably good results in the management of hyperglycemia with cardiac or nephron-benefits. For this reason, it is recommended for individuals with cardiovascular diseases in many clinical guidelines. As an incretin hormone, glucagon-like peptide-1 (GLP-1) possesses multiple metabolic benefits such as optimizing energy usage, maintaining body weight, β cell preservation, and suppressing neurodegeneration. However, recent studies indicate that oral antidiabetic medications interact with endogenous or exogenous GLP-1. Since these drugs are transported to distal intestine portions, there are concerns whether these oral drugs directly stimulate intestinal L cells which release GLP-1, or whether they do so via indirect inhibition of the activity of dipeptidyl peptidase- IV (DPP-IV). In this review, we discuss the metabolic relationships between oral anti-hyperglycemic drugs from the aspect of gut, microbiota, hormones, β cell function, central nervous system, and other cellular mechanisms. The findings of these studies provide the rationale for the development of new drugs or formulations, which can ensure better glucose metabolism.

Keywords: Type 2 diabetes mellitus; GLP-1; oral antidiabetic medications; metabolic relationships.

1. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is now the commonest type of diabetes mellitus in most economically developed nations. Type 2 DM is the most common form of DM characterized by hyperglycemia, insulin resistance, and relative insulin deficiency [1]. Type 2 DM results from interaction between genetic, environmental and behavioral risk factors [2-4]. In recent years, the incidence of obesity and metabolic syndrome among younger populations has risen. T2DM is therefore emerging as a major public issue because it is not only difficult to control but also leads to multiple cardiovascular complications. According to the epidemic report by the International Diabetes Federation (IDF) published in 2017, there are estimated 425 million people currently affected by diabetes. The report predicts that the number of people affected by diabetes will reach 629 million in the next 20 years [5]. In 2017, prevalence rate of adult T2DM exceeded 10.9% in China, being highest among overweight and obese people [6]. Obesity is considered a health disaster in both developed and developing countries [7,8]. To effectively control the high burden of diabetic nephropathy, coronary heart disease and stroke in diabetic patients, new antidiabetic medications should be developed through preclinical and clinical research. In recent years, many antidiabetic drugs have been put into the market and several others are in the development pipeline most of which have shown good clinical benefits. Some of such drugs include incretin-based therapies and sodium

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glucose co-transpotor-2 inhibitors (SGLT-2i). GLP-1 is an incretin that improves β cell function. This review describes the possible mechanisms and relationships between the currently used oral antidiabetic medications and GLP-1 (Fig. 1). In so doing, we provide knowledge that can be used to guide formulation of optimal treatment combinations for clinical management of T2DM.

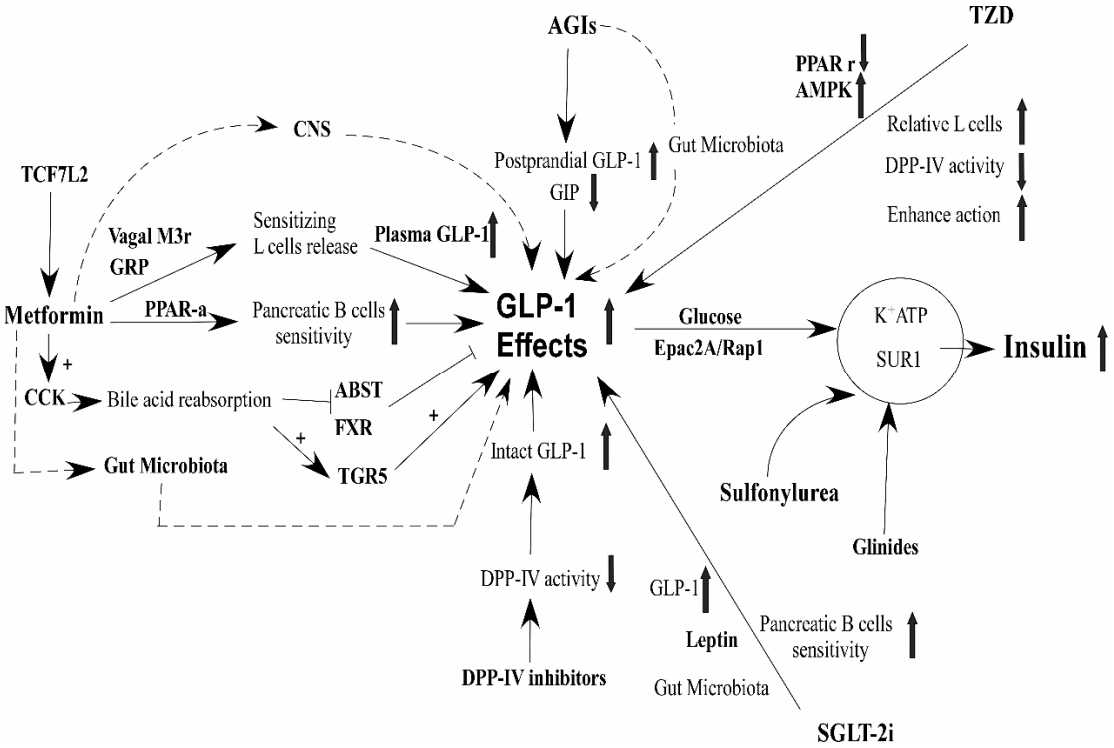

Fig. 1. An overview of possible mechanisms of current major types of oral antidiabetic medications (OADs) on GLP-1 effect: including metformin, AGIs, TZD, SU, Glinides, SGLT-2i, DPP-IV inhibitors

CNS: Central nervous system; M3r: muscarinic receptor 3; GRP: gastrin-releasing peptide; PPAR-α: peroxisome proliferators-activated receptor ; CCK: Cholecystokinin; ABST: apical sodium-dependent bile acid transporter; FXR: farnesoid X receptor; TGR5: Takeda G protein-coupled receptor; AGIs: α-Glucosidase inhibitors; DPP-IV: Dipeptidyl peptidase-IV; TZD: Thiazolidinedione; AMPK: Adenosine 5'-monophosphate (AMP)-activated protein kinase; Epac2A: The exchange protein directly activated by cAMP; Rap1: Ras-associated protein 1; K^+ATP: ATP sensitive potassium channel; SUR1: sulfonylurea receptor-1; SGLT-2i: sodium-glucose co-transporter-2 inhibitor

2. PHYSIOLOGY OF GLP-1

In the early 1900s, scientists discovered that the pancreas can produce endocrine substances which lower blood glucose levels in response to the intestinal factors that triggered by meal stimulating [9]. These intestinal factors were subsequently named "incretin". Later in the 1960s, scholars described the "incretin effect" further confirming the existence of such endocrine factors. This effect states that oral glucose elicits higher insulin level than intravenous administration in the context of the same level of blood glucose level [10]. It was later reported that this effect account for 50-70% of total insulin secretion. GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) were later isolated from intestines, which led to the recognition that GLP-1 is responsible the majority activity of "incretin effect". GLP-1 and glucagon are derived from a common pre-proglucagon gene but show tissue-specific patterns. After posttranslational processing by prohormone convertase2 (PC2) or prohormone convertase 1/3 (PC1/3), respectively, pre-proglucagon gene generates several protein products such as Glicentin-related Pancreatic Polypeptide (GRPP), glucagon, major proglucagon fragment (MPGF), Intervening Peptide-1 (IP-1), few GLP-1 and GLP-2 in pancreatic α cells. In L-cells or PPG of

brainstem, the keys products formed from proglucagon gene include glicentin, oxyntomodulin, GLP-1 and GLP-2 [11]. So far, only one receptor has been recognized to bind GLP-1. The classical GLP-1 receptor (GLP-1R) is a GPCR receptor widely expressed in several cells of the body. Its activation triggers diverse signaling cascades influencing various cellular functions (Fig. 2). GLP-1R couples with adenylate cyclase thereby elevating intracellular cAMP levels leading to activation of protein kinase A (PKA and cAMP-regulated guanine nucleotide exchange factor II (cAMP-GEFII, also known as Epac2) [12]. In this way, GLP-1 modulates the metabolic functions of target cells. The general effects of GLP-1 on metabolism include insulin release, inhibition of glucagon, β cell preservation, suppression of gastric emptying, anorexigenic, body weight reduction, bone formation, and organ protection (brain, heart, kidney) [13].

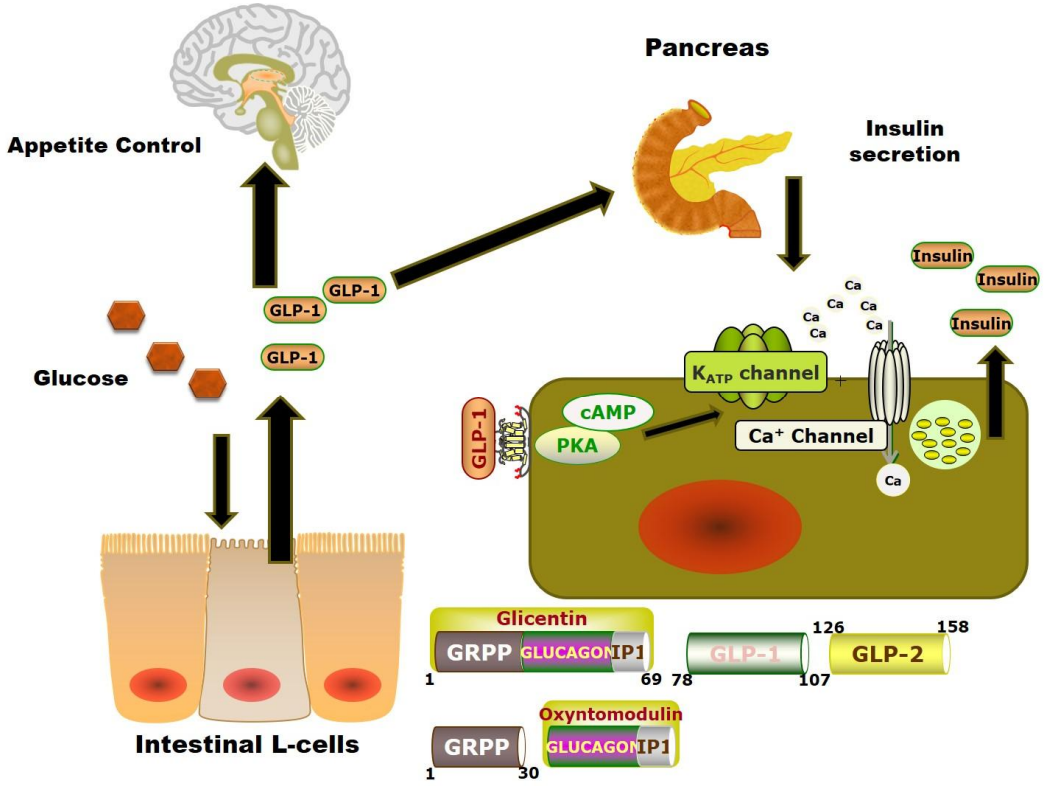

Fig. 2. A brief review of the physiology of GLP-1

The intestinal GLP-1 can be secreted by the intestinal L cells under the stimulation of glucose. Then, the GLP-1 can bind to GLP-1 receptors of pancreatic β-cell. Its downstream action leads to inhibition of ATP sensitive potassium channel, which results in the activation of calcium channel, and the accumulation of calcium in the cell promotes the secretion of insulin. On the other hand, the GLP-1 in the circulation can access the brain which affect the appetite or energy control. cAMP: Cyclic Adenosine monophosphate; PKA: Protein kinase A; GRPP: Glicentin-related Pancreatic Polypeptide

Previous studies have demonstrated that oral drugs with GLP-1R agonists or DPP- IV inhibitors result in unprecedented lowering of glucose compared to monotherapy and therefore improve metabolism. This has necessitated investigations into whether current oral medications have similar metabolic targets with GLP-1 signaling. In this review, we summarize the metabolic outcomes of oral antidiabetic medications with GLP-1-like effects.

3. METFORMIN AND GLP-1

Absorption of metformin is a transporter-dependent process that mainly occurs in the small intestine [14]. Its uptake is through apical (luminal-facing) surface of enterocytes via bidirectional transporters.

Efflux across the basolateral surface of enterocytes is limited with passive diffusion through paracellular uptake resulting in presence of metformin in the portal circulation. Several transporters have been identified including organic cation transporter (OCT) 1–3, plasma membrane monoamine transporter (PMAT), multidrug and toxin extrusion protein 1–2, serotonin transporter [15] and high-affinity choline transporter [15]. Genetic variation in OCT1 has been investigated by a number of groups and these have generated useful insights into its pharmacokinetics, efficacy and GI intolerance.

The mechanism of action of metformin with regards to its role in reducing hyperglycemia entails lowering of hepatic glucose output and subsequently enhancing glucose uptake by skeletal muscles [16]. One of the critical genetic factors involved in this process is AMP-activated protein kinase (AMPK) [17]. Pathways involved in lowering of glucose have been shown to be dependent on AMPK. For instance, its interaction with GLP-1 has been found to improve response to GLP-1 of pancreatic β cell via PPAR-α [18]. Meanwhile, metformin further enhances GLP-1 in the plasma. For instance, Mannucci et al. [19,20] reported that metformin increases plasma concentrations of active GLP-1 in obese, nondiabetic, as well as in obese, diabetic subjects. In addition, the two trials (CAMERA and DIRECT) also reported that in non-diabetic individuals, metformin increases total GLP-1 in a sustained manner independent of changes in weight or glycaemia [21]. To date, it is still unclear whether this interaction is mediated by DPP- IV inhibition or GLP-1 secretion of metformin.

A recent study in which metformin (5-) and AMPK activator aminoimidazole carboxamide ribonucleotide (100-1000 Mm) were administered to murine human NCL-H716 and rat FRIC L cells revealed that neither of these agents stimulated GLP-1 secretion, indicating that metformin does not act on L cells to directly elicit secretion of GLP-1 [22]. Similarly, rodents orally or subcutaneously administered with metformin (300 mg/kg) and AMPK activator (250 mg/kg) respectively showed an 2-8 fold increase in total plasma GLP-1 over a 2 hour period with no effect on the activity of DPP- IV. This indicates that the effect of metformin on *in vivo* GLP-1 plasma concentrations is regulated by increased peptide secretion. Moreover, pretreatment with a selective muscarinic 3 receptor antagonists also decreased GLP-1 levels. Bilateral subdiaphragmatic vagotomy revealed that only the basal GLP-1 levels were suppressed, and that a gastrin-releasing peptide acts as an antagonist blocking the metformin-induced GLP-1 secretion. These findings demonstrate that metformin stimulates release of GLP-1 through a mechanism that involves muscarinic (M3) and GRP receptor-dependent pathways but is independent of both DPP-IV enzyme and direct effects on the intestinal L cell. Similarly, Thondam et al. [23] reported that chronic administration of metformin increased GLP-1 levels but with no effect on DPP- IV activity and Ghrelin levels. In addition, Vardarli and colleagues [24], while comparing effect of sitagliptin and metformin on incretin, found that fasting significantly increased while an oral glucose challenge decreased total GLP-1 levels. However, intact GLP-1 increased in both fasting or post-load by sitagliptin. Interestingly, only sitagliptin significantly augmented insulin secretion (in monotherapy and as an add-on to metformin) while the incretin effect was not changed numerically. This study suggests that metformin-induced GLP-1 release may not directly act on islet insulin secretion or via DPP- IV inhibition.

One of the links between metformin and GLP-1 release have been described using bile acids with a recent trial conducted to examine whether metformin suppressed bile acid reabsorption leads to GLP-1 secretion [25]. Here, it was reported that type 2 diabetic patients orally administered with metformin (1,500 mg) or placebo in combination with intravenous infusion of cholecystokinin (CCK) (0.4 mol/kg/min) or saline, showed an enhanced plasma GLP-1. It is understood that CCK induced excretion of bile acids that elicited release GLP-1 through action of a single dose of metformin. However, GLP-1-mediated induction of insulin or release of glucagon were not observed [25]. This work further indicated that acute doses of metformin combined with bile acids improve glycemia and enhance GLP-1 release. Several *in vitro* studies have also shown that metformin can suppress the active reabsorption of bile acids in the terminal ileum by inhibiting apical sodium-dependent bile acid transporter (ASBT) [26,27]. Subsequent processes such as inhibition of nuclear farnesoid X receptor (FXR) and activation of the cell surface Takeda G protein-coupled receptor (TGR5) elicit GLP-1 secretion from L cells. Trabelsi et al. [28,29] reported that activation of FXR in L cells inhibits intracellular glycolytic pathways resulting in reduction of GLP-1, whereas activation of TGR5 induces GLP-1 secretion through increased ATP/ADP ratio and cyclic AMP levels in the L cells. The role

played by TGR5 on GLP-1 secretion is best described by [30,31] whose findings showed that plasma GLP-1 levels increased in patients with type 2 diabetes when bile acids were administered to the rectal or colon. In human trials, the specific ASBT inhibitors resulted in reduced levels of plasma glucose while GLP- 1 concentrations increased in rodents [32,33].

The rs7903146 T allele in transcription-factor-7-like-2 (TCF7L2), strongly associated with T2DM, has been described as an additional link between metformin response and GLP-1 levels [34]. Its factor binds to the promoter region of the proglucagon gene that encodes glucagon, GLP-1, and GLP-2. TCF7L2 exhibits an altered incretin signaling; potentially the mechanism of action with which it uses to increases T2D risk. As a result, it influences acute responses to both glipizide and metformin in people without diabetes. In the study, participants who carried the high-risk T allele at rs7903146 in the TCF7L2 gene showed higher fasting glucose levels during the baseline before glycemic perturbations. Similarly, high-risk T-allele carriers also had compensatory lower glucagon levels at baseline and higher GLP-1 levels displaying GLP-1 resistance.

Recently, other studies have reported that metformin may affect gut-brain-liver axis [35] and even alter the composition of gut microbiota that in turn improve hyperglycemia [36,37]. These opens up new frontiers for further explorations into mechanisms of action of metformin on blood glucose metabolism.

4. SULFONYLUREA (SUs), GLINIDES AND GLP-1

Insulin secretion is understood to be stimulated by SUs and GLP-1 of which calls for the need to unveil the common targets in the insulin secretion signaling. A recent direct target is the Epac2A/Rap1 signaling shared by GLP-1 and Sus [38]. Epac2A/Rap1 signaling is required for the first phase of glucose-induced insulin secretion. Epac2A, expressed in endocrine tissue, is both a PKA-independent pathway targets activated by GLP-1 and has binding sites for SUs. In this study, the researchers demonstrated that a combination of GLP-1 and Glibenclamide or Glimepiride augmented insulin secretion in Epac2A$^{+/+}$mice and whereas a significant reduction was observed in Epac2A$^{-/-}$mice. However, a combinatorial effect of GLP-1 and gliclazide was rather mild with the effect not altered by Epac2A ablation. A combination of an Epac-selective cAMP analog with glibenclamide or glimepiride favored activation of Rap1 but not gliclazide. On the other hand, ablation of Epac2A reduced the secretory response of insulin to co-administration of the GLP-1 receptor agonist liraglutide and glimepiride in diet-induced obese mice. This study provides explanations on the effect of combining incretin-based drugs and SUs during clinical trials as they may easily induce episodes of hypoglycemia.

According to a majority of endocrinologists, the major triggering step to insulin secretion is inactivation of K^+ATP channels and the subsequent depolarization of *β*-cells [39,40]. The K^+ ATP channel is made up of four pore-forming Kir6.2 and four sulfonylurea receptor (SUR1) regulatory subunits. The Kir6.2 subunits act as glucose/ATP sensors by binding onto ATP in a Mg^{2+}-dependent manner generate a conformational change that closes the channel. Conversely, binding of Mg^{2+}-ADP onto the SUR1 subunits opens the channel. At high glucose concentrations, the ATP/ADP ratio in the β cells increases leading to channel closure and membrane depolarization whereas a low glucose level elicits counteraction. SUs stimulates the secretion of insulin by the closing ATP-sensitive K^+ channels and also directly bind to sulfonylurea receptor-1 (SUR1) to inhibit K(ATP) channels. On the other hand, GLP-1 binds onto the GLP-1 receptor stimulating ß-cell cAMP formulation. Subsequent activation of PKA and Epac elicits downstream molecular activities related to insulin secretion including altered ion channel activity, elevation of intracellular calcium concentrations, and enhanced exocytosis of insulin-containing granules [41]. Effects of glucose and GLP-1 on insulin secretion may converge at the level of K(ATP) channels which is sensitive to present ATP levels, and PKA-mediated phosphorylation of S1448 in the SUR1 subunit. A result of this is that K(ATP) channel close via an ADP-dependent mechanism [11]. Such a phenomenon has been demonstrated *in vivo* where a targeted deletion of the SUR1 subunit resulted in elevation of cAMP levels by GLP-1 but with no further stimulation of glucose-induced insulin secretion. Stimulation of insulin secretion by GLP-1 therefore relies on glucose metabolism by pancreatic β cells. At the same time, SUs may allow GLP-1R agonists to bypass glucose dependence by triggering *β*-cell depolarization even in the absence of glucose [42]. Therefore, SUs that bind SUR1 to modulate K(ATP) channels (independently of glucose)

may influence GLP- 1R agonists to uncouple the glucose dependent manner by stimulating downstream effects ordinarily associated with increased glucose.

Glinides do not activate Epac2A but nateglinide and mitiglinide have been shown to mobilize Ca^{2+} from endoplasmic reticulum (ER) by binding onto the benzamido site of SUR1 on β cells. This is a separate site from that of the sulfonyl-binding but has a similar effect to sulfonylurea binding on the Kir6.2 channels. Despite this similarity, a relatively rapid onset and short duration of action restricts their use as prandial glucose-lowering agents [40,43].

5. α-GLUCOSIDASE INHIBITORS (AGIs) AND GLP-1

AGIs selectively inhibit α-glucosidase enzymes in the brush border of enterocytes lining of the intestinal villi. This in turn prevents cleaving of disaccharides or oligosaccharides into monosaccharides and postpones carbohydrate digestion and absorption leading to a reduction in blood-glucose and lowering prandial insulin levels [43]. Previous studies have demonstrated that AGI treatment increases postprandial GLP-1 but reduces glucose-dependent insulinotropic polypeptide (GIP) secretions [44,45]. Zhang et al. [46], when comparing treatment for T2D with acarbose and metformin, showed that both treatments notably increased levels of GLP-1 but decreased those of glucagon after 24 weeks. Other studies have reported that chronic administration of acarbose significantly increased FGF21 levels whereas insulin like growth factor-Ⅰ (IGF-Ⅰ) simultaneously decreased in serum and this could be responsible for the observed extended lifespans in rodents [47]. Both hormones are mainly produced by the liver, and this phenomenon could be attributed to enhanced action of GLP-1 on hepatocytes which increases expression of PPAR-α and Sirt1 to promote FGF21 production. Moreover, SCFAs mainly derived from undigested carbohydrates or insoluble fibers fermented by gut microbiota play an important role in AGIs treatment in patients with diabetes. Acarbose has previously been shown to increase levels of serum butyrate (an SCFA) in individuals with impaired glucose tolerance [48]. An increase in SCFAs promotes GLP-1 production [49]. Voglibose have been found to possess anti-obesity abilities which induce changes in dysbiosis in diet-induced obese mice. These compounds therefore increase bile acid metabolites and confer benefits on systemic outcomes such as cardiovascular end-points [50]. In addition, administration of miglitol increases butyric acid levels in the intestines and suppresses colon inflammation [51]. The underlying mechanisms of AGIs action in improving metabolism have been comprehensively investigated and reported [52]. These include effects of incretins, activation of neuroendocrine response to leptin, and induction of expression of the genes responsible for enhancement of energy metabolism.

6. THIAZOLIDINEDIONE AND GLP-1

Thiazolidinedione (TZDs) are agonists of peroxisome proliferator-activated receptor (PPAR); a nuclear receptor that regulates transcription of genes involved in lipid and glucose metabolism [53]. Although predominantly expressed in adipose tissues, PPAR is present in other insulin-sensitive tissues including the liver, muscle and pancreatic islet cells [54, 55]. In adipose tissues, stimulation of PPAR increases adipocyte differentiation resulting in an increased number of small, insulin-sensitive adipocytes which improves glucotoxicity. Development of these insulin-sensitive cells in turn enhances glucose uptake, improving glycemic control. TZDs also promote FA uptake and storage in adipose tissue thus reducing levels of circulating FFAs. Functionally, this has the potential to alleviate lipotoxicity. TZDs protect β cell function and prevent apoptosis by improving insulin sensitivity (indirectly) and acting on PPAR γ in β cell of pancreatic islets (directly). Despite limited knowledge on the influence of TZDs on GLP-1, there have been attempts to describe existence of metabolic relationships as well as the need to further explore abilities to preserve β cell function, affect lipid metabolism for application in prevention of prediabetes.

For instance, a previous study in Zucker diabetic fatty rats reported that treatment of rats with metformin and pioglitazone significantly decreased DPP-IV activity in serum as well as glycosylated hemoglobin. Regression analysis further indicated that DPP-IV activity in serum positively correlated with glycosylated hemoglobin and negatively with GLP-1, respectively. However, *in vitro* studies

showed that, metformin, pioglitazone, and glyburide did not influence serum DPP-IV activity indicating these medications are not competitive DPP-IV inhibitors [56]. In addition, the study also found that DPP-IV activity increased with ageing in T2DM subjects, and that kidney and liver RNA levels were unchanged. Since DPP-IV is secreted in some tissues, a potential explanation for this is that decreased DPP-IV activity could be secondary to improved glycemic control which could regulate the release of DPP-IV from T-cells, endothelial, pancreatic islet α or other types of cells. These could determine the extent to which these cells contribute to the pool of soluble DPP-IV activity.

Another research group seeking to determine whether insulin-sensitizing drugs improve secretion of GLP-1 studied catch-up growth rats that display insulin resistance and impaired incretin effect after pioglitazone treatment. They found that rats fed on high fat diets showed improved insulin resistance, high levels of circulating GLP-1 and increased relative number of intestinal L cells [57]. However, the effect of TZDs was not comparable to metformin treatments known as an incretin release enhancer. A separate study examined whether the TZD rosiglitazone had direct action on pancreatic β cells in acute or long-term rosiglitazone in clonal pancreatic BRIN-BD11 β cells maintained in standard, glucotoxic and lipotoxic cultures. The results showed that rosiglitazone (6.25 μ M) enhanced acute insulinotropic action of GLP-1, and displayed direct beneficial effects on β cell viability and function during gluco- or lipotoxicity setting [58]. Although the interaction of GLP-1 and TZDs have not been adequately investigated or elucidated so far, it is possible that this effect could be attributable to PPAR- γ agonist. This is because this compound can lead to activation of adenylate cyclase or late events in G protein coupled pathways, or the effect to modulation of AMPK activity by TZDs as previously demonstrated. This was partially demonstrated by action of SCFAs in protecting against high-fat diet-induced metabolic abnormalities regulated by PPARγ repression. It is understood that this repression subsequently increased expression of mitochondrial uncoupling protein 2 and AMP/ATP ratio which is responsible for activating AMPK, increasing oxidative metabolism in the liver and adipose tissues and elevating levels of GLP-1 [59]. Moreover, the metabolic relationship was also demonstrated by the inverse influence of GLP-1 signaling on PPAR γ during lipid metabolism. Decara et al. [60] showed that chronic administration of Liraglutide regulator changed lipid metabolism in a diet and tissue-dependent manner in rats, resulting in its decrease in the liver and muscles of high fat diet-induced obesity rats but an increase in lean rats. Moreover, it has been shown that both GLP-1 and TZDs can be utilized as treatment options in non-acholic fatty liver disease (NAFLD) [61]. To compare the effect of pancreatic β cells protection, Kimura et al. [62] found that both GLP-1 agonists, Liraglutide and pioglitazone, preserved the function and mass of β cells although this action was more profound during the early compared to advance stages in T2DM in db/db mice. In conclusion, TZDs treatment has metabolic impact upon GLP-1 action and shares some similarities despite their distinct action targets.

7. SODIUM-GLUCOSE CO-TRANSPORTER-2 INHIBITORS (SGLT-2i) AND GLP-1

Sodium-glucose co-transporter-2 inhibitors (SGLT-2i) are major proteins responsible for the glucose reabsorption in the kidney. Although mainly expressed in the proximal tubule of the kidney, they also localize in other organs or tissues of the body. A recent preliminary study using SGLT-2 antibody immunohistochemistry detected their expression in multiple areas in the central nervous system, including the hypothalamus, periaqueductal grey, nucleus of the solitary tract, among others. Administration of SGLT-2i in mice could have an impact on nuclei activities in CNS related to autonomic neural regulation (unpublished data). However, the mechanism of its associated effects has not been elucidated. And this can be attributed to limited studies on the drug. Some studies have shown that SGLT-2i could function by inhibiting the absorption of glucose in the renal tubule, thus resulting in the excretion of large amounts of carbohydrates into the urine [63]. The mechanism presents advantages and limitations. Excretion of glucose accompanied by sodium and fluids induces negative energy metabolic status that could result in weight loss, cardiac protection, and renal protection. However, chronic glucose deficit and excess ketone in the blood might lead to excess food consumption and euglycemic diabetic ketoacidosis (euDKA). Nonetheless, this drug is beneficial to the metabolism of patients with T2DM because of its insulin-independent action that releases excessive energy status or insulin resistance. The drug is also associated with a low incidence of adverse effects. Multiple clinical trials have demonstrated the efficacy and safety of a combination of DPP- IV inhibitors and SGLT-2i in the treatment of T2DM.

A few studies have shown that SGLT-2i alone can elevate the circulation of GLP-1 in patients with T2DM. Takebayashi et al. reported that canagliflozin treatment alone significantly increased the area under the curve(AUC) [0-120 min] of active plasma GLP-1 after three days compared with that at baseline and the addition of teneligliptin (DPP-IV inhibitor) resulted in a further increase [64]. In this study, canagliflozin treatment raised the active GLP-1 plasma level in the early phase (30min), probably by inhibition of SGLT-1 by low selective SGLT-1/SGLT-2 inhibitors that could have led to increased levels of GLP-1 secreted by lower intestinal L cells. Also, other neuroendocrine factors could have been at play. This was consistent with another study in which utilization of Empagliflozin (high selective for SGLT-2) also raised the plasma GLP-1 level in the early phase [65]. And SGLT-2i was found to improve the incretin sensitivity of pancreatic β cells in patients with T2DM. Chang et al. showed that in a 3-hour hyperglycemic clamp study involving incretin infusion before and after 8-week treatment with dapagliflozin, the C-peptide response to GLP-1 significantly increased and that to GIP/GLP-1 also increased (but not significantly) whereas both the insulin responses to GLP-1 and GIP/GLP-1 increased significantly [66].

Given that both SGLT-2 and GLP-1 are associated with weight control and food intake, Hiroshi et al. investigated the effect of SGLT-2 inhibitors by evaluating the effect of 16 weeks of ipragliflozin treatment on body weight and hormones related to appetite regulation in patients with BMI ≥22 kg/m^2. The treatment led to a significant reduction in fasting serum leptin levels after two weeks, which remained the same for up to 16 weeks, whereas the plasma active ghrelin level showed no significant change [67]. The reduction of the leptin level could indicate the resolution of leptin resistance accompanied with bodyweight reduction because overweight or obesity is associated with leptin resistance. Numerous studies have demonstrated that GLP-1 interacts with leptin to influence food intake and body weight. Administration of GLP-1 agonists for a short period decreased circulating and free plasma leptin levels, which led to an increase in the soluble leptin receptors thus bodyweight reduction [68,69]. Therefore, SGLT-2i and GLP-1 could partly share common neuroendocrinal mediators that influence body weight.

Concerning the effects on CNS, both SGLT-2i and GLP-1 have shown neuroprotection potential. Canagliflozin administration was shown to improve glucose and lipid metabolism by indirectly attenuating obesity-induced inflammation in the nodose ganglion, hypothalamus, and skeletal muscle of mice [70], thus reducing the symptoms of metabolic syndrome, which could have been accomplished by the GLP-1. Although the current study did not investigate the effect of SGLT-2i in the CNS, a few studies have shown that it could have anti-seizure properties because of its low molecular weight and lipo-solubility [71], and anti-degenerative brain diseases [72]. SGLT-2i could also gain entry into the brain by permeating the blood-brain barrier or via another neuroendocrinal pathway to influence autonomic activities such as cardiovascular regulation (unpublished data).

The gut has been used widely for the study of metabolic syndrome [73]. The occurrence of metabolic syndrome disorders has been associated with increased expression of SGLT-2 [74]. However, a study indicated that SGLT-2i could improve the gut microenvironment. In the study, diabetic animals treated with dapagliflozin showed a decrease in the gut microbiota composition, such as Firmicutes/ Bacteroidetes ratio and Oscillospira, and an increase in *Akkermansia muciniphila* [75], which suggested that the gut microbiota composition could be influenced by SGLT-2i and GLP-1interaction.

8. DPP- IV INHIBITORS AND GLP-1

Glucagons like peptide-1 (GLP-1) have a short life span in plasma (less than 2 minutes) because of degradation by DPP-Ⅳ and other enzymes. Therefore, incretin-based therapy in clinics uses DPP-Ⅳ inhibitors or a modified GLP-1 analog/GLP-1 receptor agonists as an antihyperglycemic option for patients with T2DM [11]. Dipeptidyl peptides-4 (DPP-4) inhibition allows GLP-1 to remain longer in plasma and exerts its insulin release stimulatory activities. The DPP-Ⅳ inhibitors include sitagliptin, saxagliptin, alogliptin, vildagliptin, linagliptin, etc. It has been reported that DPP-Ⅳ inhibition leads to a 5 to 10 pmol/L increase in meal-induced plasma concentrations of intact GLP-1. These types of medications are well tolerated, and only a few adverse effects have been observed in clinical use. Studies have shown that these drugs also confer cardiovascular benefits.

Contrary to the GLP-1 analogs, DPP-IV inhibitors have a neutral effect on body weight and therefore do not cause weight loss. Also, they do not cause hypoglycemia, particularly when combined with GLP-1 analogs. Dipeptidyl peptides-4 is also known as the T-cell activation antigen CD26 and one of the enzymes responsible for GLP-1 breakdown. It is also involved in thymic maturation and migration patterns [76]. Inhibition of DPP- IV was found to alter the expression of the immune response-related genes in the thymus, especially those related to central immunological tolerance. Inhibition of DPP- IV could also contribute to the prevention of T1DM in NOD mice. Several studies have shown that DPP-4 inhibition in patients with T2DM has the potential to treat pulmonary hypertension by exerting anti-inflammatory activity [77]. Other studies have also revealed that DPP-4 inhibition has reno-protective effects independent of blood pressure and glucose-lowering effects [78]. Dipeptidyl peptides-4 has also been implicated in cancer development because of its tendency to promote tumor cell adhesion, migration, and metastasis. Furthermore, autoimmune diseases pathophysiology such as multiple sclerosis, tuberculoid leprosy, Graves' disease, systemic lupus erythematosus (SLE), rheumatoid arthritis, and the human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), etc. are associated with CD26/DPP-IV activity [79]. Recently, reports have emerged that associate DPP-IV treatment with onset the of Bullous pemphigoid [80]. Thus, other than glycemic homeostasis, DPP- IV inhibitors play several roles in immunity. Currently, there is an increasing need for a more precise incretin regulation/better blood glucose control while minimizing adverse effects or complications.

9. THE SIDE EFFECTS AND EXPECTED SIDE EFFECTS ON COMBINATION OF OADs

At present, there are many drugs available for diabetes treatment, but most of the mechanism of drug action cannot be separated from the role of insulin. Therefore, the hypoglycemic effect of conventional hypoglycemic drugs may be weakened or even some adverse reactions may occur under the condition of severe insulin deficiency or function defect. Traditional hypoglycemic drugs include metformin, sulfonylureas, glinides, α - glucosidase inhibitors, thiazolidinediones. Among them, metformin may not be able to effectively control blood glucose because some people can't tolerate gastrointestinal reaction, liver and kidney function decline and can't reach the standard dose in case of lactate acidosis; sulfonylurea as an insulin secretagogues, although they have significant hypoglycemic effect, can cause the insecurity of hypoglycemia, and can also cause weight gain. Insulin secretagogues may also increase the load of islet cells and accelerate islet apoptosis, therefore, they cannot be applied to patients producing diabetes related antibodies, cases such as latent autoimmune diabetes in adults (LADA). In general, the combination conventional OADs with an insulin secretagogue should be avoided in some populations in case of hypoglycemia. AGIs can also cause adverse reactions of the digestive system, and hypoglycemic effects are limited. TZDs can cause sodium and water retention, thus, may increase risks of heart failure; SGLT-2i also induce potential genitourinary tract infections, or euglycemia ketonic acidosis, and may increase the risks of amputations. Finally, in type 1 diabetes and late stage 2 diabetes, these drugs will be more difficult and lack of blood glucose control. There also lack of evidence-based support for cardiovascular safety and protection of conventional OADs.

10. CONCLUSION

The global prevalence of type 2 diabetes mellitus (T2DM) is rising steadily. And because of its close association with obesity, the rise in the incidence of the condition reflects the changing lifestyle of modern society. The disease is also associated with the onset of other disorders, especially those related to the cardiovascular system. However, numerous studies show that the condition can be managed at an early stage and its complications can also be reduced at an advanced stage. The management strategies of the disease include lifestyle changes such as diet and exercise, or the use of antidiabetic medications, which are regarded as non-invasive and safe. Currently, several drugs for managing T2DM are available, and each has its unique metabolic targets and intra- or extracellular mechanisms. The algorithms for the application of these drugs in clinics have been established in many guidelines proposed by diabetes associations of many countries, and the dosage administered is based on the individual glycemic and health status. Currently, the common goal of developing new antidiabetic medications is to minimize cardiovascular complications and confer more metabolic benefits beyond glycemic control. The current incretin-based therapy affects the ominous octet or

more defects in the pathophysiology of T2DM. Many preclinical studies and clinical trials have demonstrated the efficacies and safety of this therapy or its combination treatments. Notably, studies have shown that drug combinations are more effective in reducing hyperglycemia, which may be attributed to the enhancement of the level of action of GLP-1.

In summary, the following areas were discussed in the present review. Firstly, this review concluded that metformin is a secretagogue or release sensitizer rather than a potent DPP-Ⅳ inhibitor. The AMPK, bile acid, genes related to T2DM and GLP-1, microbiota, and the gut-brain -liver axis influence the production, release, and β cell response of GLP-1. Secondly, the signaling and targets of GLP-1 and Sulfonylurea on insulin release were compared and based on the distinct action on the common K^+ATP channels; multiple studies questioned the rationale or safety of the two-insulin stimulating drug combination. Thirdly, the results on the role of α-glucosidase inhibitor were compiled, the mechanisms of which majorly involves intestine, as an enhancer of the metabolic action of GLP-1. Next, the limited study results on Thiazolidinedione (TZDs) and its relation with GLP-1 were reviewed. The PPAR signaling and GLP-1 are mutually influenced and have similar effects on insulin release, β cell protection, cardiovascular benefits, and treatment of NAFLD, etc. Moreover, the mechanism of SGLT-2i as a novel insulin-independent medication were also discussed in the present review. The unique and versatile functions of this drug and its promotion of GLP-1 release or action were summarized. Also, the effects of the drug were compared with the action of GLP-1, including energy control and bodyweight reduction, CNS activities regulation, gut micro-environment. Finally, the impact of DPP- IV inhibition on GLP-1 and its essential role in immunity was discussed. Collectively, the new mechanisms of oral antidiabetic medication on the effect of GLP-1 are listed to reveal the recent progress and perspectives on the treatment, which can provide an understanding of new mechanisms and treatment options (Table 1). The findings of these studies provide the rationale for the development of new drugs or formulations, which can ensure better glucose metabolism.

Table 1. Summarization on the relationships between oral antidiabetic medications and GLP-1

OADs	Mechanisms on interactions with the GLP-1 effects	Related target molecules
Metformin	Majorly promote GLP-1 release and inhibits DPP-IV activity; Improves response to GLP-1 of pancreaticβ cell Interact with gut-brain-liver axis; Affect microbiota	AMPK, PPAR-α; Bile acids absorption repression; TCF7L2
Sulfonylurea	Enhance insulin secretion and may induce hypoglycemia while combination	Epac2A/Rap1 of K^+ATP channels
Glinides	Enhance insulin secretion, but short duration of action	Benzamido site on SUR1 of K^+ATP channels,
α-glucosidase inhibitor	Majorly promote GLP-1 release Endocrine modulation Affect microbiota	FGF21, IGF-Ⅰ, hepatic PPAR-α and Sirt1
TZDs	Promote GLP-1 release and inhibits DPP-IV activity Endocrine modulation Improves response to GLP-1 of pancreatic β cell	PPAR- γ, AMPK
SGLT-2i	Promote GLP-1 release, endocrine modulation, affect microbiota	SGLT-2
DPP-Ⅳ i	Inhibit GLP-1 degradation	DPP-IV

OADs: Oral antidiabetic drugs; TZDs: Thiazolidinedione;
PPAR-α: peroxisome proliferators-activated receptor; DPP-IV: Dipeptidyl peptidase-IV; AMPK: Adenosine 5'-monophosphate (AMP)-activated protein kinase;
Epac2A: The exchange protein directly activated by cAMP;
Rap1: Ras-associated protein 1; K^+ATP: ATP sensitive potassium channel;
SUR1: sulfonylurea receptor-1; SGLT-2i: sodium-glucose co-transporter-2 inhibitor

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 16
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An Overview of Schwannomas –ATypical Presentation and Challenges

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ABSTRACT

Introduction: Neurogenic tumors include schwanomma and neurofibromas. They occur often in the head and neck. However their occurrence on the vagus is uncommon. A high index of suspicion is needed to order imaging in pelvic lesions that present atypically.
Discussion: Schwanommas are hypointense on T1 and heterogeneously hyperintense on T2 on MRI. The histopathological appearance with Antoni type A and type B is typical of Schwanomma.
Conclusion: Schwanommas are slow growing benign tumors that are separable from the parent nerve. Recurrence is uncommon after resection. Schwannomas arising from vagus nerve cause bradycardia and the anesthetist must be vigilant during surgical excision. Pelvic schwannomas may present with constipation. We present our cases for their challenges and uncommon presentations.

Keywords: Schwannoma; vagus; presaccral; S-100.

1. INTRODUCTION

Schwannomas are neural sheath tumors. They are commonly present in the neck, extremities and retro-peritoneum. By immunohistochemistry, schwannomas typically show diffuse, strong expression of S100 protein [1] and abundant pericellular collagen type IV, consistent with the presence of a continuous pericellular basal lamina [2,3]. The lesion grows gradually and superficial lesions are usually small at the time of diagnosis, but in case of retroperitoneum large lesions present without any signs and symptoms.Although schwannomas are generally benign lesions, they are known to increase in size 2.5 to 3mm/year. Among schwannomas, vestibular type is the the common cranial nerve schwannoma followed by Facial and Trigeminal schwannomas and then the glossopharyngeal, vagus and spinal accessory nerve schwannomas. Intraosseous schwannomas (IOS) represent an exceedingly rare subset of schwannomas, accounting for < 1% of benign bone tumors [4,5].

2. CASE 1

A 71 years old male presented with slowly progressive swelling on the right side of the neck for 1 year,which was painless. Examination revealed a well circumscribed oval(6*4cm) swelling(firm in consistency),non pulsatile, located in the posterior triangle (Fig. 1). Ultra-sonogram of neck showed a complex well encapsulated predominantly solid mass,with no significant lymphadenopathy. CECT of the neck showed a large well defined thick walled hypodense lesion in the right posterior cervical space, in the region of right jugular chain(5 x4.6x7.5cm) with ill-defined relative hypodensity.It superiorly extended upto C2 vetrebra, inferiorly up to end plate of C7,laterally displacing Rt sternocleidomastoid, medially abutting the vascular chain (Fig. 2). USG guided FNAC showed spindle like cells arranged in a cellular and loose areas suggestive of nerve sheath tumor. Pre-operatively the vocal cords were mobile. Intra- operatively a firm/cystic tumor was identified arising from the vagus, posterior to the IJV and displacing the carotids medially (Fig. 3a). Intraoperatively the patient had multiple episodes of bradycardia during dissection of the tumor. The anesthetist was intimated

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and bradycardia was managed by intra venous doses of atropine. The tumor was mobilized and resection was done in toto and the specimen was sent for biopsy.

Fig. 1. Swelling in the posterior triangle of neck

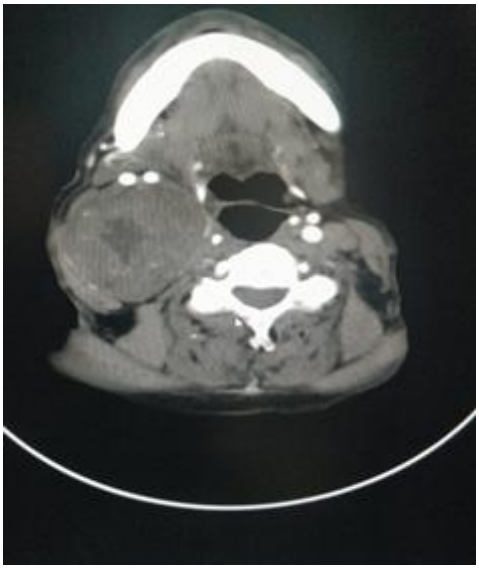

Fig. 2. CECT showing the mass displacing the carotid

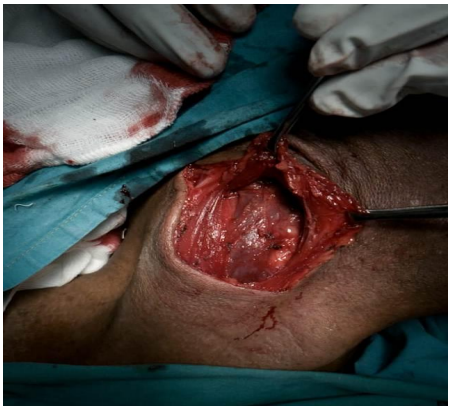

Fig. 3a. IJV splayed over the cystic lesion (Intraoperative)

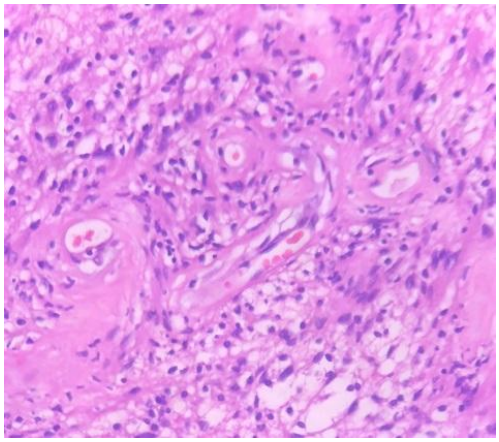

Fig. 3b. Histopathology showing schwannoma(10x resolution)

HPE: Histopathology showed cellular areas composed of spindle cells and Verocay bodies. Hyperellular area showed spindle cells in myxoid areas and congested blood vessels were seen.IHC was positive for S 100 and less than 3% for ki67 (a proliferative index marker) (Fig. 3b).

3. CASE 2

A 80 year old gentleman came with complaints of pain in the right thigh and leg for 2 months. He had history of difficulty in defecation and per rectal examination showed a extra-mucosal mass. A screening ultrasound showed a 8x7x 8cm hypoechoic lesion in the presacral region and CECT showed a large well defined heterogeneously enhancing lesion in the pre-sacral region with preserved fat plane with the adjacent structures.The lesion extended to the anterior sacral foramen and was suggestive of a neurogenic benign lesion. MRI showed a heterogeneous pre sacral lesion ,with internal hypo intense focus- (likely cystic) in the pre-sacral(S1-S4) with postero-inferior part in close contact with exiting nerve root (Fig. 4). At laparotomy a 10x7x7 cm mass was found in the retro-peritoneum. The retro-peritoneum was entered and the vessels and ureters were identified. The mass was arising from the nerves of hypogastric plexus. The tumor was excised (Fig. 5b), the troublesome pre-sacral ooze was controlled and the specimen sent for HPE.

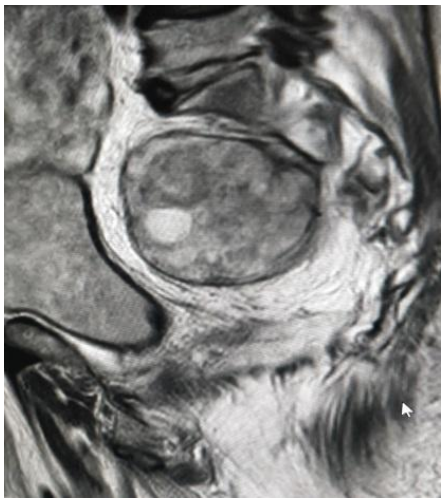

Fig. 4. MRI showing presaccral mass

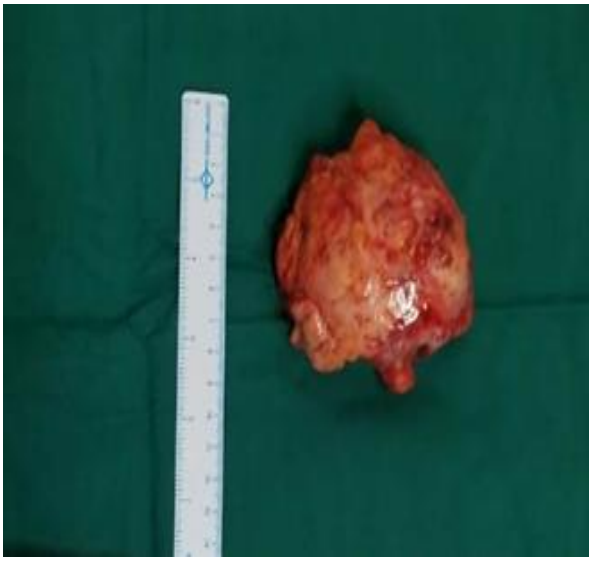

Fig. 5a. Excised presaccral mass

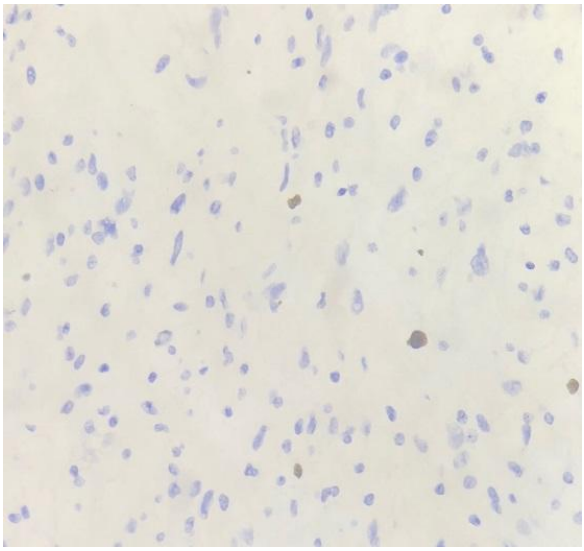

Fig. 5b. Slide shows IHC

Biopsy showed hypo-cellular areas with spindle cells having wavy nuclei and scanty eosinophilic cytoplasm. Hypercellular areas showed spindle cell with wavy nuclei with formation of Verocay bodies. Areas of focal foreign body giant cell reaction, foamy cells and dilated congested vessels were seen. Few vessels show myxoid change in the wall-consistent with schwannoma with secondary changes.IHC was positive for S-100 (Fig. 5b).

Both patients were free of symptoms at the 2 month follow up and showed no residue/recurrence at imaging.Consent was taken from the patients preoperatively for use for research purposes.

4. DISCUSSION

Schwannoma is generally defined as benign tumor of neural cells derived from nerve sheath composed of schwann cells [6]. Common sites include the head and neck, the flexor surfaces of the

extremities, and the para vertebral area of retro-peritoneum. The nerve of origin is not often made until the time of surgery. Schwannoma, originating from the vagus nerve(cervical), is very rare to occur in men between the 3rd and 6th decades of life. The most common presentation is a painless, slow-growing, lateral neck mass this appears in a large proportion of cases [7]. Rapidly growing tumors with either evidence of invasion or presenting with complete loss of nerve function should be treated by complete excision of the tumor.

About 25-33% of all the extracranial schwannomas occur in the head and neck. Chandramohan has described a case of vagal schwannoma,in which tumor was excised by intra capsular dissection thus sparing left vagus nerve.[8]Saini et al has reported intra operative bradycardia during dissection of the mass from its adjacent structure [9]. Samarakoon et al. had described a 46-year-old man who was evaluated for chronic constipation due to a giant pre-sacral schwannoma (diagnosed on magnetic resonance imaging scan).Pre-operatively they came to diagnosis of schwannoma by trans-rectal ultrasound scan (TRUS)-guided biopsy and further proceeded with excision of mass [10]. A 32-year-old man with a painless right neck mass presented with dysphagia for six months. MRI is the gold standard to assess the origin and the extent of the tumor [11].

Histologcally the cut surface appears solid, smooth, glistening and gray-white. It may show cystic and hemorrhagic areas with calcification. Histo-morphology of schwannoma characteristically shows two alternating patterns—Antoni A and Antoni B areas. Antoni A are cellular areas with compactly arranged spindle cells frequently arranged in interlacing fascicles, palisades, or in an organoid arrangement, with two compact parallel rows of well-aligned nuclei forming eosinophilic structures (Verocay bodies). Antoni B exhibits hypocellular areas consisting of a few tumor cells in loose myxomatous matrix.[12]

S100 is a marker for neural tumors and is extensively studied in schwannomas [13]. In a study Fausto J. Rodriguez has mentioned all neurogenic tumors are always strongly GFAPpositive, suggesting cross reactivity of cytokeratin antibodies with GFAP, rather than true protein expression. By immunohistochemistry, schwannomas typically show diffuse, strong expression of S100 protein and abundant peri-cellular collagen type IV, consistent with the presence of a continuous peri-cellular basal lamina [14]. Glial fibrillary acid protein (GFAP) is expressed in a subset of schwannomas. Recent markers frequently positive in schwannomas include, calretinin, SOX10 and podoplanin.

In a study conducted in Kyushu University in Japan all cervical tumors were resected through a trans-cervical approach. The nerve of origin was mainly determined by the postoperative neurological findings Complete tumor resection was performed in 11 patients, and intra-capsular enucleation of the tumor was performed in 16 patients. Intra-capsular enucleation was an effective and feasible method for preserving the neurological functions [15].

A Makni has said various techniques for excision of pre-sacral mass biopsy should be done before deciding either to start on neo-adjuvant treatment or excision [16].

5. CONCLUSION

As slow growing tumors of the head and neck, schwannomas very rarely presents as potentially morbid lesions. Although it is rare, clinicians should not forget the possibility of a nerve sheath tumor in presence of a neck mass. The preoperative bedside diagnosis is dependent on clinical suspicion and imaging modalities. Complete resection of the tumor is the treatment of choice for all benign schwannomas.

Schwannomas arising from vagus nerve cause bradycardia and the anesthetist must be vigilant during surgical excision. Pelvic schwannomas may present with constipation. We present our cases for their challenges and uncommon presentations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Biography of author(s)

Dr. R. Vijai
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He is a general and laparoscopic surgeon practicing actively in the southern state of India. He has published a number of articles in various journals, some of which are 1) A case of cavernous hemangioma of submandibular Salivary gland in adults (IOSR - 2019), 2) A diagnostic surprise of right iliac fossa mass (JCDR - 2017), 3) Tuberculous lymphadenitis and papillary thyroid cancer (IOSR - 2019), 4) Poland syndrome - a rare case report (ISJ - 2017), 5) An interesting case of anorectal foreign body (IOSR - 2018). He has also done a research study on "early enteral nutrition versus nil per oral in cases of pancreatitis" and a case series on "Pseudocyst of pancreas". He is also a member of the Association of Surgeons of India (ASI), Association of Minimal Access Surgeons of India (AMASI) and Indian Association of Gastrointestinal Endo Surgeons (IAGES). He has also been awarded in presenting "a case of perforated GIST" at ASI National conference 2017. His field of interest is in urology focusing on robotic surgery and it's evolution.

DISCLAIMER
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