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Mussel RL, De Sa Silva E, Costa AM, Mandarim-De-Lacerda CA (2003). Mast cells in tissue response to dentistry materials: an adhesive resin, a calcium hydroxide and a glass ionomer cement. J. Cell. Mol. Med. 7:171-178.

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Stanislawski L, Lefeuvre M, Bourd K, Soheili-Majd E, Goldberg M, Perianin A (2003). TEGDMA-induced toxicity in human fibroblasts is associated with early and drastic glutathione depletion with subsequent production of oxygen reactive species. J. Biomed. Res. 66:476-82.

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Case Report

Severe benzodiazepine withdrawal syndrome followed by anesthesia in elderly patient: A rare case report

Jang-Chun Lin[1], Hsin-I Ma[2] and Wei-Hsiu Liu[2]*

[1]Department of Radiation Oncology, Tri-Service General Hospital National Defense Medical Center, Taipei, Taiwan, Republic of China.
[2]Department of Neurological Surgery, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, Republic of China.

Benzodiazepine dependence is a potentially clinically serious condition and its withdrawal syndrome is complex and often protracted in time course. However, the relationship between anesthesia and benzodiazepine withdrawal syndrome is unclear. Here, we presented a 79 year-old-female who had the history of major depression and had taken hypnotic benzodiazepines for five years. The patient received total hip replacement under spinal anesthesia and during the operation, the hypnotic benzodiazepines were discontinued. One day later, the patient was suddenly dropped to coma. Brain magnetic resonance (MR) imaging demonstrated no significant finding except to brain-tissue loss over the bilateral anterior frontal lobes. Accordingly, tracheostomy was performed and ventilator dependent. Interestingly, forty days later, the patient's consciousness became alert. To increase physicians' awareness that old age, anesthesia, and cortical dysfunction may exacerbate benzodiazepine withdrawal syndrome, we presented our experience in treating one patient who rapidly stopped the hypnotic benzodiazepines.

Key words: Benzodiazepine withdrawal syndrome, anesthesia.

INTRODUCTION

Benzodiazepine withdrawal symptoms are a normal response in individuals who have chronically used benzodiazepines, and a side effect and result of drug tolerance. Symptoms typically emerge when dosage of the drug is reduced. The symptoms include insomnia, headache, nausea and vomiting, and nightmares. However, an abrupt or over-rapid discontinuation of benzodiazepines may result in a more serious and very unpleasant withdrawal syndrome that may additionally result in convulsions, catatonia, suicide, coma, hyperthermia, and delusions. Here, we reported 79 year-old-female who had the history of major depression and had taken hypnotic benzodiazepines for five years. The patient received total hip replacement under spinal

anesthesia and during the operation, the hypnotic benzodiazepines were discontinued. One day later, very serious withdrawal syndrome happened, the patient suddenly dropped to coma.

CASE REPORT

A 79 year-old-female had the history of major depression and had taken hypnotic benzodiazepines for five years. However, the patient was sustained severely, left hip pain and diagnosis was fracture of femoral neck, left hip with displacement. The patient received total hip replacement under spinal anesthesia and during the operation, the hypnotic benzodiazepines were stopping. Unfortunately, one day after the operation, the patient was suddenly dropped to coma. The laboratory examinations revealed no remarkable contribution. On neurological examination it show deep coma (Glasgow coma scale equal E1M1Vt), absent of bilateral babinski's signs, and decreased deep tendon reflex. Brain computed topography (CT) revealed no significant finding except to brain-tissue loss in the bilateral anterior frontal lobes. Brain magnetic resonance (MR) imaging demonstrated brain-tissue loss over the bilateral anterior

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*Corresponding author. E-mail: liubear0812bear@yahoo.com.tw. Tel: +886-2-8792-7177. Fax: +886-2-8792-7178.

frontal lobes and a recent lacunar infarction in the right centrum semiovale. Electroencephalogram (EEG) revealed cortical dysfunction. The psychologists suggest hypnotic benzodiazepines and flumazenil without improvement. However, forty days later, the patient's consciousness became alert (Glasgow coma scale equal E4M6Vt). Now, the patient regularly received rehabilitation, and can walk by herself.

DISCUSSION

Long-term use of hypnotic benzodiazepines is higher in older adults than younger people throughout North America, Australia and Europe (Barbui et al., 1998). It is estimated that about 15% of over 65-year-olds adults regularly take sleeping pills and, in the United Kingdom, older adults received 80% of all the prescriptions written for hypnotic benzodiazepines (Jorm et al., 2000). Hypnotics are commonly given to over 65-year-olds adults in repeat prescriptions, although benzodiazepines are not nowadays recommended for long-term used. Ticehurst (1995) concluded that after tobacco and alcohol, benzodiazepine consumption is associated with the greatest risk of abuse and dependence in the elderly. This reflects a substantial decrease in benzodiazepine anxiolytics but relatively change a little in benzodiazepine hypnotics especially among old patients (Rumble and Morgan, 1994), although there has been a reduction in scripts for benzodiazepines since the mid-eights. Holden et al., (1994) revealed patients over 65 are significantly less likely to stop benzodiazepine than younger patients by a large-scale audit of benzodiazepine prescribing and withdrawal in general practice. Moreover, it may be higher incidence in elderly patients than younger patients, but it need more research to demonstrate the hypothesis.

Some of the benzodiazepine withdrawal symptoms are identical to the symptoms for which the medication was originally prescribed. It is very important to determine the difference between relapse and rebound during the withdrawal phase and can often lead to a misdiagnosis. Therefore, many experts suggest that after withdrawal from long term or even fairly short term use of benzodiazepine drugs, at least six months should have elapsed prior to re-evaluating the symptoms and updating a diagnosis. GABA receptors are the most common receptor system in the central nervous system and use of benzodiazepines has a profound effect on almost every aspect of brain and body function, either directly or indirectly. Benzodiazepines cause a decrease in norepinephrine, serotonin, acetylcholine and dopamine. These neurotransmitters are needed for coordination, normal memory, emotional responses, mood, muscle tone, endocrine gland secretions, heart rate and blood pressure control. With chronic benzodiazepine use, tolerance develops rapidly to most of its effects, so that

when benzodiazepines are withdrawn, various neuro-transmitter systems go into overdrive due to the lack of inhibitory GABA-ergic activity. Withdrawal symptoms then emerge as a result, and persist until the nervous system physically reverses the adaptions (physical dependence) which have occurred in the central nervous system. Therefore, the cortical dysfunction may exacerbate the benzodiazepine withdrawal symptom, but it need more study to prove the hypothesis.

Benzodiazepine withdrawal symptoms include insomnia, headache, nausea and vomiting, and nightmares. However, an abrupt or over-rapid discontinuation of benzodiazepines may result in a more serious and very unpleasant withdrawal syndrome that may additionally result in coma, convulsions, catatonia, suicide, hyperthermia, and delusions.

Conclusion

The severe syndrome of benzodiazepine withdrawal syndrome may be coma. However, to the best of our knowledge the relationship between anesthesia and benzodiazepine withdrawal syndrome is unclear. In our patient, over-rapid discontinuation of benzodiazepines during anesthesia is followed by benzodiazepine withdrawal syndrome. Moreover, old age, and cortical dysfunction may exacerbate the symptom, but more study should be performed to prove the hypothesis. To increase physicians' awareness small dosage of hypnotic benzodiazepines should be maintained if elderly patient with cortical dysfunction who was chronic to hypnotic benzodiazepine use will received anesthesia.

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Full Length Research Paper

Oculocutaneous albinism and skin cancer in Calabar, Southern Nigeria

M. E. Asuquo[1]*, O. O. Otei[1], I. Bassey[2] and G. Ebughe[2]

[1]Department of Surgery, University of Calabar Teaching Hospital, Calabar, Nigeria.
[2]Department of Pathology, University of Calabar Teaching Hospital, Calabar, Nigeria.

Oculocutaneous albinism (OCA) is a congenital condition causing hypo pigmentation of the hair, skin, and eyes. Albinism and solar radiation are established risk factors for skin cancer, the commonest being squamous cell carcinoma (SCC) of the head and neck in black Africans. We present four OCA patients with histologic diagnosis of skin cancer presented to the University of Calabar Teaching Hospital from January, 2007 and December, 2008. These cases were compared with SCC and total skin malignancy seen during the same period. Forty-five patients presented skin malignancy during the study period. Four OCA patients (2 men and 2 women) with SCC accounted for 21.1% of SCC and 8.9% of skin malignancy. They ranged in age from 21 to 30 years (mean 25 years). All the lesions were on the head and neck. The tumours were excised with a recurrence in a man who also had adjuvant chemotherapy with poor response. During the evaluation period of the 7 and 12th months, the sites appeared healed with no evidence of recurrence in 3 patients. Albinism and exposure to solar radiation were identified as risk factors for skin cancer. Establishment of "albino support group" to finance early implementation of public education strategies on prevention and treatment is recommended.

Key words: Albinism, squamous cell carcinoma (SCC), skin cancer.

INTRODUCTION

Oculocutaneous albinism (OCA) is a congenital condition causing hypo pigmentation of the hair, skin, and eyes (Lund, 2005). OCA 2, the form of albinism found in Africa, is inherited, as an autosomal recessive disorder; OCA tyrosinase, the key enzyme for synthesis of melanin is present and functional. The defect in OCA 2 is in the P gene coding for a membrane protein of the melanosome leading to malfunction of melanin synthesis (Biswas and Lloyd, 1999). Non-melanoma skin cancers, basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are common among the susceptible group with albinism (Okulicz et al., 2003). SCC of the head and neck are the most common cutaneous tumours in African patients with albinism (Yakubu and Mabogunje, 1993). OCA is a stable condition with no cure and requiring life long management. Dealing effectively with the health and social issues surrounding albinos in low-income countries with limited resources is a challenge (Lund and Taylor, 2008). We

present four OCA patients to highlight SCC as the commonest skin cancer in albinos and the role of solar radiation as a risk factor in our setting.

PATIENTS AND METHODS

Clinical histories and treatment outcomes of four consecutive OCA patients seen at the University of Calabar Teaching Hospital, Calabar, during a 2-year period from January, 2007 and December, 2008 with histologic diagnosis of skin cancer were prospectively studied. The incidence was compared to total skin cancer during the same period. In total, 1978 patients were seen in the surgical out patient during the same period.

RESULTS

Forty-five skin malignancies were presented during the study period. SCC ranked first with 19 patients (42.2%), followed by Kaposi sarcoma (KS) with 15 patients (33.3%). The four albinos all with SCC accounted for 8.9% of skin malignancies and 21.1% of SCC. The ages of the four albinos: two men and 2 women ranged from

*Corresponding author. E-mail: mauefas@yahoo.com.

Figure 1. (A) Squamous cell carcinoma, (B) squamous cell carcinoma (same patient).

Table 1. Clinicopathological features and outcome of four albinos with SCC.

Patients No.	Age (years)	Gender	Clinical presentation (site)	Histology	Treatment	Outcome/ Follow-up
1	30	M	Chronic ulcer (Neck) 7 months	SCC moderate differential	Excision skin grafting, adjuvant chemotherapy, referred for radiotherapy	Recurrence 2 months, lost to follow-up
2	23	M	Chronic ulcer (Face) 7 months	SCC well differentiated	Excision	Healed 12 months
3	26	F	Chronic ulcer (Fore head) 15 months	SCC well differentiated	Excision skin grafting	Healed 9 months
4	21	F	Multiple lesions, 2 fleshy masses (Scalp), Chronic ulcer (Neck) 24 months	SCC well differentiated	Excision	Healed 7 months

21 to 30 years (mean 25 years). The ages of the SCC patients with Marjolin's ulcer ranged from 32 to 70 years (mean 48.3 years), while that of the non Marjolin's, non albinos ranged from 30 to 70 years (mean 56.6 years). The mean age of the 45 patients with skin malignancy was 46.6 years and their ages ranged from 5 to 75 years. The lesions in the albinos were located on the head and neck (Figure 1 and Table 1). The commonest site for SCC was the limbs (37%), the head, and neck ranked second (32%), while the third was external genitalia/ perineum (26%). All the patients (15 blacks, 4 albinos) were human immunodeficiency virus (HIV) sero negative (Table 2).

All the patients had excision. The outcome was satisfactory in three patients (a man and two women) with the sites healed and no evidence of recurrence. However, there was recurrence in a male, noticed 2 months post excision that persisted following adjuvant chemotherapy (cisplatin and methotrexate). He was lost to follow up after referral for radiotherapy. The histological grading of the lesion of the patient with a recurrence was moderate differentiation, while the others with satisfactory outcome were well differentiated (Table 2).

Table 2. Site distribution of SCC (2007 – 2008).

Site		Total (%)	Remark
Head/Neck		6 (32)	2 blacks and 4 albinos
Chest		-	-
Abdomen		-	-
Limbs			
Upper	1	7 (37)	All blacks
Lower	6		
External			
Genitalia/vulva	3	5 (26)	All blacks
Peritoneum	2		
Anus		1 (5)	Black
Total		19 (100)	15 Blacks and 4 albinos

DISCUSSION

Forty-five patients with skin cancer were observed during the study period (January 2007 and December 2008): 19 of the patients had SCC (42.2%) ranking first, while

Kaposi sarcoma (33.3%) ranked second. The four OCA patients accounted for 8.9% of skin malignancies and 21.1% of SCC. Reports from Datubo-Brown (1991) in Port Harcourt (Southern Nigeria) showed that albinism accounted for 16.7% of skin cancer, Yakubu and Mabogunje (1995) in Zaria (Northern Nigeria) reported 8%, while Kromberg et al. (1989) in South Africa reported 23.4%. These reports highlight albinism as a risk factor for skin cancer. The ratio of African albinos in the general population in out setting has not been reported: however, 1978 patients were seen in the surgical clinics during the period of study.

The mean age for presentation of skin cancer in OCA patients was 25 years (3rd decade), they were presented 2 decades earlier than black patients did with skin malignancy (5th decade). Black patients with Marjolin's ulcer in the SCC subset were presented in the 5th decade, while the non-Marjolin's subset of SCC excluding the albinos was presented a decade later (6th decade). In Tanzania, examining 164 patients with albinism, skin cancer were found in 25% of those with age over 20 years (Lookingbill et al., 1995). Protection from the sun must start at birth and continue throughout life as those who present with skin cancer run a risk of developing another skin cancer later in life (Lund and Taylor, 2008; Asuquo et al., 2008). OCA is a stable condition with no cure requiring life-long management; this is a challenge in a low income setting with limited resources as ours.

The lesions in the four patients were SCC located on the head and neck. The presence of the tumour in these sites highlights the role of solar radiation as a risk factor for skin cancer in albinos. Lund and Taylor (2008) in South Africa, Yakubu and Mabogunje (1993) in Northern Nigeria, Luande et al. (1985) in Tanzania, and Kromberg et al. (1989) in South Africa reported the head and neck as the most commonly affected site. This is in contrast to Caucasians in whom BCC is the most frequent skin cancer of the head and neck with solar radiation as risk factor. Perhaps other non-pigment related factors in African render them prone to SCC (Urbach, 1991; Ochicha et al., 2004). However, the commonest site of SCC in the black patients was the lower limb, followed by the external genitalia/perineum (Table 2) highlighting the role of non-solar risk factors. In white populations, SCC is the second most common cancer after BCC, affecting the head and neck with solar radiation as the risk (Gross and Monroe, 2006).

There was a recurrence in a patient whose histological diagnosis was SCC (moderate differentiation), the outcome in the other 3 patients with satisfactory outcome was SCC (well differentiated). A further study on this is required as this may predict outcome in terms of recurrence and nodal metastasis.

Albinism and environmental exposure to solar radiation were identified as risk factors for skin cancer. OCA patients presented skin malignancy 2 decades earlier than blacks. Establishment of "albino support group" to finance early implementation of public education strategies on prevention and treatment is recommended.

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Full Length Research Paper

Lactational amenorrhoea method of contraception: An in-depth study of awareness, knowledge and practice by breast feeding mothers with unintended pregnancies

Chris E. Ekpenyong[1]*, Nyebuk E. Daniel[1], Anthony Fidelis Uwah[2], Ette Okon Ettebong[3] and John O. Ibu[4]

[1]Department of Physiology, College of Health Science, University of Uyo, Akwalbom State, Nigeria.
[2]Department of Biochemistry, Faculty of Basic Medical Sciences, University of Uyo, Uyo, Akwalbom State, Nigeria.
[3]Department of Pharmacology and Toxicology, University of Uyo, Akwalbom State, Nigeria.
[4]Department of Physiology, College of Health Sciences, Benue State University, Benue State, Nigeria.

This study assessed the awareness, knowledge and practice of lactational amenorrhoea method (LAM) of post partum contraception among breast feeding mothers of 18 to 40 years of age with unintended pregnancies. It was a cross-sectional, multicenter survey of seventy one breast feeding mothers with unintended pregnancies who visited these health centers for antenatal care within the study period. These women had been on LAM as the only post partum contraception before onset of pregnancy. A two section, semi-structured, self administered questionnaires were used to survey the participants. The incidence of unintended pregnancy was 14.3% among LAM users. There was a high level of awareness, but poor knowledge and practice among users. Lactating pregnant women who properly implemented only one component had more than 4 times odds for unintended pregnancy (odd ratio (OR)=4.12, confidence interval (CI)=3.21 to 4.72, P=0.04), those who implemented two components had about 3 times odds for unintended pregnancy (OR=2.82, 95%CI=2.54 to 2.94, P=0.01), while those who did not implement any of the three components had more than 7 times odds for unintended pregnancy (OR=7.52, 95%CI=7.23 to 7.94, P=0.001). High incidence of unintended pregnancy observed in this study was associated with poor knowledge and aberrant-practice of LAM by breast feeding mothers. Therefore, improving women knowledge (especially breast feeding mothers) on the proper guidelines of LAM could be of a great help in reducing high incidence of unintended pregnancy among LAM users.

Key words: Knowledge, practice, lactational amenorrhoea method (LAM), unintended pregnancy.

INTRODUCTION

Unintended pregnancy is currently one of the greatest challenges faced by women of reproductive age in most developing countries of the world (Singh et al., 2010). It has become a public health concern in some countries like Nigeria, because its effects are not limited to women, but also the families and the society. It has negative economic, educational and social consequences for both the family and the nation (Dixit, 2012).

Although, family planning and reproductive health programs have contributed immensely to a global decline in the incidence of unintended pregnancy; however, the rates are still very high in some developing countries (Leete et al., 1999). This could be attributed to none/ inappropriate use or failure of the birth control methods of choice (Huang et al., 2012). Epidemiological studies suggest that post partum women are among the most highly vulnerable to unintended pregnancy (Huang et al., 2012),

*Corresponding author. E-mail: chrisvon200@yahoo.com. Tel: 08023347719.

probably because many of them worldwide lack access to, or do not wish to use hormonal contraceptive methods for fear of transmission of exogenous hormones to the infant.

Attempts to provide fertility awareness-based method of family planning to breastfeeding women led to the development of lactational amenorrhoea method (LAM) of post partum contraception, which is the practice of using breastfeeding to prevent pregnancy. For this method to be effective, the following three basic criteria must be met: using the method within the first six months post delivery; when menstruation has not returned; and the mother is exclusively breastfeeding (that is, the infant is breastfed frequently throughout the day and night; every 4 h during the day and every 6 h during the night, with no other food or beverage). For a breastfeeding mother to be eligible to use LAM, she must satisfy the three aforementioned criteria (Labbok et al., 1994).

There is a growing amount of evidence that, when used perfectly (correct and consistent), LAM provides 98 to 99% contraceptive effectiveness, while typical use (incorrect and inconsistent) provides 95% effectiveness (Kazi et al., 1995; Trussell, 2004). This means that 5 of 100 women who used LAM typically may become pregnant unintended within the first six months post-partum, whereas less than 1 to 2 may become pregnant within the same period with perfect use. A higher rate of unintended pregnancy is found among defaulters who are unable to adhere strictly to the tenets for the proper use of LAM as approved by World Health Organization (WHO) and entrenched in the "Bellagio Consensus" in Italy in 1995 (WHO, 2004). While very few women may choose to carry the unintended pregnancies to term, a greater number of them may opt for abortion to avoid risk of their lives and health, psychological trauma and socio-economic turmoil. Of the estimated 210 million pregnancies that occur throughout the world each year, about 38% are unintended, 22% of unintended pregnancies end in abortion (Amin et al., 2009). Worse still where there is lack of access to safe abortion, the women's health is endangered especially if they resort to unsafe methods to terminate their pregnancies. It is stated that 19 million of the estimated 46 million induced abortions each year are performed in unsafe conditions and/or by unskilled providers and result in the death of an estimated 68,000 women. This represents 13% of all pregnancies related death (The Alan Guttmacher Institute (AGI), 1991). Almost all unsafe abortion takes place in developing countries, and is where 99% of abortion related deaths occur (US CDC, 2003; WHO Report, 2004).

Previous studies have shown that the problem of unsafe abortion is more in Sub-Saharan Africa due to limited uptake of family planning, a shift towards the use of traditional contraceptive methods instead of modern methods, decreased effective use of contraception (Fatima et al., 2005), highly restrictive abortion laws and poor access to safe abortion services. In Nigeria, unintended pregnancy and unsafe abortion are critical public health problems (Onche, 2011). An estimated one in five pregnancies in Nigeria is unintended, because of low knowledge and practice of contraception, desire for smaller families, growing urbanization, increase participation of women in paid labour force and diminish ability of families to support many children (Akinrinola et al., 2006). For those women who may want to maintain the unintended pregnancy, they may face a lot of adverse social and economic impact on the family. The women's hope for better life for herself, education and prosperity for her family may be marred by unintended pregnancy (Etuk and Ekanem, 2003). Increased incidence of family disharmony is common, increased incidence of child abandonment (child neglect), battered baby syndrome, juvenile delinquency and increased number of street children are also associated with unintended pregnancies (Etuk and Ekanem, 2003).

Special attention should therefore be focused on breastfeeding mothers who still want to fulfill their marital obligations to their spouses but are ignorant of an ideal contraceptive method during lactation. The fear of effect of the hormonal contraceptives on the breastfeeding baby left them with no choice than to choose LAM which is natural, readily available, cheap and the easiest. For this method to be effective, the three basic criteria must be strictly adhered to.

This study was therefore carried out as one of the initiatives to reduce the incidence of unintended pregnancy among women practicing LAM in Uyo Metropolis, South-South Nigeria. The aim was to establish the knowledge and practice of LAM by breastfeeding mothers with unintended pregnancies due to LAM or users' failure. We hope that the outcome of this study will help unfold the required interventions necessary to improve the quality of LAM services and reduce complications associated with unintended pregnancies, unsafe abortion in our community and similar communities in other parts of the world.

SUBJECTS AND METHODS

Selection of participants

This was a cross-sectional multi-center survey conducted in two secondary and eight primary health care facilities within Uyo Metropolis in South-South Nigeria. Of the 3720 pregnant women (aged 18 to 40 years) who visited these health facilities for ante-natal care within the study period (between April 2010 and June 2011), 497 (14.6%) had unintended pregnancy. Pregnancies were grouped as either intended or unintended at conception using new definitions established by the Institute of Medicine in 1995. Pregnancies were considered intended if a woman had stopped using birth control measures, because she wanted to become pregnant. Unintended pregnancies were classified as either mistimed: wanted pregnancies that occurred sooner than desired or unwanted pregnancies that occurred while a woman was using contraception and had not ever wanted to have another baby. Seventy

Table 1. Distribution of breastfeeding mothers (n= 3,720).

Participants	n	%
Those initially excluded	305	8.2
Actual participants	3415	91.8
Intended pregnancy (Excluded)	2918	85.4
Those with unintended pregnancy	497	14.6
Unintended pregnancy not on family planning (Excluded)	340	68.4
Unintended pregnancy on LAM (Included)	71	14.3
Methods other than LAM (Excluded)	76	15.3
Intention to terminate the pregnancy	10	2.0

one (14.3%) of them claimed they were on LAM, and were included in the study. Those who practiced other forms of post partum contraception were illegible to participate. Written informed consent was obtained from each participant and the study protocol was approved by the institutional research and ethics committee.

Assessment measures

A two section semi-structured questionnaire developed by the authors based on prior studies on the practice of LAM (Khadiga et al., 1996; Hight-Laukaram et al., 1996; Brogen et al., 2006) was used to survey the participants. The first part of the questionnaire contained 10 open ended questions aimed at obtaining information on the socio-demographic characteristics of the participants such as age (years), education level, employment status, type of family, religion, ethnicity, marital status, parity and area of residence.

Based on age, participants were classified into four groups: they were also classified into low and high education levels to represent participants with up to high school and more than high school education, respectively. Also, based on employment status, family type, religion, ethnicity and marital status, were classified into employed and unemployed, extended and nuclear family, Christians and non-Christians, Ibibios and non-Ibibios and married and singles, respectively.

The second part of the questionnaire contained 17 questions aimed at obtaining information regarding respondents' level of awareness, knowledge and practice of LAM. To assess their level of awareness, open ended questions were asked. These included questions such as: are you aware of the fact that exclusive breast feeding could be used to prevent pregnancy? How did you learn about it and how would you grade your acceptance of the practice?

To assess participants' knowledge, question were asked to assess their understanding of the meaning of LAM, the basic criteria for the effective and full implementation of LAM such as, when it should be commenced and ended, its effectiveness after weaning, provision of protection against sexual transmitted infections, effects on infants' and mothers' health and also the benefits and adverse health outcomes when practiced properly. To assess respondents' practice of LAM, questions were asked to find out the components of LAM actually implemented by them. On this basis, respondents were classified into four groups: those who could not implement any of the three basic criteria, those who implemented only one, two or all three components. For the purpose of this survey, lactational amenorrhoea post partum contraception is defined as the practice of using breast feeding to prevent pregnancy during post partum period. The three basic criteria for its full implementation include the following: exclusive breast feeding, post partum amenorrhoea and less than six months since child birth.

Respondents who observed only one, two or all components were regarded as having implemented one, two or all components, respectively.

Statistical analysis

Frequencies and simple percentages were computed for categorical variables. Chi-square test was used to compare the number of women with unintended pregnancy with each level of the demographic variables as well as the number of breastfeeding women with unintended pregnancy with correct and incorrect responses on the knowledge of LAM. Moreover, the number of components of LAM properly implemented by these women was compared statistically using chi-square test. Finally, logistic regression between cases of unintended pregnancies and implementation of LAM guidelines were performed. Odds ratio (OR) and its corresponding 95% confidence interval (CI) were estimated. All statistical computations were enhanced using Statistical Package for Social Sciences (SPSS) 17.0. A probability value less than 0.05 (P<0.05) was considered to be statistically significant.

RESULTS

Of the three thousand seven hundred and twenty (3,720) pregnant women recruited for the study, 305 (8.2%) were initially excluded for failure to comply with research guidelines. This made actual participants to be 3,415 (91.8%). Four hundred and ninety-seven (14.6%) of the participants had unintended pregnancies, 71 (14.3%) of the 497 pregnant women who had unintended pregnancies were on LAM and hence constituted the study participants; all others were excluded from the study. Detailed results are shown in Table 1.

In addition, 3 (4.2%) of those who were on LAM were between ages of 18 and 24, 23 (32.4%) were between the ages of 25 and 30, 36 (50.7%) were between the ages of 31 and 35 and 9 (12.7%) were between the ages of 36 and 40 years. Most of the women had high education (66.2%), employed (71.8%), from nuclear family (62.0%), Christians (95.8%), were of Ibibio ethnicity (50.7%) and resides in the urban (60.6%) and multiparous 56 (78.9). Results are shown in Table 2.

Most participants accepted the method at first contact with the medical staff that happens to be the first source

Table 2. Demographic characteristics of breast feeding mothers on LAM, n=71.

Demographic characteristic	n (%)
Age	
18-24	3 (4.2)
25-30	23 (32.4)
31-35	36 (50.7)
36-40	9 (12.7)
Education	
High education	47 (66.2)
Low education	24 (33.8)
Employment	
Unemployed	20 (28.2)
Employed	51 (71.8)
Type of family	
Extended	27 (38.0)
Nuclear	44 (62.0)
Religion	
Christians	68 (95.8)
Muslim	3 (4.2)
Ethnicity	
Ibibios	35 (49.3)
Non-Ibibios	36 (50.7)
Area of residence	
Rural	28 (39.4)
Urban	43 (60.6)
Marital status	
Married	55 (77.5)
Single	7 (9.9)
Devoiced	9 (12.7)
Parity	
1	15 (21.1)
2-4	21 (29.6)
>7	35 (49.3)

that help maximize the contraceptive effect of LAM. Appropriate take off time and the duration for optimum contraceptive effect were known by 45.1 and 51.8%, respectively. Also, 54.6% knew that the effectiveness of LAM decline after weaning. Good knowledge of the absence of adverse health effect on the mother, baby and absence of protection against sexually transmitted infections (STIs) for users was shown by 39, 50.7 and 78.9% respondents, respectively. However, the benefits and adverse effect of LAM were known by 31 (43.7%) and 16 (22.5%), respectively. Results are shown in Table 5.

Analysis of the components of LAM properly implemented by respondents showed that 8 (11.3%) implemented exclusive breast feeding only, 13 (18.3%) were amenorrhiec, 10 (14.1%) were less than 6 months post partum. Moreover, 9.8% implemented exclusive breastfeeding and were amenorrhiec, whereas 16.9% implemented exclusive breast feeding and were less than 6 months post partum. 12.7% were amenorrhiec and less than 6 months post partum, 10 (14.4%) implemented all the components. The remaining 2 (2.8%) implemented none of the three components. Results of analysis also revealed that the number of pregnant women who implemented these three components were significantly lower than those who implemented one or two of the components effectively (P<0.001). Results are shown in Table 5.

Finally, pregnant women who implemented only one component had more than 4 times odds for unintended pregnancy (OR=4.12, CI= 3.21 to 4.72, P=0.04), those who implemented two components had about 3 times (OR=2.82, 95%CI= 2.54 to 2.94, P=0.01) while those who did not implement any of the three components had more than 7 times odds for unintended pregnancy (OR=7.52, 95%CI= 7.23 to 7.94, P=0.001). Results are shown in Table 5.

DISCUSSION

In this study, the incidence of unintended pregnancy among lactating mothers practicing LAM was 14.3%. This value is relatively lower than values obtained in previous studies within and outside Nigeria. Etuk and Ekanem (2003) recorded 30% incidence of unintended pregnancy in a similar survey in Calabar, Nigeria. In Egypt, the incidence of unintended pregnancy among LAM users was 29% (Omar and Anna 2008), while in Turkey, the incidence was recorded as 32.8%. The differences observed here could probably be due to variations in some socio-demographic variables among the different populations under study. In the present study, most of the participants (83%) were highly educated, married (77.5%), multiparous (78.9%) and between the ages of 31 and 35 years (50.7%). These attributes have been consistently

of the information to majority (56.3%) of them. Others got the information from their relatives (25.4%), in school (12.6%) and from their friends (5.6%) as shown in Table 3.

About 57.5% of lactating mothers with unintended pregnancies who practiced LAM did not know its full meaning. Only 36.6% knew the three basic criteria for LAM use. About 71.6% did not know how LAM works and only 56.3% knew the optimum breastfeeding behaviors

Table 3. Sources of information on LAM.

Item	Number	%	Item	Number	%
Had been informed about LAM			**Mother's reaction to the information**		
Yes	71	100	Accepted	71	100
No	0	0	Rejected	0	0
Sources of information about LAM			**First person to inform about LAM**		
Medical staff	40	56.3	Medical staff	40	56.3
Relative	18	25.4	Relatives	18	25.4
In school	9	12.7	In school	9	12.6
Mass media	0	0	Friends	4	5.6
Friend	4	5.6	Mass media	0	0

Table 4. Knowledge about LAM by users.

S/No.	Question	Correct response (%)	Incorrect response (%)	P-value
1	What is the meaning of LAM?	30 (42.3)	41 (57.5)	0.192[NS]
2	What are the basic criteria for the effective use of LAM?	26 (36.6)	45 (63.4)	0.024*
3	The effectiveness of LAM expires when?	36 (50.7)	35 (49.3)	0.906[NS]
4	When can LAM start?	32 (45.1)	39 (49.3)	0.406**
5	Does the mother who practices LAM need to stay close to the baby?	40 (56.3)	31 (44.3)	0.690[NS]
6	How does LAM work?	18 (25.4)	53 (71.6)	0.285[NS]
7	Can LAM continue after weaning?	39 (54.6)	23 (32.4)	0.042*
8	Does LAM provide protection against STIs?	56 (78.9)	15 (21.1)	0.00**
9	How effective is LAM?	30 (42.3)	41 (57.8)	0.192[NS]
10	Does the use of LAM affect infant's health?	36 (50.7)	35 (45.3)	0.906[NS]
11	Does the use of LAM affect mother's health?	28 (39.4)	43 (60.6)	0.075[NS]
12	What are the good effects of LAM?	31 (43.7)	40 (56.3)	0.285[NS]
13	What are the bad effects of LAM?	16 (22.5)	55 (77.5)	0.00**

**P<0.01, significant at 1%; *P<0.05, significant at 5%; [NS]P>0.05 not significant at 5%.

Table 5. Distribution of LAM components property implemented by breastfeeding mothers with unintended pregnancies.

LAM component	N (%)
1 only	8 (11.3)
2 only	13 (18.3)
3 only	10 (14.1)
1 and 2	7 (9.8)
1 and 3	12 (16.9)
2 and 3	9 (12.7)
1, 2 and 3	10 (14.1)
None	2 (2.8)

Values in the parenthesis are percentages. Components: 1 = exclusive breastfeeding, 2 = absence of menstruation (amenorrhoea), 3 = less than 6 months post partum.

shown in previous surveys to improve knowledge and practice of LAM among users. Thus, in a study carried out by Susu et al. (1996), it was found that contraceptive knowledge and practice was positively influenced by education level, parity, marital status and age of users. This corroborates findings by Sandra et al. (1987) in a Bangladeshi study. These attributes were more prominent in the present study population than the aforementioned.

Also, the failure rate recorded in this study and others are significantly higher than the internationally accepted values of 1 to 2% on effective use and 5% on typical use of the method (Kazi et al., 1995). This is probably be-cause these studies have some common features, which include poverty of knowledge and aberrant practices of LAM by users. Poor knowledge of the method, and incorrect practices by users were among the key factors identified as being responsible for the high failure rate of LAM recorded in prior studies (Valdes et al., 2000; Miriam et al., 1997).

In a study in Northern Nigeria, Audu et al. (2006) observed low level of awareness of LAM among users

Table 6. Logistic regression between the component implemented and the likelihood of having unintended pregnancies (odds ratio and 95% confidence interval).

LAM implementation	OR	95% CI	P
Number of component implemented	1.0	-	-
3 components	2.0 (reference)	-	-
2 components	3.0 2.82	2.54-2.94	0.01*
1 component	4.12	3.21-4.72	0.04*
0 component	7.52	7.23-7.94	0.001**

Adjusted for socio-demographic characteristics and post partum maternal behaviors. *P<0.005, significant at 5%; **P<0.01, significant at 1%.

(42.1%), poor utilization and wrong use of the method. These contributed to the high incidence of LAM failure according to that survey. A similar study in Turkey demonstrated low knowledge of LAM by users, more than half (52%) were not aware of the contraceptive property of breastfeeding, 48.16% did not know the importance of frequency and duration of sucking on efficacy of LAM (Vural et al., 1999). Another survey in Turkey by Turk et al. (2010) showed that only 17.2% of women using LAM fulfilled the LAM criteria and 82.8% did not. Two of the three criteria necessary for LAM to be effective were not met by users. These include absence of menstruation within the first six months (43.8%) and exclusive breastfeeding (70.3%).

Similar poor knowledge was observed among users of LAM in the present study. Fifty-seven percent of them did not know the full meaning of LAM. Forty-four percent were ignorant of the optimal breastfeeding behaviors that help maximize LAM efficacy. Fifty-five percent did not know the take-off time and duration, while 46% were ignorant of the effect of weaning and the time when LAM should be switched over to another method or addition of a complementary contraception. Consistent with present finding, Brogan et al. (2006) in their study in India found that only 47.6% of the respondent knew the tenets for effective practice of LAM.

A discordant result was obtained by Miriam et al. (1997) in a multi-center study. In that study, a good level of awareness and practice with 98% efficacy of LAM was recorded. That result yielded insight on the possibility of continuing the use of LAM beyond six months. LAM was found to be highly effective as an introductory post-partum method when offered to a variety of cultures, healthcare settings, socio-economic strata and industrial and developing country locales. The parameters studied in that survey yielded high efficacy, and the basic tenets of the 1995 Bellagio consensus on LAM was re-confirmed. LAM was therefore recommended for incorporation into hospitals, maternity, family planning, maternal

and child health and other primary healthcare setting (Miriam et al., 1997).

The present study also revealed the aberrant practices among LAM users. Only 14.1% actually implemented the three components of LAM. About 2.8% did not implement even one component, while 43.7 and 39.4% implemented 1 and 2 components, respectively. Results of multiple logistic regression analysis showed that those who implemented only one component had 4 times odds for unintended pregnancy while those who implemented two components had 3 times likelihood of having unintended pregnancy. Those who did not implement even one component had about 7 times odds for unintended pregnancy.

Earlier studies have shown that the socio-demographic characteristics of the study population are factors to be considered in assessing the knowledge and practice of LAM and associated incidence of unintended pregnan-cies. Compelling evidences have shown that occupation outside home (employed) and previous awareness of LAM emerge as predictors of its acceptance (Vural et al., 1999; Khella et al., 2004). This has reflected in the present study, most participants (71.8%) were employed and virtually all of them were previously aware of LAM. Majority of them got the information from their physicians while others were either informed by their friends or rela-tions. Existing research indicates that these two factors can impact a negative influence on the practice of LAM. Studies have shown that occupation outside home has encouraged passive practice of LAM. Even when practice of expressed breast milk is intensified, the natural sucking stimulus that initiate the signal that result in suppression of fertility is absent. The sucking stimulus is regarded as a major behavioral and physiological para-meter that account for the variation in the timing of return of fertility. One study showed that mothers who were separated from their infants (but expressed milk to provide 100% breast milk for their babies) had a higher pregnancy risk (5.2%) during the first 6 months (Valdes,

2000), while another study demonstrated that mothers who practiced exclusive breastfeeding had a zero chance of being pregnant during the first 3 months, less than 2% between 3 and 6 months, and about 6% after 6 months (assuming the menstrual periods have not yet returned (Jen O'Quinn, 1998, 1999).

Review of literatures revealed that in areas where breastfeeding is practiced physiologically, that is, frequently, day and night with little or no supplementation given, the continuous breast feeding after menses had returned was associated with significant continued delay in fertility. Such is unrealistic in a breast feeding mother in a job outside home. A high rate of unintended pregnancy among our respondents is therefore substantiated, as majority of them were employed. In conflict to these findings, Miriam et al. (1997) observed that LAM was effective in all settings, even when exclusive breastfeeding may not be fully implemented, but the most important factor according to that survey was the duration of each breastfeeding. Also, studies have shown that previous knowledge of LAM does not guarantee its active practice. This was the observation in the Republic of Kazakhstan (a member of Soviet Union) intervention studies. Knowledge of LAM as a contraceptive method was high among study participants interviewed after de-livery, and 70% of all women reported that they planned to use LAM as protection from unintended pregnancy. However, correct knowledge about the method was very low. Only 35% of the women knew all the three criteria for effective LAM use. A follow-up study revealed that among the LAM users who became pregnant post-partum, none of them knew all the three criteria for successful LAM implemented (Shamil et al., 2004).

A high incident of unintended pregnancy was recorded among participants in the present study despite the fact that they were all previously informed about the method. This therefore underscores the important of effective practice of LAM if its efficacy is to be maximized. Other contributing factors observed in this study were: maternal age, education level, marital status, religion, parity and area of residence. Most respondents (98%) were within the ages of 20 and 40 years, and this appears to be the most sexually active and fertile age bracket. This corroborates the findings by Akinrinola et al. (2006), who observed that more than one quarter (27%) of all Nigerian women fell within similar age bracket and this appears to be the peak age for sexual exuberant and fertility. They observed that women in this age bracket do not want many children soon, but were unwilling to use contraception.

Consistent with the findings in the present study, Sandra et al. (1987) observed that the median duration of amenorrhea was less in high educated than low educated women, which could have accounted for the higher failure rate of LAM among the highly educated participants. Even though some population professionals have accepted

that LAM is too difficult for women to learn and apply correctly (Bracher, 1992; Trussell and Santow, 1991), but no basis for this assertion has ever been provided. It seems logical to suppose that it will not be too difficult for a population made of highly educated women to learn and implement LAM properly. However, effect of other confounders should be considered; this is probably why LAM failed in a study population that is made up of highly educated participants such as ours.

With regards to the effect of marital status on practice of LAM, married mothers were more likely than the unmarried ones to violet the tenets of LAM; this could be explained by the fact that married lactating mothers are found to engage in more sexual activities with their husbands to fulfill their marital obligations than the un-married ones (Ndifon et al., 2006). Also, previous studies have shown that one in every two married lactating women would not want a child soon or wanted no more children, but were not using a contraceptive method effectively or use tradition contraceptive methods (Fatima et al., 2005). These contradict the findings by Ermalynn et al. (1996). Also, ethnic variation in practice of LAM as observed in the present study, corroborates the finding in prior studies (Wiemann et al., 1998; Novotny et al., 2000; Li and Grummer-Strawn, 2004), and may reflects the heterogeneity in culture/norms with regards to contraception.

Conclusion

High incidence of unintended pregnancy observed in this study was associated with poor knowledge and aberrant-practice of LAM by breast feeding mothers. Therefore improving women knowledge (especially breast feeding mothers) on the proper guidelines of LAM could be of a great help in reducing high incidence of unintended pregnancy among LAM users.

ACKNOWLEGDEMENTS

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Full Length Research paper

Antibacterial effects of crude extract of *Azadirachta indica* against *Escherichia coli, Salmonella* spp and *Staphylococcus aureus*

MAMMAN, Paul Habila[1]*, MSHELIA, Wayuta Philip[2], SUSBATRUS, Sosthenes Chidama[1] and SAMBO, Kankani Wonder[3]

[1]Department of Veterinary Microbiology, Ahmadu Bello University, Zaria, Nigeria.
[2]Department of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.
[3]Veterinary Teaching Hospital, Ahmadu Bello University, Zaria, Nigeria.

Most agents used by humans in the treatment of diseases are of plant origin. *Azadirachta indica* (neem) is a tree which has been found to possess antibacterial, antifungal, anti-inflammatory, anti-tumour properties and is also used as a pesticide. In this work, antibacterial effect of methanolic and aqueous extracts of the stem bark of *A. indica* was determined using minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and Kill-time of extracts as indices. Clinical bacterial isolates such as *Escherichia coli*, *Salmonella* spp and *Staphylococcus aureus* were used as test organisms. For the aqueous extracts, a minimum concentration of 43.75 mg/ml was found to inhibit the growth of *E. coli* in nutrient broth. *S. aureus* was inhibited by 87.50 mg/ml and *Salmonella* spp by 175 mg/ml. *Salmonella* spp and *S. aureus* were killed by a minimum concentration of 175 mg/ml but at 1200 and 130 s respectively; *while E. coli* was killed at 87.50 mg/ml at 600 s. For methanolic extract, 43.75, 87.50 and 175 mg/ml concentrations inhibited the growth of *E. coli, Salmonella* spp *and S. aureus* respectively in nutrient broth. Both *Salmonella* spp *and S. aureus* were killed by 175 mg/ml at 600 and 60 s respectively in nutrient broth, while *E. coli* was killed by 250 mg/ml at 1200 s. A comparison of the effects of commonly used antibiotics and the extracts of *A. indica* at 1, 3 and 5 mg per disc on the bacterial isolates by disc diffusion method, showed that the extracts had similar effects on the bacteria as the fluoroquinolones. The crude extracts of *A. indica* were able to inhibit the growth of bacterial isolates *in vitro,* it therefore means that the plant has antibacterial properties. It is recommended that further work be done to identify the specific ingredient(s) responsible for the effect, purify it and standardize same as a drug against bacteria.

Key words: *Azadirachta indica, Escherichia coli*, *Salmonella* spp, *Staphylococcus aureus*, fluoroquinolones.

INTRODUCTION

Medicinal plants have a long history of use and their use is widespread in both developing and developed countries. According to reports of the World Health Organization, 80% of the world's population relies mainly on traditional therapies which involve the use of plant extracts or their active substances (WHO, 1993). Microorganisms have developed resistance against many antibiotics due to the indiscriminate use of antimicrobial drugs (Ahmad et al., 1998). Furthermore, antibiotics are sometimes associated with side effects (Cunha, 2001), whereas there are some advantages of using antimicrobial compounds of medicinal plants, such as fewer side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature (Vermani and Garg, 2002). It is

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*Corresponding author. E-mail: mphabila@yahoo.com. Tel: 07037107993.

known that more than 400, 000 spp. of tropical flowering plants have medicinal properties and this has made traditional medicine cheaper than modern medicine (Odugbemi, 2006). Some plant decoctions are of great value in the treatment of diarrhoea or gastrointestinal disorder, urinary tract infections, skin infections, infertility, wound and cutaneous abscesses (Ergene et al., 2006). The tree, *Azadirachta indica* of the family Maliaceae; popularly known as neem tree or dogon yaro (Hausa) is an evergreen tree, native to the Southeast Asia and found in most tropical countries. It has been in use since ancient times, to treat a number of human ailments and also as household pesticide (Chattopadhyay et al., 1993; 1996; Chattopadhyay and Bandyopadhyay, 2005). Extracts from the bark, leaves, fruits and roots have been used to control leprosy, intestinal helminthosis and respiratory disorders (Ketkar and Ketkar, 1995). Every part of the neem tree has been used as traditional medicine for house-hold remedy against various human ailments from antiquity. The tree is still regarded as 'Village dispensary'. It is a plant known over 2000 years as one of the most versatile medicinal plants having a wide spectrum of activity (Larkshmanan and Subramanian, 1996). *Enterobacteriaceae*, the enteric bacteria are facultative anaerobic Gram-negative rods that live in the intestinal tract of animals in health and diseases. The *Enterobacteriaceae* are among the most important bacteria medically. A number of genera within the family are human and animal intestinal pathogens (for example, *Salmonella, Shigella* and *Yersinia*). Several others are normal colonist of the human gastrointestinal tract (for example, *Escherichia coli*, *Enterobacter*, *Klebsiella*), but these bacteria, as well, may occasionally be associated with diseases in humans and animals (Todar, 2008). This study was therefore carried out to determine the antibacterial activity of crudely extracted *A. indica* on clinical isolates of *E. coli, Salmonella* spp. and *Staphylococcus* spp.

MATERIALS AND METHODS

Isolation and characterization of microbes

The test organisms: *E. coli*, *Salmonella* species and *Staphylococcus* species were clinical isolates obtained from the Microbiology Diagnostic Laboratory of Ahmadu Bello University Veterinary Teaching Hospital, Zaria. *E. coli* and *Salmonella* species were subcultured on Brilliant Green Agar (BGA) and MacConkey agar, while *Staphylococcus* species was on sheep blood agar and incubated at 37°C for 24 h. Colonial morphology was observed and Gram's staining was carried out.

Herbal extraction

Source of stem bark and leaves for extraction

The stem bark and leaves were harvested from a neem tree in Ahmadu Bello University environment, close to the Ahmadu Bello University Biological Garden and indentified at the Department of Biological Sciences, Ahmadu Bello University, Zaria. These were properly dried at room temperature, grinded and taken to the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria for extraction.

Extraction by the maceration method

The powdered form of the stem bark and leaves were dissolved in Methanol. The mixture was properly agitated and allowed to stand for 24 h. This was then filtered and the filtrate was poured into an evaporating dish and concentrated to dryness over a water bath at 40°C. A knife was used to scrape the extracts into containers and preserved. The sediments after methanolic extraction were soaked in water for 24 h and same procedure as of the methanolic extraction was followed.

Antibacterial screening test

10 and 50% solutions of methanolic and aqueous extracts of both stem bark and leaves were prepared by dissolving 1 and 5 g in 10 ml each of distilled water. 10 ml each of the prepared concentrations were pipetted into sterile test tubes. Bacterial aliquots of the test organisms were made by scooping 2 colonies each of a 24 h growth of the bacteria into 4 ml of sterile distilled water. 0.2 ml of each of the aliquots containing approximately 5×10^4 bacterial cells or colony forming units was transfered into both of the extract concentrations and allowed to stand for an hour for reaction to take place between the extracts and the bacterial organisms. The mixtures were then inoculated on separate nutrient agar plates and incubated at 37°C for 24 h. Methanolic and aqueous extracts of the leaves at 10 and 50%, and 10% methanolic and aqueous extracts of stem bark had a suppressive antibacterial effect. 50% concentration of both methanolic and aqueous stem bark extracts had inhibitory effect, hence chosen as the working concentration.

Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and kill-time of crude extracts on Isolates

Working concentration

50% of methanolic and aqueous stem bark extracts were subjected to double fold serial dilution in 3 sets of 7 test tubes each containing 5 ml of double strength nutrient broth. The nutrient broth served as diluent and media. 0.1 ml of each of the test organisms' aliquot was added to each of the serially diluted extracts and incubated at 37°C for 24 h. MIC was read as the concentration equivalent to the last test tube showing visibly complete clearance. Contents of 3 consecutively retrospective test tubes from the MIC test tubes, were plated on nutrient agar and incubated at 37°C for 24 h. The concentrations of extract in the last plates that showed no growth were taken as the MBC. To obtain the Kill-time, a dilution of the extract using normal saline was made to the MBC equivalents obtained. 0.1 ml aliquot of each of the test organisms were added to the respective extract concentration and plated on nutrient agar at time intervals of 10 s, 20 s, 30 s, 1 min, 2 min, 5 min, 10 min, 20 min, 40 min and 60 min. This was to determine the contact time needed by the crude extract to kill the bacteria. The plates were incubated at 37°C for 24 h. The first plates that yielded no growth were recorded against their corresponding time.

Sensitivity test using the disc diffusion method

Paper discs were made by cutting filter papers into various suitable

sizes and weighed. The discs were immersed into the solution of the extracts, dried and weighed. The amount of extract adsorbed by the discs were determined by subtracting the initial weights of the discs from the final weights of the discs. The discs of different concentrations were then placed on nutrient agar whose surfaces were evenly smeared with the isolates and incubated at 37°C for 24 h and results read by measuring the diameter of clearance around the discs.

RESULTS

Table 1 showed that discs containing extracts concentrations of 1 mg, 3 mg and 5 mg of *A. indica* had no inhibitory effect on *E .coli.*

Table 2 showed discs containing 1, 3 and 5 mg of extracts having 10 to 20 mm zone of inhibition. Table 3 showed that the disc containing 5 mg of extracts produced the largest zone of inhibition of 10 to 20 mm, while discs containing 1 and 3 mg produced less than 10 mm each.

Table 4 showed that *E. coli* required the highest concentration (250 mg/ml) of the plant extract to be able to cause a bactericidal effect and also took the longest time of 20 min before the effect could be seen. *Salmonella and Staphylococcus* needed an equal concentration of 175 mg/ml for bactericidal effect but *Salmonella* took more time (10 min) as opposed to *Staphylococcus* (1 min)

From Table 5 it could be seen that all the bacteria were resistant to enrofloxacin at 5 μg and septrin at 25 μg. All the bacteria were susceptible, at different degrees, to Nitrofurantoin. The highest susceptibility (32 mm diameter) was recorded by chloramphenicol on *Staphylococcus aureus* (plate 3) and Erythromycin on *Salmonella* spp.

DISCUSSION

From the antibacterial screening tests of the crude extracts of *A. indica* carried out on the selected bacterial isolates (*E. coli*, *Salmonella* spp and *S. aureus*), the methanolic and aqueous extracts of the leaves, up to the concentration of 500 mg/ml were not able to inhibit the bacterial growth on nutrient agar (Figure 1 and plate 1). However, the stem bark extracts, even at 100 mg/ml had inhibitory effects on the bacterial isolates (Tables 2, 3 and Figure 2). This implied that the stem-bark extracts were more effective than the leave extract in terms of antibacterial activity. This may also explain why the stem bark is bitterer than the leaves, meaning it contains more of Nibidin which Biswas et al. (2002) showed in their work to be the main active anti-bacterial ingredient of *A. indica*. The zero zone of inhibition shown in Figure 1 on *E. coli* at concentrations of 1, 3 and 5 mg also agreed with reports by Yagoub et al. (2007) who in their preliminary screening for anti-microbial activity of different plants against different organisms, methanolic extracts of *A.*

Table 1. Inhibitory effect of methanolic leave extracts of *Azadirachta indica* on *Escherichia coli* by disc diffusion method.

Concentration of extract (mg/disc)	Zone of Inhibition (mm)	Interpretation
1	0	Resistant
3	0	Resistant
5	0	Resistant

Table 2. Inhibitory effect of methanolic extracts of the stem-bark of *Azadirachta indica* on *Staphylococcus aureus* by disc diffusion method.

Concentration of Extract (mg/disc)	Zone of Inhibition (mm)	Interpretation
1	10-20	++
3	10-20	++
5	10-20	++

Table 3. Inhibitory effect of methanolic extracts of the stem-bark of *Azadirachta indica* on *Salmonella spp* by disc diffusion method.

Concentration of extract (mg/disc)	Zone of Inhibition (mm)	Interpretation
1	<10	+
3	<10	+
5	10-20	++

Table 4. MIC, MBC and kill-time of methanolic stem-bark extract of *A. indica* on bacterial isolates.

Isolate	MIC (mg/ml)	MBC (mg/ml)	Kill-time (sec)
Salmonella spp	87.50	175.00	600
S. aureus	175.00	175.00	60
E. coli	143.75	250.00	1200

indica produced zero zone of inhibition against *E. coli.* It is important to state here that the Clinical and Laboratory Standard Institute (CLSI 2010) method for interpreting MIC was not applied here owing to the fact that we were dealing with crude extracts here while the CLSI system deals in purified active ingredients. So because there are quite a number of other ingredients in these crude extracts we could not determine the concentration of the active ingredients. Therefore our measurements here were in mg instead of μg.

Both the aqueous and methanolic stem-bark extracts of *A. indica* had anti-bacterial effects. The growth of *E. coli* in nutrient broth was inhibited by the least concentration of 43.75 mg/ml of the stem-bark extracts of *A. indica*; This was followed by *S. aureus* at 87.50 mg/ml and highest concentration of 175 mg/ml was needed by *Salmonella* spp. *Salmonella* spp. had the highest MIC of the aqueous stem bark extract, and its MBC was at the

Table 5. Susceptibility of bacteria to commonly used antimicrobials.

Antimicrobial	Conc. (µg)	*Salmonella* spp	*E. coli*	*S. aureus*
Septrin (SXT)	25	R	R	R
Chloramphenicol (C)	30	R	S	S
Nitrofurantoin (F)	50	S	I	S
Ciprofloxacin (CIP)	5	I	R	R
Enrofloxacin (ENR)	5	R	R	R
Augmentin (AMC)	30	R	R	I
Erythromycin (E)	10	S	I	R
Tetracycline (TE)	30	S	I	R

Key: S=sensitive (zone diameter of bacterial inhibition of ≥ 15 mm for TE; ≥ 16 mm for SXT, E & F ; ≥18 mm for C & AMC; ≥ 21 mm for CIP & ENR). I = intermediate (zone diameter of bacterial inhibition of 12 to 14 mm for TE, 11 to 15 mm for SXT, E & F: 13 to 17 mm for C & AMC; 16 to 20 mm for CIP & ENR). R = resistant (zone diameter of bacterial inhibition ≤ 11 mm for TE, ≤ 10 mm SXT, E & F; ≤ 12 mm C & AMC; ≤ 15 mm CIP & ENR) (CLSI, 2010).
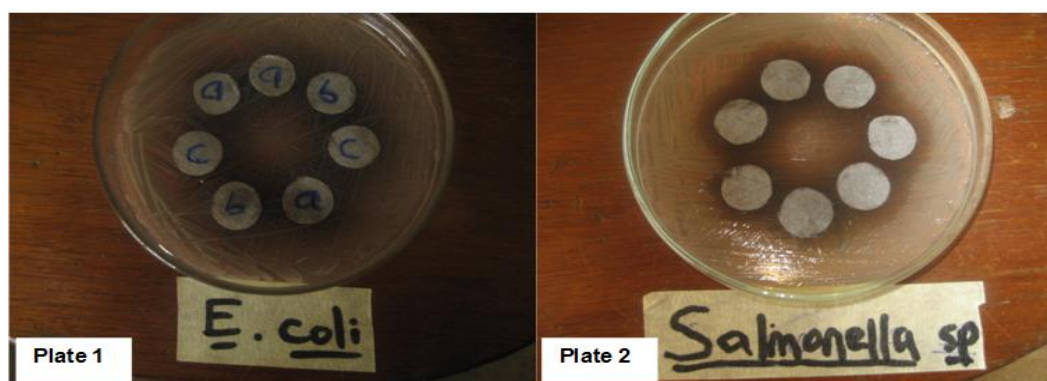
Figure 1. Plate 1; Inhibitory effect of methanolic leaf extracts of *A. indica* on *E. coli* by disc diffusion method. It showed no zone of inhibition i.e. *E. coli* was resistant. Plate 2; Inhibitory effect of methanolic extracts of the stem bark of *A. indica* on *S. aureus* by disc diffusion method. It showed zones of inhibition i.e. *S. aureus* was moderately susceptible.
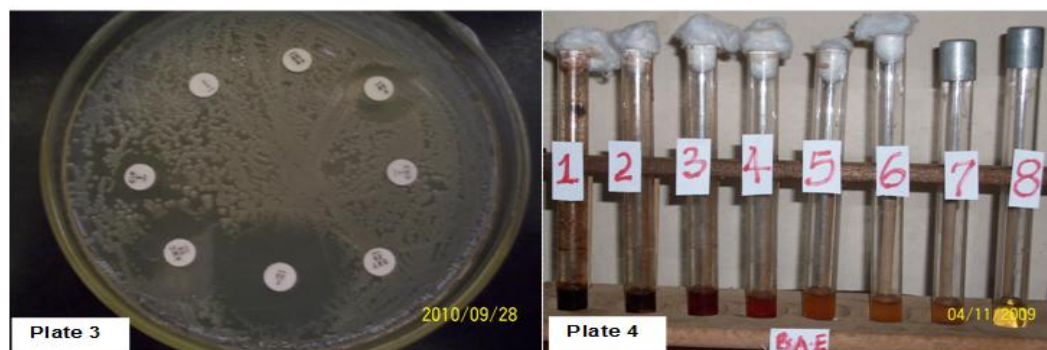
Figure 2. Plate 3; Susceptibility of *S. aureus* to commonly used antimicrobial agents. It showed the highest susceptibility by chloramphenicol. Plate 4; MIC of the stem-bark extracts of *A. indica* on *E. coli* MIC was at tube no. 3, i.e. the tube with least concentration showing clearance.

interval of 130 s. *E. coli* was killed at the least minimum concentration of 87.5 mg/ml within 600 s. The same concentration but it needed the longest time of 1200 s taken to have the killing effect. The MBC of *S. aureus* was 175 mg/ml but it required the shortest time consistency in the effects of these extracts on the test

organisms, implied that neither of the solvents of extraction had a negative effect on the active ingredient. The difference in the effect of this plant extracts within the organisms suggested that there are different antibacterial compounds in the plant extracts and that the compound that acted on one may not be the same as the one that acted on the others since antibacterial agents have different modes of action (Aliu, 2007). This phenomenon of varied susceptibility was also observed by Ergene et al. (2006). The kill-time of both the methanol and aqueous extracts of *A. indica* on Gram-negative organisms was much longer than on Gram-positive organisms. This might be due to the more complex nature of the cell wall of Gram negative organisms as compared with Gram positive organisms. The cell wall of Gram-positive organisms is single-layered; while that of Gram-negative bacteria is multilayered and also bound by an outer cell membrane (Yoa and Moellering, 1995).

In the antibiotic sensitivity tests, all the isolates were resistant to septrin and enrofloxacin. The varying susceptibility of the various bacterial isolates to commonly used antibiotics could be attributed to indiscriminate and irrational use of these drugs in the animals which usually results in resistance developed by the microbes.

CONCLUSION AND RECOMMENDATION

The present work has shown that *Salmonella* spp., *S aureus* and *E .coli* were susceptible to crude extracts of *A. indica in vitro* which means the plant has antibacterial property. It is hereby recommended that further research be done towards isolating, purifying and standardizing the active antibacterial ingredients in *A. indica*. Also more work should be carried out to determine the pharmacokinetics, pharmacodynamics and possible toxicity of the pharmacoactive ingredient(s).

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Full Length Research Paper

Descriptive study of cholelithiasis with chemical constituents' analysis of gallstones from patients living in Baghdad, Iraq

Mohammed A. Taher

Department of Clinical Laboratory Sciences, College of Pharmacy, University Of Baghdad, Iraq.
E-mail: Mohammed_taher43@yahoo.com.

The aim of this study was to describe the main clinical features of patients with cholelithiasis, and chemical analysis of stone in relevance to development of gallstone formation. The total number of patients with gallstone in this study was 75 (66 females and 9 males). The ratio of females: males was (7.3:1), this high ratio may be due to multiple factors, including high percentage of multiparity (63.64%), the use of contraceptives (46.97%), family history (44%), obesity (53.3%) and sedentary life style (non-workers) (72%). The stones were classified into cholesterol, pigment and mixed stones. Cholesterol stone was the most prevalent type of stone; its percentage was 49.3 greater than mixed and pigment stones which had percentages of 33.3 and 17.3, respectively. Cholesterol stone showed significantly higher cholesterol content ($P < 0.05$) than pigment stones; though not significantly higher than mixed stones. Cholesterol content in mixed stone was significantly higher ($P < 0.05$) than pigment stones. Total bilirubin content in pigment stones was significantly higher ($P < 0.05$) than mixed and cholesterol stones. The bilirubin content in cholesterol stones was higher [but not significantly ($P > 0.05$)] than mixed stones. Calcium content in pigment stone was significantly higher ($P < 0.05$) than cholesterol stones and higher [but not significantly ($P > 0.05$)] than mixed stones. Inorganic phosphate content in cholesterol stones was significantly higher ($P < 0.05$) than mixed and pigment stones. The content of inorganic phosphate in mixed stones was insignificantly higher than pigment stones. In conclusion, cholesterol stone was the most common type of stone but interestingly, there was a high ratio of females: males, suggesting efforts to reduce all variable risk factors which lead to cholelithiasis, especially among females.

Key words: Cholelithiasis, chemical constituents, gallstones, Baghdad.

INTRODUCTION

Cholelithiasis is a real problem in Baghdad, Iraq because increasing number of females are attending hospitals due to gallstone complications; the disease seems to be more prevalent than expected. The current study was carried out in the biggest medical center in Iraq and the patients came from different residences; so they may represent the real sample of Iraqi patients living in Baghdad. Reports about gallstone types are rare, and there are no reports concerning the analysis of chemical composition of gall stone in Iraq. This problem is probably related to obesity, cardiovascular disorders, metabolic syndrome and dietary habits, especially consumption of diet which is known to contain large amounts of meat. Obese individuals with Body mass index (BMI) > 30 kg/m^2 have 95% cholesterol-dominant gallstones and are at higher risk of cholesterol stone (Schafmayer et al., 2006). Other risk factors include marked family history of gallstone disease, ageing, multiple parity, cholecystitis and sedentary lifestyle (Cuevas et al., 2004).

Gallstone chemical analysis gives important evidence for origin, etiology, and the metabolic basis of its formation, and helps in the identification of risk factors that predispose certain individuals to the calculi formation. Chemical classification and location of biliary calculi differ in various parts of the world and change over time because of nutritional, socio-economic and demographic

Table 1. Age distribution.

Age group (years)	Female	Male	Total
20-30	14	0	14
31-40	11	6	17
41-50	12	3	15
51-60	24	0	24
>61	5	0	5

Table 2. Sex distribution.

Gender	Number of the patients
Female	66
Male	9
Female: Male	7.3:1

Table 3. Dempographic data of the patients.

Characters	Number of the patients (%)
Multiparity*	42(63.64)
Oral contraceptives*	31(46.97)
Family history	33(44)
Renal stone	11(14.6)
Obese	40(53.3)
Non-workers	54(72)

*only for the females.

factors (Bashir and Meshref, 2011). The identification of the components of gallstone is essential as it provides information that could be useful to medical practitioners to find out the underlying cause of gallstone and to decide whether to treat gallstone patients surgically or therapeutically (Kafia et al., 2009). Moreover, analysis of the chemical composition of gallstones can provide a significant reference to the treatment and prevention of their recurrence (Wang et al., 2007).

Major elements involved in the formation of human gallstones are cholesterol, bilepigment and calcium (Moosavi et al., 2006). With regard to chemical composition, majority of the human gallstones are cholesterol and pigment stones (Vivek et al., 2008). Other substances found in gallstones include calcium salts of phosphate, mucin, glycoprotein, phospholipids and some metals (Pundieir et al., 2002). It has been reported that some elements play a significant role in the formation of gallstones. It was observed that 12 elements namely, sulfur, chloride, potssium, vanadium, chromium, manganase, iron, nickel, copper, zinc, bromide and lead were present in the gallstones collected from Indian farmers (Rautary et al., 2006).

The aim of the present article was to study the clinical features of gallstone patients, with identification of the

constituents of different types of gallstones obtained from patients living in Baghdad city, Iraq.

MATERIALS AND METHODS

A total number of 75 patients with gallstone were admitted in Medical City Hospital in Baghdad (surgery unit) from October, 2010 to May, 2011. There were 66 female and 9 male patients, with mean age (44.53 ± 1.39). All patients were with symptomatic gallstone, having a history of pain in the upper quadrant and epigastric regions from the past 3 to 6 months. An abdominal ultrasonography is the standard diagnostic test for gallstone detection (Trowbridge et al., 2003). Gallstones were obtained from all the patients. Diagnosis of renal stones was made on the basis of information obtained from the history, physical examination, urinalysis and radiographic studies (Mjaland et al., 1998)

In this study, the stones were analyzed by classical chemical method in which stones were collected and powdered in pestle and mortar. To determine the total cholesterol and bilirubin, 30 mg of the powder was dissolved in 3 ml of chloroform in a test tube. The tube was kept in boiling water bath for 2 min. Aliquot from these samples were used for determination of total cholesterol and total bilirubin. To determine calcium, an inorganic phosphate, 30 mg of powdered stone was dissolved in 3 ml of hydrochloric acid in graduated 10 ml tube and then the volume made up to 10 ml with distilled water. The tubes were kept in a boiling water bath for one hour.

Total cholesterol was estimated by a colorimetric enzymatic method (Biocon Diagnostic, Germany) (Allian et al., 1974), total bilirubin by Accurex Biomedicals (Ganmino and Meiter, 1965), calcium by o-cresoiphthalein complexone (OCPC) kit, (Biocon Diagnostic) (Young et al., 1975). Inorganic phosphate was determined according to Fiske and Subbarow (1925).

Statistical analysis

The results were expressed as mean ± standard error of mean (SEM). Student's t-test was used to examine the degree of significance; values less than 0.05 were considered significant. To compare between stone types, analysis of variance (ANOVA) was also used. The statistical analysis was performed using the Statistical package for social sciences, version 17 (SPSS 17).

RESULTS

The total number of cholecystectomy specimen (stones) studied were 75. In this study, the age ranged from 20 to 64 years. The sex distribution in this study is shown in Table 1. In Table 1, the greater number of patients was in the range of 51 to 60, which included only females, so as in the age greater than 61 years. Gallstones were predominantly seen in females (66) as compared to males (9), therefore the ratio of female: male was 7.3:1 (Table 2). Multiparity was seen in 42 (63%) cases, oral contraceptives were used by 31 (46.97%) female patients longer than 6 months, and the number of the patients with family history was 33 (44%). The gallstone was associated with renal stone in 11 (14.6%) patients. Most patients in this study were obese 40 (53%) and non-workers 54 (72%) (Table 3). The stones were divided into

Table 4. Percentage of each type of gallstone in stone former.

Stone types	Gallstone carriers (n=75) (%)
Cholesterol stone	37(49.3)
Mixed stone	25(33.3)
Pigment stone	13(17.3)

n = number of gallstone carriers.

Table 5. Chemical constituents changes among different types of gallstones.

Chemical constituents (mg/l)	Cholesterol stones (n=30)	Mixed stones (n=25)	Pigment stones (n=13)
Cholesterol	593.66±3.1[a]	545.39±3.7[a]	507.16±18.76[b]
Total bilirubin	8.35±0.46[a]	4.09±0.16[a]	26.93±0.83[b]
Calcium	2.91±0.02[a]	10.52±0.02[b]	17.01±0.02[b]
Inorganic phosphate	26.3±0.28[a]	11.45±0.27[b]	1.68±0.06[b]

n = number of stones, different letters refer to significant difference between means ± SE of different type of stones ($P < 0.05$), similar letters refer to non-significant difference between means ± SE of different type of stones ($P > 0.05$).

3 groups, depending upon their color; variations from yellow and white stones were identified as cholesterol stones, dark brown and black as pigment stones, and brownish yellow or green as mixed stones (Table 4).

The total cholesterol was significantly higher in cholesterol stone compared to pigment stone ($P < 0.05$), and in mixed stones as compared to pigment stones ($P < 0.05$) (Table 5). However, there was an insignificant difference between total cholesterol content of cholesterol stones and mixed stones ($P > 0.05$). The total bilirubin concentration was highest in pigment calculi and lowest in mixed calculi. It was significantly higher in pigment calculi compared to mixed calculi ($P < 0.05$) and insignificantly higher in cholesterol stones as compared to mixed stones ($P > 0.05$). The mean calcium content was highest in pigment stones and lowest in cholesterol stones. The calcium content was significantly higher in pigment calculi and mixed stones, as compared to cholesterol calculi ($P < 0.05$) but the content was insignificantly higher in pigment calculi than mixed calculi ($P > 0.05$). The calcium content in various gallstones was in the following order: pigment calculi > mixed calculi > cholesterol calculi (Table 5).

The inorganic phosphate content was highest in cholesterol calculi and lowest in pigment calculi. There was no significant difference in inorganic phosphorous content between mixed and pigment calculi ($P > 0.05$). However, there was a significant difference between cholesterol calculi and pigment calculi and between cholesterol and mixed calculi ($P < 0.05$).

DISCUSSION

In this study, with a total of about 75 cases, the mean age at presentation was 44.53 ± 1.39. Also, the study found that maximum number of the patients was within the range of 51 to 60 (Table 1). This may be attributed to the fact that the patients in this age group were females who were more liable to develop cholelithiasis; also, increasing age may contribute to the development of gallstone by increase in dilatation and therefore volume of gallbladder (Caroli-Bosc et al., 1999). Of the 75 females, 42 (63.64%) were multiparous, having 4 or more pregnancies, and 31 (46.97%) of the females used oral contraceptive longer than 6 months. Parity appears to be a factor in the development of gallstone women with more pregnancies, and longer lengths of fertility periods appear to have a higher likelihood of developing gallstones than those who remain nulliparous (Valdivieso et al., 1993).

The number of the patients with family history in present study was 33 (44%) out of 75. In family studies, history of gallstones in first-degree relatives increases by 3-folds to the risk of gallstone disease (Ko et al., 2005). Eleven patients (41.6%) have both renal stone and gallstone in the present study. A recent study found that patients with cholelithiasis were at a risk of developing nephrolithiasis, and the opposite is true, and that both types of calculi may be associated; the study did not explain the reasons for this association (Erick et al., 2005).

Most of the patients in this study were obese (40 out of 75) (53.3%). The reason for increased risk of gallstones in obese patients is due to an increased hepatic secretion of cholesterol (Shaffer, 2006). Obesity is recognized as a major gallstone risk factor associated with gallbladder dysmotility (Vezina et al., 1990).

In this study, gallstone disease was predominantly seen in females (88%) as compared to males (12%); the

ratio of female: male was 7.3:1 (Table 3). In accordance with the findings of previous studies (Hui et al., 2009), the present study may suggest that female sex is a major risk factor for gallstone disease. It had been reported that women had two fold higher risks for cholelithiasis compared to men (Henry et al., 2005). The high ratio in Iraqi patients may be due to many factors which act together to elevate the ratio. About 46.97% of the female patients were taking contraceptives for longer than 6 months and 72% of the patients were non-workers (drivers, office workers and housewives), in addition to high percentage of obesity in the patients included in this study. All these factors may participate to deviate the present ratio from most studies (Pacchinoni et al., 2002).

Results in Table 5 showed that in 75 gallstone, 37 (49.3%) were cholesterol stones, 25 (33.3%) mixed stone and 13 (17.3%) pigment stone. Cholesterol stone was the predominant type in this study which is in agreement with other studies (Bashir and Meshref, 2011) but is different from a Libyan study which revealed that most stones were pigment (Abdulla et al., 2010). The differences may be attributed to different dietary conditions and habits, and different socio-economic status of the people in these areas.

This study also explained that the mean of chemical constituents of stones was different in the three types, as shown in Table 5. Total cholesterol was significantly higher in cholesterol calculi compared to pigment stone (P < 0.05), and in mixed stones as compared to pigment stones (P < 0.05). However, there was an insignificant difference between total cholesterol content of cholesterol stones and mixed stones (P > 0.05). Gallstones are believed to form when the concentration of cholesterol exceeded that which can be held in mixed miceller solution with bile acids and phospholipids. Supersaturation of cholesterol is believed to be due to abnormal production of bile from liver. The concept of cholesterol supersaturation as a basis for gallstone formation has been emphasized for cholesterol stones which are composed of mainly cholesterol. The high level of cholesterol in cholesterol calculi has been related to high carbohydrate diet (Cuevas et al., 2004). The total bilirubin concentration was highest in pigment calculi and lowest in mixed calculi. It was significantly higher in pigment calculi compared to mixed calculi and cholesterol calculi (P < 0.05), and insignificantly higher in cholesterol calculi as compared to mixed calculi (P > 0.05). These observations are partly in agreement with other studies carried out in Haryana (Pundir et al., 2011).

The color of the pigment stones could be attributed to color of bilirubin which forms salt with calcium to form calcium bilirubinate (Ostrow, 1984). It is known that β-glucuronidase of bacterial origin hydrolyses conjugated bilirubin into free bilirubin which forms salt with calcium as calcium bilirubinate (Swidsinski and Lee, 2001). The mean calcium content was highest in pigment calculi and lowest in cholesterol calculi. It was significantly higher in

pigment calculi and mixed calculi as compared to cholesterol calculi (P < 0.05). However, there was an insignificant difference between calcium content of pigment calculi as compared to mixed calculi (P > 0.05).

The calcium content in various gallstones was in the following order: pigment calculi > mixed calculi > cholesterol calculi. It is in agreement with Libyan article where the calcium was highest in pigment stones (Abdulla et al., 2010). Other study also found that calcium carbonate was identified as the most frequently occurring compound in pigment stones. This could be attributed to the suggestion that copper and iron may act as chelating agents for calcium bilirubinate. The central aggregates of calcium salts constitute hard foreign bodies which may lead to ulceration of gallbladder mucosa and microscopic hemorrhage. The iron released by this process may be another source of its deposition in gallstones. Injury to gallbladder mucosa also provides an opportunity for release of epithelium β-glucuronidase, an additional enzyme contributing towards precipitation of calcium bilirubinate (Verma et al., 2002).

The inorganic phosphate content was highest in cholesterol calculi and lowest in pigment calculi. There was no significant difference in inorganic phosphorus content between mixed calculi and pigment calculi (P > 0.05). However, there was a significant difference between cholesterol calculi and mixed calculi (P < 0.05) and cholesterol calculi and pigment calculi (P < 0.05). This is in conflict with the findings from North India (Pundir et al., 2011), where mixed calculi had highest inorganic content. Inorganic phosphate might be playing an important role in the formation of cholesterol gallstones by forming salt with calcium. Reports from different parts of the world indicate that the incidence of gallstones is correlated with socio-economic conditions and dietary factors (Mjaland et al., 1998).

Conclusion

An interesting finding in this study is that the females are at higher risk of cholelithiasis development than males, in a ratio of female: male (7.3:1). This finding is attributed to high percentage of patients with high risk factors such as multiparity, use of oral contraceptives, obesity, family history and sedentary lifestyle (non-workers). However, this observation needs further randomized studies to confirm it. Cholesterol stone was the most common type of stones and cholesterol seemed to be the major component in all types of stones, so it is regarded as a major key in controlling cholelithiasis.

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Full Length Research Paper

Health effects of lead exposure among Jua Kali (informal sector) workers in Mombasa, Kenya: A case study of the "Express" Jua Kali workers

Jalab Janmohamed Ashraph*, Robert Kinyua, Fred Mugambi and Ahmed Kalebi

Occupational Safety and Health, Jomo Kenyatta University of Agriculture and Technology, Mombasa, Kenya.

The objective of this study is to analyze the effects of lead exposure among the Jua Kali workers. Correlation study: relationship between lead exposure and its effects on blood lead levels (BLL), kidney function and haemoglobin levels between the lead-exposed versus the lead un-exposed workers. 162 adult Jua Kali workers participated. Out of 119 exposed workers, 8 (6.72%) were aware of lead while only 3 of them (2.5%) used protective equipment. The highest BLL in the test group was 32 µg/dl with 16 of them (13.45%) having BLL above 10 µg/dl. The mean BLL in this group was 6.76 ± 5.96. In the control group, the highest BLL was 9 µg/dl with none having BLL above10 µg/dl. The mean BLL was 2.58 ± 1.69. The spearman's correlation coefficient was 0.272 significant at 0.05 level. 14 out of 119 (13.45%) exposed workers had impaired glomerular filtration rates (GFR). The mean GFR in the exposed workers was 104.85 ± 16.485. In the un-exposed workers, 1 out of 43 (2.4%) had impaired GFR. The mean GFR was 109.98 ± 15.408. The spearman's correlation coefficient was -0.113, not statistically significant. 21 out of 119 (17.6%) lead-exposed workers had haemoglobin (HB) less than 13 g/dl with mean HB of 14.12 ± 1.600. 1 out 43 (2.3%) in the control group had an HB of less than 13 g/dl with the mean HB of 14.37 ± 1.34. Spearman's correlation coefficient of negative 0.321 (P<0.05) implying significant inverse relationship. Recommendations: education on lead and its effects, provision of protective equipments, medical facilities to diagnose and manage lead and other heavy metal toxicity.

Key words: Lead exposure, blood lead levels, glomerular filtration rates, haemoglobin levels.

INTRODUCTION

Lead is a naturally occurring element, the most accessible of the heavy metals in the earth's crust. It is widely distributed and used in numerous industrial processes and domestic appliances (ATSDR, 2005). It is a poison whose effects have been known for nearly 3000 years and written about by historical figures from the Greek botanist, Nicander, who described the colic and paralysis seen in lead-poisoned people (Pearce, 2007) to the Greek physician, Dioscodes, who wrote that lead makes the mind "give way" (Henretig, 2006). It is one of the largest environmental medical problems in terms of numbers of people exposed and public health toll it takes (Pokras and Kneeland, 2008). In adults, occupational exposure is the main cause of lead poisoning (Needleman, 2004). Exposures to lead can occur through inhalation, ingestion or occasionally skin contact. Lead may be taken in through direct contact with the mouth, nose and eyes (mucous membrane) and through breaks in the skin (Patrick, 2006). About 35 to 40% of inhaled lead dust is deposited in the lungs and about 95% of that goes into the bloodstream. Of the ingested lead, 15% is absorbed

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*Corresponding author. E-mail: jashraph@yahoo.com. Tel: +254733 831194.

Abbreviations: ACIH, American Conference of Industrial Hygiene; **BLL,** blood lead levels; **CBD,** central business district; **CDC,** Center for Disease Control; **DHHS,** Department of Health and Human Services; **dl,** deciliter; **DOSHS,** Directorate of Occupational Safety and Health; **GFR,** glomerular filtration rate; **HB,** haemoglobin; **ml,** millilitre; **µg,** microgram; **NIOSH,** National Institute of Occupational Safety and Health; **PPE,** personal protective equipment; **WHO,** World Health Organization.

into the bloodstream (Karri et al.,2008). The main body compartments that store lead are the blood, soft tissues and bone (Karri et al., 2008). 94% of absorbed lead is stored in bones. Half life of lead in bone is 20 to 30 years and bone can introduce lead in the bloodstream long after the initial exposure is gone (Patrick, 2006).

Lead circulating in the blood can accumulate in the kidney. The toxic effect of lead causes kidney failure by impairing the function of the proximal convoluted tubules (Rubin and Strayer, 2008). Kidney failure is a medical condition in which the kidneys fail to filter out unwanted/ toxic materials from the blood. It is biochemically detected by an elevated blood creatinine levels. Lead poisoning of the kidney inhibits the excretion of uric acid predisposing one to gout (Lin and Huang, 1994). Anaemia is a decrease in the number of red blood cells or less than normal quantity of haemoglobin in the blood. Anaemia leads to hypoxia (lack of oxygen) in the organs, because haemoglobin carries oxygen from the lungs to the tissues. Anaemia is one of the most characteristic symptoms that indicate high and chronic exposure to lead. It is due to inhibition of a number of enzymes that are involved in haem-synthesis, and as a result, haem-synthesis is impaired and anaemia develops. Lead also affects iron carrier system through cell membrane of red blood cell (Piomelli, 1981).

Diagnosis and treatment of lead exposure is based on blood lead level (the amount of lead in the blood), measured in microgram of lead per deciliter of blood (µg/dl). The US Centre for Disease Control and Prevention and the World Health Organization state that a blood lead level of 10 µg/dl or above is a cause for concern; however, lead may impair development and have harmful health effects even at lower levels, and there is no known safe exposure level (Shaver and Tong, 1991). Authorities such as the American Academy of Pediatrics define lead poisoning as blood lead levels higher than 10 µg/dl (Ragan and Turner, 2009).

The Jua Kali sector is referred to as the "informal sector" or the micro and small enterprise. Jua Kali literally means hot sun indicating the severe conditions under which the micro entrepreneurs go through. This sector is ever increasing as a result of reduced formal employment in the country. The sector consists of small scale workers who lack access to credit, proper equipments, education, training and good working conditions. They are involved in labour-intensive activities providing cheap goods or services while surviving on low income. Originally restricted to artisans, the term has come to include a number of professions including auto-mechanics, painters, hawkers, water kiosks, repair of goods and market vendors (Orwa, 2007). The researcher conducted a case study on the "Express" Jua Kali workers. According to Mugenda and Mugenda (2003), a case study is an in depth investigation of an individual, group, institution or phenomenon. Most case studies are based on the premise that a case can be located, that is, typical of many other cases. The primary purpose of this case study is to determine factors and relationships that have resulted in the behavior under study. The "Express" Jua Kali workers are located between Express House along Moi Avenue, Liwatoni Road, Pandya Road and the railway station in Mombasa.

Control group of Jua Kali workers in this study are those who are not occupationally exposed to lead. They were selected along Digo and Nkrumah Roads in Mombasa's Central business district. The purpose of this study was to look into the effects of lead emissions at work-place and its effects on the worker. It specifically sought to find out the effects of lead on the kidney function and haemoglobin levels.

MATERIALS AND METHODS

Research design

This study was done using correlational design: establishing the nature and degree of relationship between lead exposure as the independent variable and blood lead levels, kidney function and haemoglobin levels as the dependent variable. This study compared the dependent variables, that is, BLL, kidney function and haemoglobin levels between the test group versus the control.

Study subjects

The study area was the Express Jua Kali area located between Express Building along Moi Avenue, Liwatoni Road and the railway station in Mombasa as the test population. There were 281 Jua Kali workers located at this site. Out of these workers, 223 of them were occupationally exposed to lead (target population). Occupations considered as lead-exposed are those that directly deal with lead at work, these include:

1) Radiator repairers: use lead alloys to fix the radiators
2) Lead-acid battery recyclers: both the positive and negative plates contain lead
3) Painters: some of the paints contain lead
4) Welders: in welding leaded products
5) Mechanics: involved in many of the aforementioned jobs

A sample of 141 workers was selected from the population of 223. The figure 141 was arrived at using Atchley's formular (Saunder and Thornhill, 2009).

$$n = \frac{Z^2\,pq}{d^2}$$

where n=the desired sample size (target population> 10,000); z=standard normal deviate at the required confidence level; p=proportion in the target population estimated to have the measured character; q=1-p; d=the level of statistical significance set.

In this study, z-statistic is 1.96, and desired accuracy is at the 0.05 level.

$$n = \frac{(1.96)^2 \times (0.5) \times (0.5)}{(0.05)^2} = 384$$

Table 1. Sample population (exposed group).

Occupation	Population	Lead exposure	Sample
Mechanics	86	Yes	54
Painters	27	Yes	17
Panel beaters	42	No	-
Welders	98	Yes	63
Battery recyclers	4	Yes	2
Radiator repairers	8	Yes	5
Glass cutter	6	No	-
Exhaust fitters	10	No	-
Total	281	223	141

Table 2. Sample population un-exposed group.

Occupation	Population	Sample
Second hand book sellers	34	21
Second hand cloth sellers	26	16
Food/Water vendors	8	5
Others	23	14
Total	91	56

Gender   Male ▼

Black ethnicity?   Black ▼

Age in years   [ ]

Plasma creatinine umol/L [ ]

Reset

Estimated GFR (ml/min/1.73 m^2): [ ]

Interpretation (CKD Stage) Normal or stage 1

Figure 1. Estimated glomerular filtration rate calculator, abbreviated MDRD equation.

Since the target population is less than 10,000, the required sample size will be much smaller. In such case, a final sample estimate (nf) is calculated using the formula as follows:

$$nf = \frac{N}{1 - n/N}$$

where n = the desired sample size; N = the estimate of the population.

$$\frac{384}{1 + \frac{384}{223}} = 141$$

The calculation assumes that data will be collected from all cases in the sample and is based on:

1) How confident you need to be that the estimate is accurate.
2) How accurate the estimate needs to be.

The sample was selected by stratified random sampling (each strata was represented by the profession e.g. stratum for painters, welders, mechanics) from the 223 exposed workers to represent the test population. To select a sample of 141 workers, we divided 223 by 141 and got a factor of 1.58. The population of each strata was divided by this factor (1.58) to obtain the sample population in Table 1.

A sample of 56 from a target population of 91 in the control group (un-exposed Jua Kali population) was selected again by stratified random sampling along Digo Road and Nkrumah Road on Mombasa Central business district. The figure 56 was also obtained through Atchley's formula (Table 2).

Permission from the directorate of occupational safety and health services, ethical approval from Kenyatta National Hospital and University of Nairobi Ethics and Research Committee (KNH-UoN/ERC) and Jomo Kenyatta University authority were required to conduct the research. Consents from the respondents was requested after they had been informed about the purpose, benefits and risks of the study, and their right to withdraw at any point during the study.

Research instruments

Interviews were done on one on one basis, conducted by well-trained research assistants. Information regarding age, nature and duration of occupation, awareness of lead toxicity, use of protective equipments was sought. Biological monitoring-blood samples were collected by qualified and professional laboratory technologists from Lancet, Kenya. 12 cc of blood sample from each respondent were collected and kept in three labeled bottled containers of 4 cc each for assessing blood lead levels, creatinine levels and haemoglobin levels. The samples were taken to the laboratory on the same day having been kept in a cold box. The samples for lead levels were taken to Lancet Laboratories in South Africa for analysis and results were returned by e-mail as attachments. Creatinine values obtained were converted into estimated glomerular filtration rates (eGfR) using the eGFR calculator. It is the abbreviated MDRD equation (MDRD means modification of diet in renal disease study) which is recommended by NICE and renal association of UK (Figure 1). The data collection was done in August 2012.

Data analysis

Data collected from the interview and the laboratory results (blood lead levels, haemoglobin levels and eGFR) were entered in the excel program, tabulated and analyzed in Microsoft Excel, Statistical Package for Social Science (SPSS) and statistical methods. The mean, median and standard deviations (descriptive analysis) were calculated and the correlation coefficient (inferential analysis) was used to assess the existence of the relationship. The aim of the blood analysis was to compare blood lead levels, haemoglobin levels and kidney function among the test and control group.

Table 3. Relationship between occupations and BLL.

Occupation	N	BLL > 10 µg/dl	Percentage	Mean BLL
Mechanics	48	2	4.2	4.06
Painters	12	2	16.67	6.42
Welders	51	6	11.76	7.47
Battery recyclers	2	0	0	7.00
Radiator repairers	6	5	83.3	22.83

Among those occupationally exposed to lead, the radiator repairers had 83.3% of their workers with high BLL followed by the painters.

Table 4. Relationship between occupations and GFR.

Occupation	N	GFR < 90 ml/min	Percentage of GFR < 90 ml/min	Mean GFR
Mechanics	48	7	14.58	106.58
Painter	12	0	0	109.40
Welder	51	7	13.72	103.25
Battery recyclers	2	0	0	94
Radiator repairers	6	3	50	99

The radiator repairers were the most affected with 50% of them having impaired kidney function.

RESULTS

Response rates

Out of the 141 test Jua Kali workers, 119 consented to participate, giving a response rate of 84.4%. In the control, 43 out of 56 consented to the study giving a response rate of 76.7%.

Awareness of lead toxicity

Out of the 119 exposed Jua Kali workers, only 8 were aware of lead toxicity. This represented 6.72% awareness levels.

Awareness in relation to education

Out of those who were aware of lead toxicity, 3 out of a possible 50 were from primary school representing 5%. 3 out of a possible 44 were from secondary school representing 6.8% and 2 out of a possible 11 were from college, representing 18%.

Use of protective equipments

Only 2.5% of the exposed Jua Kali workers use some form of protective equipment. The maximum blood lead levels (BLL) in the test group was 32 µg/dl, with 16 out of the 119 exposed workers having BLL more than the recommended levels of 10 µg/dl as per the World Health Organization/Center for Disease Control (WHO/CDC), representing 13.45%. The highest BLL among the control group was 9 µg/dl. None in this group of workers had BLL above 10 µg/dl. The mean BLL of the test group was 6.76±5.926, while the mean of the control was 2.58±1.679. Spearman's correlation coefficient was 0.272 (P<0.05; 1-tailed) (Table 3).

Out of 119 lead-exposed workers, 14 had impaired glomerular filtration rates (GFR) of less than 90 ml/min representing 13.3%. 2 out of the 14 had severe kidney damage GFR of 60 and 10 ml/min. Only 1 out of the 43 lead-unexposed workers had impaired GFR representing 2.4%. The mean GFR in the exposed group of workers was 104.85±16.485, while the mean in the un-exposed group was 109.98±15.408. The spearman's correlation coefficient was negative 0.113. Correlation was found not significant (Table 4).

The lowest haemoglobin among the test group was 6.10 g/dl. There were 21 lead-exposed workers with haemoglobin below the WHO recommended levels of 13 g/dl representing 17.6%. The lowest haemoglobin levels in the lead-unexposed group was 10 g/dl, only 1 worker was having haemoglobin below 13 g/dl representing 2.3%. The mean haemoglobin levels in the test group was 14.12±1.600, while the control being 14.37±1.34. Spearman's correlation coefficient was -0.321, (P<0.05) indicating significant inverse relationship between lead exposure and haemoglobin levels. Hence, null hypothesis was rejected (Table 5).

Table 5. Relationship between occupations and Hb levels.

Occupation	N	HB < 13	Percentage	Mean Hb
Mechanic	48	6	12.5	14.375
Painter	12	1	8.3	14.35
Welder	51	11	21.55	14.13
Battery recycler	2	0	0	14.60
Radiator repairer	6	2	33.3	13.87

The radiator repairers were the most affected with 33.3% of them having haemoglobin of less than 13 g/dl.

DISCUSSION

This study looked at 2 groups of Jua Kali workers: (1) those occupationally exposed to lead (test group) and (2) those not occupationally exposed to lead (control group).

Amongst the test group, this study realized very low awareness level of lead and its effects among the Jua Kali workers (6.7%). This is despite all the international campaigns to ban or reduce lead levels in products and in the environment since 1970s. With education, however, this study revealed that there is a significant improvement of awareness amongst these groups of workers with 18% of those with tertiary level of education being aware of lead and its effects as compared to 5% of those with primary level of education. Since the worker's awareness of lead and its effects is low, it is expected, as was shown in this study, that the use of protective equipment was very low (2.5%).

This study compared the blood lead levels of the 2 groups of workers to see if occupational exposure to lead translates to higher blood lead levels. The study indicated higher BLL in the test group as compared to the control group with over 13% of the test group having BLL above 10 µg/dl (recommended levels by WHO/CDC), while in the control group none had BLL above 10 µg/dl. Although, there was a significant difference in lead levels between the test and the control, previous studies had indicated much higher lead levels. Study done by Njoroge et al. (2008) on environmental and occupational exposure to lead, done in Nairobi, Kenya, the highest BLL was 65 µg/dl with 89% of the test group having high BLL and a mean of 22.5 µg/dl, while 15% of the control group having high BLL, with a mean of 6.75 µg/dl. The high BLL in the earlier study (Njoroge et al., 2008) could be explained by:

1) Nairobi is a more industrial town than Mombasa, possibly with more lead emissions
2) The study was done much earlier in 2006, at that time, international awareness and actions against lead emissions and toxicity was less than at this present times. Hence, most products like paints and petroleum had higher lead levels compared to the present times.

This study also compared the kidney function of the test versus the control group. Lead is a known poison to the kidney causing injury to the proximal tubules of the nephron. 13.3% of the test group had impaired GFR as compared to 2.4% of the control, with a correlation coefficient of -0.113; the difference is not statistically significant. Related study done by kemal et al. (2005) on the effects of lead exposure among battery workers, in Addis Ababa, Ethiopia, showed no significant correlation between lead exposure and kidney function. Similarly, Karimooy et al. (2010) in Mashad, Iran, found no significant correlation between lead exposure and kidney function while studying workers at the traditional tiles factory, on effects of occupational lead exposure on kidney function.

This study also looked at the effects of lead on haemoglobin by comparing the 2 groups of workers. Lead inhibits certain enzymes necessary for haem-synthesis. In this study, 17.6% of the test population had haemoglobin levels less than 13 g/dl as compared to the control which had 2.4%, showing that lead had an effect on haemoglobin levels.

As much as the occupation of lead-exposed workers is concerned, the radiator repairers were the most affected with more than 80% of them having high BLL, 50% of them with impaired kidney function and more than 30% with HB less than 13 g/dl. This is because in repairing the radiator, these workers heat the lead until it melts with lead vapour, then mix with tin to form an alloy. During this process they inhale the lead vapour. To make it worse, the radiator repairers were located in a small, poorly ventilated room.

Conclusion

This study looked at the effects of lead exposure on Jua Kali workers. It specifically sought to find out:

1) The level of awareness of lead and its effects among Jua Kali workers
2) The effects of lead exposure on the blood lead levels.
3) The effects of lead exposure on the kidney function.
4) The effects of lead exposure on the haemoglobin levels.
5) The prevalence of lead toxicity amongst different occupations of the Jua Kali workers.

In view of the findings of this research, the study concluded that:

1) There is low level of awareness of lead and its effects amongst Jua Kali workers, with those of lower educational level more affected.
2) There is a significant relationship between lead exposure and blood lead levels, with 13.5% of the exposed workers having high BLL (>10 µg/dl). None of the workers had >40 µg/dl.
3) There is no significant relationship between blood lead levels and kidney function although 13.3% of the exposed workers had impaired kidney damage, 2 of them having

severe kidney disease (GFR of 60 and 10).

4) There is a significant inverse relationship between lead exposure and haemoglobin levels with 17.6% of exposed workers having anaemia, 1 having severe anaemia (hb of 6.10).

5) Amongst the exposed workers, radiator repairers had the highest BLL (83% of them with >10 µg/dl) followed by painters 17%. The radiator workers were the most affected with 50% having impaired kidney function and 33% being anaemic.

RECOMMENDATIONS

Intervention measures need to be put in place to protect Jua Kali workers from over exposure to lead. These measures include:

1) Workers education on lead and its effects.
2) Provision of free or subsidized personal protective equipments (PPEs) e.g. facial mask, gloves and gowns
3) Provision of water for washing of the protective clothing and bathing.
4) Establishment of engineering controls
5) Regular medical examination to assess lead levels and its effects in the exposed population.
6) Establishing a specialized medical center as a refferal for the diagnosis and management of lead and other heavy metal toxicity.
7) A detailed study to be done to assess the actual amounts of lead levels at the place of work (occupational lead levels) and comparing these levels with the environmental lead levels.

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