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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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International Journal of Medicine and Medical Sciences

Table of Contents: Volume 5 Number 6 June 2013

ARTICLES

Case Report

Research Articles

International Journal of Medicine and Medical Sciences

Table of Contents: Volume 5 Number 6 June 2013

ARTICLES

Research Articles

International Journal of Medicine and Medical Sciences

Table of Contents: Volume 5 Number 6 June 2013

ARTICLES

Research Articles

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International Journal of Medicine and Medical Sciences

Case Report

Multiple oesophageal coin-like foreign bodies appearing like one: A caution for otorhinolaryngologist

Segun-Busari Segun, Afolabi Olushola Abdulrahman*, Alabi Biodun Sulyman, Adebola Stephen Oluwatosin

Department of Otorhinolaryngology,University of Ilorin Teaching Hospital,P. O. Box 2400, Ilorin, Kwara State, Nigeria.

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Ingestion of foreign bodies is a common pediatric problem. Majority of ingested foreign bodies pass freely without causing any injury. Ingestion of multiple foreign objects and recurrent episodes are uncommon. Thus we present a case of multiple foreign body ingestion to caution otorhinolaryngologist. We present a six year old boy admitted eight hours following ingestion of a multiple roundish metallic object. There is associated dysphagia to solids and liquid feeds with drooling of saliva. He has had two episodes of induced non-projectile vomiting which contains recently ingested feeds prior to admission. There is no cough or difficulty in breathing. Examination of the neck revealed a positive pointing sign. Plain radiograph of the soft tissues neck revealed a spherical radio-opaque object of metallic density within the oesophagus at the level of C5 to C7. Patient had rigid oesophagoscopy under general anaesthesia with extraction of the multiple metallic foreign bodies. It was uneventful both post operatively and on follow up. The study thus revealed though multiple oesophageal foreign body is rare, there is need for caution among the otorhinolaryngologist when extracting the oesophageal foreign bodies and also stressed the need to double check again following extraction, that is, to repeat endoscopy.

Key words: Multiple, oesophageal, foreign bodies, endoscopy, otorhinolaryngologist.

INTRODUCTION

Ingestion of foreign body is relatively common in the aero-digestive tract among the paediatric population world wide. However some of them get impacted because of either their large size or shapes (Amadasun 1995; Hawkins 1990; Crysdal et al., 1991; Davey and Burkitt 1989; Jackler and Kaplan 1989), while some may pass through the gastrointestinal tract freely uneventful (MacManus 1941). Aerodigestive foreign body ingestion among the paediatric population is a preventable problem (Afolabi et al., 2009). Management of foreign body ingestions varies and it depends upon the object ingested, its location, the patient's age and past history. Once a foreign body is impacted in the throat, it become associated with dramatic presentation of dysphagia and or drooling of saliva which may be a source of apprehension to the patient, to the care giver and the healthcare providers.

Ingestion of multiple foreign objects and repeated episodes are uncommon. Thus we present our case to caution otorhinolaryngologist who is providing the rescue services to have a second and third look.

CASE REPORT

Patient I.A. is six year old school boy who was admitted through the emergency Paediatric Unit eight hours post foreign body ingestion. The patient was said to have swallowed a roundish metallic object,

*Corresponding author. E-mail: droaafolabi@yahoo.com. Tel: +2348035727069.

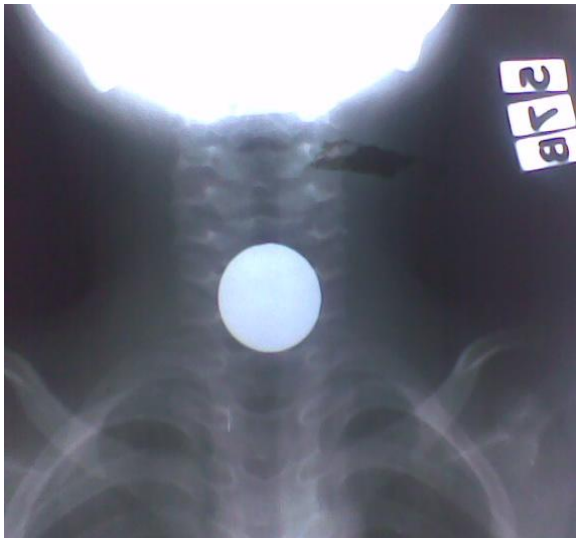

Figure 1. Plain radiographs of the soft tissues of the neck (anterior-posterior view).

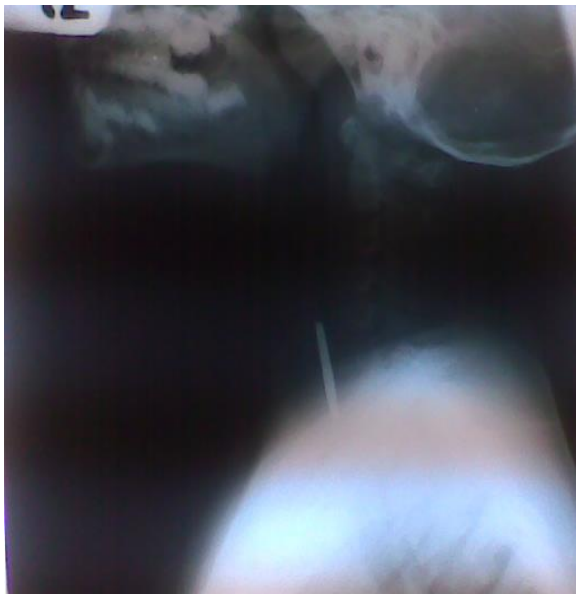

Figure 2. Plain radiographs of the soft tissues of the neck (lateral view).

while he was playing. There is associated odynophagia, dysphagia to solids and liquid diet and there is drooling of saliva. He has had two episodes of induced non-projectile vomiting of which contains recently ingested food prior to admission. There was no cough and difficulty in breathing. There was no abnormal neck swelling. There were no otologic or nasal symptoms. The patient was not a known psychiatric or asthmatic patient. He is from a monogamous family and the first child of the family. There was noticed failed attempt at removal using bolus of meal.

Clinical examination revealed a boy not in any obvious cardio-respiratory distress, not pale, afebrile, but with drooling of saliva and a positive pointing sign at the level of cricothyroid cartilage joint.

The patient has good oro-dental hygiene but with pooling of saliva in the oral cavity; dentition was intact with no use of dentures, no foreign body visualised in the oropharynx. He has a granular posterior pharyngeal wall.

Otologic and anterior rhinoscopy was essentially normal. There was tenderness at the front of the neck on palpation and scarification marks were noticed at these points.

A diagnosis of impacted pharyngo-oesophageal foreign body ingestion was made. Plain radiographs of the soft tissues of the neck (AP and lateral view) revealed a spherical radio-opaque object of metallic density within the oesophagus at the level of C5 to C7, with air-oesophagogram in which the lower limit cannot be delineated (Figures 1 and 2).

The patient's heamogram and blood biochemistry were all within normal limits. Patient had rigid oesophagoscopy using paediatric Karl Storz oesophagoscope with gentle extraction of foreign body under general anaesthesia with small size 3.0 endotracheal tube intubation. The surgical findings were multiple, silver coloured spherical metallic objects of the same size were extracted at about 12 to 14 cm from the upper incisor, at the level of the crico-pharyngeus. These objects were superimposed on one another. Repeat endoscopy was done to ensure no other foreign bodies were left out.

Post operatively, patient was uneventful. Patient commenced oral intake, 48 h post-operatively with no evidence of oesophageal per-foration. The patient was discharged home 72 h post operatively. Follow up revealed he has remained well for six months now.

DISCUSSION

The majority of foreign body ingestions occur in the paediatric population with a peak incidence between ages six months and six years (Afolabi et al., 2009; Webb 1995; Cheng and Tam 1999; Hachimi-Idrissi et al., 1998).The age group which our index case presented falls into, may be associated with peer group influence. In children, the true duration of the foreign body in the oesophagus may be unknown until it is discomforting; however in the index case the duration was said to be about eight hours when the attention of the care giver was drawn to it. Nevertheless, the exact timing of insertion may be difficult. Male are at risk of foreign body insertion or ingestion as in the index case reported, this may be associated with the increased risk of exploration of all cavities and high level of inquisitiveness among the male children than the female children similar to previous reports (Afolabi et al., 2009).

In adults, true foreign object ingestion occurs more commonly among those with psychiatric disorders, mental retardation or impairment caused by alcohol, and those seeking some secondary gain with access to a medical facility (Webb 1995; Blaho et al., 1998; Kamal et al., 1999). Edentulous adults are also at greater risk for foreign body ingestion, including their dental prosthesis (Blaho et al., 1998; Abdullah et al., 1998).

The most common site of foreign body impaction in the oesophagus is the cervical oesophagus at the level of the cricopharyngeus which is similar to our finding; in the report presented, the foreign body lodged was lodged at

Figure 3. Multiple metallic foreign body.

Figure 4. Diameter of multiple metallic foreign body.

about 12 to 14 cm from the upper incisors. The next commonest site is the thoracic oesophagus at the level of the aortic arch (Nandi and Ong 1978). This radiographic investigation done in our index case if combined with the clinical findings of his pointing sign as it corresponded to the location of the foreign body increases the specificity. (Lue et al., 2000) reported a sensitivity and specificity of 39 and 72%, respectively while a recent study quoted 54.8 and 100% (Akazawa et al., 2004), for their plain radiographs. The present report does not report a certain percentage as it was a single case report.

Two radiological views as is customarily done in our centre was buttressed by Whelan-Johnson S, Hall CE, who equally stated that two radiological views are recommended in the assessment of oesophageal foreign bodies (Whelan-Johnson and Hall 2009).

Oesophageal foreign bodies are commonly encountered in otolaryngology practice. Such circumstances are often compounded by pre-existing psychiatric problems such as bulimia and/or anorexia nervosa (Sastry et al., 2008). Patients with bulimia may often present with a very similar pattern of multiple episodes of ingestion of large foreign bodies. Identification of this eating disorder (especially when there is a recurrent history of large, accidentally ingested foreign bodies) and prompt psychiatric referral is essential for efficient long-term management of this condition (Sastry et al., 2008). Our patient has no psychiatric illness.

Management of the patient is influenced by the patient's age and clinical condition; the size, position of the coin if vertical or horizontal, shape, and classification of the ingested material; the anatomic location in which the object is lodged; and the technical abilities of the endoscopist (Ginsberg 1995;Faigel et al., 1997; Michaud et al., 2009). The index patient presented had the multiple foreign body that is metallic, silver coloured with a diameter of about 4 cm (Figures 3 and 4) which is equivalent to the oesophageal diameter in vertical position relative to the oesophageal lumen; thus there was no sign of airway compression and allows minimal swallowing, thus no total dysphagia. The timing of endoscopic intervention in foreign body ingestion is dictated by the perceived risks of aspiration and/or perforation base on the position/placement. In this situation, the intervention was after 12 h of injury due to investigative procedure and sourcing for finance. This extraction was done under general anaesthesia with a Karlstorz rigid oesophagoscope and foreign body removed. Further delay in the removal may predispose the patient to corrosion of the metallic object with increased risk of perforation and mucosal reaction.

Conclusion

Multiple oesophageal foreign body though rare, is an emergency with need for caution among the otorhinolaryngologists/endoscopist. As it is common among the paediatric population, all patients with foreign bodies in the aero-digestive tract should have adequate history, ear, nose and throat examination along with the pointing sign (if the child is old enough) which should be combined with the radiological investigation. Patients should be assumed to have multiple foreign bodies thus the need to scope the aero-digestive tract and re-scope

after extraction to look for a remnant if there are and to ascertain the integrity of the site of impaction of the FB, in this case the mucosa.

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Full Length Research Paper

The effect of clofibrate in near term newborns with non hemolytic jaundice

Sedigheh Hossein pour Sakha, Manizheh Mostafa Gharehbaghi* and Mohammad Ebrahim Rahbani

NICU Al Zahra Hospital, South Artesh Street, 5138665793Tabriz University of Medical Sciences, Tabriz, Iran

Accepted 23 April, 2013

The aim of this study was to evaluate the efficacy of clofibrate in reducing total serum bilirubin levels in near term neonates with non hemolytic jaundice. A randomized controlled study was carried out in the neonatal ward of Children's Hospital, Tabriz, Iran, over one year period. Sixty eight healthy near term infants with non hemolytic hyperbilirubinemia were randomized to receive phototherapy plus clofibrate (n=35) or phototherapy and placebo (n=33). There were no significant differences in the weight, gender, modes of delivery and age of neonates between two groups. Similarly, the mean total serum bilirubin (TSB) level at the time of admission was not significantly different between the two groups (19.72±1.79 versus 20.05±2.82 mg/dl, P=0.57). The mean TSB 48 h after phototherapy (8.06±1.34 versus 10.94±2.87 mg/dl P=0.02) and the mean duration of phototherapy (64.32±12.48 versus 87.84±29.76 h P<0.0001) was significantly lower in clofibrate treated group. Clofibrate is an effective adjunctive drug in neonatal hyperbilirubinemia and results to lower TSB level and reduced duration of phototherapy in near term newborns.

Key words: Clofibrate, near term neonate, non hemolytic jaundice, phototherapy.

INTRODUCTION

Most newborns experience benign hyperbilirubinemia. Severe elevation of serum bilirubin levels can result in brain damage known as kernicterus (American Academy of Pediatrics, 2004; Maisels, 2005; Halamek and Stevenson, 2006; Shortland et al., 2008; Alkalay and Simmons, 2005). It is important to promptly initiate appropriate therapy. The intensity and invasiveness of therapy is determined by the many factors such as gestational age, relative health of the neonate, the current level of total bilirubin and the etiology of jaundice. In the neonates, hyperbilirubinemia is usually due to a combination of an increased bilirubin load and decreased bilirubin elimination (American Academy of Pediatrics, 2004; Maisels, 2005; Halamek and Stevenson, 2006; Shortland et al., 2005; Alkalay and Simmons, 2005). Phototherapy and exchange transfusion are two main interventions used to decrease total serum bilirubin (TSB) (Maisels, 2005; Halamek and Stevenson, 2006). Despite an understanding of the enzymatic pathways leading to bilirubin production and degradation, very few pharmacologic interventions are utilized and the mainstay of treatment remains phototherapy.

Pharmacologic agents used in the management of hyperbilirubinemia can accelerate the normal metabolic pathways for bilirubin clearance, inhibit the enterohepatic circulation of bilirubin or interfere with bilirubin formation by either blocking the degradation of heme or inhibiting hemolysis (Avery et al., 2005; Martin et al., 2006; Dennery, 2002).

Clofibrate as a hypolipidemic drug is a glucuronyl transferase inducer which accelerates bilirubin elimination (Bruns et al., 1999; Wang et al., 2007).

*Corresponding author. E-mail: gharehbaghimm@yahoo.com. Tel: +98-411-5539161. Fax: +98-411-5262280.

Table 1. Baseline characteristics of the infants in two groups.

Characteristic	Study group	Control group	P-value
Male [n (%)]	20 (57.1)	18 (54.5)	0.82
Weight (kg)	2.33±0.61*	2.39±0.45*	0.92
Cesarean section [n (%)]	16 (45.7)	18 (54.5)	0.47
Age at admission (days)	6.20±2.62*	6.03±3.14*	0.44
Gestational age (week)	35.04±1.56*	35.16±1.44*	0.72
Hemoglobin (g/dl)	16.90±2.16*	16.23±1.94*	0.18
Direct bilirubin (mg/dl)	0.86 ±0.39*	0.79 ±0.43*	0.61
Reticulocyte count	1.22±1.46*	2.00 ±0.21*	0.07

*Mean±SD.

Hyperbilirubinemia is a common problem in Iranian newborns and consists of approximately 1/3 of admissions in our neonatal ward. The efficacy of clofibrate has been shown in term neonates in several studies (Lindenbaum et al., 1981; Mohammadzadeh et al., 2005; Badeli et al., 2008; Zahed et al., 2007). A little is known about the usefulness of this drug in preterm infants. We hypothesized that near term infants are not very different from preterm neonates. So, this study was conducted to assess the efficacy and safety of clofibrate in the treatment of non hemolytic jaundice in near term infants.

MATERIALS AND METHODS

This study was a prospective randomized clinical trial conducted between January and December, 2008 in the neonatal ward of Tabriz Children Hospital, which is the major teaching and referral center in Northwest of Iran. Ethical committee of the institute approved the study protocol. Criteria for enrollment in this study included healthy newborns with non hemolytic jaundice, gestational age 34 to 37 weeks, jaundiced neonates who did not need urgent exchange transfusion. It is routine to use 2004 American Academy of Pediatrics (AAP) hyperbilirubinemia guidelines for management of admitted newborn infants in our neonatology department (AAP, 2004).

Neonates with major congenital anomalies, hemolytic disorders, glucose-6-phosphate dehydrogenase (G6PD) deficiency, urinary tract infection, sepsis or significant illness requiring NICU admission, dehydration and hypothyroidism were excluded. Healthy near term infants admitted for non hemolytic jaundice were eligible for the trial. Sixty eight neonates were enrolled in this study. They were randomized into the study or control groups by a random-number table sequence after informed parental consent were obtained. The allocations were contained in opaque sequentially numbered sealed envelops. Study group received phototherapy plus clofibrate (n=35) and control group was treated with phototherapy plus placebo (n=33). Corn oil was used as placebo clofibrate or placebo was administered to patients by a nurse who was not involved in the care of the infants according to orders from sealed envelope.

A single dose of clofibrate 100 mg/kg was administered orally to infants in the study group within 12 h of admission. All patients received phototherapy until discharge. Each phototherapy unit contained 8 special blue fluorescent tubes labeled TL 52/20 W (Philips, Eindhoven, Netherlands) adjusted at a 20 cm distance above infant.

Lamps of phototherapy units were changed regularly after 1500 h of usage. Total and direct serum bilirubin levels were measured 12, 24, and 48 h after admission and then daily until phototherapy became discontinued. TSB measurement was performed by spectrophotometric method using Bilimeter 3 (Germany) and direct serum bilirubin was measured by autoanalyser system (Selectra E, Netherland) and Pars Azmoon kit, Iran. Laboratory workers measuring them were blind about the type of intervention. The equipments were standardized periodically.

Routine laboratory tests such as complete blood count, total and direct serum bilirubin, reticulocyte count, direct Coombs agglutination test, maternal and neonatal blood group, G6PD level and peripheral blood smear were performed for all jaundiced infants in both groups. Our plan for discharge was TSB less than 50% of exchange level which was ordered by a physician who did not know the infants group assignment and duration of phototherapy was recorded by a nurse who was not involved in drug administration.

All infants were examined 48 h and 1 week after discharge with a careful physical examination for any probable side effects of therapy and laboratory tests for detection of rebound hyperbilirubinemia, leucopenia, and renal failure.

All data were analyzed by using Statistical Package for Social Sciences (SPSS) 14. Statistical analysis of the data was performed by Chi square and independent t-test. P values less than 0.05 were considered significant.

RESULTS

The main cause of admissions in 144 cases of 446 admitted neonates was hyperbilirubinemia (32%) during the study period. Seventy neonates had inclusion criteria. Two patients were excluded from the study, because of subsequent positive blood culture and refusal of parents for blood sampling. Remaining 68 newborn infants were assigned randomly into two groups. Of 68 neonates enrolled in this study, 33 patients in control group received phototherapy plus placebo and 35 neonates in study group treated with phototherapy plus clofibrate. There was no significant difference in gender, weight, age at admission and cesarean section rate between the two groups (Table 1). Sixty five percent of neonates were first offspring. Ninety seven percent of the studied newborns (66 cases) were exclusively breastfed.

Mean total serum bilirubin levels at the time of admission were 20.05±2.82 mg/dl (range: 15.8 to 23.6

Table 2. Laboratory tests results in patients of both groups.

Laboratory test	Study group (Mean±SD)	Control group (Mean±SD)	P-value
TSB 1	20.05±2.82	19.72±1.79	0.57
TSB 2	14.77±2.73	14.23±3.09	0.44
TSB 3	9.60±2.99	10.21±3.65	0.46
TSB 4	8.06±1.34	10.94±2.87	0.02*
Duration of phototherapy	64.32±12.48	87.84±29.76	<0.0001*

TSB 1: At the time of admission; TSB 2: 12 h after initiation of treatment; TSB 3: 24 h after initiation of treatment; TBS 4: 48 h after initiation of treatment.

mg/dl) in the study group and 19.72±1.79 mg/dl (range: 16.5 to 23.9 mg/dl) in the control group (P=0.57). Mean duration of phototherapy was significantly shorter in the study group in comparison with the control group [64.32±12.48 h (range: 45 to 90 h) versus 87.84±29.76 h (range: 70 to 210 h) P<0.0001]. The results of the laboratory tests of patients in the two groups are shown in Table 2. None of the cases in the present study required exchange transfusion.

None of the babies receiving clofibrate developed vomiting, diarrhea or had other side effects of drug. Only one case of rebound hyperbilirubinemia was noted from the control group in the follow up of neonates. White blood cell count, blood urea nitrogen (BUN), and creatinine levels were normal one week after discharge.

DISCUSSION

Clofibrate has been used for many years in adults. It is an activator of peroxisome proliferator-activated receptors (PPARS) which decreases serum cholesterol and triglyceride levels (Bruns et al., 1999). Liver fatty acid binding protein expression is known to be regulated by PRAR agonists such as clofibrate. In the study of Wang (2007), the up regulation of liver fatty acid binding protein was associated with a significant decrease in serum bilirubin and alanine aminotransferase by reduction of hepatic oxidative stress and improvement of hepatic function in bile duct ligated rats (Wang et al., 2007).

There are reports (Lindenbaum et al., 1981; Mohammadzadeh et al., 2005; Badeli et al., 2008; Zahed et al., 2007) that clofibrate treatment resulted in the decrease of the duration of jaundice and a lowered use of phototherapy in term infants, but there are few studies in preterm infants (Mohammadzadeh et al., 2008; Lindenbaum et al., 1985). A few studies showed the usefulness of clofibrate in neonates with G6PD deficiency (Zahedpasha et al., 2008), but were excluded from the study.

In a double blind controlled study of infants without ABO incompatibility, 47 infants treated with a single dose of clofibrate demonstrated significantly lower bilirubin levels after 16 h of treatment as compared to 46 controls given corn oil alone (Lindenbaum et al., 1981). Many studies showed significant decrease of total serum bilirubin 12 h after clofibrate administration for full term infants (Mohammadzadeh et al., 2005; Badeli et al., 2008). In our study, serum bilirubin was significantly decreased only after 48 h in clofibrate group. One possibility for the cause of difference in time needed for bilirubin reduction is lower gestational age of our patients that may affect the time needed for clinical response to drug. There was a 24 (Mohammadzadeh et al., 2008) and 48 h (Lindenbaum et al., 1985) interval for bilirubin reduction in other studies of clofibrate efficacy in premature neonates.

This study shows the usefulness of this drug in near term infants' jaundice. Occurrence of hyperbilirubinemia results in the prolonged hospital stay with increased cost. In developing countries, this intervention would reduce costs of treatment and hospitalization.

A common side effect of clofibrate is nausea. Other gastrointestinal (GI) disturbances including vomiting and loose stools have been reported. Although, complications such as muscle cramping, fatigue, pruritus, alopecia, leukopenia, renal failure and peripheral neuropathy have been described (Wazir et al., 2006), but they are very rare with single dose of this drug (Bruns et al., 1999). Side effects were not found in the studied neonates during hospitalization and until one week after discharge, but the long term safety of clofibrate is debatable and need more studies for longer period follow up and with lower doses and lower doses before recommending wide spread use of this drug as adjunctive therapy for neonatal hyperbilirubinemia.

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Full Length Research Paper

The comparison of intradermal versus intramuscular vaccination of hepatitis B in healthcare workers who fail to respond to previous repeated intramuscular vaccines

Masoud Mardani[1] and Amir Roudgari[2]*

[1]Shahid Beheshti University (M C), Chamran Expressway, Tabnak Ave, Tehran, Iran.
[2]Resident of Infectious Diseases, Shahid Beheshti University (M C), Loghman Hospital, Kargar Street, Tehran, Iran.

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Vaccination is recommended for all healthcare workers (HCWs) at risk of exposure to blood and body fluids to prevent occupational acquisition of hepatitis B virus (HBV). However, about 5% of HCWs after 6 doses of intramuscular injections of vaccines fail to develop protective antibody levels (anti HBs). These groups are named non-responder. To compare the humoral immune responses of intramuscular (IM) and intradermal (ID) vaccination in non-responder HCWs, we designed an open, prospective trial. In this trial, we chose HCWs who did not respond to several booster doses of IM hepatitis B vaccine. Three doses of IM or ID recombinant vaccine were injected with two weeks interval in each group. Hepatitis B antibody responses (anti HBs) were assessed one month after last injection. Seroconvertions were observed in 95.5% of ID group and 85% of IM group of these non-responder subjects after vaccinations. ID recombinant hepatitis B vaccination induced protective antibody responses in more than 95.5% of HCWs non-responsive to repeated IM hepatitis B vaccination and can be considered for all non-responder HCWs.

Key words: Vaccination, healthcare workers, nonresponder, hepatitis B virus.

INTRODUCTION

Vaccination is recommended for all healthcare workers (HCWs) at risk of exposure to blood and body fluids to prevent occupational acquisition of hepatitis B virus (HBV) (Playford et al., 2002; Centers for Disease Control and Prevention, 1997; National Health and Medical Research Council, 1997). However, about 12% of HCWs who receive primary intramuscular (IM) vaccination fail to develop protective hepatitis B surface antibody (anti HBs) concentration (≥ 10 mIU/mL) (Roome et al., 1993; Wood et al., 1993) and remain at risk for occupationally acquired HBV infection (Playford et al., 2002; Szmuness et al., 1980).

Some risk factors for vaccine non response include male gender, older age, cigarette smoking (Roome et al.,1993; Wood et al., 1993; Yen et al., 2005), renal failure (Stevens et al., 1984), chronic liver disease (Aziz et al., 2006), intragluteal vaccine administration (Lindsay et al., 1985; Chen and Gluud, 2005, 2008), certain human leukocyte antigen (HLA) haplotype (Alper et al., 1989; McDermott et al., 1998), and height and weight (body mass index) (Playford et al., 2002; Weissman et al., 1988).

Current guidelines (Centers for Disease Control and Prevention, 1997; National Health and Medical Research Council, 1997) for management of HCWs who fail to respond to primary IM vaccination recommend additional

*Corresponding author. E-mail: roudgari@yahoo.com. Tel: +989121544083. Fax: +9821 55416170.

Table 1. Characteristics of study subjects.

Subject	Gender	Age (years)	BMI (kg/m^2)[1]	No of prior boosters[2]	Time since last dose (years)	Vaccination route	Anti HBs levels after vaccination	Smoking (P/Y)[3]
1	F	50	41.4	9	1	ID	200*	0
2	F	41	21.0	7	10	IM	200*	0
3	F	50	16.4	6	2	ID	179	0
4	F	33	15.2	6	6	ID	200*	0
5	F	49	20.3	6	2	ID	200*	0
6	F	33	21.2	6	5	ID	200*	0
7	F	48	24.9	6	5	ID	200*	0
8	F	50	19.7	6	5	ID	92	0
9	F	38	17.9	6	3	ID	200*	0
10	F	36	15.6	6	2	ID	4	0
11	F	35	17.6	6	3	ID	200*	0
12	F	25	18.2	6	0.8	ID	200*	0
13	F	50	24.2	6	2	ID	200*	0
14	F	40	15.8	6	1	ID	9	0
15	F	46	22.4	6	6	ID	70	0
16	F	41	33.2	6	2	ID	88	0
17	F	47	17.8	5	15	ID	150	0
18	F	41	18.1	5	10	ID	73	0
19	F	34	23.4	5	2	ID	172	0
20	F	38	20.8	5	7	ID	200*	0
21	F	50	18.7	4	5	ID	200	0
22	F	32	19.6	4	3	ID	44	0
23	F	30	17.0	4	0.6	ID	125	0
24	F	28	19.5	4	1	ID	40	0
25	F	42	19.4	4	2	ID	19	0
26	F	46	18.6	3	2	ID	28	0
27	F	38	17.5	3	10	ID	170	0
28	F	27	16.4	3	1	ID	200*	0
29	M	51	21.6	6	8	ID	200*	0
30	M	52	27.4	6	3	ID	200*	0
31	M	35	19.7	6	1	ID	150	0
32	M	49	18.2	6	2	ID	38	0
33	M	42	21.1	6	1	ID	200*	0
34	M	41	26.7	6	1	ID	200*	0
35	M	32	20.4	6	3	ID	200*	0
36	M	40	19.3	6	2	ID	181	20
37	M	52	22.9	6	1	ID	76	7
38	M	56	14.3	6	10	ID	131	5
39	M	48	22.8	5	2	ID	200*	0
40	M	57	19.7	5	2	ID	46	0
41	M	25	20.2	4	3	ID	118	0
42	M	38	25.7	4	2	ID	200*	0
43	M	53	23.2	4	3	ID	200*	0
44	M	46	25.0	3	2	ID	200*	25
45	F	23	15.1	7	3	ID	200*	0
46	F	23	18.0	6	4	IM	200*	0
47	F	34	16.3	6	1	IM	9	0
48	F	33	19.4	6	7	IM	80	0
49	F	46	19.5	6	1	IM	200*	0

Table 1. Contd.

50	F	46	19.3	6	6	IM	100	0
51	F	26	15.8	6	5	IM	86	0
52	F	47	25.0	6	1	IM	200*	0
53	F	49	21.5	6	8	IM	86	0
54	F	48	19.8	5	2	IM	125	0
55	F	38	24.1	5	3	IM	200*	0
56	F	44	27.3	5	1	IM	46	0
57	F	32	15.0	5	2	IM	161	0
58	F	49	22.7	4	1	IM	66	0
59	F	22	20.3	4	4	IM	200*	0
60	M	42	22.2	6	1	IM	0	0
61	M	37	22.4	5	5	IM	200*	0
62	M	30	23.0	4	5	IM	200*	0
63	M	34	24.0	3	1	IM	4	1
64	M	30	25.6	3	4	IM	44	1

[1]Body mass index, [2]number of booster vaccine doses in addition to three primary vaccine doses, [3]pack years, * ≥ 200 mIU/ml.

IM doses (booster doses), although response rate are gene-rally less than 50% (Alper et al., 1989; McDermott et al., 1998; Weissman et al., 1988; Struve et al., 1994; Chen and Gluud, 2008). In contrast, response rate of about 90% have been reported with ID hepatitis B vaccine using either recombinant (Playford et al., 2002; Levits et al., 1995) or plasma-derived vaccine (Nagafuchi et al., 1991).

We design this study for further assessing the humoral immune responses, safety and tolerability of ID re-combinant hepatitis B vaccination and comparing with IM vaccination in HCWs non-responsive to repeated IM vaccination.

MATERIALS AND METHODS

All HCWs in three hospitals in Tehran were assessed (cluster sampling). Any HCWs who have no protective anti HBs levels (≥ 10 mIU/ml) were chosen. Inclusion criteria for this study were HCWs who had:

1. Previously received a primary of at least 3 booster doses (equal to at least 6 doses) of hepatitis B vaccine;
2. Failed to respond to vaccines as documented by appropriately timed post vaccination serology;
3. Received all previous vaccine doses IM into the deltoid muscle;
4. Not received a vaccine dose within the previous 6 months;
5. Negative baseline hepatitis B core antibody (anti HBc) and hepatitis B surface antigen (HBs Ag);
6. Anti HBs levels of less than 10 mIU/ml;
7. No history or laboratory evidence of immunodeficiency, renal failure or hepatic dysfunction.

Subjects were divided randomly in two groups and then vaccinated either with ID or IM route. There was no statistical difference be-tween the two groups in variables such as gender, age, body mass index (BMI), number of previous injections, time from last injection and smoking.

The used vaccines were Hepavax-Gen, a Korean recombinant hepatitis B vaccine, and all subjects were injected by one physician. Intradermal group subjects were vaccinated with 5 microgram (0.25 ml) and a 1 ml insulin syringe (29-gauge needle) to the arm, and intramuscular group subjects were vaccinated with 20 microgram (1 ml) and a 2 ml syringe (22-gauge needle). A total of 3 doses were given every second week to both groups. Direct questioning of subjects and inspection of the injection site 48 to 72 h after each dose was done to detect local reactions, tenderness and other side effects such as fever.

A total of 134 HCWs were included in our trial, sixty five in ID group and sixty nine in IM group. Sixty four subjects (43 women and 21 men) finished our trial (Table 1). In final participants, the median age were 41 (range 22 to 57) years. The median BMI were 20.01; (range 14.29 to 41.38) kg/m^2). 4.7% of subjects had BMI more than 30.1 kg/m^2. 6 (9.4%) were current cigarette smokers. Subjects had received previously between 3 to 9 IM injections of boosters of hepatitis B vaccines (mean 6 doses). The mean range of last injections was 3.5 (0.6 to 15) years. HLA patterns were not included in our study. Concentrations of anti HBs were determined at baseline and one month after last vaccine dose by Enzyme-linked immunosorbent assay (ELISA) method. Levels of 200 mIU/ml or greater were not further quantified; these were treated as equal to 200 mIU/ml for calculations of mean antibody concentration. Sta-tistical analysis was done between two groups by independent sample t-test.

RESULTS

All of the subjects received 3 doses of vaccine, 44 (68.2%) intradermal and 20 (31.8%) intramuscular. In ID group subjects, 22 (50%) had concentration of anti HBs more than 200 mIU/ml, 9 (20.5%) between 100 to 199 mIU/ml, 11 (25%) between 10 to 99 mIU/ml, 2 (4.5%) below 10 mIU/ml and total mean antibody level of 145.5 mIU/ml (Tables 2 and 3). In IM group subjects, 8 (40%) had concentration of anti HBs more than 200 mIU/ml, 3 (15%) between 100 to 199 mIU/ml, 6 (30%) between 10

Table 2. Separative antibody response in the study subjects.

Subjects group \ Antibody response		<10	10-99	100-199	≥200	Total
Intradermal	Female	2	8	5	13	28
	Male	0	3	4	9	16
	Total	2	11	9	22	44
Intramuscular	Female	1	5	3	6	15
	Male	2	1	0	2	5
	Total	3	6	3	8	20

Table 3. Antibody levels after vaccination.

Parameter	N	Valid	44
		Missing	0
Intra dermal	Mean		145.52
	Median		190.50
	Mode		200
	Standard deviation		68.838
	Variance		4738.720
	Range		196
	Minimum		4
	Maximum		200
	N	Valid	20
		Missing	0
Intra muscular	Mean		120.35
	Median		112.50
	Mode		200
	Standard deviation		76.626
	Variance		5871.608
	Range		200
	Minimum		0
	Maximum		200

to 99 mIU/ml, 3 (15%) below 10 mIU/ml and total mean antibody levels of 120.3 mIU/ml (Tables 2 and 3). In summary, 42 (95.5%) of ID subjects and 17 (85%) of IM subjects had anti HBs levels of more than 10, a protective level against hepatitis B. No statistical differences were observed between two groups by independent sample t-test analysis (P value = 0.46). Intradermal vaccination was generally well tolerated. Most of the responding subjects had skin reactions at the vaccine site. One subject had a local reaction of about 15 mm after third injections that subsided with local care. Non responding subjects had no such reactions. No other complications were seen.

DISCUSSION

In this study, 42 of 44 (95.5%) subjects had protective anti HBS Ab levels following ID vaccinations. These HCWs were previously considered unlikely ever to respond to hepatitis B vaccination, all having failed to seroconvert, despite repeated previous intramuscular vaccine doses and must be considered as nonresponder and having risk factors associated with non response.

Primary ID hepatitis B vaccination has been associated with poorer seroconvertion rates and lowers anti HBs levels than IM vaccination (Center for Disease Control and Prevention, 1991), possibly reflecting lower vaccine doses (2 µg versus 5 µg), inadvertent subcutaneous vaccine administration, or both. Similar to others (Playford et al., 2002; Nagafuchi et al., 1991), in this study we sought to optimize vaccine responsiveness by using higher vaccine doses and one physician ex-perienced in administering ID injections. The antibody responses elicited by ID route in this study were high: half of the subjects had maximum responses more than 200 mIU/ml and 95.5% of subjects had responses more than lower limits of protective antibody. These concentrations are higher than those of other studies of ID vaccination of HCWs nonresponsive to intramuscular vaccination (Levits et al., 1995; Nagafuchi et al., 1991).

We also compare ID route with IM route for better evaluation of ID route. Although no statistically significant differences exist between two groups of ID and IM subjects, but a response rate of 95.5% with mean increase of 145.5 mIU/m in HCWs who did not respond to any previous IM injections was very significant. Two reasons of this problem are: first, low sample size and second the IM group subjects received vaccine with two weeks interval, same as ID group, which is not a recommended schedule (0, 1 and 6 months) (Chen and Gluud, 2008). This means that our control group probably was not a true control.

The duration of protection from HBV infection conferred by intradermal vaccination is uncertain. It is currently believed that prolonged protection from clinically significant infection independent of subsequent waning antibody concentrations is conferred to individuals who have an initial protective response to intramuscular vaccination. We would expect that HCWs who respond to ID vaccination should also experience prolonged protection (Playford et al., 2002; Chen and Gluud, 2008; West and Calandra, 1996; Assad and Francis, 1999), despite the lower peak

antibody responses elicited by ID vaccination (Bryan et al., 1992).

Playford et al. (2002) reported that more than 90% of previously non-responsive HCWs responded to ID recombinant hepatitis B vaccine with protective anti HBs levels without comparison with control group, and to our knowledge, we did not find any published study in this regard. So, on the basis of our results, we recommend that non-responder HCWs receive three ID vaccine doses followed by assessment of anti HBs levels. For persistent non-responders, it is unclear whether additional doses would induce seroconvertion.

We have demonstrated that ID recombinant hepatitis B vaccination induced protective antibody responses in more than 95.5% of HCWs nonresponsive to recommended IM hepatitis B vaccination. Also, IM recombinant hepatitis B vaccination with two weeks interval induced protective antibody level of about 85% of such subjects. These vaccinations are safe and well tolerated, and thus can be considered for all nonresponder HCWs.

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Full Length Research Paper

Antibacterial activity of Schiff base ligands containing pyridine and disulphide moieties against some chosen human bacterial pathogens

Sabina Jhaumeer Laulloo[1]*, M. G. Bhowon[1], S. Ravikumar[2], A. Kalaiarasi[2], M. Raja[3] and V. Vijayakumar[2]

[1]Department of Chemistry, Faculty of Science, University of Mauritius, Reduit, Mauritius.
[2]School of Marine Science, Centre for Marine Pharmacology, Alagappa University, Thondi- 623 409, Ramanathapuram District, Tamil Nadu, India.
[3]Department of Chemistry, Sethupathy Government Arts College, Ramanathapuram, Tamil Nadu, India.

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The objective of the study was to evaluate the antibacterial activities of N,N'[1,1'-dithiobis(phenylene)]bis(benzyldeneimine), referred to as L1 and *o,o'*-(N,N-dipicolinyldene) diazadiphenyldisulfide, referred to as L2, containing disulfide moieties against some ophthalmic pathogens (*Klebsiella* species, *Escherichia coli*, *Streptococcus* species, *Proteus morganii, Pseudomonas* species, *Streptococcus pneumoniae, Acinetobacter* species, *Streptococcus pyogenes* and *Streptococcus viridans*), urinary tract infectious pathogens (*P. morganii, E. coli, Pseudomonas* spp., *Enterobacter* species, and *Klebsiella* spp.) and antibiotic resistant pathogens (*Staphylococcus* species, *Streptococcus*, *Pseudomonas* spp., and *Klebsiella* spp.) for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The MIC for the ophthalmic pathogens and antibiotic resistant pathogens were found to be 400 to 500 µg/ml, while for the urinary tract infectious pathogens a lower MIC value (200 µg/ml) was obtained. The MBC for the compounds against all the pathogens tested was 400 to 500 µg/ml. The synthesized Schiff L1 and L2 showed the MIC values for all the tested ophthalmic and antibiotic resistant bacterial pathogens more or less similar. Further studies are needed to prove the safe and efficacy needed for these compounds to develop as a drug after completing successful preclinical and clinical tests.

Key words: Antibacterial activity, minimum bactericidal concentration (MBC), minimum inhibitory concentration (MIC), N, N'[1,1'-dithiobis(phenylene)]bis(benzyldeneimine), ophthalmic pathogens, Urinary tract infectious pathogens (UTI), antibiotic resistant pathogens.

INTRODUCTION

Soon after each antibacterial agent entered into clinical practice, resistance was reported in at least one bacterial pathogen (Bell et al., 1998). Resistance determinants had already accumulated in the environment from which these tagents originated. The environmental resistance determinants were established shortly before the selection pressures that were being treated with the new antibiotics. The clinical utility of the antibiotic was severely

*Corresponding author: E-mail ravibiotech201321@gmail.com. Tel: +91- 9003306959.

diminished within a 5-year time span (Schmitz et al., 1999). The first documented example of the rapid selection of a resistant population was the increase in penicillin resistance from ≤8% to almost 60% in *Staphylococcus aureus* from 1945 to 1949 (Schmitz et al., 1999). That was why, the pharmaceutical industry has responded by designing new classes of drugs. Examples of these were the development of the 'penicillinase-stable penicillins' to counteract Gram-positive β-lactamases, aminoglycosides to avoid streptomycin resistance, cephalosporins to provide improved activity against Gram-negative pathogens, the ketolide telithromycin to avoid macrolide resistance in the pneumococci or linezolid in a novel class of synthetic oxazolidinones with no cross-resistance to any known antibiotic class. However, resistance has emerged to the new agents and thus repeating the cycle (Bush, 2004).

In the history of the antibiotic world, one might imagine that all resistance mechanisms could be overcome by some agents. Indeed, some have stated that virtually all infections can be treated by combination of effective drugs. Therefore, it is not necessary to develop new agents. Unfortunately, that is not the case, as evidenced by the multidrug-resistant enteric bacteria and the pan-resistant pseudomonas that are currently being treated with the toxic membrane-disruptive polymyxins (Evans et al., 1999). With the approval of the three most recent antibacterial agents, namely, linezolid in 2000, daptomycin in 2003 and telithromycin in 2002 to 2004, three new classes of agents have been introduced into the market place. However, resistance has already been reported for all the three agents (Evans et al., 1999), thus providing an opportunity for additional agents in these classes to overcome the new resistance.

However, there will be also unpredictable factors such as the appearance of new diseases, or the newly recognized association of established diseases with infectious agents (Paul, 2010). Finally, this will lead to the threat of bioterrorism due to epidemic outbreaks of resistant organisms. Although, the mail attacks of anthrax in the USA was fortunately conducted with a highly susceptible *Bacillus anthracis* strain in 2001 (Dalton, 2001). It is now well established that antibiotic resistant strains can be readily selected *in vitro* (Brook et al., 2001), thus leading to the possibility of more resistant strains appearing in the future. Opportunities are available to approach this disease more creatively, with the development of new drugs to attack the toxin and the use of classical antibacterial agents to eradicate the bacteria, because inhalation anthrax is the most frequently causing fatal disease due to rapid toxin production (Shen et al., 2004). Development of safe and effective medicines is still an option that is frequently ignored. Hence, this study has been focused on the development of safe and effective medicines. Derivatives of pyridine (Mei-Ying et al., 2005; Li-Xia et al., 2006; Tarafder et al., 2001) and diphenyl-disulphides (Bhowonet al., 2005; Jiang et al., 2003) are known to possess significant antibacterial, antifungal, anticancer and anti-human immunodeficiency virus (HIV) properties. This gave a great insight to search for potential pharmacologically active Schiff bases containing pyridine and disulphide moieties.

MATERIALS AND METHODS

Chemistry

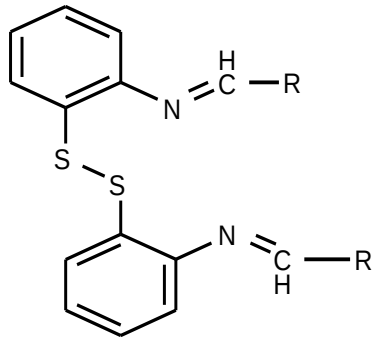
Melting points were uncorrected and were determined on a Stuart Scientific Electric Melting Point Apparatus. Infrared (IR) spectra (KBr) were recorded on a Mattson 1000 Fourier transform Infrared (FTIR) spectrometer in the range of 400 to 4000 cm^{-1}. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker spectrometer at 250 MHz.

Synthesis of phenylenediimines N,N'[1,1'-dithiobis(phenylene)]bis(benzyldeneimine) (L1) and *o,o'*-(N,N-dipicolinyldene) diazadiphenyldisulfide (L2)

To a vigorously stirred solution of bis (2-aminophenyl) disulphide (0.9 mmol) in EtOH (40 ml), benzaldehyde or 2-pyridine carboxaldehyde (1.9 mmol) was added. The yellow mixture was allowed to stir at room temperature. The precipitated product was collected by filtration, washed thoroughly with EtOH (10 ml) and dried *in vacuo*. **L1**: mp: 140°C (Krebs, 1995); ^{1}H NMR: δ 8.74 (s, 2H, 2-CH=N), 8.02 (m, 4H, Ar-H), 7.59-7.55 (m, 8H, Ar-H), 7.34-7.24 (m, 6H, Ar-H); ^{13}C NMR: δ 118.4, 125.7, 127.7, 128.0, 129.5, 131.5, 132.5, 136.3, 148.8, (benzene carbons), and 161.5 ppm (2 imine carbons); UV-Visible: 258 (4507), 324 (11,646). **L2: m**p: 139 to 140°C (lit 139 to 140°C); ^{1}H NMR**:** δ 8.71 ppm (d, J=5, 2H, 2-pyridyl-H), 8.66 (s, 2H, 2-CH=N), 8.33 (d, J=8, 2H, 2-Ar-H); 7.81 (dt, J=16, 8, 1, 2H, 2-pyridyl-H), 7.61 (dd, J=7, 2, 2H, 2-pyridyl H), 7.32 ppm (dt, J=12, 5, 1Hz, 2H, 2-pyridyl-H), 7.14 (m, 6H, 6-Ar-CH); ^{13}C NMR**:** δ 122.2, 126.0, 136.8, 147.8, 154.5 (pyridyl –carbons) δ 117.3, 125.5, 127.1, 127.9, 132.7, 149.7 (benzene carbons), and 160.4 ppm (2 imine carbons); UV-Visible: 260 (31,382), 285 (25,947), 349 (11,295).

Minimum Inhibitory Concentration (MIC)

Different concentrations of stock solutions were prepared by dilution with sterile distilled water so as to obtain 200, 300, 400, 500 and 600 µg/ml of L1 and L2. MIC determination was carried out by mixing 5 ml of varied concentration of L1 and L2 and mixed with 0.5 ml of nutrient broth. 50 µl of bacterial inoculums of ophthalmic pathogens, namely, *Klebsiella* species*, Escherichia coli*, *Streptococcus* species, *Proteus morganii, Pseudomonas* species, *Streptococcus pneumoniae, Acinetobacter* species, *Streptococcus pyogenes* and *Streptococcus viridans,* obtained from Aravind Eye Hospital, Madurai, India; Urinary tract infectious pathogens (UTI), namely, *P. morganii, E. coli, Pseudomonas* spp., *Enterobacter* species, and *Klebsiella* spp. obtained from Vivek Laboratories, Nagercoil, India and antibiotic resistant pathogens, namely, *Staphylococcus aureus*, *Streptococcus* spp., *Pseudomonas aeruginosa*, *Streptococcus pneumoniae* and *Klebsiella* spp. resistant to co-trimoxazole, gentamycin, ampicillin, tetracyclin, chloramphenicol, ciprofloxacin, and kanamycin, obtained from Vinayaga Mission Hospital, Salem India. Nutrient broth alone served as negative control. Whole setup in duplicate was incubated at 37°C for 24 h. The MIC was the lowest concentration of the synthetic compounds that did not permit any visible growth of bacteria during 24 h of incubation after inoculation examined on the



L 1 = C_6H_5
L 2 = C_5H_5N

Figure 1. Structure of L1 and L2. L1: R=Ph; L2: R= 2-pyridyl.

basis of turbidity (Hammond and Lambert, 1978).

Minimum bactericidal concentration (MBC)

To avoid the possibility of misinterpretations due to the turbidity of insoluble compounds if any, the MBC was determined by sub-culturing the MIC serial dilutions after 24 h in Nutrient agar plates using 0.01 ml loop and incubated at 37°C for 24 h. MBC was regarded as the lowest concentration that prevents the growth of bacterial colony on this solid media (Hammond and Lambert, 1978).

RESULTS

Chemistry

The Schiff bases L1 and L2 (Figure 1) were synthesized by the reaction of bis (2-aminophenyl) disulphide with benzaldehyde (Bhowon et al., 2007) and 2-pyridine carboxaldehyde (Uma and Palaniandavar, 1993) in ethanol. The IR spectrum of L1 and L2 showed a sharp peak at 1623 to 1624 cm^{-1} (Bhowon et al., 2005) indicating the presence of imines group, whereas the peak at 1583 cm^{-1} was due to C=N of the pyridine in L2. The other spectral data of L1 and L2 as described in the experimental section were concordant with the proposed structure. The mass spectrum of L1 showed peaks at m/z 425 and 214 corresponding to the molecular formula $C_{26}H_{20}N_2S_2$ (100%) and a fragment ion $C_{13}H_{10}NS$ (12%). The mass spectrum of L2 showed peaks at m/z 427 and 428 accounting for the molecular ion $[C_{24}H_{18}N_4S_2]^+$ (M^+, 100% base peak) and for $[C_{24}H_{19}N_4S_2]^+$ (M+1, 37.2% base peak) ion peaks. Other peaks at m/z 245 were assigned to the ion $[C_{12}H_9N_2S_2]^+$ (5.7% base peak).

Determination of MIC of synthesized compounds L1 and L2 were carried out against 9 ophthalmic bacterial pathogens, 5 urinary tract infectious bacterial pathogens and 5 antibiotic resistant bacterial pathogens. In addition, multidrug resistant *S. aureus* strains are often isolated from human clinical specimens (Oplachenova and Obreshkova, 2003). The results reported in Table 1 show differential sensitivity of the investigated bacterial pathogens. The concentration of L1 and L2 required to inhibit bacterial growth were higher for the chosen bacterial pathogens. Moreover, the MIC values for all the tested ophthalmic and antibiotic resistant bacterial pathogens were quite similar (400 to 500 µg/ml) for L1 and L2 compounds. Lower MIC concentration was reported against urinary tract infectious bacterial pathogens (200 µg/ml). MIC values for *S. aureus* were similar for all tested fractions, while MIC for *Bacillus subtilis* varied between 0.15 and 2.50 µg/ml, depending on the fraction applied (Dragana et al., 2005). Lower sensitivity of *S. aureus* compared to *B. subtilis* to sage essential oil was also reported by other authors (Carvalho et al., 1999). MBC results for all the tested ophthalmic pathogens, UTI pathogens, and antibiotic resistant pathogens were more or less similar (400 to 500 µg/ml) for L1 and L2 compounds.

Table 1. MIC and MBC determinations of 2 synthesized compounds (L1 and L2) against chosen ophthalmic, UTI and antibiotic resistant pathogens.

Pathogen	MIC (µg)		MBC (µg)	
	L_1	L_2	L_1	L_2
Ophthalmic pathogens				
Klebsiella species	500	400	500	500
Escherichia coli	400	400	400	500
Streptococcus species	500	500	500	500
Proteus morganii	400	500	400	400
Pseudomonas species	500	400	500	500
Streptococcus pneumoniae	500	400	500	500
Acinetobacter species	500	500	500	400
Streptococcus pyogenes	500	500	500	500
Streptococcus viridans	400	500	400	500
UTI Pathogens				
Proteus morganii	400	300	500	500
Escherichia coli	300	400	500	500
Pseudomonas species	200	400	500	500
Enterobacter species	400	300	500	500
Klebsiella species	300	400	400	400
Antibiotic resistant pathogens				
Staphylococcus aureus	500	500	500	500
Streptococcus species	400	400	400	400
Pseudomonas aeruginosa	500	500	500	500
Streptococcus pneumoniae	500	500	500	500
Klebsiella species	400	400	400	500

Moreover, the differential MIC value is due to the differences in their physiology as well as in the variations in their sensitivity. Although, the MIC test is considered more accurate for quantitative evaluation of antimicrobial activity, it does not represent an absolute value either. MIC is somewhere between the lowest test concentration which inhibits the bacterial growth and the next lower test concentration.

DISCUSSION

Bactericidal activity of antimicrobial agents can also be assessed by performing a MBC test. Table 1 presents data on the MBC values of tested compounds L1 and L2 against chosen bacterial pathogens. Both of the investigated compounds exhibited a strong bactericidal effect against the tested bacteria. However MBC is slightly higher compared with the MIC values due to the varying in the incubation period. Moreover, the survival of the tested pathogens at higher concentrations of MBC is probably due to the presence of endospores, which are resistant to conditions to which vegetative cells are intolerant. This study was designed to study the MIC and MBC against some ophthalmic pathogens, UTI and anti-biotic resistant pathogens. The synthesized Schiff bases L1 and L2, showed the MIC values for all the tested ophthalmic and antibiotic resistant bacterial pathogens quite similar. Therefore, both compounds could score as putative drugs.

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Full Length Research Paper

Patterns of antibiotic use among children

Hala Abdullah Hussien Abu Romman

Preventive Medicine Department, Royal Medical Services, Amman, Jordan.

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Misuse of antibiotics is considered one of the phenomenon that exist among mothers. This research is aimed at investigating the attitudes and conviction of parents for antibiotic use for their children. Random sample was selected from parents from Amman governorate. The sample composed of (40) family respondents. The results of the research revealed low awareness of parents on the use of antibiotics for their children. Also mothers' awareness is weak for the use of antibiotics during pregnancy, nursing and lactation. The results showed that pharmacists and physician contributes to some extent for the distribution of use of antibiotics despite of the wrong impression among parents that most of the common diseases can be treated through antibiotic.

Key words: Antibiotic, children, patterns.

INTRODUCTION

The use of antibiotics is very wide without any medical supervision. Wise et al. (1998) and Cars et al. (2001) approved that about 80% of the antibiotics are used in the community. Wise et al. (1998) reported that about 20 - 50% of antibiotic used are inappropriate. This behavioral pattern increases the risk of adverse effects and the resistance of antimicrobial community pathogens (Cizman, 2003). The constraints of antibiotic use should be determined with high controls and with the regulation of relationship of patients with physicians. The high demand of patients for antimicrobials shows increase of unnecessary prescriptions (Bauchner, 1997). The relief the patients feel when they take antibiotics increases the pressure on physicians to describe antibiotics.

Josta et al. (2012) showed that the use of antibiotic is connected to parents' use of antibiotics. Their research has shown that the high use of antibiotics among parents has contributed to high use of antibiotics among their children. Haung et al. (2012) revealed that while most antibiotics about 66.66% is given to children according to prescriptions, the rest of antibiotics are given without prescriptions. Bajcetic and Jovanovic (2012) observed lack of knowledge and skill in administering antibiotics among children. The authors indicated that parents lack essential knowledge to deal with antibiotics they give to their children. They indicated also that parents did not consider the physiological and physical characteristics of their children. Consequently, this paper will test the distribution of antibiotic use.

METHODS

Study design

The purpose of this paper was testing the use of antibiotics among children. To accomplish this objective, cross-sectional study was used. Random sample of parents was selected. About (40) parents of fathers and mothers were selected to study the frequency of antibiotic use among children. Questionnaire was used as a tool for data collection. The questionnaire included 57 questions. Part of these questions care for the frequency of antibiotic use, while the rest asked about the demographic characteristics of parents. The validity of the questionnaire was tested using a group of mothers

E-mail: aboromman.papers@gmail.com.

Table 1. Demographic characteristics of sample (n=40).

Characteristic	%
Gender	
Male	17.5
Female	82.5
Marital status	
Married	87.5
Divorced	7.5
Widow	5.0
Age (years)	
Less than 18	0
18 - 25	7.5
26 - 35	45.0
36 - 45	30.0
46 - 55	17.5
56 - 65	0
More than 65	0
Household income	
Less than 200 JD	2.5
200 - 500 JD	35.0
500 - 1000 JD	27.5
More than 1000 JD	35.0
Education	
Primary	0.0
Up to grade 9	2.5
High School	20.0
College	7.5
Bachelor	55.0
Postgraduate	15.0
Place of residence	
Amman	97.5
Irbid	2.5

Table 2. Demographic characteristics of children (n=40).

Characteristic	%
Number of children per family	
One	48.5
Two	30.3
Three	18.2
Four	3.0
Age	
Average age of first child	9.78
Average age of second child	8.17
Average age of third child	6.43
Average age of fourth child	3.00

outside the sample of this research.

Statistical analysis

The collected data through questionnaires were coded and entered to Statistical Package of Social Sciences (SPSS) version 18. Descriptive statistics using frequencies, percentages and cross tables were used to get information about each question. Chi-square testing was used to find out if there are any significant differences among the questions of the research.

RESULTS

Demographic characteristics of sample

Respondents included 17.5% males and 82.5% females. The females of the sample were married or divorced mothers. The current married women were 87.5%, while the divorced were 7.5% and the widows were 5.0% of the sample. The marital status of females at least reflected their ability and experience to deal with the needs of children. The dominant age of the sample was 26 - 36 years, followed by 36 - 45 years which is the age of pregnancy (Table 1).

Most of the income ranged from 200 – 500 JD (0.7 JD=$1) and 500 – 1000 JD, while the dominant education was the bachelor, which indicates that most of the sample are well educated and live in Amman (Table 1).

Demographic characteristics of children

The percentage of mothers with one child was 48.5%, while the percentage of mothers with two children is 30.3%. The percentage dropped for mothers with three children to 18.2% and 3.0% for mothers with four children. The average age of first child was 9.78 years, second child 8.17 years, third child 6.43 years, and fourth child 3.00 years.

Patterns of antibiotic use practiced by parents for children

Most of parents (62.5%) indicated that they give their children medical treatment through their physicians. On the other hand, about 20.0% of the sample indicated that they give their children treatment after checking the private clinic, while about 17.5% of the parents indicated other conditions to give their children treatment (Table 3).

The majority of parents indicated that they pay for their children's treatment (42.5%), while about 30.0% indicated that they have private insurance and 22.5% have national insurance. The majority of the sample indicated that their children has been treated by private physicians while the rest of the sample indicated that they receive medication through urgent care center, pharmacist or accident care

Table 3. Patterns of antibiotic use among children (n=40).

Characteristic	%
Medical treatment	
Physician	62.5
Private clinic	20.0
Others	17.5
Type of payment	
National insurance	22.5
Private insurance	30.0
Self pay	42.5
Other	5.0
Have/has your child(ren) been on an oral (by mouth)	
Yes	52.2
No	47.5
Who treated them?	
Urgent care center	7.5
Private physician	47.5
Pharmacist	7.5
Accidents and emergency room physician	2.5
Community clinic	12.5
Others	2.5
Frequency	
1	15.0
2	32.5
3	20.0
4	10.0
5	2.5
Source of antibiotic	
Pharmacy	85.0
Physician	15.0

and emergency room physician, community clinic or others. The most frequent visit for a physician was twice per year with 32.5% followed by three visits with frequency of 20.0. The source of antibiotics was mostly through the pharmacy with 85.0 and 15.0% provided by physician directly (Table 3).

Table 4 shows the causes and types of antibiotics taken by children. Most of antibiotics were used to treat strep throat and ear infection with 42.5% and bronchitis with 30.0%. The most frequent antibiotic used was Amoxicillin with 42.5% followed by Augmentin/Amoklan with 30.0%. The third highest percentage was reported for Pencillin with 10.0% (Table 4). The major cause for starting antibiotic was sore throat with 20.0% and cough, sore throat, runny nose, and earache with 10%. The other causes distributed for fever diarrhea, abdominal pains and others.

About 65.0% of respondents indicated that they changed the physician to another one because he did not prescribe antibiotics. About 27.5% of respondents indicated that the treatment of cold was handled through antibiotics. About 17.5% of respondents reported that the physician of their children guided them to antibiotics as a treatment through phone consultation. The cause of consultation was sore throat infections with 10 and 5% for cough cases (Table 4).

More than half of respondents believe that infection associated with fever should be treated with antibiotics. Parents used antibiotics to treat their children in cases of bacterial infection (37.5%), fever (12.5%), and viral and bacterial infections 10%, while about 20% of respondents indicated that they use antibiotic for previous conditions. About 40% of the respondents indicated that they use antibiotics according to their accumulative experience with them. Based on non production of the desired effect of antibiotics, 45% of the sample indicated that non completion of the course of antibiotic will decrease the desired effect of antibiotic. Other factors was related to the use of unnecessary antibiotic or non completion of the course of antibiotic with 30 and 15% of respondents justifying the low response for the use of antibiotic with physician consultation (Table 5).

75.0% of respondents indicated that antibiotic can be used to treat child stomachache, diarrhea and vomiting, while 45% of respondents indicated that antibiotic can be used to treat cold, cough or nasal congestions. Also, 65% of respondents reported that their children require antibiotic for the treatment of cough, cold or flu symptoms before they take them to physician (Table 5).

About 65% of respondents indicated that they use antibiotics for their children more than 3 times per year, while about 25% of respondents indicated that they use the antibiotic more than once per month for their children. Some parents (27.5%) reported that they request the physician to prescribe them antibiotic for their children in case it lacks that. 20.0% parents used to change the physician if he refused to prescribe antibiotic for the treatment for their children (Table 6).

35.0% parents depend on others' experience (e.g, relatives) and advice to give their children antibiotic. About 55% of respondents indicated that Amoxicillin and Bactrim can be used to treat the symptoms of common cold. Moreover, about 27.5% of respondents show that Biaxin, Bactrim and Amoxicillin can be used to treat viruses (Table 6).

The majority of parents prefer the imported antibiotics over the local ones. About 55% of parents consider the price when purchasing antibiotics. About 12.5% of parents use antibiotics for their children as prophylaxis measure. More than half of respondents indicated that they use antibiotic from one child to another. About 32.5% of respondents indicated that they use leftover reconstituted antibiotic suspension from one child to

Table 4. Reasons and types of antibiotics taken (n=40).

Variable	%
Reason for taking antibiotics	
Bronchitis	30.0
Ears infection	12.5
Pneumonia, Bronchitis	7.5
Strep throat, Ear infection	42.5
Urinary tract infection	5.0
Wound infection	2.5
Type of antibiotic	
Amoxicillin	42.5
Augmentin/ Amoklan	30.0
Cefix	2.5
Ciprofluxacin,Augmentine/Amoklan	2.5
Keflex	5.0
Penicillin	10.0
Zithromax	7.5
Have you ever given your child/children "left-over"?	
Yes	47.5
No	52.5
Specify: why you start did the antibiotic?	
Abdominal pain	2.5
Cough	2.5
Cough, Sore throat, Runny nose, Earache	10.0
Diarrhea	2.5
Earache	2.5
Fever	5.0
Fever, Diarrhea	2.5
Fever, Sore throat	2.5
Painful	2.5
Runny nose	7.5
Sore throat	20.0
Did you ever go to another physician or emergency department to obtain antibiotics for your child/children, when the first physician that you saw did not prescribe antibiotics?	
Yes	65.0
No	32.5
Does your physician routinely treat your child's/children's cold symptoms with antibiotics?	
Yes	27.5
No	72.5
Has your physician ever told you over the phone to start antibiotics for your child without examining him/her?	
Yes	17.5
No	82.5
If you answered YES, what was the reason (why)?	
Abdominal pain, fever	2.5
Cough	5.0
Cough, sore throat	2.5
Earache	2.5
Runny nose	2.5
Sore throat	10.0
Urinary tract infection, earache	2.5

Table 5. Parents convictions about antibiotic (n=40).

Convictions about antibiotic	%
Do you think that all infections associated with fever should be treated with antibiotics?	
Yes	52.5
No	47.5
In what disease condition(s) do you usually use antibiotics for your child(ren)?	
Bacterial infection	37.5
Fever	12.5
Non	2.5
Fever, Bacterial infection	2.5
Fever, viral and bacterial infections	7.5
Viral and bacterial infections	10.0
Viral infection	7.5
All	20.0
If an antibiotic was effective in treating an infection 5 years ago, do you think it will always be effective against the same infection in the future?	
Yes	40.0
No	60.0
Why do you think that antibiotics sometimes do not produce the desired effect (that is, do not work)?	
Taking the antibiotics before meals	5.0
Using antibiotics without doctor's prescription	15.0
I don't know	2.5
Not completing the full course of antibiotics	45.0
Using antibiotics unnecessarily, not completing the full course	30.0
Using the same antibiotic with a different brand	2.5
Do you think antibiotics are necessary for treating your child's stomachache, diarrhea or vomiting?	
Yes	75.0
No	22.5
Do you think antibiotics are necessary for treating your child's common cold, cough or nasal congestion?	
Yes	45.0
No	52.5
Did your child develop an infection that was hard to treat because she/he was taking antibiotics when was needed?	
Yes	2.5
No	92.5
Did you know that your child needs antibiotic treatment before you take her/him to the doctor for cough, cold or flu symptoms?	
Yes	65.0
No	30.0

another. About 15% of respondents indicated that they use this behavior regularly (Table 7).

For the preservation of antibiotics, about 70% reported that they use refrigerators, while the rest use medicine cabinet to reserve medicine. Less than half of respondents reported that they suspended the antibiotics at home and these suspensions left at home took care of emergencies (Table 7).

The majority of sample (65%) reported their knowledge that children may develop allergic reaction to antibiotic and that may cause death. One-third of respondents asked the children physicians to test them for antibiotic allergy, while two-thirds did not. Less than one-third of the sample reported if they know that one of the family

Table 6. Parents impression about the use of antibiotic (n=40).

Impression about use of antibiotics	%
How often do you use antibiotics for your children per year	
3 times year	65.0
Every 2 weeks	5.0
Monthly	2.5
More than monthly	25.0
Have you ever requested antibiotics prescription from your physician even if it was not included in the treatment plan for your child's illness?	
Yes	27.5
No	70.0
Have you ever consulted another doctor to prescribe an antibiotic for your child's illness if the previous doctor did not prescribe any?	
Yes	20.0
No	80.0
Have you ever selected and gave antibiotic(s) for your child(ren) based on relatives' advice?	
Yes	35.0
No	65.0
Do you believe that antibiotics like Amoxicillin and Bactrim will effectively treat and limit the duration of symptoms from the common cold?	
Yes	55.0
No	45.0
Do you believe that antibiotics like Biaxin, Bactrim, and Amoxicillin are effective against viruses?	
Yes	27.5
No	72.5

Table 7. Preference of antibiotic, use and reservation (n=40).

Variable	
Preference of antibiotics, use and reservation	**%**
Do you prefer local or imported antibiotics?	
Local	17.5
Imported	82.5
Do you consider antibiotic price when buying?	
Yes	55.0
No	45.0
Had you ever used antibiotics for your child as a prophylaxis measure?	
Yes	12.5
No	87.5
Had you ever used siblings (brothers and sisters) antibiotic(s) from one child to another?	
Yes	52.5
No	47.5
Did you ever use leftover reconstituted (prepared) antibiotic suspension from one of the children for any of the other siblings (children)?	
Yes	32.5
No	67.5

Table 7. Contd.

If your answer YES, how often?	
Rarely	7.5
Sometimes	2.5
Usually	15.0
If you answered YES, when was the suspension prepared?	
2 weeks	5.0
3 weeks	5.0
A month	10.0
A week ago	5.0
Do not know	7.5
Where do you usually store the prepared antibiotic suspension?	
Drug cabinet	30.0
Refrigerator	70.0
Do you prepare antibiotic suspension at home?	
Yes	47.5
No	50.0
If your answer NO, do you ask your pharmacist to do so?	
Yes	52.5
No	17.5
Do you keep antibiotic suspension at home for emergency use?	
Yes	45.0
No	55.0

members has antibiotic allergy. Mothers were the highest allergic for antibiotics. More than 52.5% of the sample reported their knowledge of the harmfulness of antibiotics for children's teeth. About 27.5% of mothers reported their intake of antibiotic through pregnancy, while 17.5% reported that it is safe to take it during pregnancy. Moreover, about 22.5% of mothers reported that it is safe to take antibiotic during nursing and 20.0% reported it is safe during lactation.

DISCUSSION

The aim of this research is to assess parents' knowledge, attitudes and experience regarding antibiotic use for their children. The results of the research revealed misuse of antibiotics of parents for their children as well as some misbehavior of physicians to satisfy the confidence of parents.

Despite the fact that more than 62.5% of parents reported that they consult physician to treat their children, the results show that parents' first believe that antibiotics is considered a major treatment of different common disease such as cold symptoms, sore throat, diarrhea, running nose and others. Parents show some contradiction concerning their awareness of the negative impact the antibiotic could leave for children and the patterns of using antibiotic to treat their children.

Despite parents' knowledge of the decrease in effect of antibiotics as a result of frequent use, some parents give their children antibiotic once per month or more. Other parents reported that they use antibiotic as prophylaxis procedure for their children. These results indicate high misuse of antibiotics a well as the emotional dealings with children in this respect.

Pharmacies are the major source of antibiotics. The low restriction on pharmacist for the purchase of antibiotics makes it possible for parents to get antibiotics for their children any time. In addition, physicians contribute to the increased use of antibiotics through providing them directly to parents. Physicians do not practice their role in increasing the knowledge about the use of antibiotics. Some physicians introduce consultations by telephone without diagnosing the health status of children. Other physicians prescribe antibiotics for the satisfaction of parents. The responsibility of physicians is to sensitize parents on the allergicity of antibiotics; however this is not practiced fully.

Table 8. Parents' knowledge of antibiotics (n=40).

Knowledge of antibiotics	%
Do you know that some children might develop allergic reaction to antibiotics causing death?	
Yes	65.0
No	35.0
Did you ever ask your physician to test your child for antibiotic allergy?	
Yes	32.5
No	67.5
Do you know if any of your family members are allergic to some antibiotics?	
Yes	30.0
No	70.0
If your answer to question 46 was Yes, what is his/her relation to you?	
Father	5.0
Grandparents	5.0
Mother	12.5
Sister	2.5
Uncles	7.5
Do you know that some antibiotics are harmful for children's teeth?	
Yes	52.5
No	47.5
Have you ever taken antibiotics during pregnancy?	
Yes	27.5
No	70.0
Do you think it is safe to use antibiotics during pregnancy?	
Yes	17.5
No	80.0
Do you think it is safe to use antibiotics while nursing?	
Yes	22.5
No	75.0
Have you ever taken antibiotics during lactation?	
Yes	20.0
No	77.5

One of parents' misbehavior is the storage of antibiotics for long time or not completing the antibiotic course for their children and reserving it for future use. Parents are not knowledgeable enough to deal with suspension antibiotics. Most parents did not realize that when an antibiotic is suspended its validity for use will have to be determined and its method of reservation has to be taken into consideration.

The majority of parents indicated correct procedure for the reservation of treatment, but the antibiotic reserved is not used properly, because antibiotic should be used completely by the child as a treatment course, or suspending antibiotic will alter its maximum period of use. Both of these facts make parents deal with antibiotic wrongly despite their knowledge that non completion of treatment course may cause decrease of the desired effect of antibiotics.

Parents believe that antibiotic is the only treatment that can be used for various diseases. They believe that antibiotic is a proper treatment for bacterial infections, fever,

fever, viral infections, and some parents believe that antibiotic is necessary in any of the previous cases. Even though parents show improper intake of antibiotics, mothers reported that they use antibiotic during pregnancy, nursing and lactation and they believe that it is safe for the health of the baby. These convictions contradict the medically approved ethics of the effect of medicine on infant or baby health during pregnancy, nursing and lactation.

Depending on personal experience or other experience in antibiotics was a contributor for the determination of the type of antibiotic to be used for children. Such conviction indicates that the parents depend on history of dealing with antibiotic to draw their future attitudes.

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Full Length Research Paper

Health care seeking among pulmonary tuberculosis suspects in Wuhan: A community-based study

Qiong-hong Duan[1], Peng Wang [2], Jing Lv [2], Rong Zhong [2], Wei-hua Wang [1] and Qing-zhi Zeng[1]*

[1]Department of TB Control, Wuhan TB Center of Hubei Province, 430030, Wuhan, China.
[2]Department of Epidemiology and Health statistic, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China.

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The aim of this study was to investigate the patterns of health care seeking behavior and to identify the influential factors of the same behavior among community level tuberculosis (TB) suspects in Wuhan city, and to provide an appropriate method and relevant references to increase the detection rate for TB. We followed a cluster proportional sampling procedure to select the four study communities (clusters), a total of 5878 people above 15 years old. Subjects with continuous sputum coughing for more than two weeks, or subjects, who were found to be hemoptysis and with blood sputum, six months prior to the survey were included as research subjects, whom would be participants of an interview with questionnaires and receive X-ray examination, three sputum smear and sputum culture check from May to July in 2010. Chi-square test and logistic regression were used to analyze the influencing factors of health care seeking behavior among TB suspects. A total of 270 cases of TB suspects were interviewed and 259 questionnaires were eligible. 79 cases (30.5%) did not seek any health care, 86 cases (33.2%) have sought medical assistance from primary health care centers or general hospitals, 89 cases (34.4%) self-medicated, while 5 cases (1.9%), consulted a private practitioner. A logistic regression analysis revealed that the presence of health insurance (odd ratio (OR)=3.405, 95% confidence interval (CI)=1.018 to 11.392) was relevant to the formal visitation of TB suspects; the difference was significant (P<0.05). With the severe or chronic respiratory symptom (OR=4.959, 95%CI=2.548 to 9.652), no stigmatization (OR=2.528, 95%CI=1.317 to 4.853) were relevant to the formal visitation of TB suspects; the difference was highly significant (P<0.01). The proportion of TB suspects who had actively sought health care was low. The health insurance, severe or chronic respiratory symptom and stigmatization were the main influential factors upon heath care seeking behavior among community level TB suspects.

Key words: Tuberculosis, suspects, health care seeking behavior.

INTRODUCTION

At present, tuberculosis (TB) remains one of the greatest public health problems in the world. China ranked the second among the world's 22 TB high-burden countries, with the number of TB cases up to 1.5 million in 2010 according to World Health Organization 2011 Global Tuberculosis Control report (WHO, 2011). Detecting and curing TB patients are currently the two major applications for the prevention and control of TB. The cure rate for TB patients with new smear positive increased quickly and reached up to 94% in 2010 with the coverage of Directly Observed Treatment Short-course (DOTS) in most China area. However, the detection rate of tuberculosis patients is not satisfactory, with only 10.5% according to the Fifth National Epidemiological Survey of Tuberculosis (National Technical Steering Group of the Epidemiological Sampling Survey for Tuberculosis, 2012).

The issues that led to the low detection rate could be

*Corresponding author. E-mail: zqz1339719@163.com. Tel: 86-27-836-02190. Fax: 86-27-836-60081.

summarized as follows: the main way to detect TB patients is passive case finding and proportion of TB suspect to seek health care actively is not high. The fourth national TB epidemiological survey showed that among the detection ways of tuberculosis patients, visitation accounted for 94.3% and only 57.2% of symptomatic cases seek health care actively (National Technical Steering Group of the Epidemiological Sampling Survey for Tuberculosis, 2002). The fifth national TB epidemiological survey showed that the proportion of symptomatic cases to seek health care was only 46.8% (National Technical Steering Group of the Epidemiological Sampling Survey for Tuberculosis, 2012). The pattern of TB suspect to seek health care will influence the level of the patient detection. In order to improve the detection rate of TB and control the spread of TB, we must improve the pattern of health seeking behavior among TB suspects.

Studies have shown that the seeking behavior is influenced by seeking motivation, medical conditions, medical standards and reputation, social status, economic factors and health-care system and multifactors. In China, there are some studies (Fei et al., 2006; Hu et al., 2004; Long et al., 2007) about the factors to impact diagnosis delay of TB suspects. But the aforementioned studies were hospital-based, in which subjects with suspicious symptoms surveyed are actively seeking medical care, thus their conclusions could not represent all of the TB suspect in the community because of a large part of TB suspect among the community had not sought health care actively. There is only one community-based (Fei et al., 2006). This study was conducted in rural area Yangzhong, and therefore cannot represent the TB suspect in urban community.

From May to July, 2010, by the use of probability proportionate cluster sampling method, our study was conducted in Wuhan city, to understand the health seeking behavior of TB suspect in the community, and to provide the basis of further improving the detection rate of TB patients.

MATERIALS AND METHODS

Study subjects

This study was conducted from May 19, 2010 to July 10, 2010 in the Wuhan city, located in the center of China. The central city population is estimated to be 8,355,527 with a male/female ratio of 1.06:1.

We followed a probability proportionate cluster sampling procedure to select the study community (clusters). The sample size was calculated using the following formula to estimate the simple random sample size:

$$n = \frac{\mu_\alpha^2}{\delta^2} p \quad 1 - p$$

Assuming that TB suspect prevalence was above 15 year in Wuhan central city p=2.5/100, α- 0.05, thus μ_α=1.96, coefficient of variation (CV)=10%, thus permissible error δ = 1.96×p×CV= 0.0049, the minimum sample size(n) was calculated to be 3900 individuals. In this study, taking into account the probability proportionate cluster sampling method to determine the survey sites the sample size was expanded to 7800. This was equivalent to 6 clusters with an estimated 1500 people above 15 years per community. We reduced the sampling size to be 4 clusters, because of the cost and resources were limited. The 4 clusters had a population of 5878 above 15 years of age. People who had been a TB suspect in the last 6 months and of age>15 years old were selected as our subjects.

Data collection

First of all, the investigating group consists of community hospitals and community committee staffs that verified the information and informed the location and timing of the inspection door to door. After verification, the verification results were entered in a timely manner to the software, and each subjects personal data was printed out for on-site examination. Survey settings were located in the community health center. Residents to attend the investigation were ask to first register and verify personal information, then receive symptom questionnaire, followed by going to the survey room. For TB suspects in the last 6 months, the investigators interviewed suspects using the “health care seeking behavior questionnaire”. After that, they were requested to provide three sputum samples for bacteriological analysis, receive chest radiology, and finally receive 15 yuan transport subsidies.

In this study, the seeking behavior questionnaire included six parts: general, no action, treatment situation, self-medication situation, TB-related knowledge, attitudes and behavior. "General" section included sex, date of birth, residence, educational level, occupation, marital status, monthly family net income, health insurance, etc. "No action" section included the reason for taking no action and the next intention. "Treatment situation” section included the reason to see doctor, the initial symptoms, time of onset of symptoms, the treatment process (including treatment time, treatment, medical institutions, diagnosis, treatment and prognosis of the situation, etc). "Self-medication situation" section included the reasons for self-medication, drug type, source and treatment; "TB-related knowledge and attitude and behavior" section included TB-related knowledge, attitudes and behavior.

Definition of variables

TB suspect was defined as an individual with a history of cough for 2 weeks or more, chest pain, difficulty in breathing or hemoptysis in the last 6 months prior to the investigation time.

Formal visitation was defined as visiting to community and outside health services. Self-medication referred to TB suspect without a physician's prescription choice, the use of drugs to deal with the symptoms of self-awareness to and disease.

Statistical analysis

The collected questionnaire would be checked by two different investigators. Data entry and check were done by Epidata3.0 databases. Chi-square test and multivariate logistic regression was used to analyze the risk factors of TB suspect’s health care seeking action. A two-tailed P<0.05 was used as the criterion of statistical significance. All statistical analyses were performed by Statistical Package for Social Sciences (SPSS) v18.0 software.

RESULTS

Status of health care action

A total of 270 questionnaires, after questionnaires of

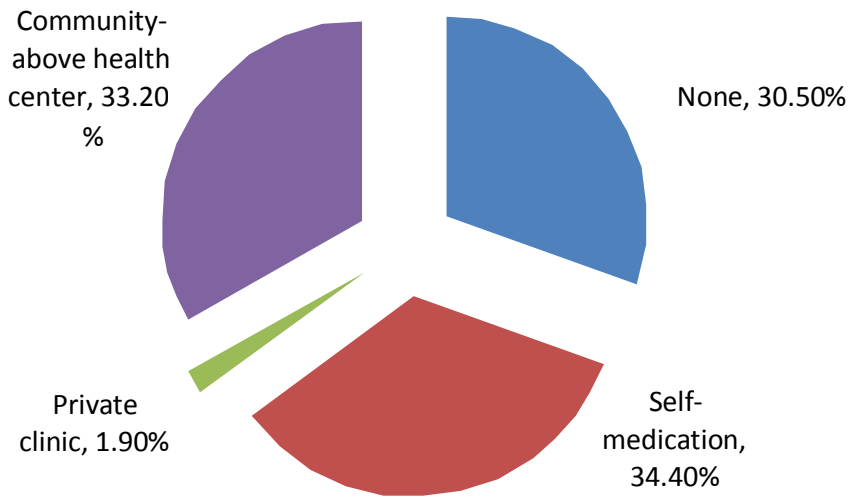

Figure 1. Action taking among TB suspects.

those not completed were cancelled, 259 questionnaires were eligible. The response rate was 95.9%. A total of 259 TB suspects (139 males and 120 females) were enrolled in this study, with mean age of 34.13±13.56 years (ranging from 15 to 85 years); 88.8% of the respondents had various forms of health insurance (public health, medical insurance for urban workers, the new rural cooperative health insurance, etc).

Majority, 180 (69.5%) of the TB suspects took different forms of health care actions for their symptoms. 86 (33.2%) visited community-above health center. 5 (1.9%) visited private clinic. 89 (34.4%) had self medication and 79 (30.5%) did not seek any form of health care for their symptoms (Figure 1).

Of the total 86 (33.2%) TB suspects who sought health care from medical health providers, only 15 (17.4%) were reported having given a sputum sample for Acid-Fast Bacilli (AFB) during their visit. Among 86 (33.2%) TB suspects who visited community-above health center, only 8 (9.3%) were reported to have been referred to the next level of health care for further investigation and management.

The clinical features

The most commonly reported symptom for the 259 pulmonary TB suspect was cough or expectoration (97.7%), others included hemoptysis or bloody sputum (7.3%), fever (19.7%), weight loss (10.4%), chest pain (14.3%), night sweats (7.3%), dyspnea (12.0%), and other symptoms (such as chest tightness, etc) (1.9%).

After the TB suspect having the aforementioned symptoms, 136 cases (52.5%) consider themselves to have cold, 15 cases (5.8%) had pneumonia, 50 cases (19.3%) had bronchitis or bronchitis, 7 cases (2.7%) had tuberculosis, 7 cases (2.7%) had asthma, three cases (1.2%) had emphysema, 23 cases (8.9%) had other reasons due to smoking, lit, etc.

Case detection

After the end of the field investigation, 7 in 259 cases were diagnosed with active pulmonary tuberculosis, 1 case with smear-positive pulmonary tuberculosis, and 1 case was a known patient who was in treatment. In 7 patients, duration of cough expectoration was 22 to 170 days. Among 7 patients, 2 cases did not take any form of health care finding, 2 cases took self-medication, and 3 cases went to medical units above the community level. In 7 patients, 1 case was referred by community medical institutions to TB control center, the rest were newly detected.

Reasons for seeking no health care

After the onset of symptoms, 79 cases (30.5%) did not have any form of health seeking behavior. The main reason for it (57 cases, 72.2%) was the self-inductance of less severity, thus there was no need for treatment or medication. Other reasons were economic difficulties in 7 cases (8.9%), being troubled (7 cases, 8.9%) and bad attitude of the doctor (1 case, 1.3%).

Reasons for self-medication

In 259 cases, 89 cases (34.4%) had self-medication. The main reason for choosing self-medication was self-inductance as mild disease and no need to see the doctor (37 cases, 41.6%). Other reasons were had experience (25 cases, 28.1%), suffered from a similar disease, according to doctors' previous prescription towards it to purchase medicaments (14 cases, 15.7%), economic difficulties (5 cases, 5.6%), had no time (6 cases, 6.7%), other (2 cases, 2.2%) such as feeling trouble, etc.

Table 1. Influential factors of formal visitation by univariable analysis.

Factor	Formal visitation			
	No	Yes	χ^2	P
Sex				
Male	84	36	1.035	0.309
Female	89	50		
Age (year)				
60~	58	32		
40~	66	33	0.549	0.760
15~	49	21		
Residence				
Migrant	20	12	0.304	0.582
Resident	153	74		
Education				
Junior middle school and lower	93	46	0.002	0.967
Senior middle school and above	80	40		
Occupation				
Unemployed/laid off	27	14		
Employed	61	32	1.211	0.750
Retired	63	33		
Student	22	7		
Marriage				
Divorced/widowed/separated	23	12	0.342	0.843
Married	121	62		
Unmarried	29	12		
Average per capita income (yuan)				
<1000	82	53		
1000~	67	26	3.825	0.148
>3000	17	6		
Health insurance				
With	24	5	3.752	0.053
Without	149	81		
Chronic or severe* respiratory symptoms				
No	139	41	28.927	0.000
Yes	34	45		
Score of TB-related knowledge				
<9	37	24	1.504	0.220
≥9	130	58		
Stigma				
No	95	59	5.290	0.021
yes	72	23		

Determinants of health care seeking behavior

Univariate analysis showed (Table 1) that the factors influencing the formal visitation were with chronic or severe respiratory symptoms (χ^2 = 28.927, P = 0.000) and no stigma (χ^2 =5.290, P = 0.021). Multivariate logistic analysis showed (Table 2) that the factors influencing the formal visitation were the presence of health insurance

Table 2. Influential factors of formal visitation by multivariable logistical regression analysis.

Factor	B	SE	Wald	P	OR	95%CI
Health insurance (yes=1, no=0)	1.225	0.616	3.955	0.047	3.405	1.018~11.392
Chronic or severe respiratory (yes=1, no=0)	1.601	0.340	22.210	0.000	4.959	2.548~9.652
Stigma (yes=0, no=1)	0.927	0.333	7.767	0.005	2.528	1.317~4.853

(odd ratio (OR)=3.405, 95% confidence interval (CI): 1.018 to 11.392), with chronic or severe respiratory symptoms (OR=4.959, 95% CI: 2.548 to 9.652) and no stigma (OR=2.528, 95% CI: 1.317 to 4.853).

DISCUSSION

This study found out that after the onset of symptoms among the TB suspects in communities, 30.5% had not taken any action, 33.2% visited community-above level healthcare center, 1.9% visited private clinic, and 34.4% used self medication. Tupasi et al. (2000) study on Philippines found out that 43.0% of TB suspects did not take any action, 31.6% took self-medication, and 25.4% went to various hospitals for treatment. In a study in rural India by Fochsen et al. (2006), 30.9% of the TB suspects did not take any action and 69.1% sought any form of medical care including self-medication.

After the onset of symptoms, 79 (30.5%) cases did not seek any form of health care. The main reason for it was that they considered that the symptoms were light and there was no need to have treatment or medication. The fourth national epidemiological survey of tuberculosis in 2000 and the fifth in 2010 showed that the first reason why TB suspects did not seek treatment was "they did not care", the ratio was 56.2 and 76.0%, respectively (National Technical Steering Group of the Epidemiological Sampling Survey for Tuberculosis, 2002, 2012). Rumman et al. (2008) also found out that the reason why 80.7% of TB suspects did not seek health care was because they thought that the symptoms were not severe. Thus, because cough, expectoration, and fever are very common in the population; most people will consider it as a cold and cold in most cases will not affect people severely. Therefore, we must make people aware of the seriousness of symptoms ("cough, sputum for two weeks") and raise the doubts of tuberculosis.

Our study found that when the suspects had chronic or severe respiratory symptoms such as hemoptysis or dyspnea, the possibility of TB suspects to seek health care was 4.959 (95% CI: 2.548 to 9.652) times as high as those without such symptoms. The results of this study were supported by the previous studies by Tupasi et al. (2000) study in Philippines, Nair et al. (1997) research in Mumbai, Ponticiello et al. (2001) in Italy and Lambert et al. (2005). The results were consistent with the results of Lewis et al. (2003) in London. TB suspects who had chronic cough and (or) other lung diseases are less likely to delay.

This study found out that health insurance is also an influential factor of TB suspects to seek health care. Those with health insurance were more likely to see doctor. Wang et al. (2008) also found out that TB suspects without health insurance often experience longer delay. Fei et al. (2006) study also found that health insurance affected the TB suspect's seeking health care behavior and patient delay. Uninsured people often had low income, and must pay for the treatment by themselves. Even if there is a case of cough and expectoration more than two weeks, people without health insurance usually delay treatment, even not to see doctor, or take way to relieve symptoms from self medication. Therefore, we should further expand health insurance coverage, and then prompt TB suspects to seek health care.

Our study also found out that stigma affected the presence or absence of TB suspects to take formal health care seeking action. The possibility to have a formal treatment of those without a sense of shame is 2.528 times as those with the sense of shame. A study by Yan et al. (2006) also showed a high frequent phenomenon in the common population that stigma or discrimination towards tuberculosis lead to fear and prejudice and to hinder the individual to seek professional health care, thus increasing the spread of TB in the population.

The present study has limitation. Our subjects were TB suspects in the last 6 months. Outcome variable of health care action taken was self-reported. Recall bias may exist. To minimize this bias, we asked about the onset of the major symptoms (cough) and how long did it take to take an action. In addition, we had used local calendar, listing the main festival and national days to confirm the date of symptom onset.

In addition, the sample size was calculated by simple random sampling size formula to be 3 clusters. Due to the use of probability proportionate cluster sampling method, the theory should expand the sample size to be 6 clusters. However, due to limited human, material and financial resources, in this study, only four clusters had been selected, which may lead to a certain degree of sampling error.

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Perception of the public on the common zoonotic diseases in Jimma, Southwestern Ethiopia

Dawit Tesfaye[1]*, Daryos Fekede[1], Worku Tigre[2] Alemayahu Regassa[1] and Amene Fekadu[1]

[1]School of Veterinary Medicine, Hawassa University, Ethiopia.
[2]College of Agriculture and Veterinary Medicine, Jimma University, Ethiopia.

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This study was conducted with the objective of assessing the perception of the public on common zoonotic diseases in Southwestern Ethiopia using a face-to-face interviewing technique. The respondents were stratified into four groups, namely: farmers (n=48), smallholder dairy farmers (n=44), butchers (n=34) and city residents (n=49). Many of them (97.1%) knew rabies was a zoonotic disease contracted via the bite and contact with saliva of a rabid dog. There was no statistically significant difference (P>0.05) in the level of awareness about rabies in the four respondents groups. Taeniasis was the second most recognised zoonotic disease (84.3%). Anthrax was known by 55.4% of respondents, whereas only 29.1% of them knew tuberculosis can be transmitted from cattle to humans. There was a significant (P<0.05) difference in the level of awareness with regard to zoonotic tuberculosis in the study groups where small holder dairy farmers had the highest awareness and traditional farmers had the lowest awareness. Majority of the respondents consume raw milk (66.8%), however, only 11.4% of them have knowledge about transmission of TB through raw milk and meat consumption. Awareness about echinococcosis was lower (4%) than other zoonotic diseases. The zoonotic importance of brucellosis was reported by none of the respondents. In general, the present study revealed a very low level of awareness by the public about major zoonotic diseases, signifying the need for public health promotion through education and inter-disciplinary one health approach with close collaboration among veterinarians, public health practitioners and policy makers.

Key words: Perception, public, transmission, zoonotic, disease, Ethiopia.

INTRODUCTION

The link among humans, animal populations and the surrounding environment is very close in many developing countries, where animals provide transportation, draught power, fuel, clothing and sources of protein (that is, meat, eggs, and milk). In the absence of proper care, this linkage can lead to a serious risk to public health with huge economic consequences (WHO, 2010).

Zoonoses are defined as those diseases and infections naturally transmitted between people and vertebrate animals (WHO, 2005). Zoonoses constitute a diverse group of viral, bacterial, rickettsial, fungal, parasitic, and prion disease with a variety of animal reservoirs, including wild life, livestock, pet animals, and birds (Nkuchia et al., 2007). The transmission may occur through direct contact with the animal, through vectors (such as fleas or ticks), or through food or water contamination (James, 2005). Globally, zoonoses are said to account for 60% of all infectious disease pathogens and 75% of all emerging pathogens (WHO, 2004). In both developing and developed countries, a number of new zoonoses have emerged. This might be the result of either newly discovered

*Corresponding author. E-mail: dawit89@yahoo.com

pathogens or agents that are already known, usually appearing in animal species in which the disease had not previously been detected (Jonathan and Joshua, 2006). Many diseases that affect humans which are new, emerging and re-emerging, were caused by pathogens that originated from animals. Moreover, a number of zoonotic diseases, including rabies, brucellosis, bovine tuberculosis and echinococcosis continue to affect humans and animals in many countries, particularly developing nations (Meslin et al., 2000).

It has been observe that 75% of emerging pathogens fall within the category of zoonotic diseases (WHO, 2005). Zoonotic diseases cause mortality and morbidity in people, while also imposing significant economic losses in the livestock sector. Their burden tends to fall most heavily on poor societies (WHO, 2005). They have both direct and indirect effects on livestock health and production (Smits and Cutler, 2004). Indirect effects occur as a result of the risk of human disease, the economic impact on livestock producers through barriers to trade, the costs associated with control programmes, the increased cost of marketing produce to ensure it is safe for human consumption, and the loss of markets because of decreased consumer confidence (McDermott and Arimi, 2002).

Different studies conducted so far on animals from different districts of Jimma zone indicated the occurrence of zoonotic diseases. For example, Tolosa et al. (2009) reported the prevalence of 2.93 and 31.44% in Jimma area for *Taenia saginata* metacestodes and hydatid cysts, respectively. Prevalence values ranging from 2.93 to 4.4% was reported for bovine cysticercosis in cattle slaughtered at a Jimma abattoir (Megersa et al., 2010; Tolosa, 2010). Whereas, herd level and individual animal level prevalence of bovine tuberculosis was found to be 48.6 and 21.4%, respectively in and around Jimma (Tigre et al., 2012). Moreover, information from Jimma town health center and Jimma zone health bureau showed that rabies was one of the ten listed health problems in the area (personal communication). According to WHO (2011), rabies is a wide spread zoonotic disease that is found on all the continents, but more than 95% of human deaths occur in Asia and Africa.

The perception of the community towards zoonotic diseases plays an important role for the maintenance of life cycle and transmission of these diseases to the different arrays of their hosts. Studying the perception of the community on the risk factors, routes of transmission and life cycle of zoonotic diseases is a crucial step towards the development and implementation of appropriate disease prevention and control strategies. Though, the aforementioned zoonotic diseases were reported and found to be prevalent in Jimma and its surroundings, there was no study performed to assess of the overall perception of the community towards major zoonotic diseases in the area. Therefore, the objective of this study was to assess the public's perception regarding the major zoonotic diseases in and around Jimma town, Ethiopia.

MATERIALS AND METHODS

Study area

The study was conducted in Jimma town and its surrounding villages from November, 2011 to April, 2012. Jimma town, which is the capital of Jimma zone, is located in Oromia Regional State at 346 km South West of Addis Ababa. The town has a latitude of about 7°36° to 8°N and longitude of about 35°52 to 37°37° E, and an elevation ranging from 880 to 3360 m above sea level. The area receives a mean annual rainfall of about 1,530 mm, which comes from long and short rainy seasons. The average minimum and maximum annual temperature ranges between 14.4 and 26.7°C, respectively. According to CSA (2009), Jimma town has a total population of 120,600.

Study design and subjects

A questionnaire based cross-sectional study design was employed to look on the perception of the public on zoonotic diseases, that is, rabies, tuberculosis, anthrax, brucellosis, taeniasis and echinococcosis. The study populations were residents of Jimma town and its surrounding. The respondents were stratified into four groups, namely, farmers, smallholder dairy farmers, butchers, and city residents. Accordingly, 48 farmers, 44 smallholder dairy farmers, 34 butchers and 49 city residents were included in the study. The farmers included in the study were found in the villages around Jimma town and practice the mixed crop-livestock production system. Smallholder dairy farmers are found inside Jimma town and they are dependent on milk sell for their livelihood.

Sampling method

A simple random sampling method was employed to select the respondents. Respondents were selected during questionnaire administration in different parts of the town and peasant associations around the town. During the questionnaire administration, any member of the households who was willing to participate in the interview was taken as a sampling unit.

Study methodology

A semi-structured questionnaire was pre-tested and used for the face-to-face interview to evaluate the perception of the community about the common zoonotic disease (Unger and Munstermann, 2004). The two common local languages (Amharic and Oromiffa) were used for the interview. On average, 30 min were spent with each respondent. The questionnaire contains questions that can evaluate the perception of the respondents about zoonotic diseases' importance, their transmission cycle and major clinical signs in humans and animals. In addition, the respondents were also asked questions regarding the use of traditional medicine for the treatment of zoonotic diseases in their area. They were also asked if they had encountered anyone infected and/or that had died of zoonotic disease. According to the Oxford dictionary, perception, in this research is defined as the way in which something is regarded, understood, or interpreted.

Data management and analysis

The data collected was properly coded and entered into a spreadsheet. Descriptive statistics, namely, mean, frequencies and

Table 1. Socio-demographic characteristics of the study participants.

Characteristic		Number	%
Age group	15-34	66	27.9
	35-49	73	46.3
	50-64	30	18.7
	> 64	6	7.1
Sex	Male	123	71.0
	Female	52	29.0
Educational status	Illiterate	32	18.4
	Basic education	143	81.6

Table 2. Mode of transmission of rabies perceived by respondents.

Transmission	Mode of transmission		
	Bite	Contact with saliva	Inhalation
	n (%)	n (%)	n (%)
Dog to dog	161 (92)	21 (12)	15 (8.6)
Dog to human	165 (94.3)	23 (13)	23 (9.7)

percentages were made to summarize the results using STATA Corp (2009). Logistic regression was used to see the influence of the different factors on the level of awareness on major zoonotic diseases. The knowledge of the importance of major zoonotic diseases was presented in the form of binary variable (yes=1 and no=0) and taken as the dependent variable, whereas occupation, sex, religion and level of educations were taken as explanatory variables. A P-value <0.05 was considered to represent a significant difference.

RESULTS

Socio-demographic characteristics of respondents

All of the rural residents were people living in villages surrounding Jimma town, whereas butchers, smallholder dairy farmers and city residents were found in Jimma town. Males accounted for 71% (123) and females 29% (52) of the respondents. The highest numbers of respondents were in age group 35 to 49 years. Regarding the educational level, 81.6% completed primary, secondary or higher education (Table 1).

Knowledge and awareness about the common zoonotic diseases

Rabies

About 97.1% of the respondents said they were familiar that rabies can be transmitted from dogs to humans. The study revealed that about 95.8, 97.7, 97, and 89.8% of the traditional farmers, small holder dairy farmers, butchers, band city residents interviewed, respectively, know that rabies is a zoonotic disease transmitted to humans. The knowledge of rabies as a zoonotic disease among the different respondent groups was not sig-nificantly (P>0.05) different. The modes of transmission perceived by respondents were bites, contact with saliva, and inhalation (Table 2). The fate of an untreated person bitten by a rabid dog, as perceived by respondents, was death (82.8%), madness (33.7%), behavioral change (29%), and development of puppies in the abdominal cavity of the person (20%), but 3.43% of the respondents said they do not know what would happen to the person.

The major clinical signs of a rabid dog mentioned by respondents were salivation, biting humans and other animals, and dropping of tail (Table 3). The major combination of signs mentioned by the respondents was salivation plus human bites (Table 4). Among the persons interviewed, 46.2% of them encountered a rabid dog at least once in their life in their surroundings, and 28% of the respondents experienced a dog bite in their family.

Bovine tuberculosis

Of the total respondents, only 29.1% of them knew that tuberculosis can be transmitted from cattle to humans. The knowledge of tuberculosis as a zoonotic disease in the respondents interviewed was 50% in smallholder, 34% in city residents, 20.6% in butchers and 10 .4% in traditional farmers. The awareness of respondents about the zoonotic importance of bovine tuberculosis was significantly higher (P<0.05) in smallholder dairy farmers

Table 3. The clinical signs of rabies mentioned by the respondents.

Clinical sign	No. of respondents	%	Standard error
Salivation	63	36.5	3.65
Bite humans	62	35.6	3.64
Tail dropping	53	30.5	3.49
Bite animals	47	27	3.37
Wandering	44	25.2	3.30
Aggressiveness	35	20	3.04
Reddening of eyes	13	7.5	1.99
Madness	12	6.8	1.92
Hydrophobia	11	6.3	1.85
Anorexia	5	2.8	1.27
*Hb+Sal	48	27.4	3.4
*Ab+Hb	46	26.3	3.3
*Sal+Td+Hb	29	16.5	2.8
*Hb+Sal	15	8.5	2.1

*Combination of signs mentioned by the respondents: Sal=salivation, Td=tail dropping, Hb=human biting, Ab= animal biting.

Table 4. Proportion of respondents having awareness about transmission of tuberculosis through consumption of raw meat and milk.

Respondents' category	Number asked	Number having awareness	%	SE	P-value
Farmers	48	5	10.4	4.4	-
Smallholder dairy farmers	44	22	50	7.6	0.00
Butchers	34	7	20.6	7.0	0.207
City residents	49	17	34.7	6.8	0.007

Table 5. Mode of transmission of anthrax from animals to humans reported by the respondents.

Mode of transmission	No. respondents	%	SE
Consumption of raw meat	96	54.9	1.13
Contact	25	14.3	2.65
Inhalation	4	2.3	3.77

followed by city residents (Table 5). However, the habit of raw milk consumption was significantly higher in traditional farmers ($P<0.05$) (91.6%) as compared to 54.5% in smallholder dairy farmers, 58.8% in butchers and 59.2% in city residents. The risk of raw meat and milk consumption as perceived by respondents were diarrhea (unspecified cause), tuberculosis, typhoid, amoeba and taeniasis.

Taeniasis (*Cysticercus bovis*)

The overall proportion of respondents having knowledge of taeniasis as a zoonotic disease was 83.4%. Taeniasis was perceived by 94.1% of butchers, 90.9% of small holder, 87.7% of city residents and 64.5% of the traditional farmers as a zoonotic disease transmitted to humans when raw meat is consumed. In addition to consumption of raw meat, 31% of the respondents reported that it can be transmitted by consumption of raw milk. The infection of cattle by the eggs of *T. saginata* was known only by 27.45% (48/175) of the interviewed persons. Regarding consumption of raw meat, 69.1% (121/175) of the respondents have the habit of raw meat consumption. Among those who consume raw meat, 82.6% (100/121) of them have been infected by *T. saginata* at least once previously.

Infection of *T. saginata* was 66.6, 82.1, 87.1 and 90.3% in the traditional farmers, small holder dairy farmers, butchers and city residents, respectively. The infection rate in the farmers was significantly ($P<0.05$) lower than that of the city residents, whereas sex, level of education

and religion of respondents did not show significant level of variation in the prevalence of taeniasis. However, the probability of getting infected was observed to be associated directly and significantly (P<0.05) with age.

Many of the respondents (77.7%) mentioned that traditional medicine was effective for *T. saginata*. Traditional medicine mentioned by respondents was "Enkoko" (*Embelia schimperi*), "kosso" (*Hagenia abyssinica*) and seed of pumpkin. The knowledge of traditional medicine for *T. saginata* in the four respondent groups was not significantly different (P>0.05).

Echinococcosis

In the study area, *Echinococcosis* was known only by 4% (7/175) of interviewed persons as a zoonotic disease transmitted from dogs to humans. However, 19.4% (34/175) of the respondents said they had noticed parasitic disease in dogs, but had no information whether it can be transmitted to humans or not. The clinical signs they mentioned for parasitic infestation in dogs by respondents were reduced appetite, vomiting, excretion of adult parasites and/or proglotides of cestodes with feces and eating grass. Among the persons interviewed, 56% (98/175) owned dogs, but only 25.5% (25/98) of them vaccinate their dogs regularly. Moreover, 31.6% of them allow their dog to roam outside their compound. The feeding practice of dog owners was also assessed and the result showed that 41.8% (41/98) of the dog owners feed raw condemned visceral organs without cooking or checking for any abnormality on the organs.

Anthrax

Anthrax was known as a zoonotic disease transmitted to humans by 51, 51, 67.6, and 79.6% of farmers, small holders, butchers, and city residents, respectively. The modes of transmission reported by respondents were consumption of raw meat from infected animal, contact, and inhalation (Table 5). Most of the respondents reported that consumption of meat from infected animals was the major source of infection for humans.

Brucellosis

Respondents were asked if they were aware of a disease that can cause abortion in cattle and transmitted to humans. But none of the respondents were aware of such a disease.

DISCUSSION

The most frequently known zoonotic diseases among the respondents in the study area were rabies (97.1%), followed by taeniasis (83.4%), anthrax (55.4%), bovine tuberculosis (29.1%) and hydatidosis (4%). This study indicated a relatively lower level of awareness of the respondents in the study area as compared to the report of Girma et al. (2012) who indicated that all respondents in Addis Ababa mentioned rabies as a zoonotic disease, followed by anthrax (94.27%), taeniasis (89.06%), bovine tuberculosis (88.54%) and brucellosis (49.48%). The difference in the overall awareness between the two study sites for the common zoonotic diseases could be due to variations in the living style between the two settings, where in Addis Ababa, as a capital city, information might be acquired more easily than in the current study area.

With regard to the perception of rabies as a zoonotic disease, there was no significantly (P>0.05) different level of awareness among the different respondent groups which can imply that rabies is a well known disease in the area. This is evidenced by the fact that a higher proportion (94.3%) of the respondents knew the means of transmission of rabies from dog to humans. Moreover, 82.9% of the respondents said that untreated cases of rabies in humans can result in death. Another 25.7% of the study participants reported that at least one family member was bitten by a suspected rabid dog previously. This is in line with the work of Girma et al. (2012) who reported rabies as the most frequently (100%) mentioned zoonotic diseases in Addis Ababa. However, 20% of the respondents in the current study seem to misunderstand the consequence of untreated rabies cases in humans where development of puppies in the abdominal cavity of the person was mentioned. This signifies the need for awareness creation about rabies in the area. Among the respondents who owned dogs, only, 25.6% of them reported that they were treating or vaccinating their dogs. The poor management of owned dogs and the presence of high populations of unvaccinated stray dogs are responsible for the frequent occurrence of rabies (Deressa et al., 2010). Globally, about 55,000 persons die annually due to rabies where rabid dog bites account for 99% of the infection (WHO, 2011).

Of the total respondents, only 29.1% of them knew tuberculosis can be transmitted from cattle to humans. This is in line with the work of Amenu et al. (2010) who reported that a high number of respondents had no detailed and accurate knowledge about zoonotic tuberculosis. In this study, inhalational route and consumption of raw milk and meat were mentioned as transmission routes from cattle to humans. There was a significant (P<0.05) difference in the level of awareness with regard to the zoonotic tuberculosis in the study groups where small holder dairy farmers had the highest awareness and traditional farmers had the lowest awareness. However, traditional farmers were found as the major consumers of raw milk. This might put traditional framers as the most vulnerable group as far as zoonotic tuberculoses is concerned. The relatively higher level of awareness among the smallholder dairy farmers could be attributed to their living inside the town that might facilitate to have information about the zoonotic importance of bovine tuberculosis from private veterinary practitioners. Ashford

et al. (2001) stated that in countries where bovine tuberculosis is common and pasteurization of milk has not been practiced widely, an estimated 10 to 15% of human tuberculosis cases are caused by *Mycobacterium bovis.*

The overall proportion of respondents having the knowledge that taeniasis is a zoonotic disease was 83.4%. Raw/undercooked meat consumption as a vehicle for transmission of taeniasis to humans was indicated by 82.3%. But 31% of them said raw milk can also transmit *T. saginata* which shows lack of knowledge about the disease to some extent. Respondents in the current study area seem to have relatively lower awareness about *T. saginata* as compared to a study conducted at Arsi-Negele district, Southern Ethiopia, by Amenu et al. (2010) who indicated that 96.3% of the respondents knew that raw meat was a vehicle for disease transmission to humans. The difference could be due to lack of information about the disease in the current study area.

However, raw meat consumption in the area is wide spread (69.1%) and 82.6% of the respondents who consume raw meat had *T. saginata* previously. An infection rate of 64.2% was reported in a study conducted by Abunna et al. (2008) at Hawassa town. The level of infection in the different educational levels, sex and religious groups was not significantly different. This could be due to the deep rooted culture of consuming raw meat in the different social groups of the country, even by highly educated individuals (Abunna et al., 2008). However, the probability of infection was observed to rise with increasing age. This could be attributed to the fact that elderly individuals have the chance to consume more raw meat in different social ceremonies such as wedding and other local holy days, where raw meat is one of the major food items served almost everywhere in the country. A similar finding was reported by Megersa et al. (2010) in Jimma where older people were more infected with *T. saginata* than the younger ones. Raw meat consumption is practiced in some parts of the world as a cultural heritage passed through many generations. Countries like Russia, Cuba and many social groups on the African continent are known to consume raw and/or undercooked meat (Sua´rez and Santizo, 2005). In spite of the relative higher perception of the respondents about the zoonotic importance of taeniasis, the consumption rate of raw meat and also the infection rate of taeniasis is found to be high. This seems to be attributed to the deep rooted culture of raw meat consumption in the country. To safe guard the public, an intensive awareness creation programs should be undertaken in the area regarding the danger of raw meat consumption which not only predispose to taeniasis , but also to other very serious food borne pathogens like anthrax and bovine tuberculosis.

In the current study, only a small proportion (4%) of the participants had an awareness of echinococcosis. This disagrees with the work of Tigre (2012) who reported that 32.2% of the study participants had an awareness of echinococcosis. The variation in the level of awareness could be due to the difference in the study groups, where the previous study was conducted only on butchers and abattoir workers who might be familiar with the problem unlike our study groups which incorporates a variety of respondents. The awareness level of participants in this study was similar to that reported by Kebede et al. (2010) and Zelalem (2012) who indicated an awareness level of 0 and 8% of the households had awareness about zoonotic echinococcosis, respectively. The lower level of awareness about echinococcosis could also be due to the longer incubation period of the disease in humans, in which it takes up to 30 years to manifest clinical signs (CFSPH, 2011).

In this study, 25.5% of the participants owned dog(s) and 56% of them said they let their dogs freely roam outside their compound. The presence of large numbers of non-restricted dogs plays a crucial role not only in transmission of rabies but also in contaminating the environment with tapeworm eggs which could subsequently infect humans. Among the dog owners, 68.9% of them reported that they fed offal to their dogs regardless of the safety status of the offal. Feeding the viscera of infected slaughter animals to dogs was reported to facilitate the transmission of the sheep strain of *Echinococcus granulosus* and this was suggested to consequently increase the risk that humans will become infected (Moro and Schantz, 2009). According to a study conducted by Carmena et al. (1998), the type of feed given to dogs was found to significantly affect the prevalence of cystic echinococcosis in humans. Tigre (2012) reported that only 4.3% of the dog owners treat their dogs with anthelminthic drugs periodically around Jimma area. In order to eliminate the infection risk to humans living in close association with the infected dog, either euthanasia of such dogs or chemotherapy under strict safety precaution has been recommended (Ekerte and Peter, 2004).

The knowledge of the study population about the zoonotic importance of anthrax was limited. Transmission through the consumption of raw meat was mentioned by 54.9% of the respondents, whereas contact and inhalational transmission routes were only mentioned by 14.3 and 2.3% of respondents, respectively. The low level of awareness about the zoonotic importance of anthrax in the area is of concern given that the disease is endemic in most part of the country and the case fatality rate is very high both in humans and animals. The wide spread culture of raw meat consumption combined with the lower level of awareness about anthrax seems to put the public at a greater risk of contracting the disease.

Among the six common zoonotic diseases, only brucellosis was not known by any one of the interviewed persons. This is in contrast to Mihiret-ab (2012) who reported that 5.6% of the respondents were aware of the zoonotic importance of brucellosis in and around Dire Dawa. The absence of awareness in the present study

area might be due to poor or absent awareness creation activities that should have been given by the public health bureau of the area and the veterinary department.

Conclusively, the public awareness about some common zoonotic diseases and their means of transmission, especially bovine tuberculosis, echinococcosis, anthrax and brucellosis was very low. The level of awareness about rabies in this study was good but improvements are needed on the management and proper handling of dogs. Awareness should be created in the public regarding the life cycles of the common zoonotic diseases in the area. The public health department of the ministry of health should give due emphasis for public educating and awareness creation on preventive measures for the existing zoonotic diseases in the area at the grass root level using the health extension workers.

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Full Length Research Paper

Profile and outcome of diabetic admissions at the University of Uyo Teaching Hospital, Uyo

B. C. Unadike[1]*, I. Essien[1], N. A. Akpan, E. J. Peters[2] and O. E. Essien[2]

[1]Department of Internal Medicine, University Of Uyo Teaching Hospital, Uyo, Akwa Ibom State, Nigeria.
[2]Department of Medicine, University of Calabar Teaching Hospital, Calabar, Nigeria.

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The aim of this study was to determine the morbidity and mortality pattern amongst patients with diabetes mellitus (DM) at the University of Uyo Teaching Hospital Uyo, Nigeria over four consecutive years (June 2004 to June 2008). This study was a retrospective study. Data was obtained from the records register in the medical wards of the hospital. These records registers included admission and discharge records as well as outcome of the individual cases. Data extracted included age, sex, indication for admission and outcome. A total of 407 patients with DM were admitted during the period under review. Uncontrolled DM was the commonest indication for admission accounting for 62.1% of the total admissions. Hyperglycaemic emergencies (diabetic ketoacidosis and hyperosmolar non-ketotic coma (Honk)) accounted for 18.7% of the total admissions. The overall mortality rate was 8.1%. HONK was the commonest cause of death accounting for 24.2% of the total deaths recorded. The mortality rate of diabetic ketoacidosis was 10.3%, while for HONK it was 30.3%. DM is an important cause of morbidity and mortality in Uyo. Adequate health/diabetic education needs to be given to diabetic patients to enable them take care of their illness to prevent the poor outcome associated with the disease.

Key words: Outcome, morbidity, mortality, diabetes, Uyo.

INTRODUCTION

Diabetes mellitus (DM) is an important cause of morbidity and premature mortality in patients who have the disease and accounts for at least 10% of total health care expenditure in most countries (Centres for Disease Control and Prevention 2005). People with diabetes are at increased risk of death from causes specific to diabetes like diabetic ketoacidosis (KDA), from cardiovascular disease like ischaemic heart disease and from other causes including infectious diseases (Geiss et al., 1995; McEwen et al., 2006).

Diabetes is a leading cause of morbidity and mortality, because of its role in the development of renal, cardiovascular, neuropathic and eye complications. Approximately 987,000 deaths in the year 2002, which was 1.7% of the total world mortality was attributed to diabetes by the World Health Organization (WHO 2003).

Cardiovascular causes have been reported as the greatest cause of mortality in these patients and diabetic nephropathy contributes significantly, as one in every three persons undergoing dialysis in the United States of America is a diabetic patient (Diallo et al., 1997).

This study analysed the outcome of diabetic admissions at the University of Uyo Teaching Hospital.

MATERIALS AND METHODS

This study was a retrospective study carried out in the University of Uyo Teaching Hospital. Medical records of patients admitted on account of diabetes during the period under review were analysed. Data was obtained from the records register in the medical wards

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

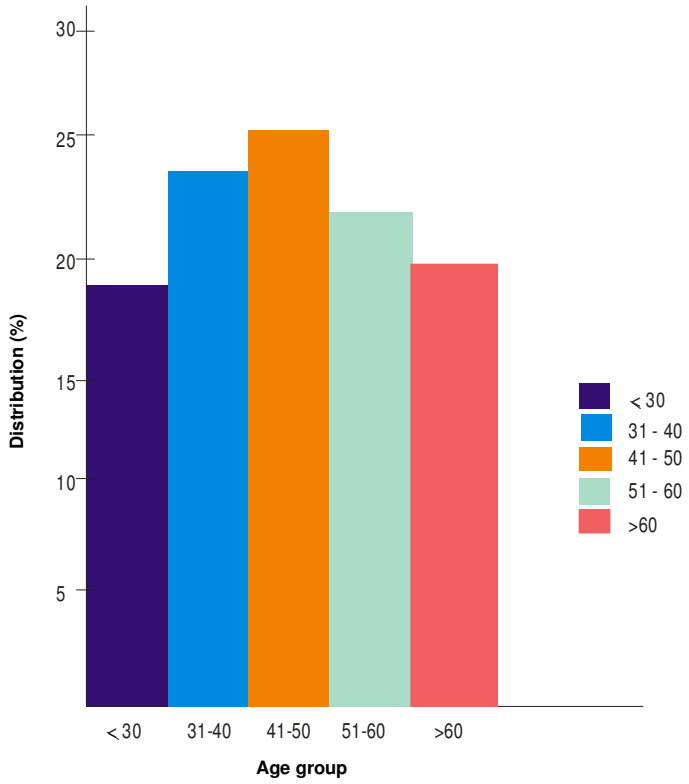

Figure 1. Age distribution of patients with diabetes mellitus.

Table 1. Mortality pattern of diabetic patients.

Cause of death	No	%
HONK	8	24.2
DKA	5	15.1
Uncontrolled DM	4	12.1
CVD	2	6.0
Hypertensive heart disease	3	9
Hypoglycaemia	3	9
DM Nephropathy	2	6.0
DM Foot Ulcer	3	9.0
Septicaemia	1	3.0
Pneumonia	1	3.0
Retroviral disease	1	3.0
Total	33	100

HONK: Hyperosmok Non ketotic Coma; DKA: diabetic keto Acidosis; CVD: cerebrovascular disease.

which included admission and discharge records.

Data collected included age, sex, indication for admission and outcome. The study was approved by the Ethical Committee of the University of Uyo Teaching Hospital. Descriptive statistics such as means and standard deviation was used to summarize qualitative variables while quantitative variables were summarized using percentages. Chi-square test was used to test the relationship between proportionate variables. Data analysis was done using Statistical Package for Social Sciences (SPSS) version 13. Results are presented in tabular form.

RESULTS

There was a total of 407 diabetic patients admitted during the period under review (188 females and 219 males). The mean age of the study subjects was 43.5±13 years. Majority of the patients were between 31 and 50 years of age (47.3%). Most patients were in the 41 to 50 year age range (24%) followed by the 31 to 40 years (23.3%), ≤ 30 years (18.6%), 51 to 60 (17.9%) and > 60 years (15.9%) (Figure 1). Uncontrolled DM accounted for 62.1% of the admissions (55.7% in males and 69% in females), while hyperglycaemic emergencies (KDA and hyperosmolar non-ketotic coma (HONK)) accounted for 18.75% of the total admissions (20.2% in females and 17.3% in males). The gender difference in admissions (219 males versus 188 females) is statistically significant ($P<0.05$)

The total number of deaths in this period was 33 (12 females and 21 males) giving a mortality rate of 8.1% overall (6.3% for females and 9.5% for males). The gender difference in diabetic deaths (21 males versus 12 females) is statistically signuificcant ($P<0.05$). The commonest cause of death was HONK accounting for 24.2% of all deaths. It was also the commonest cause of death amongst the male gender (28.5%), while DKA was the most common cause in females (33.3%).

The mortality for DKA in the study was 10.3% (16.6% for females and 4% for males), while the mortality for HONK was 30.3% (15.3% for females and 45.4% for males). The mortality for DM foot ulcer was 17.6%.

Out of the 407 patients admitted, 33 died, 364 were discharged and 10 took their discharge against medical advice. No change was identified in the pattern of diabetic morbidity and mortality over the years as uncontrolled DM was the commonest cause of morbidity and hyperglycaemic emergencies (DKA and HONK) were the commonest cause of mortality over the years in the period under review. The mortality pattern and outcome of the diabetic admissions are both shown in Tables 1 and 2, respectively.

DISCUSSION

DM is the sixth leading cause of death in the United States and age adjusted mortality in people living with diabetes is approximately twice that of people without diabetes (Anderson et al., 2001; Geiss et al., 1995). Studies from other countries have also confirmed this excess mortality attributable to diabetes (Roskinen et al., 1998; Weiderpass et al., 2001; de Marco et al., 1999). In Nigeria, non-communicable diseases, of which diabetes is one are a major cause of morbidity and mortality (Akinkugbe et al., 1997).

More males than females were admitted giving a male

Table 2. Outcome of diabetic admissions.

Event	Number	%
Death	33	8.1
Discharged	364	89.4
DAMA	10	2.4
Total	407	100

DAMA: Discharge against medical advice.

to female ratio of 1.1:1. The reason may be that males are more economically empowered than females and hence will seek treatment earlier than their female counterparts. This male preponderance is also reported amongst other non communicable diseases (Akinkugbe et al., 1997; Kadiri, 2005).

Uncontrolled DM accounted for 62.1% of the admissions. In our environment, most patients do not readily accept the fact that diabetes is a chronic illness and treatment is life long. Poor drug compliance, lack of financial wherewithal, and poor access to medical facilities may all compound this problem. Many studies have highlighted poor glycaemic control amongst persons with DM (Azab, 2001; Qari, 2005). Poor glycaemic control would necessitate admitting these patients for effective control of their hyperglycaemia.

Adequate health education should be given to patients on the long term implication of hyperglycemia usually resulting from non compliance with medications.

Hyperglycaemic emergencies (DKA and HONK) accounted for 18.7% of the total admission. This is also a reflection of poor-metabolic control in these patients, who subsequently present as diabetic emergencies. This reinforces the need for adequate health education of these patients.

HONK was the most common cause of death in the study population. HONK is usually a complication of type 2 DM accounting for about 10% of all hyperglycaemic emergencies (Rolfe et al., 1995). It however carries a high mortality since most of the patients are elderly and have other co-morbidities. The mortality in this study was 30.3%. In a similar study in South Africa, a much higher figure of 44% was reported (Rolfe et al., 1995). Early recognition and prompt treatment at peripheral clinics before subsequent referral will reduce this mortality seen.

DKA is a common diabetic emergency and carries with it, a relatively high mortality ranging from 25 to 33% in reported series from East Africa (Rwiza et al., 1986). The mortality rate in our series is 10.3%, which is still higher than the accepted mortality rate of 5 to 10%. Lack of access and high cost of insulin, delays in seeking medical attention, misdiagnosis and poor diabetic care are all contributory factors to this attendant mortality.

HONK was also the commonest cause of death in males, while DKA was the commonest cause in females in our study. In two studies from East Africa, most deaths were due to infections and hyperglycaemic emergencies (Castle and Wicks 1980; McLarty et al., 1990). Our findings agree with these reports.

Hyperglycaemic emergencies (HONK and DKA) contributed to 39.3% of the total deaths in our series. Most of these deaths would have been prevented. There are other similar other reports from within Nigeria and other places in Africa (Ogbera et al., 2002; Ahmed et al., 2000; Corrigan and Ahren 1968). The poor state of our health facilities generally is responsible for most of these preventable deaths. Provision of facilities for monitoring of blood glucose and electrolytes at the emergency centres will result in a reduction in the attendant mortality due to hyperglycaemic emergencies.

Diabetic foot ulcer is the leading cause of non-traumatic amputation and contributes significantly to diabetic morbidity and mortality (Ogbera and Ohwovoriole, 2003; Unachukwu et al., 2005). Nine percent of the total death in this study was attributed to it and most of these deaths however, can be reduced with good diabetic foot care services and education (Shaw and Boulton, 1999). Cerebrovascular disease (CVD) was responsible for 6% of the deaths. DM is a risk factor for CVD, and in co-existence with other risk factors like hypertension, dyslipidaemia, obesity and considering the fact that our patients are blacks significantly increase the risk of CVD. Efforts must be put in place to address each of these risk factors in order to reduce the risk of CVD and subsequent mortality in these patients (Olefsky, 2000).

With the increasing incidence and prevalence of DM, and the fact that diabetes is an important cause of chronic kidney disease, and with one out of every three patients on dialysis in the United States being a diabetic patient, the contribution of diabetic nephropathy cannot be under-estimated (WHO, 2003; Dirks and Robinson 2006). It was responsible for 6% of the total death in this study. However, in a resource poor setting like ours, with apparent lack of facilities for renal replacement therapy, and its high cost if available, our strategy should be primary prevention with emphasis on adequate control of DM, hypertension and other risk factors for renal disease.

Hypoglycaemia was a cause of death in 3% of the cases. Hypoglycemia is a serious complication of treatment in patients with diabetes. This is usually due to sulphonylurea or insulin therapy. In a South African study the major cause precipitating hypoglycaemia included a missed meal, alcohol use, gastro intestinal upset and inappropriate treatment (Gill and Huddle, 1993). In a Nigerian study, hypoglycaemia was responsible for 10.2% of the total deaths reported among diabetic patients (Unachukwu et al., 2006).

The limitation of this study was the fact that data on the diagnosis was obtained from the records register in the medical wards. Most patients may have developed other complications like hypoglycaemia which could have been responsible for their deaths, but this was not reflected in the ward register.

Conclusion

DM is a major cause of morbidity and mortality and contributes significantly to the burden of disease in Nigeria. Uncontrolled DM is the commonest cause of morbidity, while hyperglycaemic emergencies (DKA and HONK) are the commonest causes of death in patients with diabetes in Uyo.

RECOMMENDATION

Adequate health education is recommended for diabetic patients to reduce the morbidity and mortality associated with this disease. Facilities for adequate management of diabetes and its complications should also be provided for improved outcome. Medications used for its treatment should be made more affordable and available.

There is also need to ensure that the care and treatment of diabetes is covered by the National Health Insurance Scheme.

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Full Length Research Paper

Awareness and attitudes to voluntary counseling and testing (VCT) for human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) among law undergraduates in tertiary institutions in Anambra State, Southeast, Nigeria

S. U. Mbamara*, N. J. A. Obiechina and J. C. Akabuike

Department of Obstetrics and Gynaecology, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria.

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Nigeria, like other sub Saharan African countries, is heavily affected by human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), and the young people have the highest prevalence. It is estimated that less than one in ten people in sub Sahara Africa know their HIV status despite the fact that the present HIV intervention packages depend on the knowledge of HIV status. Voluntary counseling and testing (VCT) for HIV/AIDS is a concept that is designed to address this gap. This study was conducted to determine the knowledge and attitudes of VCT for HIV/AIDS among undergraduates of law faculty in tertiary institutions in Anambra state southeast Nigeria. This is a cross sectional questionnaire based survey conducted among law undergraduates in Anambra state using a multistage random sampling method. The respondents were all full time law students and were aged between 15 to 42 years, with a mean of 21.06 ± 3.0 years and a modal age of 20 to 24 years. Two hundred and ten (71.4%) of the students were aware of VCT for HIV/AIDS while eighty four (28.6%) of the respondents had no knowledge of it. The commonest sources of information about VCT for HIV/AIDS were electronic media 114 (38.8%), followed by churches 67 (22.8%) and print media 44 (15.0%). Although majority of respondents had positive attitude towards VCT for HIV/AIDS, 20% of them disapproved of VCT and this is quite high considering their educational status. Public health education, awareness programmes and VCT centers dedicated to young people should be established.

Key words: Voluntary counseling and testing (VCT), awareness, law undergraduates, attitude, southeast, Nigeria.

INTRODUCTION

Voluntary counseling and testing (VCT) has been recognized as an integral element of effective HIV public health primary prevention and care programme (Solomon et al., 2008). In the context of HIV/AIDS, VCT has been described as a confidential dialogue between the counselor and the patient, with the aim of assessing the risk level and encouraging the making of personal decisions to prevent infection or to enable them cope with stress and other problems related to HIV infection (SOGON, 2005). It has been estimated that each day, 7,000 people between the ages of 15 to 24 years are infected with HIV worldwide resulting, in 2.6 million new in

*Corresponding author. E-mail: Kensunny30@yahoo.co.uk. Tel: +234 8033963252.

Table 1. The biosocial characteristics of the respondents.

Sex distribution	Frequency N = 294	Percentage (%)
Male	138	46.9
Female	156	53.1
Age distribution		
15-19	48	16.3
20-24	168	57.1
25-29	54	18.4
30-34	0	0
35-39	6	2.0
≥40	18	6.1
Marital status		
Single	276	93.9
Married	18	6.1

Table 2. The sources of information about VCT for HIV/AIDS.

Source	Frequency	Percentage (%)
Electronic media	114	38.8
Churches	67	22.8
Print media	44	15.0
Friends/peers	25	8.5
Health care providers	20	6.8
Schools	18	6.1
Relatives	6	2.0
Total	294	100.0

fections per year; 1.7 million of which occurs in Africa (McClure et al., 2004).

In Nigeria, it is currently estimated that the highest seroprevalence rates of 4.2 to 9.7% is among the youths (15 to 24 years) (NACA, 2001). Similar to the Joint United Nations Programme on HIV/AIDS (UNAIDS) report (Bateganya et al., 2007) in most parts of sub Saharan Africa, fewer than one in ten people are aware of their HIV status (Matovu and Makumbi, 2007). However, many of the current interventions to prevent HIV/AIDS require people to know their status and hence require HIV testing. VCT therefore is a vital point of entry to the HIV/AIDS services including prevention of and cli-nical management of HIV and HIV related illness including, tuberculosis (TB) control, psychological and legal support and prevention of maternal to child trans-mission of HIV. VCT also enables people living with HIV to access the appropriate cure and is an effective HIV preventive strategy.

VCT is of immense benefit to those who test positive and those who test negative. It alleviates anxiety, increases client's perception of vulnerability to HIV, promotes behavioural change, facilitates early referral to care and support, including access to anti retroviral (ARV) drug therapy and it also assists in reducing stigma. VCT for young people should be recognized as a major priority with the Nigerian HIV prevention program. The utilization of VCT services among the young people how-ever depends on their knowledge about its' existence and their attitude towards it. This study therefore asseses the awareness, practice and attitude of VCT for HIV/AIDS among law students in tertiary institutions in Anambra State, Southeast Nigeria.

MATERIALS AND METHODS

This cross sectional, questionnaire based, descriptive study was conducted among law undergraduates studying in tertiary institutions in Anambra State, Southeast Nigeria between October and December 2009. The 10 tertiary institutions (5 universities and 5 polytechnics) in the state together with the courses they offer were initially identified. Nnamdi Azikiwe University, Awka and Anambra state University Uli which are the only institutions running undergraduate courses in Law in the state were selected as the area of study. Nnamdi Azikiwe University is a Federal tertiary institution located in Awka, the capital city of Anambra State, with campuses at Nnewi and Agulu while Anambra state University is a state owned university which also has a multi-campus structure, with campuses located at Uli and Igbariam. Both institutions offer a varied range of courses ranging from Agriculture to Zoology.

Prior to the onset of the study, consent was obtained from both the University administration and the Faculties of law of both institutions. By systematic random sampling, a total of 300 students were selected from both institutions in such a way that 150 students were chosen from the law faculty of each institution. The survey instrument was semi-structured, pre-test, self-administered, questionnaire which were distributed only to the respondents who gave their consent to partake in the study by the researches. The questionnaire which was divided into 3 segments obtained information on the respondents' sociodemographic data, knowledge of and attitude about VCT for HIV/AIDS. The students were requested not to write their names on the questionnaire to maintain anonymity. They were also requested not to communicate with each other while filling or compare answers after filling the questionnaire. A total of 300 questionnaires were distributed to the students by the researchers but only 294 questionnaires were properly filled or returned. These correctly filled questionnaires formed the bases of the analysis done using SSPS for windows version 15.0. Chi square test was used to assess the significance of associations between categorical variables. A p-value of 0.05 or less was considered statistically significant.

RESULTS

A total of 300 questionnaires were distributed but 294 of them were correctly filled and these formed the basis for this analysis. The respondents were all full time law students and were aged between 15 to 42 years, with a mean of 21.06 ± 3.0 years and a modal age of 20 to 24 years. One hundred and fifty six (53.1%) were females and one hundred and thirty eight (46.9%) were males. While 93.9% of the students were single, 6.1% were married (Table 1). All the students were aware of HIV/AIDS. Two hundred and ten (71.4%) of the students were aware of VCT for HIV/AIDS while eighty four (28.6%) of the respondents had no knowledge of it. The sources of information about VCT for HIV/AIDS were as shown in Table 2. The commonest source of information about VCT for HIV/AIDS 114.0 (38.8%) was from

Table 3. The understanding of respondents about VCT for HIV/AIDS.

Understanding of VCT	Frequency	Percentage (%)
Service that help make informed consent about HIV	138	47.9
Treatment to reduce spread of HIV/AIDS	114	30.3
A form of medication for PLWHA	48	12.7
Only for high risk people	12	3.2
Should be done after counselling	12	3.2
Should be done when prescribed by a doctor	10	2.7
Total	376	100.0%

Table 4. The reasons given for disapproving of VCT for HIV/AIDS.

Reasons	Frequency (F)	Percentage (%)
Cannot cope with the result	17	28.3
VCT is not confidential	14	23.3
No need for VCT since HIV has no cure	12	20.0
stigmatization	11	18.3
Sure of myself cannot get HIV	6	10.0
Total	60	100.0

electronic media followed by churches 67 (22.8%) and print media 44 (15.0%).

From Table 3, 138 (47.9%) of the respondents understood VCT as a process that enables individuals make informed decision about being tested for HIV, 114 (30.2%) a form of treatment to reduce the spread of HIV/AIDS, 48 (12.7%) understood VCT of HIV/AIDS as a form of medication for people with HIV/AIDS, and twelve (3.3%) of them think that VCT is advisable only for people with an increased risk of contracting HIV. One hundred and fourteen (31%) believe that VCT is a means of reducing the spread of HIV/AIDS while 12 (3.3%) of them believe that VCT should be done only when it is needed and not just to know someone's HIV status. Two hundred and twenty two (75.5%) of the respondents approved of VCT; 60 (20.4%) of them disapproved of VCT while 12 (4.1%) of the respondents were undecided. The various reasons why the students disapproved of VCT of HIV/AIDS are shown in Table 4. The common reasons why the students disapproved of VCT was because they felt that they would not be able to cope with the result [17 (28.3)], also, 14 (23.3%) of them felt that VCT is not confidential and 12 (20.0%) of them felt VCT was not necessary since HIV/AIDS has no cure.

Only 112 (38%) of the respondents had undergone HIV screening test. Ninety four (83.93%) of those who had undergone HIV test were tested voluntarily while 18 (16.07%) of them were not tested voluntarily. They undertook test due to medical indications and or as a requirement before church wedding. Of the 184 respondents who did not know their HIV status, 102 (55.43%) of them indicated their willingness to undergo HIV screening test while 78 (42.39%) of them were not. Sixty (32.6%) of the students who were not aware of their HIV status indicated they require counseling before visiting VCT for HIV/AIDS center. One hundred and twenty six (42.85%) of the students were aware of at least a centre where VCT for HIV/AIDS is carried out while 156 (57.1%) were unaware of any VCT centre. Majority of the students claimed not to discriminate against people living with HIV while 6 (2%) admitted to discriminating against them. There was a significant in-verse association between the knowledge of one's HIV status and discrimination against people living with HIV ($X^2 = 9.19$; $p = 0.002$), as the greater the number of the respondents' who were aware of their HIV status, the lesser the discrimination against people living with HIV. There was also a significant association between the awareness of VCT of HIV/AIDS and the knowledge of HIV status ($X^2 = 11.92$, $p = 0.00056$). The greater the number of women who are aware of VCT for HIV/AIDS, the greater the knowledge of the respondents HIV status.

DISCUSSION

This study which was conducted among law undergraduates in Anambra state had over 70% of the respondents being of age range of 20 to 24 years. This age group has been found to be at the greatest risk of HIV/AIDS because of their documented risky sexual behavior (Pettiform et al., 2005; Eaton et al., 2003) and vulnerability due to their lack of knowledge and skills required to protect themselves. The majority at risk are those who engage in unsafe sex, unsafe injection drug use, exposure to contaminated blood and blood products, skin piercing, tattooing and scarification.

In Nigeria, young people constitute about 40 million of her estimated 140 million people (National population commission, 2006) and they contribute significantly to new infections in Nigeria and other sub Saharan African countries (Federal Ministry of health, 2007). In Africa, an estimated 1.7 million young people are infected annually (WHO/UNAIDS, 2000). Globally, over 40% of all new infections in 2007 were in young people aged 15 to 24 years old, with 65% occurring among youths living in Africa (United Nations, 2008). Preventing HIV infection among the young people is particularly urgent in sub Saharan Africa where in many of her countries, young people more than 30% of the population and general HIV prevalence comprise exceeds 10% (United Nations, 1999). It is also an important commitment each country can make to its future economic and social well-being. The utilization of intervention programs however depends on their knowledge about the program and its acceptability by the young people.

This study demonstrates a high level of knowledge about HIV/AIDS and an average knowledge about VCT for HIV/AIDS. This correlates with some reports from Nigeria (Ikechebelu et al., 2006; Iliayasu et al., 2005), but contrasts with the findings from Danbar village in Northern Nigeria (Alemu et al., 2004) and findings among Tanzania healthcare professional students (Mgosha et al., 2009), and Uganda (Nuwaha et al., 2002). The differences may be due to the differences in the biosocial characteristics, behavioural and environmental factors of the study group.

Like in some studies (Mgosha et al., 2009; Ikechebelu et al., 2006), the mass media was discovered to be the major source of information about VCT of HIV. This strongly indicates that some successes are being recorded by the massive media campaign being mounted by the Government, Non-Governmental organizations (NGOs) and international agencies against the spread of HIV/AIDS and on the positive step in preventing its spread. Media campaigns have also been documented to be a veritable tool in the campaign against the spread of HIV/AIDS. It is very disappointing however that none of the respondents had VCT center as their primary source of information about HIV/AIDS and VCT for HIV/AIDS. This could be as a result of wrong location site which are far from the young people or places where the young people cannot easily assess or does not find comfortable to attend. It is necessary to establish VCT centers specifically targeting the young people because their reason of seeking VCT for HIV/AIDS services, outcome and needs following VCT for HIV/AIDS can be different from others. More so, they bear the highest burden of the disease (UNICEF, 2007).

Unlike the findings in other studies in Nigeria (Ikechebelu et al., 2006; Iliyasu et al., 2006), most of the students were aware of at least a center where VCT services are rendered. This is very much expected because of the differences in the educational status of the respondents and for the fact that most of the VCT centers are located in the urban areas where most of the university undergraduates reside. In addition, some of them would have heard VCT centers mentioned in the mass media.

The misconception about VCT among this population is a source of concern considering their level of education. The understanding of the participants about VCT for HIV/AIDS include a process that enables individuals make informed decision about being tested for HIV, a form of treatment for people with HIV/AIDS, and a form of service advisable only for people with an increased risk of contracting HIV. Although similar misconceptions have also been reported elsewhere (Iliyasu et al., 2006), this calls to question the content of health information passed to our people through various sources of information. This gap of information regarding VCT for HIV/AIDS can lead to low utilization of VCT services, increased stigmatization with resultant adverse effects on the efforts to check the spread of the disease. This study group comprises of the elites, future parents and the nation's work force consequently therefore, adequate and appropriate knowledge of VCT for HIV/AIDS will be of tre-mendous impact on the general populace.

Churches being a significant source of information to our respondents calls for the need for our health care programmers and policy formulators, NGOs, governments at all levels to establish, foster or strengthen partnership with faith based organizations and churches to facilitate access to information about VCT for HIV/AIDS.

The uptake rate of 83.93% among the respondents who are aware of VCT for HIV/AIDS is very encouraging and similar to report from Lagos, Nigeria (Ekanem and Gbadegesin, 2004) but contrasts with the report from Kano, Nigeria (Iliayasu, 2006) and South Africa (Kalichman and Saimbayi, 2003). Nevertheless, the fact that about half of the respondents who were not aware of their HIV status still express their unwillingness to undertake VCT for HIV/AIDS should be of concern to all. The reasons adduced by the respondents for their unwillingness to undertake VCT is based on false assumptions, misconceptions and the lack of adequate, proper and appropriate knowledge about HIV/AIDS. There is need therefore to intensify reproductive health education efforts among the young people. This will impact tremendously on primary prevention of HIV/AIDS and clinical management of the cases.

The finding that about a good number of the students who were not aware of their HIV status said they require counseling before they can visit VCT center(s) shows that they have not fully appreciated the extent, content and the holistic nature of services rendered at these centers. It also reveals that the students are actually one of the populations in need of the VCT for HIV/AIDS. The other reasons given by the students for not approving of or utilizing VCT for HIV/AIDS correlate with the reports from

other studies (Day et al., 2003; Iliayasu, 2006).

The significant relationship between the knowledge of the students HIV status and discrimination is worthy of note. The fact that the greater the number of students who knew their HIV status the lesser the rate of stigmatization shows that VCT for HIV/AIDS could be a veritable instrument in the fight against stigmatization and discrimination against people living with HI/AIDS. The knowledge of the students HIV/AIDS status also significantly increased with their knowledge about VCT for HIV/AIDS as people can only utilize the services they are aware of. It is therefore imperative that dissemination of information about VCT for HIV/AIDS should be intensified among the young people.

CONCLUSION

This study reveals an average knowledge about VCT for HIV/AIDS among the law undergraduates. The uptake among the aware group is high and encouraging. There is a great concern about the high proportion of students who neither approved of VCT for HIV/AIDS nor are willing to avail themselves of the services. Efforts to improve the knowledge of the young people about VCT for HIV/AIDS and its benefits should be intensified. Sexual and reproductive health education should be included in their curriculum. VCT for HIV/AIDS centers should be established in their respective campuses where the young people will readily access it and feel free to attend. The VCT staff should be trained on the peculiarities of providing repro-ductive health services to young people.

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Full Length Research paper

Seroprevalence of *Toxoplasma gondii* between couples in Ramadi city using enzyme linked immunosorbent assay (ELISA)

Mohanad Mohammad[1]*, Shehab Ahmed[2] and Abudalla Hussain[2]

[1]Education College for Pure Science, University of Anbar, Iraq.
[2]Department of Biology, Education College, Tikrit University, Iraq.

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Toxoplasma gondii **is an obligate intracellular protozoan parasite that represents an actual public health problem. This study aims to investigate the prevalence of *T. gondii* among 91 couples in Ramadi city who were examined for the presence of antibodies against *T. gondii* using enzyme linked immunosorbent assay (ELISA). The overall anti-*T. gondii* (Immunoglobin M (IgM) and Immunoglobin G (IgG)) in both couples were 38.4%, the seroprevalence in wives was only 30.7%, while that in husbands was 13.1% only. This study showed that abortive women and abnormal pregnancy had the highest percentage rates (35.7 versus 57.14%) of toxoplasmosis among those of 26 to 30 years old and the lowest was among those who have the average of age (36 to 40) years old. One group miscarriage was (50%) higher than the other groups. The group of 26 to 30 years old showed high rate of IgM antibodies of about (66.66%). The number of abortion in the first trimester was high in both patterns of antibodies IgM only and (IgM and IgG) (62.5 and 29.16%), respectively. Analysis of variance revealed that there were no significant interactions between IgM and IgG seropositivity and the gestational age of the fetus.**

Key words: *Toxoplasma gondii*, pregnancy, the couples, seroprevalence.

INTRODUCTION

Toxoplasmosis is an important zoonotic parasitic disease that affects millions of people and is caused by the protozoan *Toxoplasma gondii*. In immune competent individuals, *T. gondii* preferentially infects tissues of central nervous systems, which might be a contributing factor to certain psychiatric disorders (Reischl et al., 2003, Xiao et al., 2010). It is a ubiquitous obligate intracellular protozoan parasite, widely prevalent in humans and other animals on all continents (Dodds, 2006; Weiss et al., 2007).

In Iraq, Niazi et al. (1988) found out that the prevalence of *Toxoplasma* antibodies among women in Baghdad was 39%, whereas Niazi et al. (1992) reported low rate of 8.6% positively from eight governorates in Iraq. Mohammed and Al-Nasiry (1996) reported a prevalence rate of 20.4% toxoplasmosis in Iraqi women. In a study carried out in Basrah (Yacoub et al., 2006), the prevalence of toxoplasmosis had been shown to be 41.1 to 52.1%, whereas a previous study by Al-Hamdani and Mahdi (1997) showed low rate of 18.5% of *Toxoplasma* antibodies in Basrah population. In Duhok, North of Iraq, Razzak et al. (2005) found low *Toxoplasma* infections of about 0.97%. This result indicated that the contribution of toxoplasmosis to fetal loss is greatly overestimated. On his side, Karem (2007) found out that the seropositivity was 32.6% by enzyme linked immunosorbent assay (ELISA) between women in Sulaimania. In Baghdad, Juma and Salman (2011) found the infection of *T. gondii* in women to be 19.17%. In Tikrit, Al-Doori (2010) showed the presence of infection around 49 to 95% and higher

*Corresponding author. E-mail: shehuakuyam@yahoo.com. Tel: 08032889572, 08023599243.

Table 1. Seropositivity of anti-*Toxoplasma* IgM and IgG detected by ELISA in examined samples.

Month	Number of couples	Both couples infected		Wife infected only		Husband infected only		No anti-*Toxoplasma* abs.	
		No.	%	No.	%	No.	%	No.	%
November	10	4	40	2	20	0	0	4	40
December	11	4	63.6	5	45.4	2	18.1	0	0
January	16	5	31.2	6	37.5	3	18.7	2	12.5
February	18	5	27.7	6	33.3	3	16.6	4	22.2
March	11	8	72.7	3	27.2	0	0	0	0
April	13	8	61.5	1	7.1	3	23.0	1	7.1
May	12	1	8.3	5	41.6	1	8.3	5	41.6
Total	91	35	38.4	28	30.7	12	13.1	16	17.5

rate of infection lies among those of 25 to 31 years of age in women and their husbands.

Seroprevalence of *T. gondii* infection in man rises with age and it does not vary greatly between sexes (Montoya and Remington, 2000). The prevalence of toxoplasmosis significantly increases with age and the highest seropositivity rate 35.4% was found among pregnant women in the age group of 35 to 44 years in Slovakia (Studenicova et al., 2006).

The overall seroprevalence of toxoplasmosis in South Africa was 29/160 (18.1%). Seroprevalence in males and females were 7/42 (16.7%) and 22/118 (18.6%), respectively and the difference was not statistically significant ($P > 0.05$). The age distribution was 0.63% (1/160) for individuals of 20 years old and below, 10.6% (17/160) for those between 21 and 35 years old, and 6.9% (11/160) for individuals who were 36 years old and above (Bessong and Mathomu, 2010).

The serologic evidence of toxoplasmosis in Ethiopia was found in 60% (39/65) of them. A large number of the seropositive were females (64.1%), while in male it was (53.8%) (Negash et al., 2008). The overall anti-*T. gondii* immunoglobin G (IgG) prevalence in china was 12.3%, while the seroprevalence was 10.5% in men versus 14.3% in women (Xiao et al., 2010).

The purpose of this research is to investigate infections with *T. gondii* in couples by ELISA.

MATERIALS AND METHODS

ELISA is one of the few frequently used methods in detection of *T. gondii* infection in humans and animals. In the ELISA test, soluble antigen is coated to micro titer plates and sample serum is added to form an antigen-antibody complex (if specific antibodies are present). A secondary enzyme-linked antibody specific to the host species is added to detect antigen-antibody complex. This test requires an ELISA reader and also enzyme conjugation to the secondary antibodies. Numerous modifications of ELISA have been reported to enhance specificity and simplify the protocol of the conventional ELISA (Dubey and Beattie, 1988).

Serum samples were collected from 91 clinically and laboratory confirmed *Toxoplasma* infected patients. The samples were collected during the period from November 2010 to May 2011 from the clinical laboratory in Ramadi Hospital. Collected samples were stored at -20°C until we started ELISA test.

This assay was performed by using two kits, one for detection of IgG and another one for detection of immunoglobin M (IgM) specific antibodies against *T. gondii* antigens in the patient's serum (Biokit Diagnostics Company, Spain).

Detection of IgG and IgM titers in all samples were analyzed for *T. gondii* by the titer of IgG and IgM antibodies by ELISA kit as described (Biokit Diagnostics Company, Spain). The optical densities (OD) of the samples were measured at 450 nm, using the OD value of the blank well to correct all the OD reading from test wells (Biokit Diagnostics Company, Spain).

RESULTS

Seroprevalence data obtained are shown in Table 1. The overall percentage of positive reaction to *T. gondii* in both couples was 38.4 (35/91), while for wife infected it was only 30.7% (28/91) and husband infected was only 13.1% (12/91). The most frequent age group for abortive women and abnormal women was among those of 26 to 30 years old and it represents 35.7 and 57.14% of the total number of each group, respectively. Moreover, it was not observed to have a significant difference in the prevalence of toxoplasmosis between the age groups ($P < 0.05$) (Table 2).

In Table 3, the samples of wives were divided into three groups (abortive women, abnormal pregnancy, and normal pregnant women), and each of them was subdivided into four groups (0, 1, 2, and >3 miscarriages). A rate of one miscarriage in abortive women was 50% (35/70), while 40% (28/70) and 10% (7/70) are for the two and three miscarriages, respectively. The statistical analysis revealed a high significant difference ($P < 0.05$).

The prevalence of IgM only in the age group of 26 to 30 years shows a high percentage (66.66%) and in the age group of 36 to 40 it shows a high prevalence of IgG (60%), while in the age group of 31 to 35 it shows a high prevalence (50%) for both (IgM and IgG). Its correlation to the different age groups was not statistically significant (Table 4). The prevalence of IgM recorded the highest number with two miscarriages, it was 58.83% (10/17),

Table 2. Age group distribution in examined samples.

Age groups (year)	Abortive women		Abnormal pregnancy		Normal pregnant women	
	No.	%	No.	%	No.	%
≤ 20 - 25	21	30	1	14.28	3	21.42
26 - 30	25	35.71	4	57.14	3	21.42
31 - 35	15	21.42	1	14.28	3	21.42
36 - ≥40	09	12.85	1	14.28	5	35.71
Total	70	100	7	100	14	100
Statistical analysis	Cal. χ^2 = 5.817; Tab. χ^2 = 14.06; P ≤ 0.05					

Table 3. Ratio of previous miscarriages in examined samples.

Number of miscarriage	Abortive women		Abnormal pregnancy		Normal pregnant women	
	No.	%	No.	%	No.	%
0	0	0	5	100	2	12.5
1	35	50	0	0	7	43.7
2	28	40	0	0	6	37.5
≥3	7	10	0	0	1	6.2
Total	70	100	5	100	16	100
Statistical analysis	Cal. X^2 = 67.16; Tab. X^2 = 9.488; P ≤ 0.05					

Table 4. Seropositivity of anti-*Toxoplasma* IgM and IgG in relation to participant age.

Age group (year)	IgM^+ve		IgG^+ve		IgM^+ve and IgG^+ve		Total	
	No.	%	No.	%	No.	%	No.	%
≤ 20 - 25	7	58.33	2	16.66	3	25	12	100
26 - 30	8	66.66	2	16.66	2	16.66	12	100
31 - 35	3	50	0	0	3	50	6	100
36 - ≥40	1	20	3	60	1	20	5	100
Statistical analysis	Cal. X^2 = 8.59; Tab. X^2 = 14.06; P≤ 0.05							

then 23.52% (4/17) for IgG antibodies, while the percentage of both (IgM and IgG) recorded the highest number of one miscarriage, which was 44.44% (8/18) (Table 5). Statistical analysis revealed a non significant interaction between IgM or IgG seropositivity and the gestational age of the fetus. Comparable results of seropositivity of both IgM and IgG obtained in the first trimester gave a high percentage in IgM 62.5% (15/24) and both (IgM and IgG) 29.16% (7/24), while the number of women in their third trimester indicated no anti-*Toxoplasma* antibodies (Table 6).

DISCUSSION

The main purpose of this study was to evaluate the seroprevalence of *T. gondii* antibodies between couples of Ramadi city. The overall seroprevalence of *Toxoplasma* in this study among both couples was 38.4%, but when the previous results were compared with the published data, a decreasing seroprevalence was observed in pregnant women (Yacoub et al., 2006; Al-Rawi, 2009; Al-Doori, 2010; Juma and Salman, 2011), while the results of other studies were in agreement with the results of this study (Al-Khafajy, 2004; Al-Musauy, 2008). These variable results may be due to the differences in the specimens used by each researcher and their variable condition and data of studies.

This study found out that seroprevalence of toxoplasmosis in wives was higher than in husbands. These results were similar to the results of Negash et al. (2008), Bessong and Mathomu (2010), Xiao et al. (2010), and Sroka et al. (2010). One of the reasons for this high prevalence is related to the fact that women handle raw meat more frequently than men due to the fact that they spend more time cooking at home.

There are several causative factors responsible for both habitual and sporadic abortions. However, the prevalence of toxoplasmosis in women with bad obstetrics history is known to be significantly higher than

Table 5. Ratio of anti-*Toxoplasma* antibodies according to the number of miscarriages in couples infected.

Number of miscarriage	Pattern of antibody							
	IgM+ve		IgG+ve		IgM+ve and IgG+ve		Total	
	No.	%	No.	%	No.	%	No.	%
0	0	0	0	0	0	0	0	0
1	7	38.88	3	16.66	8	44.44	18	100
2	10	58.83	4	23.52	3	17.64	17	100
≥3	0	0	0	0	0	0	0	0
Statistical analysis	Cal. X^2 = 2.9; Tab. X^2 = 3.841 ; P ≤ 0.05							

Table 6. Seropositivity of Anti-*Toxoplasma* IgM and IgG in relation to gestational age.

Gestational age	Pattern of antibody							
	IgM+ve		IgG+ve		IgM+ve and IgG+ve		Total	
	No.	%	No.	%	No.	%	No.	%
1st trimester	15	62.5	2	8.33	7	29.16	24	100
2nd trimester	4	36.36	3	27.27	4	36.36	11	100
3rd trimester	0	0	0	0	0	0	0	100
Statistical analysis	Cal. X^2 = 2.97 ; Tab. X^2 = 3.841 ; P ≤ 0.05							

in normal. The seroprevalence in pregnant women on worldwide scale varies from 7 to 51.3% and in women with abnormal pregnancies and abortions; the seroprevalence varies from 17.5 to 53.3% (Kumar et al., 2004). The seropositivity rate of abortive women in age group (26 to 30) years old was obviously higher (53.71%) than in other groups, which was similar to the results reported in Iraq (Shani, 2004; Kadhim, 2006; Al-Rawi, 2009; Juma and Salman, 2011). This is presumably due to the high presence of cats, climatic, hygienic, and socioeconomic conditions in the regions. However, it is acknowledged that seroprevalence increases with age, as seen in studies conducted in various countries (Dodds, 2006).

Women who may get infection during pregnancy may show a variety of clinical signs and symptom depending on many factors, such as the number of parasites, virulence of strain, and the time period the mother acquires infection (Tenter et al., 2000). If the mother is infected in the first trimester, the result is abortion, stillbirth or severe disease of fetus (Lin et al., 2000).

On the other hand, IgM antibodies titer to *T. gondii* was found to be more than IgG antibodies. Clearly, the overall prevalence of IgM antibodies was interpreted as a diagnosis of the acute form of the disease. In the present survey, it was shown that chronic form (shows prevalence of IgG antibodies) increased with age (16.66 to 60%). These results reflected the contact with cats or infected materials and vegetables in these age groups.

This results pointed out that most IgM and both IgM and IgG patterns of antibodies were increased in first trimester (62.5 and 29.16%, respectively), these patterns of antibodies were absent in the third trimester. The severity of disease decreases if the infection occurs in the second or third trimester, but the risk for transmission from mother to fetus increase (Romand et al., 2001). In pregnant women, the primary infection of *T. gondii* may cause abortion, neonatal malformation, neonatal death, or severe congenital deficiency, such as mental retardation, retinochoroiditis, and blindness (Kravetz and Federman, 2005). In addition, toxoplasmosis is one of the main causes of fetal abortion, stillbirth, and neonatal mortality in domestic animals, resulting in significant economic loss in the farming industry (Mcallister, 2005).

Congenital toxoplasmosis is most severe when the mother becomes infected in the first trimester, then approximately 10 to 20% of fetuses are infected. If the infection is acquired in the second trimester, 30 to 40% of fetuses are infected, but the disease is mild or asymptomatic at birth. These differences in transmission may be related to the placental blood flow, size of uterus, virulence of the parasite or to the immunocompetence of the mother (Singh, 2003).

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Impact of lymphatic filariasis elimination programme by study of advances and challenges using mathematical model

Vaibhav Changediya* and Subhash Devdhe

Department of Pharmaceutics, Yash Institute of Pharmacy, Aurangabd, 431134 India.

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Mathematical simulation models for transmission and control of lymphatic filariasis are useful tools for studying the prospects of lymphatic filariasis elimination. Two simulation models are currently being used. The first, EPIFIL, is a population-based, deterministic model that simulates average trends in infection intensity over time. The second, LYMFASIM, is an individual-based, stochastic model that simulates acquisition and loss of infection for each individual in the simulated population, taking account of individual characteristics. The two main challenges for future modeling work are: (1) quantification and validation of the models for other regions, for investigation of elimination prospects in situations with other vector-parasite combinations and endemicity levels than in Pondicherry; (2) application of the models to address a range of programmatic issues related to the monitoring and evaluation of ongoing control programmes. The models' usefulness could be enhanced by several extensions; inclusion of different diagnostic tests and natural history of disease in the models is of particular relevance. Nevertheless, published estimates of the duration of mass treatment required for elimination differed, due to the use of different indicators for elimination (EPIFIL: microfilaraemia prevalence < 0.5% after the last treatment; LYMFASIM: reduction of microfilaraemia prevalence to zero, within 40 years after the start of mass treatment).

Key words: EPIFIL model, LYMFASIM model, microfilaraemia, mathematical simulation.

INTRODUCTION

Lymphatic filariasis is a mosquito-borne parasitic disease and an important cause of chronic morbidity in tropical countries. In 1998, the Global Programme to Eliminate Lymphatic Filariasis (GPELF) was initiated, aiming at worldwide elimination of this parasitic disease as a public health problem. The main strategy in the global programme is to interrupt transmission by annual population treatment with antifilarial drugs (diethylcarbamazine or ivermectin plus albendazole). In addition, morbidity management should reduce the suffering of patients who have chronic manifestations (Anderson and May, 1985).

Thirty-two countries had started elimination programmes in 2002 and this number is still growing. The goal of elimination is ambitious. Past mass treatment programmes had varying degrees of success. In some areas, transmission was apparently interrupted (Anderson and May, 1999). *Aedes polynesiensis* is arguably the most important Lymphatic filariasis (LF) vector in the Pacific, in part because it exhibits a characteristic known as 'limitation', whereby the percentage of microfilaria (mf) which develop to stage 3 larvae increases with decreasing densities of mf. For this reason *Ae. polynesiensis* may pose the greatest

*Corresponding author. E-mail: v.changediya@rediffmail.com.

Table 1. LYMFASIM predictions of the number of annual mass drug treatment rounds required to achieve elimination in an area like Pondicherry, with 99% probability.

Drug(s)	Adult worms (%)	Microfilariae (%)	65%	80%
Ivermectin + albendazole	25	100	8	5
Diethylcarbamazine	45	60	6	3
Diethylcarbamazine + albendazole	60	60	4	2
Doxycycline	70	0	2	2

challenge to LF elimination in the region.Mathematical models can help to clarify these issues and application of such models is considered important for support of GPELF. Mathematical models have been used widely in parasitology. They help to understand the complex transmission dynamics of parasitic diseases and are useful tools for planning and evaluating control programmes (Bryan and Southgate, 1988). Targeted models, which consider part of the processes involved in transmission, helped, for example, to clarify the role of acquired immunity and the macrofilaricidal effects of treatment.

This paper concentrates on so-called 'full transmission models', which relate the rate of transmission to the intensity and distribution of infection in a human population and can be used to predict the impact of interventions on transmission and the probability of elimination. To our knowledge, three full transmission models have been described in the literature (Bryan and Southgate, 1988). The first was specifically developed for the evaluation of a vector control programme and is not considered here. The two other models, called EPIFIL and LYMFASIM are both being used for planning and evaluation of elimination programmes. After a brief introduction of the processes involved in transmission and control of lymphatic filariasis, we describe the basic structure of these models, compare and discuss some critical model predictions, and outline future research priorities (Boakye et al., 2004).

Processes In lymphatic filariasis transmisson and control

Models for lymphatic filariasis control basically describe the main biological processes involved in transmission. To study the dynamics of transmission and how intervention affects transmission, it is specifically important to take account of density dependence and heterogeneities. Density dependence means that the outcome of a process depends on the abundance of the parasite stages involved. Several limitation mechanisms may reduce transmission when the average worm burden increases (Churcher et al., 2005). For example, the proportion of microfilariae (mf) that develop into infectious L3 larvae saturates in *Culex quinquefasciatus* when the mf intake is higher, limiting the transmission of infection. Further, the survival probability of mosquitoes is reported to reduce with their infection load.

Acquired immunity may limit infection intensity in the human host. Different mechanisms for this have been proposed, but evidence for the operation of such immunity is inconclusive (Das and Subramanian, 2002). These limitation mechanisms all negatively affect the impact of interventions because transmission becomes relatively more efficient when infection levels are lower. Density dependence, however, may also occur in the opposite direction (called facilitation). The probability that a female worm mates with a male worm increases with higher worm burdens (Duerr et al., 2005). Further, in some anopheline mosquito species, larval development might increase with higher mf intake.

It is unknown whether density dependence, either limitation or facilitation, occurs in parasite establishment and survival in humans, their fertility, and mf survival. The term heterogeneity points at variation between individuals. Individuals differ for example in genetic background, nutritional status and behaviour, which may cause differences in exposure to mosquitoes, susceptibility to infection, and survival, maturation and fecundity of parasites (Duerr et al., 2003). Therefore individuals may be predisposed to heavy or light infection, leading to an aggregated or overdispersed distribution of parasites (with a few hosts harbouring the majority of the parasites). Individuals also differ in compliance and responsiveness to treatment, which may also contribute to aggregation of parasites. Heterogeneity may also occur in the parasite population for example with respect to the lifespan and resistance to treatment (Esterre et al., 2001).

AVAILABLE MODELS

The two available models for lymphatic filariasis transmission and control, EPIFIL and LYMFASIM, mainly differ in the amount of detail included. Specific variants of both models have been developed for *Wuchereria bancrofti* transmitted by *Culex quinquefasciatus*, using data from an integrated vector management control programme carried out in Pondicherry, India, 1981 to 1985. These 'Pondicherry model variants' are described below. Table 1 gives the quantification of several key biological parameters of the models (Goodman, 1994).

Table 2. Prediction of number of yearly mass treatment rounds required to reach a 0.5% microfilaraemia prevalence threshold, using a combination of diethylcarbamazine plus albendazole in relation to endemicity and coverage.

Pretreatment Mf prevalence	60%	70%	80%	90%
EPIFIL				
2.5%	7	6	5	4
5%	9	7	6	5
7.5%	10	8	7	6
10%	12	9	8	7
LYMFASIM				
10%	10	8	6	5

Mf: microfilariae.

EPIFIL model

EPIFIL simulates the average course of infection over age and time in a human population by a set of differential equations. The mf prevalence is calculated using a negative binomial distribution, assuming a certain amount of aggregation of parasites in the human population. The model can be used to simulate the impact of vector control or mass treatment (Habbema et al., 1996). The human population is constant in size and age-structure. Limitation in the transmission of infection by culicine mosquitoes is taken into account, so that the number of infectious L3 larvae that can develop in mosquitoes saturates at higher mf intensities.

Acquired immunity is included as a second limitation mechanism: it is triggered by incoming L3 larvae and reduces the probability that new larvae develop into adult worms. Heterogeneity is only included by age-related exposure to mosquitoes that is, the risk of infection increases with age, until a maximum level is reached at the age of 9 years. Vector control is assumed to reduce the mosquito biting rate (Habbema et al., 1992). Mass treatment leads to killing of a proportion of adult worms or mf and to temporal infertility of worms, depending on the proportion of the population that receives treatment and characteristics of the treatment regimen. The design of this population-based deterministic model is based on a general differential equation framework describing the dynamics of macroparasitic infections (Krishnamoorthy et al., 2004).

LYMFASIM model

LYMFASIM simulates the acquisition and loss of worms over age and time in a discrete number of human individuals, using stochastic microsimulation. Individuals interact through biting mosquitoes and together they form a dynamic population of which the size and age-structure may change over time. Like EPIFIL, LYMFASIM takes account of limitation in the proportion of engorged mf that develop into L3 larvae inside the mosquito and of acquired immunity in human hosts (Molyneux and Zagaria, 2002). Parasites may vary with respect to their lifespan (about ten years on average). Individual mf intensities are translated into the number of mf than the number of mf that would be counted in a 20 µl blood smear, taking account of random variability in these counts and reduced sensitivity of diagnostic tests at lower mf densities.

Two model variants were developed for Pondicherry which differed with respect to the type of acquired immunity: ‘anti-L3’ immunity is triggered by incoming L3 larvae and reduces the probability of successful adult worm establishment; ‘anti-fecundity’ immunity is triggered by incoming L3 larvae and reduces the probability of successful adult worm establishment; ‘anti-fecundity’ immunity is triggered by the presence of adult worms and reduces the rate of mf production by female worms. By considering individual worms in individual hosts, the model automatically takes account of the declining mating probability of female and male with lower average infection intensities (Michael et al., 2004). Age-dependent exposure is included, assuming that exposure increases until a maximum is reached at about 20 years of age. The mf prevalence and (geometric or arithmetic) mean mf intensity can be directly calculated from the smear counts, using data from all simulated individuals or specific subgroups (Michael and Bundy, 1998).

EPIFIL versus LYMFASIM: Predictions

We focus here on model predictions of the coverage and duration of annual mass treatment programmes required for elimination. All published predictions were based on the Pondicherry variants of the models, although acquired immunity was left out of the model in the EPIFIL predictions. LYMFASIM's predictions were based on the models with anti-L3 or anti-fecundity immunity, with a population size of about 3000 to 4500 individuals. Both EPIFIL and LYMFASIM have been used to predict the impact of control measures and assess prospects for elimination by mass treatment (Michael et al., 2001). From the predictions of both models we can conclude that it is possible to eliminate lymphatic filariasis by yearly mass treatment but that the number of treatment rounds largely depends on coverage, precontrol mf prevalence and the macrofilaricidal effects of drugs (Norman et al., 2000). This is illustrated in Tables 2 and 3.

Results in Table 1 are shown for four different drugs or drug combinations and two coverage levels. Predictions are based on the anti-L3 variant of the model for Pondicherry, with a precontrol microfilaraemia prevalence of 8.5%. Elimination is defined as zero microfilaraemia prevalence 40 years after the start of treatment.

The combination treatment (Table 2) is assumed to kill 55% of all adult worms and 95% of the microfilariae, and

to interrupt microfilaria production for six months. EPIFIL simulations were published10 and concerned a model without acquired immunity. LYMFASIM results from the model with anti-L3 immunity were added for comparison for an average pretreatment microfilaraemia prevalence of 10%.

The prevalence just needs to be brought under a threshold or breakpoint level, below which the acquisition rate of new worms is lower than the death rate of existing worms (by treatment or natural death), so that the worm population will eventually die out. In many situations, the predicted number of yearly treatment rounds required for elimination was higher than 4 to 6, which was hoped to be sufficient when GPELF was initiated. As an alternative to longer programmes, one might consider more frequent mass treatment (for example, half-yearly) or the additional application of vector control (Plaisier et al., 1998).

The predictions for Pondicherry-like situations indicate that elimination can be achieved within a reasonable timeframe. Clearly, the quantitative predictions should be interpreted with some care. Achieving elimination would for example be more difficult, if the macrofilaricidal effects of treatment are lower, if the adult worms live longer, if there is stronger aggregation of worm-burdens, or if density-dependent mechanisms operate that enhance parasite transmission at low infection intensities. In fact, the required time period is shorter than the mean adult worm lifespan. This is possible, because the antifilarial drugs are thought to have strong macrofilaricidal or sterilizing effect. Moreover, not all worms need to be killed or sterilized in order to achieve elimination. Further, different criteria elimination were used: in EPIFIL elimination was assumed to occur if the mf prevalence after treatment was below 0.5%; in LYMFASIM elimination was defined as a zero mf prevalence 40 years after the start of control in 99% of the runs (Plaisier et al., 1999).

To allow better comparison of the models, we did a series of additional simulations with LYMFASIM for mass treatment with the combination of diethylcarbamazine plus albendazole, using the same assumptions on drug-efficacy and the same criterion for elimination as in published EPIFIL predictions. In spite of these uncertainties, the predictions give important information on the determinants of elimination. The predictions of EPIFIL and LYMFASIM cannot be compared directly because the original publications reported results for different treatment regimens, with different assumptions on efficacy of the drugs, and different precontrol mf prevalence levels.

The two models come to comparable conclusions regarding the number of treatment rounds required to bring mf prevalence below 0.5%, although LYMFASIM's predictions are slightly more optimistic than EPIFIL's at higher coverage levels. This finding of nearly equal predictions is not straightforward (Plaisier et al., 1990). The LYMFASIM model contains several assumptions and mechanisms, which, relative to EPIFIL, limit the impact of the intervention on transmission: (1) a longer adult worm life span (10 versus 8 years); (2) acquired immunity; (3) heterogeneities in exposure to mosquitoes, in compliance to mass treatment, and in adult worm life span.

However, the limiting effect of these assumptions and mechanisms on the impact of mass treatment is apparently counteracted by the enhancing effect of a reduced mating probability of worms at lower average worm burdens in LYMFASIM (Rochet, 1990). It is reassuring that both models come to comparable conclusions regarding the number of treatment rounds required to achieve elimination, although LYMFASIM predictions are slightly more optimistic than EPIFIL predictions when population coverage is high. This finding of nearly equal predictions is not straightforward. The LYMFASIM model contains several assumptions and mechanisms, which, relative to EPIFIL, limit the impact of the intervention on transmission: (1) a longer adult worm lifespan (about 10 years versus 8 years); (2) acquired immunity; (3) heterogeneities in exposure to mosquitoes, compliance to mass treatment, and adult worm lifespan. However, the limiting effect of these assumptions and mechanisms on the impact of mass treatment is apparently counteracted by the enhancing effect of a reduced mating probability of worms at lower average worm burdens in LYMFASIM (Stolk et al., 2005).

Criteria for elimination

EPIFIL predictions were based on the assumption that transmission will not continue when the mf prevalence falls below 0.5%. The choice for this threshold is somewhat arbitrary in the absence of evidence from the field. Given its individual-based structure, LYMFASIM is more suitable for examining in how many runs infection is ‘truly’ eliminated, as indicated by zero mf prevalence 40 years after the start of control (Stolk et al., 2003). For example, in the runs with 10% pre-control, prevalence rounds were required to bring the average mf prevalence below 0.5% (Table 3). However, only 87% of the runs did this result in zero mf prevalence 40 years after the start of control. It is clear that to be 99% certain of elimination (as was the criterion in Table 2), much longer continuation of mass treatment would be required. This threshold level (or threshold levels) will depend on local transmission dynamics and mosquito biting rates, immigration of parasite carriers or infected mosquitoes, but also on heterogeneities and population size in view of the stochastic processes involved (Stolk et al., 2004).

APPLICATION OF MODELS FOR OTHER REGIONS

The existing model variants were all quantified for

Table 3. Quantification of several key biological parameters in the EPIFIL and LYMFASIM model variants for Pondicherry, where *Wuchereria bancrofti* is transmitted by *Culex quinquefasciatus*.

Parameter	EPIFIL	Anti-L3 immunity	Anti-fecundity immunity
Parasite lifecycle			
Average adult worm lifespan in years (type of distribution)	6	11.2	12.8
Average mf lifespan in months (type of distribution)	10[b]	10[b]	
Premature period in months			
Exposure variation by age			
Exposure at age zero as fraction of maximum exposure	0	0.26	0.40
Age in years at which maximum exposure is achieved	9	19.1	21.3
Density dependence in mosquitoes			
Maximum number of L 3 larvae that can develop in mosquitoes at high mf intensities	6[d]	6.6[e]	6.6[e]
Acquired immunity	Lifelong	9.6[f]	11.2[f]
Duration of acquired immunity in years			
Other parameters			
Monthly biting rate	5760	2200	2200
Proportion of L 3 larvae in mosquitoes that enter the human host when a mosquito bites	0.414×0.32=0.13	0.1	0.1
Proportion of inoculated L 3 larvae that develop successfully into adult worms (×103)	0.113	1.03 g	0.42
Mf production per worm	2	0.61 h	4.03 h,i

transmission of *W. bancrofti* by *Culex quinquefasciatus* and tested against data from Pondicherry. Biological parameters are not expected to vary much between regions. Most importantly, this concerns the relationship between mf density in the human blood and the number of L3 larvae developing in mosquitoes. Unfortunately, few data are available to quantify this relationship for the different mosquito species involved. Especially for the anopheline mosquito species responsible for trans-mission in large parts of Africa, more field research is needed. However, our understanding of the biology of infection (in spite of in-depth model based analysis of the Pondicherry data) is incomplete and there is uncertainty about the quantification of several key parameters, such as the parasite lifespan or role of acquired immunity.

The basic structure of the models is generalizable to other areas, but various model parameters may take different values. Other parameters that may need requantification relate to the composition of the human population, mosquito biting rates and heterogeneity in exposure, and operational characteristics of interventions (Southgate and Bryan, 1992). Therefore, it is crucial to continue testing the validity of existing and new model variants against epidemiological data. Testing models against age specific data may help to determine the role of acquired immunity or other processes.

Trends during vector control are especially informative about the adult worm lifespan. Trends during mass treatment may give information about the effects of drugs on worm survival and productivity (Snow and Michael, 2002) and trends after cessation of control may help to determine whether density-dependent mechanisms have appropriately been included in the model. Better

information on all these aspects should eventually come from field research. Using combinations of available diagnostic tests (mf and antigen detection, ultrasound to visualize adult worms), it may be possible to further increase the validity of our existing models.

The LYMFASIM model has been applied to age-patterns observed in an area of South-East India that has the same vector-parasite combination and presumably the same transmission dynamics as Pondicherry. This led to the development of new model variants with less strong or no immunity (Subramanian et al., 2004). Comparison of predictions from the new LYMFASIM model variant and EPIFIL with observed trends during mass treatment in this region indicated that assumptions regarding efficacy of drugs, or possibly coverage and compliance patterns, had to be adapted (Subramanian et al., 1998).

CHALLENGES IN THE EVALUATION OF CURRENT ELIMINATION PROGRAMMES

Predictions of the number of treatment rounds required for elimination were only a first step. However, specific programmes also need to be monitored and evaluated. For example, the observed results can be compared with model predictions to see whether progress is as expected. If results lag behind, programmes can be adapted (Schlemper Jr. et al., 2000). Also, the models could help to determine when mass treatment can be stopped with low risk of recrudescence, taking account of the specific local conditions, local coverage and compliance levels, and the achieved reduction in mf prevalence and intensity.

Analogously, models can help to determine cost-effective surveillance strategies for early detection of recrudescence of infection after cessation of control and measures to be taken to stop this recrudescence. In some situations, focus may shift to reducing the public health problem without explicitly eliminating infection. To address this with the models, more attention is required for the development of disease (Woolhouse, 1992). Simple mechanisms of disease development are included in both models, but this has received little attention in published work until now. To address the discussed issues on monitoring and surveillance, the models must be extended to include the results of antigen detection, which is widely used in monitoring and surveillance by ongoing control programmes. Although discussion until now focused on the elimination of transmission, this goal may be difficult to achieve in some areas (Vanamail et al., 1996).

Conclusion

These models give more or less similar predictions on the number of treatment rounds that will be required for elimination, at least in Pondicherry-like situations. There are currently two models for lymphatic filariasis transmission and control, LYMFASIM and EPIFIL that have been used in predicting the impact of mass treatment programmes. The models differ however in defining when elimination occurs, which leads to different advice on the duration of mass treatment. In view of current elimination programmes, it is crucial to obtain better criteria for when to stop control, taking account of stochasticity in the eventual outcome of elimination. Antigen tests should be included in the model, and the disease part of the models may need more attention. Model variants that are adjusted to local situations are powerful tools to aid decision-making in current control programmes.

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Comparison of efficacy and safety of mifepristone-misoprostol combination with ethacridine lactate in mid-trimester termination of pregnancy

Smiti Nanda and Anshu Paul*

Department of Obstetrics and Gynaecology, 32/11J, Medical Campus, PGIMS, Rohtak, 124001, Haryana India.

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This study was done to compare the effectiveness and safety of mifepristone/misoprostol versus extra-amniotic injection of ethacridine lactate for the termination of second trimester pregnancy. Sixty women requesting voluntary termination of pregnancies, between 13 and 20 weeks of gestation, were randomly assigned into two groups. Group 1 (MM) received a single oral dose of 200 mg mifepristone and 48 h later 400 mcg vaginal misoprostol every 4 h, with up to five additional doses. Group 2 (EL) received an extra-amniotic injection of 150 ml of ethacridine lactate with 250 mcg of PGF2α. The primary outcome was successful abortion rate. Secondary outcomes included the difference in the induction-to-abortion interval and the frequency of adverse events. Both MM and EL regimens were effective, with successful abortion rates of 96.67 and 93.33%, respectively (P value > 0.05, NS). The complete abortion rates were 90 and 86.66%, respectively. The induction-to-abortion interval was longer in the MM group than in the EL group that is, (58.31 ± 3.62 h) versus (32.28 ± 9.94 h), respectively, P < 0.001, VHS). Both treatments were safe, although there was a significant difference in duration of hospital stay between the two groups. Both MM and EL regimens were effective with high success rates and were safe for the termination of second trimester pregnancy.

Key words: Mifepristone, misoprostol, pregnancies, abortion, treatments.

INTRODUCTION

Second trimester termination of pregnancy carried out from 13 to 20 weeks can be physically and psychologically traumatic for the patient. Surgical termination of pregnancy is of high risk for the woman's health and medical ways are required. Important reasons for termination of pregnancy include fetal demise, pregnancy induced hypertension, fetal anomalies; where termination of pregnancy has to be performed to safeguard maternal health.

An ideal method for termination of pregnancy should be safe, easy and effective and associated with less complications, morbidity and mortality. The need for termination of pregnancy in second trimester has resulted in inventing various methods and the research continues since ancient days till date for example, intracervical laminaria tents, surgical evacuation, intraamniotic instillation of hypertonic saline, ethacridine lactate and prostaglandins (World Health Organization, 1997).

MATERIALS AND METHODS

In a retrospective analysis of prospectively collected data, 60 healthy women over the age of legal consent, coming for second trimester termination of pregnancy, between 13 to 20 weeks, were recruited over 2 years. They were counseled regarding various methods, their side effects, dosage schedules and need for

*Corresponding author. E-mail: Anshupaul81@gmail.com. Tel: 09050425513.

subsequent follow up. Termination was done only after the opinion of two registered medical practitioners were soughted. Patients with anemia, scarred uterus, allergy, low lying placenta and coagulation disorders were excluded from the study. Women were randomized in two groups by using random number tables, each group comprising 30 women.

In Group 1, each woman received a single oral dose of mifepristone 200 mg on day 1 followed by 400 mcg of vaginal misoprostol, 48 h later. This was followed by vaginal misoprostol 400 mcg every 3 h for a maximum of five doses. In Group 2, 150 ml of ethacridine lactate was instilled in extra amniotic space by foleys catheter; along with this, one ampoule of PGF2 alpha was added. The catheter was removed after 24 h, unless expelled spontaneously. Side effects including nausea, vomiting, diarrhea, headache, dizziness, rash, fever, shivering and pain were recorded. The induction abortion interval was calculated from the time of administration of mifepristone/ethacridine lactate to the time when fetus and placenta aborted. Also, induction abortion interval after misoprostol administration was calculated and final outcome was assessed at 48 h and classified as complete, incomplete, or failure. If placenta was found to be incomplete, suction evacuation or check curettage were performed. If fetus was not expelled, oxytocin infusion of 10 units in 500 ml of ringer lactate was used. Comparisons of two groups were done in terms of (a) induction abortion interval from the time of start of mifepristone/ethacridine lactate, (b) intensity of side effects, (c) evacuation required for incomplete abortion.

Comparison of group 1(mifepristone-misoprostol combination) and Group 2 (ethacridine lactate, 0.1%) was assessed by applying chi square test. The difference was said to be significant when probability was less than 0.05.

RESULTS

The present study was carried out on 60 pregnant women with the aim to compare the efficacy and safety of mifepristone-misoprostol combination with ethacridine lactate (0.1%) and 250 µg of injection PGF2x combination in second trimester termination of pregnancy between 13 to 20 weeks. Patients were randomly divided into two groups, each comprising 30 patients. The most patients (63.33%) were Group 1 between 21 to 25 years and 56.67% patients in Group 2 between 26 to 30 years. The mean age of Groups 1 and 2 were 24.43 ± 2.44 years and 25.4 ± 2.40 years, respectively ($p > 0.05$, NS).

Successful abortion was achieved in 96.67% patients in Group 1 and 93.33% patients in Group 2 ($p > 0.05$). Success of induction was not related to age and parity. Abortion was complete in 90% patients in Group 1 and 86.67% patients in Group 2. There was one case of failure in Group 1, and 2 cases of failure in Group 2 ($p > 0.05$). Mean induction abortion interval was 10.51 ± 4.46 h in Group 1 and 32.28 ± 9.94 h in Group 2 ($p < 0.001$, VHS). However in Group 1, mean mifepristone to abortion interval was 58.31 ± 3.62 h. The maternal side effects to either medication included nausea, vomiting, diarrhea, fever, abdominal cramps. The incidence of side effects was more in Group 2, as compared to Group 1. There were no cases of rupture uterus or hyper-stimulation in both the groups. Mean number of days of hospital stay in Groups 1 and 2 were 4.13 ± 0.77 and 3.06 ± 0.78 days, respectively ($p < 0.001$, VHS).

There was no significant correlation between bishop score and induction to abortion interval. In Group 1, 8/9 (88.88%) primigravidae delivered within 24 h, while 100% multigravida (21/21) delivered within 24 h. However in Group 2, only 12.5% primigravidae and 18.18% (4/22) multigravidae delivered within 24 h. However, this data did not reach up to a significant p value ($p > 0.05$, NS).

DISCUSSION

According to WHO (1997), the preferred medical method for induction of abortion after 14 weeks of pregnancy is a combination of antiprogestogen, followed 24 to 48 hours later by a prostaglandin. Hence, newer methods of second trimester abortion like mifepristone and misoprostol have almost replaced the traditional methods and very few studies have compared them to currently recommended methods. Ethacridine lactate (EL) method is one of them. Contrary to other countries, ethacridine lactate is still the first line method for second trimester abortion in China. This is because EL is an inexpensive, effective and safe method that offers an alternative to mifepristone-misoprostol regimen in countries or areas, where mifepristone is unaffordable or unavailable.

In the present study, two groups were comparable in terms of age, parity and initial Bishop score. The primary outcome measure was the achievement of successful abortion and induction abortion interval. The success of induction was defined as complete abortion occurring within 48 h of administration of ethacridine lactate or within 24 h of administration of first dose of misoprostol. In the present study, 96.67% patients (29/30) had successful abortion in Group 1 (MM) and 93.33% patients in Group 2 that is, EL (28/30). Bhatena et al. (1999) reported a success rate of 92% with 0.1% ethacridine lactate which increased to 98% after addition of 250 µg of PGF2α extraamniotically. However, Kalekci et al. (2006) reported a reduced success rate of 70.6% with 0.1% ethacridine lactate which improved to 80.4% on addition of oxytocin infusion. Almost similar successful abortion rates were shown by other authors (Shukla et al., 1984; Zauva et al., 1989; Sofat et al., 1994).

Ashok and Templeton (1999) studied the efficacy and safety of mifepristone misoprostol combination in patients requiring second trimester termination of pregnancy and reported a successful abortion rate of 97% which is similar to the present study. Similar successful abortion rates were reported by other authors (le Roux et al., 2001; Bartley and Baird, 2002).

In the present study, the mean induction abortion interval was 58.31 ± 3.62 and 32.28 ± 9.94 h in Groups 1 and 2, respectively ($p < 0.001$, VHS) (Table 1) and difference was highly significant. However, misoprostol to abortion interval was significantly shorter that is, 10.51 ± 4.46 h (Table 2). Bhatena et al. (1990) reported median IAI of 35 h with EL alone and 19 h with EL and PGF2α combination which is not comparable to the present

Table 1. Induction abortion interval (mifepristone abortion).

Induction abortion time (h)	Group 1 (MM) Mifepristone-abortion interval (%)	Group 2 (EL) (%)
<24	0	5 (16.66)
24-36	2 (6.67)	20 (66.66)
37-48	4 (13.33)	2 (6.66)
49-60	18 (60)	3 (10)
>60	6 (20)	0
Total	30	30
Mean±SD	58.31±3.62	32.28±9.94
p value	< 0.001 very highly significant	

MM: Mifepristone/misoprostol; EL: ethacridine lactate.

Table 2. Induction abortion interval (misoprostol abortion)

Induction abortion time (h)	Group 1 (MM) Misoprostol-abortion interval (%)	Group 2 (EL) (%)
<12	21 (70)	0
12-24	8 (26.67)	5 (16.67)
25-36	1 (3.33)	20 (66.67)
37-48	0	2 (6.67)
49-60	0	3 (10)
Total	30	30
Mean±SD	10.51±4.46	32.28±9.94
p value	P value < 0.001 VHS	

MM: Mifepristone/misoprostol; EL: ethacridine lactate.

study. Kelekci et al. (2006) reported an IAI of 17.3 h with EL alone and 15.8 hrs with EL and oxytocin infusion. IAI of EL is comparable to the study by Chaudhari et al. (2004) who compared extraamniotic instillation of 0.1% ethacridine lactate with misoprostol 400 µg 12 h and reported an induction abortion interval of 31.3 h, which is similar to the present study, with a successful abortion rate of 95% (Chaudhuri et al., 2004). Purandre et al. (1977) showed an induction abortion interval of 29.54 h and a complete abortion rate of 82% with 0.1% ethacridine lactate which is almost similar to the present study. Similarly, Sofat et al. (1994) also reported 92% success rate within 48 h and 98% within 72 h following ethacridine lactate instillation for second trimester MTP. The mean induction abortion interval was 31 h 31 min which is comparable with our results. Almost similar results were reported by Hou et al. (2010). He showed a significant difference between the two groups in term of mean induction to abortion interval.

The mean time from initial drug administration to fetal expulsion was 50.57 ± 6.80 h in mifepristone-misoprostol group versus 43.02 ± 8.74 h in EL group ($p < 0.001$). However, the mean time from administration of misoprostol to fetal delivery in mifepristone-misoprostol group was 10.54 ± 5.81 h which is almost similar to the present study that is, 10.51 ± 4.46 h (Table 2).

In the present study, two groups were also compared for the number of days of hospital stay. In Group 1 (mifepristone-misoprostol) 60% patients (18/60) had a hospital stay of 4 days whereas in Group 2 (EL) 60% of patients (18/60) had a hospital stay of 3 days. This may be due to the fact that in Group 1, all the patients were admitted 48 h prior to induction with misoprostol, for mifepristone administration, which had probably led to increase in the number of days of hospital stay in mifepristone-misoprostol group.

The induction abortion interval was significantly shorter in parous women than in nulliparous in Group 1. In Group 1 8/9 (88.88%) delivered within 24 h while 100% (21/21) multigravida delivered within 24 h. However in Group 2, 12.5% primigravida and 18.18% multigravida delivered within 24 h. Hou et al. (2010) also reported that mean induction abortion interval between the two groups was significantly shorter in parous women than in nulliparous women ($p < 0.05$).

In the present study, the ethacridine lactate group experienced more gastrointestinal side effects as compared to mifepristone misoprostol group. 13 patient (43.33%) experienced abdominal cramps, requiring analgesia and 11 patients (36.67%) experienced nausea

Table 3. Side effects of two groups.

Side effect	Group I (MM) (%)	Group 2 (EL) (%)	χ^2, (p value)
Nausea/vomiting	4 (13.33)	11 (36.67)	4.35, (<0.05 S)
Headache	3 (10)	1 (3.33)	1.07, (>0.05 NS)
Fever	6 (20)	1 (3.33)	4.04, (<0.05 S)
Abdominal cramps	1 (3.33)	13 (43.33)	13.41, (<0.001 VHS)
Diarrhoea	2 (6.60)	0	2.06, (>0.05 NS)
Hyperstimulation	0	0	
Rupture	0	0	
Others	0	0	
Total	16	26	

MM: Mifepristone/misoprostol; EL: ethacridine lactate; S: significant; NS: non significant; VHS: very highly significant.

Table 4. Effectiveness of procedure.

Measure of effectiveness	Group 1 (MM) (%)	Group 2 (EL) (%)
Successful	29 (96.67)	28 (93.33)
Failure	1	2
Total	30	30
P value	χ^2 = 0.350, p>0.05 NS	

Table 5. Hospital stay (number of days).

Number of days	Group 1 (MM)	Group 2 (EL)
2	0	6
3	5	18
4	18	4
5	5	2
>5	2	0
Total	30	30
Mean±SD	4.13±0.77	3.06±0.78
p value	<0.001 very highly significant	

and vomiting in EL group (Table 3). There was only 1 case of fever. There were no cases of hyperstimulation or rupture uterus. Increase in the number of gastrointestinal side effects in ethacridine lactate can be attributed to the extraamniotic injection of PGF2α; which has known side effects of nausea, vomiting and abdominal cramps. Hou et al. (2010) reported higher incidence of side effects in mifepristone misoprostol combination as compared to ethacridine lactate group.

In mifepristone misoprostol group, 28.57% patients experienced nausea, vomiting, as compared to 3.81% in EL group, 17.14% patients experienced diarrhea, 36.19% patients had fever, chills and rigors. EL group reported minimal side effects in the form of nausea, vomiting (3.8%) and fever (8.57%) (Table 3). Mifepristone-misoprostol group experienced fewer side effects in the form of nausea/vomiting (13.33%), headache (10%), fever (20%) and diarrhea (6.60%) (Table 3). On the basis of these findings, the present study has shown that both ethacridine lactate and mifepristone-misoprostol combination are safe and effective for the termination of second trimester pregnancy (Table 4).

Though ethacridine lactate has a longer abortion interval compared to mifepristone misoprostol combination, total number of days of hospital stay was lesser with ethacridine lactate than with mifepristone-misoprostol regimen, as the patients were admitted at the time of administration of mifepristone (Table 5). Thus, ethacridine lactate offers an alternative to the mifepristone-misoprostol regimen in countries where mifepristone is either unavailable or unaffordable.

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Full Length Research Paper

Inhibition of ubiquitin-proteasome pathway: A possible treatment of hepatocellular carcinoma

Abdulkareem Albekairy, Abdulmalik Alkatheri and Mahmoud Mansour*

College of Pharmacy, King Saud bin Abdulaziz University for Health and Science. P. O. Box 22490, Riyadh 11426, Kingdom of Saudi Arabia.

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Alterations in ubiquitination and deubiquitination reactions have been directly implicated in the etiology of many malignancies. In general, specific cancers can result from stabilization of oncoproteins or destabilization of tumor suppressor genes. Proteasome inhibitors (PIs) represent potential novel anticancer therapy. These agents inhibit the degradation of multi-ubiquitinated target proteins, that is, cell cycle regulatory proteins such as cyclins and cyclin-dependent kinase inhibitors that regulate cell cycle progression. Following the successful application of Bortezomib as an effective treatment for multiple myeloma (MM), a number of next-generation proteasome inhibitors have been developed with the goals of improving efficacy, overcoming drug resistance, minimizing dose-limiting toxicity such as peripheral neuropathy (PN), and improving convenience of administration. The recent accelerated approval of carfilzomib exemplifies the success of this approach, with other four inhibitors currently under study both preclinically and clinically. The role of PIs in hepatocellular carcinoma (HCC) has been demonstrated for the first time in 2004 that MG-132 induced apoptosis in human HCC cells through caspase-dependent cleavage of β-catenin and inhibition of β-catenin-mediated trans-activation. In addition, effect of Bortezomib on HCC was investigated and concluded that Bortezomib induced apoptosis in HepG2 cells as a model of HCC by stimulating both the extrinsic and intrinsic apoptotic pathways. Moreover, it has been shown that treatment with MG132 in combination with celecoxib resulted in synergistic anti-proliferative rather than anti-inflammatory and proapoptotic effects against liver cancer cells, providing a rational basis for the clinical use of this combination in the treatment of liver cancer.

Key words: Hepatocellular carcinoma, ubiquitin-proteasome pathway, proteasome inhibitors.

PROTEASOME STRUCTURE AND ORGANIZATION

In structure, the intact 26S is a large multi-subunit complex, which is approximately 2000 kilodaltons (KDa) in molecular weight and it composed of one 20S core particle structure and two 19S regularity caps (Figure 1). That core is a hollow cylindrical-shaped structure that provides an enclosed chamber in which proteins are destructed. The openings at each end of the core cylinder make it possible for the target protein to enter (Wang and Maldonado, 2006). Each side of the barrel-shaped structure is attached to a cap structure which has several ATPase active sites and ubiquitin binding sites. This cap structure regulates the recognition of the polyubiquitinated targeted substrates and translocates them to the proteolytic cavity of the core particle. 11S particle, which is an alternative form of regulatory subunit, can be attached to the 20S core particle in exactly the same way as the 19S particle (Wang and Maldonado, 2006).

*Corresponding author. E-mail: mansour1960us@yahoo.com. Tel: 0096618011111, 0966502997605. Fax: 0096612521011.

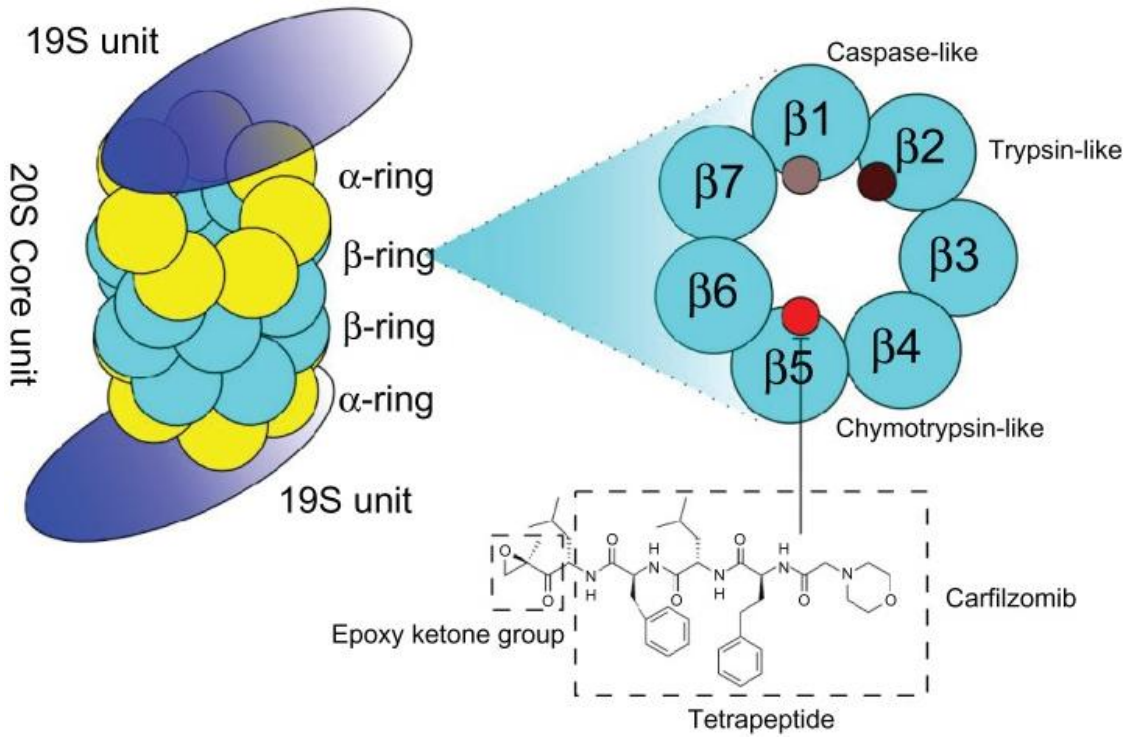

Figure 1. Composition of the 26S proteasome, carfilzomib chemical structure and it is binding site in the 20S proteasome and immunoproteasome. The 26S proteasome comprises a hollow cylindrical 20S proteolytic core and one or two 19S regulatory particles. The 19S unit recognizes poly-ubiquitynated substrates, and prepares them for proteolysis, which occurs inside the 20S cores. The 20S core comprising 2 pairs of 14 different polypeptides arranged in 4 stacked rings. Six subunits carry catalytic residues for the proteolytic sites: two are chymotrypsin-like (β5), two trypsin-like (β2), and two caspase-like (β1).

20S core particle

The type of organism is an important factor in which the number and diversity of subunits contained in the 20S core particle depends on. The distinct and specialized subunits is smaller in unicellular than multicellular organisms and smaller in prokaryotes than in eukaryotes. All 20S core particles comprise four stacked seven-membered ring structures. These structures consist of two different types of subunits: α subunits and β subunits. The α subunits have structural nature while β subunits are basically catalytic in nature. Each of the exterior two rings in the stack composed of seven α subunits. These subunits acts as docking domains for the regularity caps and the α subunits N-termini form a gate which restricts unregulated passage of proteins to the lumen of 20S core particle. Each of the interior two rings composed of seven β subunits and they have multiple catalytic sites which are responsible for the proteolysis reactions (Smith et al., 2007).

The archaebacterial 20S proteasome such as *Thermoplasma acidophilum* has just one type of α subunit in the outer two rings in the stack and one type of β subunit in the inner two rings in the stack while the 20S core particle in the eukaryotic proteasome consist of seven distinct but homologous α subunits and seven distinct but homologous β subunits. This core particle in the eukaryotic proteasome has at least three distinct proteolytic activities. Despite sharing a common mechanism, they have three distinct substrate specificities known as chymotrypsin-like (β5), trypsin-like (β2) and post-glutamyl peptide hydrolase-like (caspase-like, β1) (Heinemeyer et al., 1997; Adams, 2003). Alternative β forms denoted β1i also known as low molecular mass polypeptide 2 (LMP2), β2i also known as multicatalytic endopeptidase complex subunit (MECL1) and β5i, also known as low molecular mass polypeptide 7 (LMP7) can be shown in hemato-poietic cells mainly monocytes and lymphocytes upon stimulation with inflammatory signals like cytokines, especially interferon-gamma. The immunoproteasome is the proteasome assembled with these alternative β subunits (Altun et al., 2005; Nandi et al., 2006).

19S regulatory particle

In eukaryotes the 19S regulatory particle composed of at least 19 different subunits and can be detached further into two subcomplexes. The first is a 10-protein base that binds directly to either of the outer two rings of the 20S core particle, and the second is a 9-protein peripheral lid in which polyubiquitin chain is bound. In the base of the

19S regulatory particle six of the ten proteins are ATPase subunits which belong to the AAA family, and an evolutionary homologous of these ATPase subunits exists in archaea. It was called Proteasome-Activating Nucleotidase (PAN) (Zwickl et al., 1999). The binding of ATP to 19S ATPase subunits achieves the assembling of the 19S and 20S particles. The assembled complex degrading folded and ubiquitinated proteins need ATP hydrolysis. Note that ATP-binding alone can support all steps needed for protein destruction (e.g., complex assembly, gate opening, translocation, and proteolysis) except the unfolding of substrate required energy from ATP hydrolysis (Smith et al., 2005; Liu et al., 2006). Opening the gate in the α ring of the 20S core particle which prevents the access of substrates into the interior cavity is one role of the 19S regulatory ATPase subunits (Köhler et al., 2001). The way by which the 19S regulatory ATPase subunits opens the gate in the α ring of the 20S core particle has been recently explained (Pathare et al., 2011). 20S gate opening allows substrate entry into the proteolytic cavity of the core particle and requires the C-termini of the 19S regulatory ATPase subunits that has a certain motif known as hydrophobic-tyrosine-X (HbYX) motif. The proteasomal ATPases C-termini bind to pockets in the top of the 20S core particle. Tying these C-termini into these 20S pockets by themselves induces α ring conformational changes which subsequently lead to 20S gate opening in the same way that a key in a lock opens the door (Smith et al., 2007).

11S regulatory particle

A second type of regulatory cap that can be associated with 20S core particle is the 11S regulatory particle. It is a seven-membered structure that does not contain any ATPases. This heptameric structure is also known as PA28 or REG. It can facilitate entry and degradation of short peptides but not of complete proteins. It is supposed that this is because the proteasome assembled with this alternative regulatory particle cannot unfold larger substrates. It is binds to the 20S core particle through C-termini of its subunits and promotes α ring conformational changes which subsequently lead to 20S gate opening (Forster et al., 2005). 11S particle is induced by interferon gamma. By the union of the immunoproteasome β subunits it is responsible for the generation of certain peptides for major histocompatibility complex class I (MHC-I) presentation (Wang and Maldonado, 2006).

PROTEASOME FUNCTION: THE PROTEIN DEGRADATION PROCESS

Ubiquitylation and targeting

The proteins destined for destruction by the proteasome are marked covalently by a polyubiquitin chain. The ubiquitination of protein is carried out by the coordinated action of a cascade of enzymes: ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligases (E3) (Figure 2). In the first step of this cascade, in an adenosine triphosphate ATP-dependent manner, ubiquitin is activated by an ubiquitin-activating enzyme. This step involves hydrolysis of ATP, adenylylation of an ubiquitin molecule and the covalent binding of adenylylated ubiquitin to cysteine in the active site of an ubiquitin-activating enzyme in concert with the adenylylation of a second ubiquitin (Haas et al., 1982). After activation, the first adenylylated ubiquitin is then transferred to the active site cysteine of an ubiquitin-conjugating enzyme. In the third and last step, adding ubiquitin to the target protein is catalyzed by a member of many E3s. This E3 recognizes the specific protein to be ubiquitinated and catalyzes the transfer of ubiquitin from the active site cysteine of an ubiquitin-conjugating enzyme to a lysine residue on this target protein. A target protein must be labeled with a polybiquitin chain of at least four units before it is recognized by the proteasome lid (Thrower et al., 2000). The high substrate specificity of this system lies in the diversity of different E3s that can identify a specific substrate (Risseeuw et al., 2003). The organism and cell type determine the number of E1, E2 and E3 proteins. Here we must say that in humans there are a huge number of targets for the ubiquitin proteasome system because there are many different E3 enzymes (Li et al., 2008).

It is not fully understood how a polyubiquitinated protein is shuttled to the proteasome. Recent work has shown that ubiquitin-receptor proteins contain an N-terminal ubiquitin-like (UBL) domain and one or more ubiquitin-associated (UBA) domains. The UBL domains are recognized by the 19S regulatory particle while the UBA domains tie ubiquitin by three-helix bundles (Elsasser and Finley, 2005).

Unfolding and translocation

After a protein has been tagged with a polybiquitin chain, it is recognized by the 19S regulatory cap. This occurs in an ATP-dependent binding step. After that the tagged protein must then access the interior cavity of the 20S core particle to come in direct contact with the protease active sites contained in the β-ring of the proteasome. The protein substrates must be at least partially unfolded before their entry into the catalytic cavity; that is because the 20S core particle's central channel is narrow and locked by the α subunits N-termini. The journey of the unfolded substrate to enter the proteolytic chamber of the 20S core particle is called translocation and it occurs after removing the attached ubiquitin molecules (Liu et al., 2006). Zhu et al. (2005) reported that the order in which substrates are deubiquitinated and unfolded is not yet clear. However, the specific substrate decides on which of these processes is the rate-limiting step in the

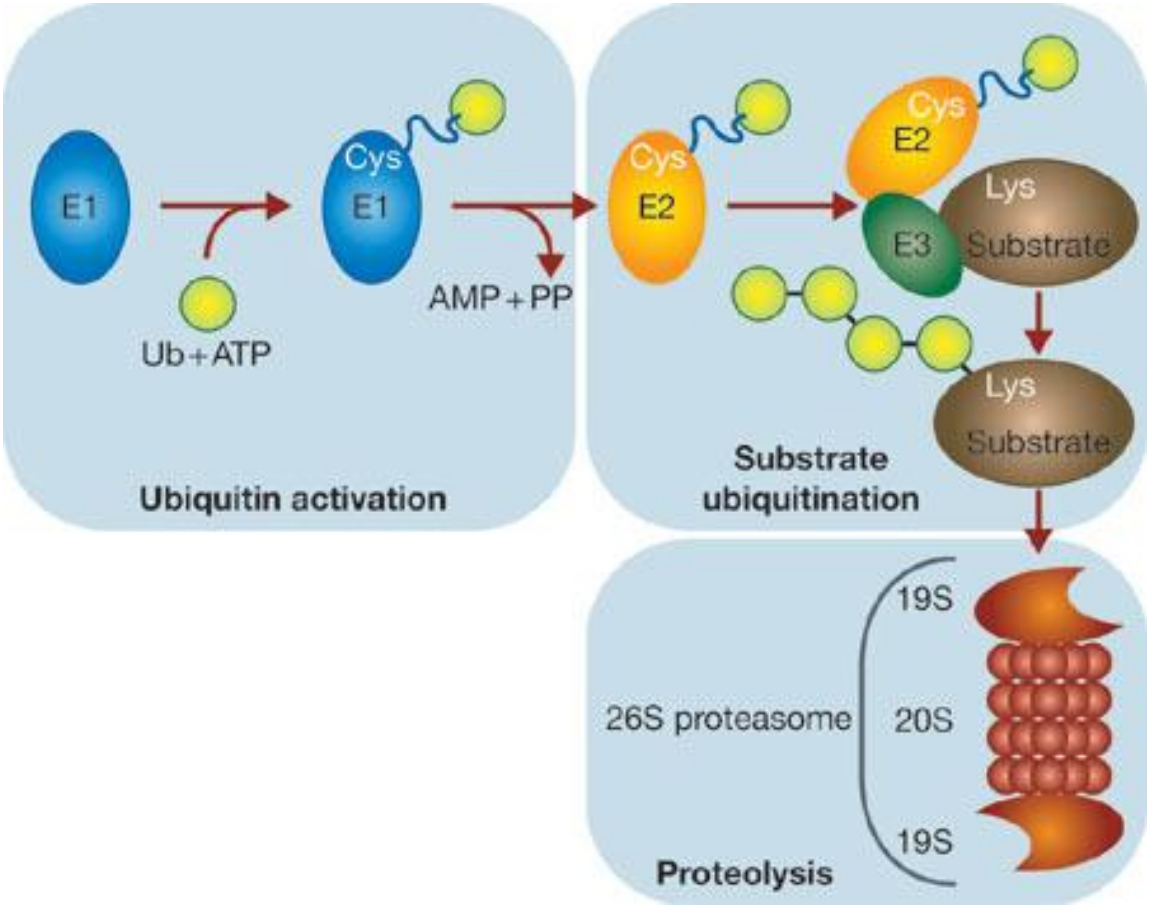

Figure 2. The protein ubiquitination pathway. A cascade of enzymatic reactions leads to ubiquitination of lysine residues of the substrate. First, the ubiquitin-activating enzyme (E1) hydrolyses ATP and forms a high-energy thioester linkage between its active site cysteine and the carboxy terminus of ubiquitin. Activated ubiquitin is then transferred to a member of the family of ubiquitin-conjugating enzymes (E2). E2 enzymes together with ubiquitin protein ligases (E3) attach ubiquitin to lysine residues of substrate proteins. In most cases, E3s function as substrate-binding factors that align the substrate and E2 in a way that facilitates ubiquitination (Meusser et al., 2005).

overall proteolysis reaction. The unfolding process for some proteins, for example, is rate-limiting while for other proteins deubiquitination is the slowest step (Smith et al., 2005).

Peptides longer than about four residues are prevented from entering the lumen of the 20S particle by the gate consisted of the α subunits N-termini. The ATP molecules, which are bound before the recognition of the tagged protein, are hydrolyzed before translocation. Energy from ATP hydrolysis is not required for translocation while it is needed for protein unfolding. The assembled complex can destruct unfolded substrates in the existence of a non-hydrolysable ATP analog. However, it cannot destruct folded substrates. This implies that energy from ATP hydrolysis is used for protein unfolding (Smith et al., 2005; Liu et al., 2006). If the 19S regulatory particle is in the ATP-bound state, the passage of the unfolded protein through the opened gate happens via facilitated diffusion (Smith et al., 2006).

Proteolysis

In protein destruction by the catalytic β-subunits of the 20S core particle, its mechanism is considered to be the threonine-dependent nucleophilic attack. Destruction happens in the middle of the core particle channel of the two β rings. In most cases, it does not generate partially destructed products, instead it cleaves protein substrate into short polypeptides of about 7 - 9 amino acids long, although they can vary between 4 to 25 amino acids in length depending on the organism and substrate protein. The biochemical process that specify the length of the peptide in the decomposition products is not fully clear yet (Voges et al., 1999). Although the catalytic activity of three β subunits shares a common destruction

mechanism, they have slightly different substrate specificities which are considered chymotrypsin-like, trypsin-like, and caspase-like. These differences in substrate specificity due to the interatomic contacts with local residues are near the active sites of each subunit. Also each catalytic β subunit contains a conserved lysine residue required for proteolysis (Heinemeyer et al., 1997; Rape and Jentsch, 2002; Adams, 2003).

PROTEASOME INHIBITORS

Proteasome inhibition treatment can induce cell cycle arrest and apoptosis. Inhibition of the proteasome by specific inhibitors has been shown to induce apoptosis in a variety of mammalian cells, just like neurons (Qiu et al., 2000), epithelial cells (MacLaren et al., 2001), aortic endothelial cells (Drexler et al., 2000), vascular smooth muscle cells (Kim, 2001) and tumor cells (Shah et al., 2001). The mechanism(s) through which proteasome inhibitors induce apoptosis is not clear and several factors seem to be important in different cells.

Tumor suppressor protein p53 is a short-life transcription factor that is normally degraded via ubiquitin-mediated proteolysis (Scheffner, 1998). Biochemically, Mdm2 acts as an ubiquitin ligase that covalently attaches ubiquitin to p53 and thus targets p53 for proteasomal degradation (Haupt et al., 1997). Accumulation of p53 will induce growth arrest, DNA repair or apoptosis. Bcl-2–associated X protein (Bax) (Chang et al., 1998) and an active form of BH3-interacting domain death agonist (tBid) (Breitschopf et al., 2000), which are examples to pro-apoptotic proteins, are also substrates of the proteasome. Inhibition of proteasomes could possibly induce apoptosis by causing an accumulation of these pro-apoptotic proteins. In addition, proteasome inhibition prevents degradation of inhibitor of kappa B (Iκ B), thereby preventing NF-κB transcriptional activity, which is involved in the induction of various anti-apoptotic Bcl-2 family members and some members of the inhibitor of apoptosis (IAP) family that directly prevent activation of caspases (Orlowski and Baldwin, 2002). Since proteasome does not only destruct pro-apoptotic proteins, but also enhance the expression of anti-apoptotic proteins, we can deal with proteasome as an anti-apoptotic factor. The accumulation of transcriptionally active p53 (Chen et al., 2000) and other pro-apoptotic Bcl-2 family members like Bax (Li and Dou, 2000), lead to the release of cytochrome c from mitochondria into the cytoplasm, which activates downstream caspase members have all been documented in the proteasome inhibitor-induced apoptosis. In addition, proteasome inhibitors caused a steady increase in activity of c-jun NH2-terminal kinase (JNK), which translocates to mitochondria and induces the release of cytochrome c and second mitochondrial activator of caspases (Smac), followed by caspase-9 activation (Chauhan et al., 2003). Besides the above-noted signaling events, endoplasmic reticulum stress and unfolded protein response, inhibition of angiogenesis and impairment of DNA-damage response have all been reported to contribute to the apoptotic affect of proteasome inhibitors in tumor cells (Crawford et al., 2011).

PIs can be classified into three groups by chemical properties and targets into: peptide boronates (Bortezomib), peptide epoxyketones (carfilzomib/PR-171), and β-lactones (NPI-0052/marizomib).

Peptide boronates

Bortezomib

Bortezomib (originally PS-341 and marketed as VELCADE by Millennium Pharmaceuticals), is the first proteasome inhibitor to enter clinical practice as a chemotherapeutic agent. It is approved by FDA for the treatment of relapsed multiple myeloma (Richardson et al., 2003), as well as mantle cell lymphoma (Fisher et al., 2006). Bortezomib is a dipeptide boronic acid analogue that reversibly inhibits the chymotrypsin-like (CT-L) and caspase-like (C-L) active sites, with minimal effect on trypsin-like (T-L) activity of the proteasome (Dick and Fleming, 2010). As a result of this approval, several second-generation proteasome inhibitors, such as carfilzomib have been developed and entered clinical trials in an attempt to overcome resistance to Bortezomib and improving safety profile.

Peptide epoxyketones

Carfilzomib

Carfilzomib (formerly known as PR-171) is a tetrapeptide epoxyketone analog (Figure 1) of the microbial natural product, epoxomicin 3, that was discovered initially as antitumor agents in animals and later on it was shown to be a potent inhibitor of the proteasome (Demo et al., 2007; Bennett and Kirk, 2008). Carfilzomib selectively binds to and inhibits the chymotrypsin-like activity of the proteasome via the β5 and LMP7 proteasome subunits, and has minimal cross-reactivity with the trypsin-like or caspase-like activities of the proteasome (Figure I). Carfilzomib exhibits mechanistically irreversible proteasome inhibition and requires new proteasome complexes synthesis for recovery of cellular proteasome activity. As a result, we can say that carfilzomib provides prolonged proteasome inhibition if we compared it with that of the slowly reversible inhibitor, Bortezomib. Also the epoxybutane pharmacophore of carfilzomib shows a high level of selectivity for the NH2-terminal threonine residue of the proteasome active sites, most potently the CT-L active sites of the constitutive proteasome (β5

subunit) and immunoproteasome (LMP7 subunit) (Arastu-Kapur et al., 2011; Jain et al., 2011). In several phase 2 clinical trials, single-agent carfilzomib has demonstrated tolerability and significant anti-tumor activity (O'Connor et al., 2009; Martin et al., 2010; Siegel et al., 2012; Vij et al., 2012).

β-lactones

Marizomib (NPI-0052)

NPI-0052 has been evaluated in a number of phase I trials in patients with advanced hematologic and solid malignancies. The initial data from dose-escalating studies of once weekly intravenous administration had shown rapid, broad and potent dose-dependent proteasome inhibition, with a favorable safety profile and some efficacy (Hofmeister et al., 2009; Spencer et al., 2004). The common adverse events include mild-to-moderate fatigue, with no significant PN, neutropenia or thrombocytopenia (Richardson et al., 2009).

The results from a phase I trial of once weekly NPI-0052 in combination with the histone deacetylase inhibitor vorinostat in solid tumor patients showed marked synergistic effect in a number of cell lines *in vitro*. Moreover, the administration in patients appeared to be safe and tolerable as well, without any drug–drug interaction (Millward et al., 2012).

PROTEASOME INHIBITORS AND HEPATOCELLULAR CARCINOMA

Treatment of human cancers is limited by the systemic toxicity of chemostatic or chemotoxic anti-cancer agents and also by the existence of drug resistance mechanisms. HCC is one of the most common malignancies in the world with an estimated annual incidence of greater than 1 million new cases per year (Schafer and Sorrell, 1999). Although several alternative therapies other than radical operation have been employed such as a transarterial embolization, there is still no satisfactory improvement in the prognosis of HCC to date (Schafer and Sorrell, 1999). One of the reasons for the poor prognosis of HCC is the high rate of recurrence. This high recurrence rate, even after curative therapy, has been shown to be due to intrahepatic metastasis or multicentric development of each respective neoplasm clone (Ikeda et al., 2000). It has been shown that the existence of liver fibrotic changes promotes hepatocarcinogenesis (Sakaida et al., 1998).

Emanuele et al. (2002) showed that MG132 reduced the viability of HepG2 cells in a time- and dose-dependent manner. The effect was in tight connection with the induction of apoptosis, and was accompanied by a remarkable increase in the production of H_2O_2 and a reduction in mitochondrial transmembrane potential ($\Delta\psi m$). In addition cell death was prevented by antioxidants such as GSH, N-acetylcysteine or catalase.

Western blot analysis showed that HepG2 cells contain a very low level of Bcl-2 and a much higher level of Bcl-XL, another antiapoptotic factor of the same family. When the cells were exposed to MG132 the level of Bcl-XL diminished, while a new band, corresponding to the expression of the proapoptotic protein Bcl-XS was detected (Emanuele et al., 2002). MG132 also caused the release of cytochrome c from mitochondria and the activation of caspase-3 with the consequent degradation of poly-ADP ribose polymerase (PARP). Cervello et al. (2004) supported the therapeutic potential of the proteasome inhibitors in HCC. He confirmed the induction of apoptosis by the proteasome inhibitor MG132 in human HCC cells by caspase-dependent cleavage of beta-catenin and inhibition of beta-catenin-mediated transactivation.

TRAIL exhibits potent anti-tumor activity on systemic administration in mice. Because of its proven *in vivo* efficacy, TRAIL may serve as a novel anti-neoplastic drug. However, approximately half of the tumor cell lines tested so far is TRAIL resistant, and potential toxic side effects of certain recombinant forms of TRAIL on human hepatocytes have been described.

Previous study have demonstrated that inhibition of proteasome function effectively sensitizes cells to TRAIL by regulating several factors, normally reduced in neoplastic cells through enhanced proteasome degradation (Zhang et al., 2007). Ganten et al. (2005) in this issue of hepatology reported that proteasome inhibitors can also sensitize hepatocellular carcinoma cells, but not primary human hepatocytes, to TRAIL-induced apoptosis.

The mechanism of increased TRAIL sensitivity in HCC cells have been investigated by Inoue et al. (2006). He examined surface expression of TRAIL and its receptors in different HCC cell lines. MG132 up-regulated both TRAIL and its receptors (TRAIL-R1 and -R2) in SK-Hep1 and HLE, parallel with down-regulated the expression of X-linked inhibitor of apoptosis protein (XIAP) in SK-Hep1 and HLE, and survivin in all three cell-types. Furthermore, MG132 down regulated phospho-AKT and its down-stream target phospho-BAD, indicating that MG132 activated the mitochondrial apoptosis pathway by inhibiting phosphorylation of AKT and BAD.

Lauricella et al. (2006) elucidated the molecular mechanism of apoptosis induced by Bortezomib in HepG2 cells and ascertain the reasons for the insensitivity to Bortezomib shown by Chang liver cells. Bortezomib induced apoptosis in HepG2 cells by stimulating both the extrinsic and intrinsic apoptotic pathways.

Moreover, Chen et al. (2008) investigated the role of Bortezomib on Akt signaling as a major molecular mechanism in determining Bortezomib-induced apoptosis in HCC cells and showed the suppression of tumor growth with down regulation of P-Akt in Huh-7 tumors but

not in PLC5 tumors.

Combinational therapy for molecular targeted therapy has been a common approach to improve responsiveness in cancer therapy. Cusimano et al. (2010) assessed the effects of celecoxib in combination with MG132 on the growth of two HCC cell lines regarding cell viability, apoptosis and ER stress response, and concluded that combination treatment with celecoxib and MG132 resulted in synergistic antiproliferative and proapoptotic effects against liver cancer cells, providing a rational basis for the clinical use of this combination in the treatment of liver cancer.

Deleted in liver cancer 1 (DLC1), a tumor suppressor gene has been identified in a primary human heaptocellular carcinoma. Luo et al. (2011) showed that, intracellular stability of DLC1 protein is regulated by the 26S proteasome in human hepatocellular carcinoma cell line Hep3B and demonstrated that DLC1 is an unstable protein that is rapidly degraded by the 26S proteasome in human hepatocellular carcinoma Hep3B cells. The protein levels of endogenous DLC1 were significantly higher in HEK293 and Hep3B cells after treatment with the proteasome inhibitor MG132. The protein levels of exogenous DLC1 were also increased by inhibition of the 26S proteasome, suggesting that both endogenous and exogenous DLC1 proteins are degraded by the 26S proteasome.

CONCLUSION

These findings suggested that proteasome inhibitors may have a pivotal role in hepatocellular carcinoma and therefore the increasing evidence of multiple roles for the Ubiquitin-Proteasome System within the pathogenesis of HCC suggests that it may prove to be fertile ground on which to develop novel therapies that will prove effective in the treatment of this most devastating disease.

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