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ORIGINAL ARTICLE

Management of complex ankle fracture: A Ghanaian experience

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Ankle fractures are among the most common conditions for surgical emergencies in most developing countries including Ghana. Despite the fact that many ankle fractures are uncomplicated, a high proportion may require surgical intervention. Decision-making depends on recognition of the fracture pattern, availability of surgical implants and anaesthetic materials. In resource-limited settings where patients are unable to afford the cost of surgical implants and anaesthetic materials associated with ankle fractures, suggested modification of the open reduction and internal fixation (ORIF) technique have proven to yield satisfactory results. This study retrospectively assessed the effectiveness of the modified ORIF method among Ghanaians living within the Tamale metropolis, a resource-limited setting located in the Northern Region of Ghana. The study reviewed 70 cases of bimalleolus fractures which were either treated using the ORIF based on the Association for the Study of Internal Fixation (ASIF) protocol or a modified version of the ORIF which involves internal fixation of the malleolus without screws. The findings indicate that the modified method is as good as ORIF (based on ASIF protocol) with added benefits such as shorter operation time, reduced risk of anaesthetic complications and cost of operation (anaesthetic agents and orthopaedic implant cost) as well as reduced number of foreign bodies (implants) leading to a lower risk of wound infections. The use of this method however demands that foot and ankle joint must be handled with extreme care so as not to dislocate the tibia malleolus post-operatively.
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Keywords*:* Bimalleolus, surgical, open reduction, internal fixation, bone fracture, Ghana

INTRODUCTION

As a weight-bearing joint, the ankle can absorb great amount of shock and pressure up to about 6 times the individual's body weight (Carr *et al.*, 2003). Excessive amount of energy across the ankle joint can lead to fracture. Ankle fractures are considered the most common of all fractures treated in hospitals (Yang *et al.*, 2011). In the United States of America (USA), the incidence per year of ankle, tibia and fibula fractures is about 492,000. (Praemer *et al.*, 1992; Weening and Bhandari, 2005) and in the United Kingdom (UK), documented incidence rate of fractures is 14.8% per 10,000 persons per year (Van Staa *et al.*, 2001). In Africa however, the incidence is expected to be higher due to additional high incidence of road traffic accidents (Twagirayezu *et al.*, 2008). Road traffic accidents are said to account for about 46.3% of ankle fractures in Nigeria (Ifesanya and Alonge, (2012) and about 71.5% of lower limb fractures in Rwanda (Twagirayezu *et al.*, 2008)

The management of ankle fracture in the general populace has been documented to range from non-operative restriction to open reduction and internal fixation (ORIF). Irrespective of management method, anatomic alignment of the ankle joint and complete healing are major factors which can ensure long-term treatment success (Dahners, 1990; Egol *et al.*, 2000) and to prevent arthritis due to abnormal pressure distribution because of malunion of the ankle fracture (Ramsey and Hamilton, 1976). The quality of bone and related cartilage injury, age and alignment of the joint surface (Walheim and Akerman, 1936; Klossner, 1962) as

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well as the type of fracture (Lindsjo, 1985) are other factors to consider during management. However, ORIF is said to offer a potential for more rapid recovery than non-operative treatment (Lindsjo, 1985; Porter *et al.*, 2008). Congruent joint, fracture union, functional motion, normal strength and optimal recovery time are the main objectives of any management method of ankle fracture (Jelinek and Porter., 2009). In a resource-limited setting where availability and accessibility to surgical implants and anaesthetic materials is a major problem coupled with the fact that the community dwellers may not be able to afford the cost of surgical implants and anaesthetic materials, a modification of the method may be necessary. This study retrospectively assessed the effectiveness of the modified ORIF method among Ghanaians living within the Tamale metropolis and its surrounding environs, a resource-limited setting located in the Northern Region of Ghana.

PATIENTS AND METHODS

Study site and participants

A total of 70 patients who received complex ankle fracture or bimalleolus fracture management at the Tania Specialist Hospital between September 2005 and September 2010 were included in this retrospective study. Patient characteristics (age and gender), fracture type, mechanism of injury and treatment type were retrospectively reviewed. After review of patient data, the subjects were grouped into two based on method of treatment. Group one (n = 35) was treated with open reduction and internal fixation (ORIF) using principles of the Arbeitsgemeinschaft Osteosynthesefragen (AO/ASIF) group. The second group (n = 35) was treated with modified ORIF without syndesmosis and medial malleolus lag screws. All surgical cases were performed by a consultant orthopaedic surgeon and were usually done after initial physical and photographic assessment of the patient to confirm the position and 'personality' of the fracture. Radiographs taken at 2 and 6 weeks of treatment in both methods were also reviewed.

Treatment Procedure

All patients treated by ORIF with AO/ASIF principles followed the under listed protocol;

a. Open reduction of laterally dislocated tibia malleolus and stabilization with lag screw(s)
b. Open reduction of fibular fracture with unstable syndesmosis
c. Reduction of lateral dislocated Talus
d. Fixation of syndesmosis with screw(s)
e. Application of below knee splint for 7-10 days
f. Application of knee circular POP after 7-10 for six weeks
g. Removal of POP and start of physiotherapy and partial body weight bearing (15 kg) for start increasing over six more weeks

The major outcome is the anatomic fixation of the ankle joint which allows for early return to functional range of motion.

The rest of the patients who were treated with modified ORIF without bimalleolus lag screws followed the under listed procedure;

a. Open reduction of laterally dislocated tibial malleolus and stabilization with Vincryl-2 suture (first as pair-string and fortified with Z-shape suture) over deltoid ligament without lag screws.
b. Reduction of laterally dislocated Talus
c. Fixation of syndesmosis with screw(s)
d. Application of below knee splint with extreme care for 10 days
e. Application of knee circular POP for six weeks
f. Removal of POP and start of physiotherapy and partial weight bearing of 15 kg body weight for the start, increasing over six more weeks.

Statistical Analysis

All categorical variables were expressed as proportions and were compared using Fisher's exact test. In all statistical tests, a value of $P<0.05$ was considered significant. All analysis was performed using GraphPad Prism 5.10 for windows (Graphpad software, San Diago, CA. USA).

RESULTS

From this study, plate 1A shows a radiograph of pre-operative fracture (*in the direction of the black arrow*). Plate 1B shows a radiograph of the ankle joint after stabilization using the modified ORIF method (*in the direction of the white arrow*) compared to Plate 1C which is a radiograph of the fracture after heal-

ing following the ORIF (AO/ASIF) (*indicated with a white arrow*).

From the retrospective review of available data, the general cause of injury was road traffic accident which accounts for 88.6% (62/70) of all recorded ankle fracture cases followed by direct blow (10.0%) and falls from heights (1.4%) as shown in Table 1.

Table 1: Aetiology of fractures

Aetiology	No. (%)
Road traffic accident	62 (88.6)
Direct blow	7 (10.0)
Fall from a height	1 (1.4)
Total	70 (100)

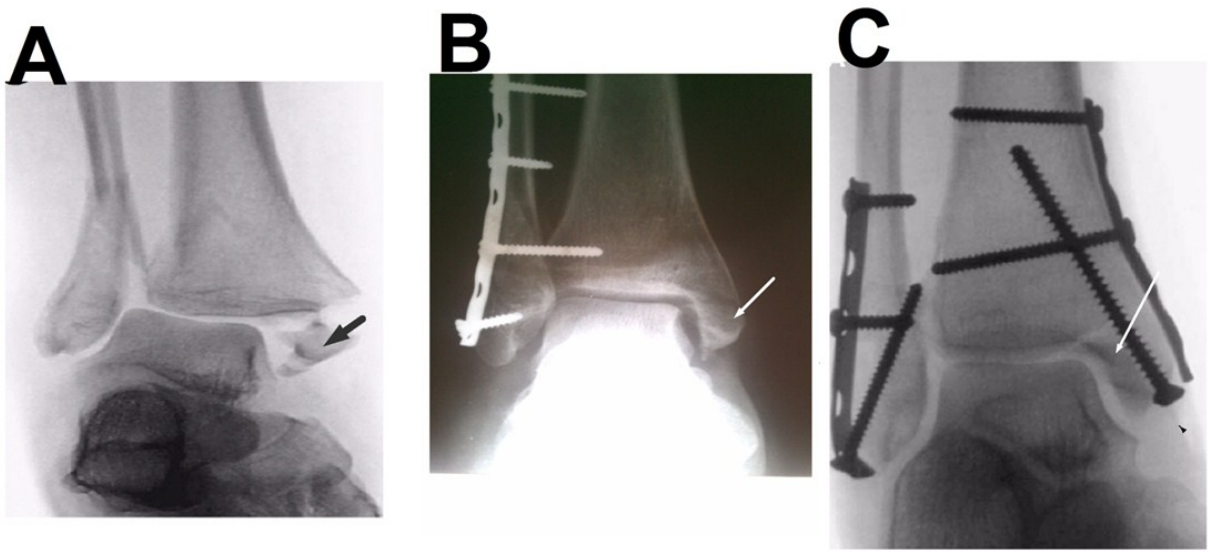

Plate 1: Radiographs of **A)** pre-operative fractures with an arrow (black) showing the medial malleolus; **B)** post-operative treatment without medial malleolus screw(s) indicated by the arrow (white); **C)** post-operative treatment with medial malleolus screw(s) indicated by arrow (white).

In this study, 85.7% (60/70) of the patients were males with only 14.3% (10/70) being females. The mean age of the patients was 36 ± 9 years with a range of 11-65 years. Majority of the patients (62.9%) were within the 31-50 year age bracket as shown in Table 2. The recovery period for the patients ranged from within 3 months to 6 months with about 67.4% (47/70) gaining full recovery within 3 months and the remaining 32.9% (23/70) recovering within 3-6 months post-operation. When the rate of recovery between the subjects treated with the AO/ASIF was compared to those treated without the malleolus lag screws, there was no significant difference (P = 0.8075). There was no significant difference in the recovery period between men and women as shown in Table 2. Although younger subjects tendered to heal early there was no statistical significant difference in healing among the subjects with respect to patient age. After one year of follow up there were no post-operative complications such as deep wound infections and reflex sympathetic dystrophy in both groups.

DISCUSSION

This study reports ankle fracture incidence of 88.6% resulting from vehicular road traffic accident (RTA). This high figure could be attributed to the high number of motor cycles in the three Northern regions. According to the Regional motor traffic unit majority of users of these motor cycles are without driving licence leading to careless and reckless driving culminating in the high incidence of

Table 1: Relationship between fracture healing time and patient age, method of fixation and gender

Variable	Healing time		Total	P value
Age (Yrs)	Within 3 months	3-6 months		
10-20	2(100.0%)	0(0.0%)	2	
21-31	9(52.9%)	8(47.1%)	17	
31-40	12(57.1%)	9(42.9%)	21	
41-50	13(56.5%)	10(43.5%)	23	
51-60	3(60.0%)	2(40.0%)	5	
61-70	0(0.0%)	2(100%)	2	
Method of stabilization				
ORIF(AO/ASIF)	22(62.9%)	13(37.1%)	35	P = 0.8075
ORIF (without screws)	20(57.1%)	15(42.9%)	35	
Gender				
MALE	35(58.3%)	25(41.7%)	60	P = 1.000
FEMALE	6(60.0%)	4(40.0%)	10	

road traffic accidents (*personal communication*). The high vehicular related ankle injury reported in the present study is in conformity with results of similar studies in other parts of Africa which indicated that road traffic accidents are the leading cause of ankle fractures in Africa (Twagirayezu *et al.*, 2008).

Proponents of open reduction and internal fixation suggest that restoration of the normal anatomy will reduce the risk of subsequent osteoarthritis due to incongruence (Weber, 1966). In areas where surgical implants are not readily available or accessible, surgeons find it difficult to manage complex ankle fractures leading to complications such as arthritis and bacterial infections (Ifesanya and Alonge, 2012). According to Steiner and Kotisso (1996), it is not clear whether or not internal fixation should have a place in Africa. It is often argued that there would be too many infections and other complications because in Africa there is neither adequate training in internal fixation nor adequate infrastructure in the operating theatre (Steiner and Kotisso, 1996). This perception is however changing with the establishment of specialist hospitals around Africa. In this study internal fixation of bimalleolus fracture without lag screw has proven successful with added benefits such as a shorter duration of surgical process, reduced risk of anaesthetic complications, reduced cost of operation (anaesthetic agents and orthopaedic implant cost) and reduced number of foreign bodies (implant) leading to a lower risk of wound infections. Clinical studies have consistently failed to show any difference in outcome between fractures treated operatively and those managed conservatively. The findings of the present study is similar to Yde and Kristensen (1980) who compared operations based on ASIF techniques with closed treatment and immobilisation in a plaster cast and found no difference in outcome at a minimum follow-up of three years. The modified ORIF procedure without lag screws also ensures early restoration of anatomical function similar to the ORIF based on ASIF principles. There were no post operative complications such as deep wound infections and reflex sympathetic dystrophy which are usually associated with the ASIF procedure as reported by Paudel (2011) giving this procedure an added advantage.

CONCLUSION

The results of this retrospective study suggest that the modified ORIF treatment protocol for complex ankle injuries is safe, satisfactory and equally effective with good functional outcome similar to the AO/ASIF ORIF treatment protocol which uses screws for internal fixation. The use of the modified ORIF protocol however demands that foot and ankle joint must be handled with extreme care so as not to dislocate the tibia malleolus post-operatively.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Anti-diarrheal activity of leaf extract of *Juniperus procera* and its effect on intestinal motility in albino mice

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This study was designed to evaluate the anti-diarrheal property of *Juniperus procera* using albino mice. An aqueous extract of *J. procera* leaves was administered to albino mice at 150, 300, and 450 mg kg^{-1} (*p.o*). Wet feces, intestinal accumulation (enteropooling) and intestinal motility were recorded. The aqueous extract of *J. procera* significantly ($p < 0.0001$) decreased the mean number of wet faeces produced by the albino mice in a dose dependent manner as well as decreasing the distance travelled by the charcoal meal ($p < 0.0001$) from 28.5 ± 1.1 cm when treated with 150 mg kg^{-1} to 11.8 ± 0.5 cm when treated with 450 mg kg^{-1} through 20.0 ± 1.0 cm when treated with 300 mg kg^{-1}. Results obtained for the extract especially the 450 mg kg^{-1} dose was almost equivalent to diphenoxylate and atropine sulphate (the reference drugs used). In conclusion, aqueous extract of *J. procera* demonstrated anti-diarrheal activity and could be an inexpensive and readily available anti-diarrheal remedy.

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Keywords*: Juniperus procera*, diarrhea, intestinal motility, castor oil, enteropooling

INTRODUCTION

Diarrhea is a gastrointestinal tract (GIT) dysfunction, which is considered as a common symptom of infection and one of the causes of intestinal motility disorder (Maresca *et al.,* 2003). It causes loss of water and important nutrients from the GIT in addition to increasing intestinal motility (Jimba *et al.,* 2002). The rate of material movement through the intestinal lumen is directly associated with its motility. As diarrhea causes high intestinal motility the increased motility also heightens diarrheal effects through increasing the rate of movement of intestinal content (Qnais *et al.,* 2005; Hejazian *et al.,* 2007).

Diarrhea is the cause of death in about 2.2 million people each year (Guerrant *et al.,* 2001; Meite *et al.,* 2009) despite the availability of synthetic drugs. Medicinal plants have been recommended as good alternatives due to their cost as well as availability. Chebula, swertia, and black pepper are some medicinal plants that are used in India and China to treat diarrheal (Das *et al.,* 2009). Many species of the Genus *Juniperus* belonging to the family *Cuperssaceae* are claimed to cure diarrhea. The anti-diarrheal properties of *J. phoenicia*, *J. communis*, *J. oxycedrus* and *J. thurifera* have been validated (Cosentino *et al.,* 2003; Karaman *et al.,* 2003, Qnais et al., 2005). Also, WHO has encouraged the use of traditional medicinal plants for the treatment and prevention of diarrhea since the 1980s (Syder and Merson, 1982; Park, 2000).

Castor oil is known to induce GIT enteropooling similar to that observed in diarrhea (i.e. accumulation of substances in the gut lumen) (Galvez *et al.,* 1993; Gorard *et al.,* 1994; Akomolafe *et al.,* 2003). Its effect is mediated by ricinolic acid that can induce a hypersecretory response by the gut wall leading to diarrhea (Capasso *et al.,* 1994; Chitme *et al.,* 2000; Das *et al.,* 2009). In this study *J. procera*, an evergreen indigenous gymnosperm in Ethiopia is

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tested as an antidiarrheal plant. This plant has a wide range of traditional uses including charcoal and timber productions, fire wood, fencing etc. Its leaves are smoked to deter insects (personal observation) in rural areas of the country. In the northern parts of the country people are known to use this plant to treat menorrhagia, emmenagogue, constipation, toothache, gum pain, and biliousness (Abebe and Ayehu, 1993).

MATERIALS AND METHODS

Plant Material Collection

Fresh leaves of *J. procera* were collected from the campus of Natural Science College of Addis Ababa University (AAU) at an attitude around 2450 m a.s.l. in June 2010. The collection was made after identification and taxonomic authentication by the help of a botanist and sample specimen is kept in the Herbarium of Faculty of Life Science, AAU under voucher № 006. The collected leaves were allowed to dry under shade for 20 days and the air dried leaves were then ground.

Preparation of the extract

A measured amount of the ground leaves of *J. procera* was dissolved in warm distilled water in 1:10 (w/v) with continuous stirring for 30 min according to the method in Qnais *et al.,* (2005) and Oben *et al.,* (2006). The solution was filtered using cotton and filter paper. The filtrate was completely lyophilized under reduced pressure. The resultant powder was weighed and dissolved in Tyrode, a physiological salt solution. This physiological salt solution was prepared daily with the following compositions (mM): 118 NaCl, 4.7 KCl, 25 $NaHCO_3$, 1 $NaH_2PO_4.H_2O$, 0.5 Na_2HPO_4, 11.1 glucose, 2.5 $MgCl.6H_2O$, and 2.5 $CaCl_2.2H_2O$. The pH used during this preparation was 7.4.

Experimental animals

Adult albino mice weighing between 35-45 g were used. All mice were provided with a standard pellet food and water *ad libitum*. The mice were starved for 18 h before the experiment but were provided with water.

Drugs and chemicals

A reference anti-diarrhoeal drug (diphenoxylate), castor oil (laxative agent), atropine sulphate and charcoal meal were used. All the chemicals were of pharmacological grades and obtained from BDH Merck Ltd, UK.

Experimental Procedures

Anti-diarrheal test

Five groups of mice (n=6) were set for the experiment and labeled A-E. Group A serving as a negative control was given 0.2 ml PSS. Groups B, C and D were given the extract at doses of 150, 300 and 450 mg kg^{-1} respectively. Diphenoxylate was given to Group E, the positive control at a dose of 5 mg kg^{-1}. All administrations were by gavage. Castor oil (1 ml) was given orally to all mice an hour before the treatment (described above). Observations were made for 4 hrs and the number of both wet and dry feces was recorded. The experiment was performed in triplicate according to standard procedures. Average number of feces was taken to calculate percentage diarrheal inhibition according to the following formula (Oben *et al.*, 2006).

$$\%\ inhibition = \frac{No.of\ WFC - No.of\ WFT}{No.of\ WFC}\ x\ 100$$

WFC = wet feces in control and WFT = wet feces in test group

Intestinal motility test

Intestinal motility test was done according to the methods of Qnais *et al.,* (2005) and Meite *et al.,* (2009) with slight modifications. Five groups of mice (n=6) were organized and made to fast for 18 hrs. Group A served as a control and received 0.5 ml of PSS. The reference drug, atropine sulphate (5 mg kg^{-1}) was given to group E that had served as a positive control. Groups B, C and D received the extract at a dose of 150, 300 and 450 mg kg^{-1} of body weight respectively. All administrations were made orally by gavage. Mice were given 1 ml of charcoal meal (5 g of activated charcoal suspended in 50 ml PSS) 30 min later through the same route.

After another 30 min all mice were sacrificed and their abdomen was open. The experiment was performed in triplicate according to standard procedures. The distance traveled by the charcoal meal from the pylorus to the caecum was measured and the percentage of inhibition of movement was calculated as follow (Oben *et al.,* 2006):

$$\% \, Inhibition = \frac{MTLI - MDCC}{MTLI} \, x \, 100$$

MTLI = mean total length of the intestine and MDCC = mean distance covered by the charcoal

Anti-enteropooling test

As in test for antidiarrheal and intestinal motility, triplicate experiments were conducted to test the anti -enteropooling property of the plant. Four groups of mice (n=6) were assigned as A, B, C, and D. Group A served as a control receiving PSS (0.5 ml) by oral administration. Group B, C and D respectively received *J. procera* leave extract at a dose of 150, 300 and 450 mg kg^{-1} by the same route. Castor oil (1 ml) was given orally to the mice after an hour. Two hours later all mice were sacrificed to isolate the small intestine. Intestinal contents were collected by mixing the intestine content and the volume was measure using graduated cylinder.

Statistical analysis

Continuous variables were presented as mean ± SEM and categorical variables presented as proportion. To compare differences between groups, *one way Analysis of Variance* (ANOVA) was performed followed by Tukey test as *post hoc*. In all test p value < 0.05 was considered significant.

RESULTS

Anti-diarrheal activity

As shown in figure 1A, the aqueous extract of *J. procera* significantly ($p < 0.0001$) decreased the mean number of wet faeces produced by the albino mice in a dose dependent manner. Even though, the mean reduction in the number of wet faeces produced when the extract was administered at 450 mg kg^{-1} was not as much as that produced when 5 mg kg^{-1} of the standard drug was administered, the difference did not reach significant level ($p = 0.74$). The extract also in a dose dependent manner increased the percentage inhibition of wet faeces production as the treatment dose was increased from 150 through 300 to 450 mg kg^{-1} of *J. procera* ($p < 0.001$) (Figure 1B). The percentage inhibition induced by the 450 mg kg^{-1} of the extract was not significantly different from the inhibition induced by 5 mg kg^{-1} of the standard drug ($p = 0.45$) (Figure 1B).

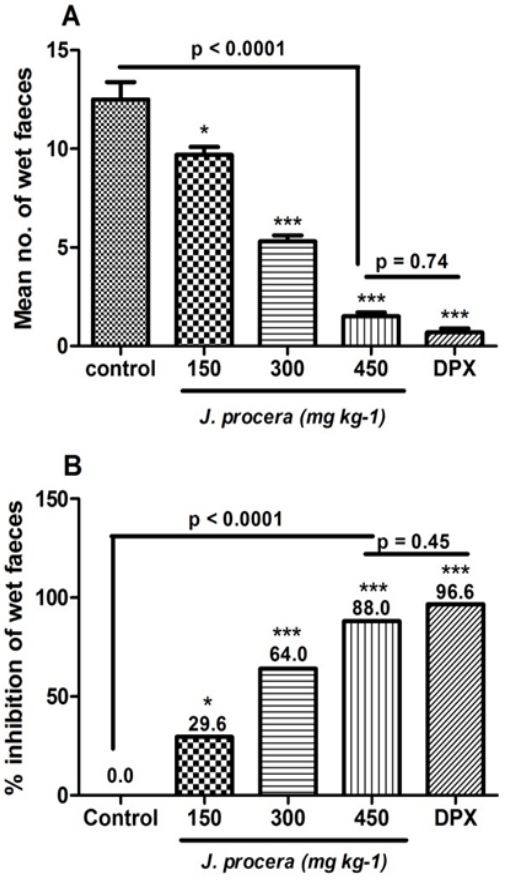

Figure 1: Effects of *J. procera* (150, 300 and 450 mg kg^{-1}) and 5 mg kg^{-1} diphenoxylate (DPX) treatment 1 hr after castor oil (1 ml) induced diarrheal on the number of wet faeces produced (A) and the percentage inhibition of wet faeces (B). Data are presented as mean ± SEM and proportion. Significantly different from control: *p<0.05 and ***p<0.001 by Tukey post hoc test (n = 6).

Table 1: Effects of *J. procera* (150, 300 and 450 mg kg^{-1}) and 5 mg kg^{-1} atropine treatment 30 minutes before administration of 1 ml of charcoal meal on the distanced as well as % inhibition of charcoal movement.

Test Group	Total distance of the intestine (cm)	Distance Traveled by charcoal meal (cm)	P value	% Inhibition
Control	69.0 ± 1.0	61.0 ± 1.1		11.6
Extract (150 mg kg^{-1})	68.5 ± 1.0	28.5 ± 1.1	0.00	58.4
Extract (300 mg kg^{-1})	67.5 ± 1.1	20.0 ± 1.0	0.00	70.4
Extract (450 mg kg^{-1})	68.7 ± 1.0	11.8 ± 0.5	0.00	82.8
Atropine (5 mg kg^{-1})	69.0 ± 1.2	9.3 ± 0.5	0.00	86.5

Data are presented as mean ± SEM and proportion. P values are significantly different from control using Tukey post hoc test (n = 6).

Effect of the extract on intestinal motility

As presented in Table 2, the length of the intestine of the albino mice in all the groups was similar. Using one way ANOVA, the aqueous extract of *J. procera* was able to decrease the distance travelled by the charcoal meal in a dose dependent manner ($p < 0.0001$) from 28.5 ± 1.1 cm when treated with 150 mg kg^{-1} to 11.8 ± 0.5 cm when treated with 450 mg kg^{-1} through 20.0 ± 1.0 cm when treated with 300 mg kg^{-1} (Table 1). Inversely, the percentage inhibition of the charcoal meal movement also significantly increased ($p < 0.0001$). There were no significant differences when the highest dose of the extract was compared to the 5 mg kg^{-1} of the standard reference drug (Atropine) in terms of the distance travelled as well as the % inhibition (Table 1).

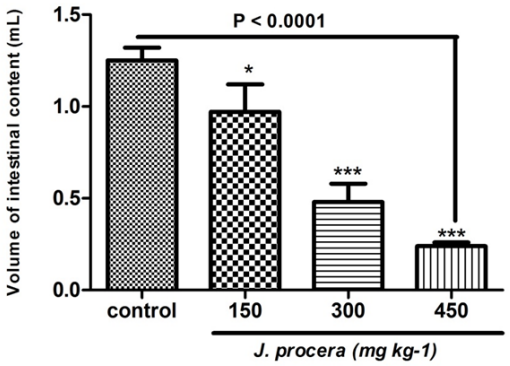

Figure 2: Effects of *J. procera* (150, 300 and 450 mg kg^{-1}) on the volume of intestinal content. Data are presented as mean ± SEM. Significantly different from control: *p<0.05 and ***p<0.001 by Tukey post hoc test (n = 6).

Anti-enteropooling property

From the one way ANOVA using treatment as a factor, the extract significantly reduced ($p < 0.0001$) the content of the animal intestine from 0.97 ± 0.45 mL when treated with 150 mg kg^{-1} through 0.48 ± 0.10 mL when treated with 300 mg kg^{-1} to 0.24 ± 0.02 mL when treated with 450 mg kg^{-1} in a dose dependent manner (Figure 2).

DISCUSSION

The results from this study clearly reveal that the aqueous extract of *J. procera* possesses anti-diarrhoeal property. The aqueous extract of the leaves of this plant may contain different agents that effectively reduced diarrhoea that was induced by a potent diarrhoeal agent, castor oil. Diarrhoea can be characterized by different phenomena including frequent out flow of wet (waterish) faeces, high intestinal motility, high accumulation of important nutrients in the lumen of the intestine, and others (Capasso *et al.,* 1994; Jarbur and Sjovall, 2000). The findings from the present study are in agreement with previous works by Qnais *et al*., (2005). As reported by Venkateran *et al*., (2005), Oben *et al*., (2006) and Das *et al*., (2009) the anti-diarrhoeal properties of plant extracts are expressed by their action of reducing intestinal motility and enhancing intestinal reabsorption, which can be done through inhibition

of prostaglandin release.

A high rate of intestinal absorption might lead to a decrease in intestinal accumulation and together with reduced intestinal motility may result in increased transit time (Jarbur and Sjovall, 2000). This in turn might give chance for further absorption as evidenced by small volume of intestinal contents recorded in this study. Hence, the obtained anti-diarrhoeal activities of *J. procera* in this study might be due to possession of chemicals that facilitate the aforementioned actions. Phytochemical groups like flavonoids, tannins, alkaloids and saponins have been reported to show anti-diarrhoeal activities (Langana *et al.,* 2000; Venkateran *et al.,* 2005; Salgado *et al.,* 2006). Moreover, these substances have also been reported in other *Juniperus spp*. (Qnais *et al.,* 2005). Though further analysis is needed to assert the presence or otherwise of these aforementioned phytochemicals, the positive result of the present study indicates that these secondary metabolites might exist in the leaves of *J. procera*. Reductions in the volume of intestinal contents were also recorded in this study that might be correlated to the ability of the extract to increase intestinal absorption (Oben *et al.,* 2006). In addition to this, the extract might have tannate that can make the intestinal mucosa more resistant and reduce secretion, which is similar with reports made for *J. phoenicia* by Qnais *et al*., (2005).

CONCLUSION

The present study clearly shows that like other members of the genus this ever green plant may contains phytochemicals with anti-diarrhoeal properties. Hence, further studies are needed not only to isolate the active principles but also to find such property in parts other than its leaves.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

HIV co-infection and mortality pattern of purulent meningitis: A 5 year retrospective autopsy study at the Korle-Bu Teaching Hospital

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This study utilized retrospective autopsy data to examine the relationship between HIV co-infection and mortality pattern of purulent meningitis. All autopsy log books and available hospital files were reviewed for information on purulent meningitis for which autopsies were performed in 2005 through 2009 at the Pathology Department of the Korle-Bu Teaching Hospital, Accra, Ghana. The mean ± SD of the studied population was 34.6±19.5 years and the prevalence of HIV co-infection among this population was 4.3%. Female participants died at a significantly younger age (31.9±19.7 years; p=0.0103) compared to their male counterparts (36.1±19.2 years). Most of the cases in HIV negative purulent meningitis death had purulent meningitis as the primary cause (i.e. 87.9% *vs* 18.5%) whereas most of the death in HIV co-infection cases had purulent meningitis as the secondary cause of death to other conditions such as CVA, pneumonia, head injury due to road traffic accidents and various malignancies (i.e. 81.5% *vs* 12.1%). Purulent meningitis secondary to other conditions is more likely to be the cause of death in aged subject. The prevalence of purulent meningitis with HIV co infection was low in this study. The mortality pattern is related to the age and gender of the studied population and whether the purulent meningitis is primary or as a co infection with HIV.

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Keywords*:* Autopsy, Purulent meningitis, HIV, Co-infection, opportunistic infections

INTRODUCTION

Purulent meningitis is a life threatening acute bacterial inflammation of the meninges of the brain and spinal cord. It mostly affects children, the aged or the immune-compromised. Ghana lies within the meningitis belt in sub-Saharan Africa and as such outbreaks of bacterial meningitis are not uncommon (Molesworth *et al.*, 2002).

Human immune deficiency virus (HIV) infected patients have a defective immunity and so are susceptible to numerous opportunistic infections caused by both bacteria and fungi including purulent meningitis (Hakim *et al.*, 2000). The association of the meninges has the potential to worsen any opportunistic brain infections. Furthermore, primary infection of HIV is complicated by meningitis thus it is appropriate to expect co-infection of purulent meningitis and HIV especially in places where both conditions are endemic. A number of studies (Silber *et al.*, 1999; Gordon *et al.*, 2000; Hakim *et al.*, 2000) have examined various aspects of the relationship between HIV and purulent meningitis including the mortality pattern looking at the role played by the various opportunistic organisms. However, there is paucity of data on the relationship between these two conditions in Ghana. This study therefore aims to use retrospective autopsy data to examine the relationship between HIV co-infection and the mortality pattern of purulent meningitis.

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MATERIAL AND METHODS

Study Site

All data were gathered from the Department of Pathology, Korle-Bu Teaching Hospital, Accra, Ghana. It is the nation's foremost teaching hospital and has the largest mortuary in the country. This mortuary performs 3,000 to 6,000 autopsies a year. Cases are received primarily from the Accra Metropolis, neighbouring towns and districts, and in some circumstances, from other regions across the country. However, autopsy was not done on all meningitis related deaths within the catchment area. This is because deaths that occurred in the communities and were not reported to the police did not have autopsy done on them.

Data Collection and Analysis

All autopsy log books and available hospital files of subjects who underwent autopsy were reviewed from the period of 2005 through 2009, and all cases of meningitis related deaths were recorded. Data were collected and cross-checked to prevent double entry. For each case of meningitis death, data were collected on age, gender, and category of death by location (Coroner's request or hospital). Coroner's cases are deaths that occurred in the community or within 24-hours of admission to a health facility, where no definitive diagnosis was arrived at before death. Hospital deaths, on the other hand, are deaths that occurred in a health facility while the subject was receiving care for a given diagnosis. The diagnosis of meningitis was based on the ante-mortem clinical and laboratory diagnosis as well as macroscopic autopsy findings. The HIV status was from ante-mortem testing. All cases of tuberculosis (TB) and Cryptococcus meningitis were excluded.

RESULTS

Gender distribution of the studied population

In 2005 through to 2009, a total of 24,787 autopsies were performed. Out of this number, purulent meningitis was recorded as the cause of death in 621 (2.5%) cases. As shown in Table 1, the mean age of the studied population (i.e. the 621 cases with purulent meningitis) was 34.6 ± 19.5 years. Majority of the deaths occurred within the communities with the primary cause of death in recorded. Coroner's cases (85.7%) being purulent meningitis (84.9%) and 95.7% of recorded cases without HIV co-infection. Death within the health facility was found in 14.3% of recorded cases and purulent meningitis was found to be the secondary cause of death to other conditions such as CVA, pneumonia, head injury due to road traffic accidents and various malignancies in about 15% of the studied population. The prevalence of HIV co-infection among this population was found to be 4.3% (Table 1).

When the studied population was classified based on gender, the females died at a significantly ($p = 0.0103$) younger age (31.9 ± 19.7 years) as compared to the male counterpart (36.1 ± 19.2 years). No significant differences were observed in the place of death, type of purulent meningitis as well as HIV co-infection among both sexes (Table 1). As noted in the total population, majority of deaths in both genders are Coroner's cases with purulent meningitis being the primary cause without HIV co -infection (Table 1).

Impact of HIV co-infection on the studied population

From Table 2, HIV related meningitis deaths occurred at a significantly ($p = 0.0432$) older age (41.6 ± 10.1 years) as compared to those without HIV co -infection (34.0 ± 19.3 years) among the total population. Significantly ($p < 0.0001$), HIV negative purulent meningitis death was more associated with Coroner's death (i.e. 87.5% *vs.* 44.4%) whereas HIV related meningitis deaths were more associated with death within the health facilities (i.e. 55.6%*vs.* 12.5%). Majority of the cases that occurred in HIV negative purulent meningitis deaths had purulent meningitis as the primary cause (i.e. 87.9% *vs.* 18.5%) whereas majority of the death that occurred in HIV co-infection cases had purulent meningitis as the secondary cause of death to other conditions such as CVA, pneumonia, head injury due to road traffic accidents and various malignancies (i.e. 81.5% *vs.* 12.1%) (Table 2). These same pattern of results were noted when the studied population

Table 1: General characteristic of the studied population stratified by gender

Variable	Total (n = 621)	Female (n = 220)	Male (n = 401)	P value
Age (yrs)	34.6 ± 19.5	31.9 ± 19.7	36.1 ± 19.2	0.0103
Place of death				
Coroner	532(85.7%)	183(83.2%)	349(87.0%)	0.1905
Permission	89(14.3%)	37(16.8%)	52(13.0%)	0.1905
Type of purulent meningitis				
Primary cause of death	527(84.9%)	187(85.0%)	340(84.8%)	1.0000
Secondary cause of death	94(15.1%)	33(15.0%)	61(15.2%)	1.0000
HIV co-infection				
Yes	27(4.3%)	10(4.5%)	17(4.2%)	0.8398
No	594(95.7%)	210(95.5%)	384(95.8%)	0.8398

Continuous data is presented as mean ± SD and analyzed using unpaired t-test whilst categorical data are presented as proportion and analyzed using Fischer's exact test

were stratified based on HIV co-infection and gender, except for the fact that age was not a significant factor among the male population (Table 2).

Impact of age on the studied population

When the total studied population was stratified based on age in Table 3, generally, fewer proportion of the female deaths occurred at older ages whereas higher proportion of male deaths occurred as subjects aged as indicated by the Chi-square test for trend. Coroner's death was negatively associated with age as opposed to the positive association between permission death and age (Table 3). Purulent meningitis was less likely to be the primary cause of death as the subjects aged whereas purulent meningitis secondary to other conditions was more likely to be the cause of death as the subjects aged (Table 3).

As the subjects aged, the prevalence of HIV co-infection assumed a "bell-shaped" curve whereas prevalence of HIV negative purulent meningitis assumed a "U-shape" curve. The highest prevalence of HIV co-infection of 8.8% was noted among the 35-44 years group, followed by 8.6% in 25-34 years group, 6.0% in 45-54 years group, 3.9% in 55-64 years group and 2.2% in 65 years and above group. No HIV co-infection was noted up to the age of 24 years (Table 3).

Stratification of the studied population based on age and gender in Table 4 indicated similar patterns of results as in the total population with regards to place of death and type of purulent meningitis. However, the highest prevalence of HIV co-infection of 11.1% was noted among the 55-64 years group followed by 10.0% in 25-34 years group, 8.3% in 35-44 years group, 6.7% in ≥ 65 years group and 3.2% in 45-54 years group among the female. No HIV co-infection was noted among females up to the age of 24 years (Table 4). Among the males, the highest prevalence of HIV co-infection of 9.0% was found among the 35-44 years group, followed by 7.5%, 7.2% and 2.4% among the 25-34 years group, 45-54 years group and 55-64 years group respectively. No male subject up to the age of 24 years and ≥ 65 years had HIV co-infection (Table 4).

DISCUSSION

Opportunistic infections including meningitis are common in HIV infection. This study sought to establish the relationship between HIV co-infection and the mortality pattern of purulent meningitis by examining retrospective autopsy data at the Korle-Bu Teaching Hospital (KBTH). The prevalence of purulent meningitis and HIV co-infection was 4.3% and factors such as age and gender affected the prevalence of purulent meningitis HIV co-infection

Table 2: Distribution of the type of purulent meningitis as well as the place of death of the studied population stratified by HIV co-infection and gender

Variables	HIV positive	HIV negative	P values
Total	***n = 27***	***n = 594***	
Age (yrs)	41.6 ± 10.1	34.0 ± 19.3	0.0432
Gender			
Female	10(37.0%)	210(35.4%)	0.8398
Male	17(63.0%)	384(64.6%)	0.8398
Place of death			
Coroner	12(44.4%)	520(87.5%)	< 0.0001
Permission	15(55.6%)	74(12.5%)	< 0.0001
Type of purulent meningitis			
Primary cause of death	5(18.5%)	522(87.9%)	< 0.0001
Secondary cause of death	22(81.5%)	72(12.1%)	< 0.0001
Female	***n = 10***	***n = 210***	
Age (yrs)	42.7 ± 14.3	30.0 ± 18.0	0.0294
Place of death			
Coroner	3(30.0%)	180(85.7%)	0.0002
Permission	7(70.0%)	30(14.3%)	0.0002
Type of purulent meningitis			
Primary cause of death	1(10.0%)	186(88.6%)	< 0.0001
Secondary cause of death	9(90.0%)	24(11.4%)	< 0.0001
Male	***n = 17***	***n = 384***	
Age (yrs)	40.9 ± 7.0	35.9 ± 19.6	0.2912
Place of death			
Coroner	9(52.9%)	340(88.5%)	0.0005
Permission	8(47.1%)	44(11.5%)	0.0005
Type of purulent meningitis			
Primary cause of death	4(23.5%)	336(87.5%)	< 0.0001
Secondary cause of death	13(76.5%)	48(12.5%)	< 0.0001

Continuous data is presented as mean ± SD and analyzed using unpaired t-test whilst categorical data are presented as proportion and analyzed using Fischer's exact test.

Table 3: The impact of age on purulent meningitis among the total studied population

Variables	≤ 18	19-24	25-34	35-44	45-54	55-64	≥ 65	P value
	n = 149	***n = 69***	***n = 93***	***n = 114***	***n = 100***	***n = 51***	***n = 45***	
Age (yrs)	10.6 ± 6.0	21.6 ± 1.7	30.1 ± 3.0	38.5 ± 2.9	48.9 ± 2.8	58.9 ± 8.1	74.4 ± 2.9	
Gender								
Female	62(41.6%)	27(39.1%)	40(43.0%)	36(31.6%)	31(31.0%)	9(17.6%)	15(33.3%)	0.0039
Male	87(58.4%)	42(60.9%)	53(57.0%)	78(68.4%)	69(69.0%)	42(82.4%)	30(66.7%)	0.0039
Place of death								
Coroner	135(90.6%)	66(95.7%)	84(90.3%)	98(86.0%)	78(78.0%)	40(78.4%)	31(68.9%)	< 0.0001
Permission	14(9.4%)	3(4.3%)	9(9.7%)	16(14.0%)	22(22.0%)	11(21.6%)	14(31.1%)	< 0.0001
Type of purulent meningitis								
Primary cause of death	137(91.9%)	68(98.6%)	83(89.2%)	90(78.9%)	79(79.0%)	40(78.4%)	30(66.7%)	< 0.0001
Secondary cause of death	12(8.1%)	1(1.4%)	10(10.8%)	24(21.1%)	21(21.0%)	11(21.6%)	15(33.3%)	< 0.0001
HIV co-infection								
Yes	0(0.0%)	0(0.0%)	8(8.6%)	10(8.8%)	6(6.0%)	2(3.9%)	1(2.2%)	0.0396
No	149 (100.0%)	69(100.0%)	85(91.4%)	104(91.2%)	94(94.0%)	49(96.1%)	44(97.8%)	0.0396

among the study population.

The 4.3% prevalence of purulent meningitis recorded in this study is compares closely to the 5% prevalence reported in the Western world (Currie and Casadevall, 1994) but contrary to the 20-30% reported in other sub-Saharan African countries and in Eastern Asia (Pinner *et al.*, 1995). The low prevalence recorded among this population could be due to the fact that most HIV-infected patients die of other complications hence the low prevalence.

Palleres *et al.,* (1995) reported that there is no difference in relation to mortality rate between patients with only HIV infection and bacteria and HIV co-infection. This assertion however is inconsistent with observations made from this study. The inclusion of both HIV 1 and 2 patients in our study could account for differences in the age of mortality.

Several studies have examined the relationship between gender, prevalence and mortality rate of purulent meningitis. This study established a trend that fewer females died of purulent meningitis at an older age compared to males. This finding is in conformity to the study of Pfister *et al.,* (1993) who found purulent meningitis to be more prevalent in males. The longer life expectancy of females in Ghana could be the reason for the low mortality rate among the female participants.

Recent studies have reported higher mortality rates in meningitis without HIV co-infection compared to purulent meningitis HIV co-infection (Lynch and Kapila, 1996) which confirms the findings of this study. The low mortality rate among subjects with purulent meningitis HIV co-infection could partly be due to the reduced immunologic response that characterizes HIV infection. This phenomenon is known to reduce inflammatory responses produced in the brain in the initial stages of meningitis infection which is known to be linked directly to mortality (Almirante *et al.*, 1998).

Various reports have established purulent meningitis due to various bacteria as the primary cause of

Table 4: Association between increase in age and place of death, type of purulent meningitis as well as HIV co-infection among the studied population stratified by gender

Variables	<19	19-24	25-34	35-44	45-54	55-64	>64	P value
Female	***n = 62***	***n = 27***	***n = 40***	***n = 36***	***n = 31***	***n = 9***	***n = 15***	
Age (yrs)	10.4 ± 5.8	20.8 ± 1.6	29.9 ± 3.0	38.4 ± 2.9	49.3 ± 3.4	60.8 ± 1.8	76.9 ± 8.2	
Place of death								
Coroner	57(91.9%)	26(96.3%)	34(85.0%)	29(80.6%)	23(74.2%)	5(55.6%)	9(60.0%)	< 0.0001
Permission	5(8.1%)	1(3.7%)	6(15.0%)	7(19.4%)	8(25.8%)	4(44.4%)	6(40.0%)	< 0.0001
Type of purulent meningitis								
Primary cause of death	58(93.5%)	27(100.0%)	33(82.5%)	29(80.6%)	24(77.4%)	6(66.7%)	10(66.7%)	0.0002
Secondary cause of death	4(6.5%)	0(0.0%)	7(17.5%)	7(19.4%)	7(22.6%)	3(33.3%)	5(33.3%)	0.0002
HIV co-infection								
Yes	0(0.0%)	0(0.0%)	4(10.0%)	3(8.3%)	1(3.2%)	1(11.1%)	1(6.7%)	0.067
No	62(100.0%)	27(100.0%)	36(90.0%)	33(91.7%)	30(96.8%)	8(88.9%)	14(93.3%)	0.067
Male	***n = 87***	***n = 42***	***n = 53***	***n = 78***	***n = 69***	***n = 42***	***n =30***	
Age (yrs)	10.5 ± 6.1	22.1 ± 1.6	30.3 ± 3.0	38.5 ± 2.9	48.7 ± 2.5	58.5 ± 3.0	73.2 ± 7.9	
Place of death								
Coroner	78(89.7%)	40(95.2%)	50(94.3%)	69(88.4%)	55(79.7%)	35(83.3%)	22(73.3%)	0.0029
Permission	9(10.3%)	2(4.8%)	3(5.7%)	9(11.5%)	14(20.3%)	7(16.7%)	8(26.7%)	0.0029
Type of purulent meningitis								
Primary cause of death	79(90.8%)	41(97.6%)	50(94.3%)	61(78.2%)	55(79.7%)	34(81.0%)	20(66.7%)	< 0.0001
Secondary cause of death	8(9.2%)	1(2.4%)	3(5.7%)	17(21.8%)	14(20.3%)	8(19.0%)	10(33.3%)	< 0.0001
HIV co-infection								
Yes	0(0.0%)	0(0.0%)	4(7.5%)	7(9.0%)	5(7.2%)	1(2.4%)	0(0.0%)	0.2124
No	87(100.0%)	42(100.0%)	49(92.5%)	71(91.0%)	64(92.8%)	41(97.6%)	30(100.0%)	0.2124

Continuous data are presented as mean ± SD and categorical data are presented as proportion. The association between age and other variables was access using Chi and analyzed using Chi-square test for trend.

death in the aged (Bruyn *et al.*, 1989; Durand *et al.*, 1993; McMillan *et al.*, 2001). Findings of from this study, however, contradict these observations as purulent meningitis secondary to other infections was the major cause of mortality amongst the aged subjects in this study.

As the participants advanced in age, the prevalence of HIV co-infection assumed a "bell-shape" with the total prevalence among the various age groups being far less than the 80% prevalence recorded among participants in a study in Malawi (Kelly *et al.*, 2012). The lower prevalence rate of HIV in Ghana coupled with the up scaling and wide provision of antiretroviral therapy (ART) to these patients which boosts their immunity could have accounted for reduced prevalence rate observed in this study.

CONCLUSION

The prevalence of purulent meningitis and HIV co-infection among studied population was 4.3%, with mortality rate being related to whether the purulent meningitis was primary or secondary to HIV infection. Primary infection with purulent meningitis was associated with a high mortality rate.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Antibacterial activity of the fruit extract of *Physalis angulata* and its formulation

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The inhibitory activity of zinc oxide-ointment formulation as well as the unformulated crude extract of fruits of *Physalis angulata* was investigated against clinical wound isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The zinc oxide-ointment formulation and the unformulated *P. angulata* crude extract were found to be ineffective against *P. aeruginosa* at all concentrations used, but potent against *S. aureus* at varying degrees. The zinc oxide-ointment (100 mg g^{-1}, 125 mg g^{-1} and 150 mg g^{-1}) and *P. angulata* crude extract/zinc oxide-ointment (100 mg g^{-1}, 125 mg g^{-1} and 150 mg g^{-1}) formulations were only slightly active against *S. aureus* at the highest concentration of 150 mg g^{-1}. The unformulated *P. angulata* crude extract alone exhibited the highest inhibitory activity against *S. aureus* at all concentrations used with zones of inhibition between 34.5 mm and 50.5 mm, followed by a formulation of the extract with only oleaginous base (ointment), with zones of inhibition between 12.8 mm and 20.3 mm. A one-way analysis of variance (ANOVA) of these values compared with the activity of Chloramphenicol (positive control) indicated significant inhibitory activity by the unformulated *P angulata* crude extract and the extract and ointment formulation against *S. aureus* thus suggesting their efficacy in treating staphylococcal infections.
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Keywords*:* Oleaginous base, *S. aureus*, *P. aeruginosa*, zone of inhibition

INTRODUCTION

Wound is defined simply as the disruption of the cellular and anatomic continuity of the tissue (Edwards and Harding, 2000). Wound may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue (Raina *et al.*, 2008). The development of wound infection depends on the integrity and protective function of the skin. It has been shown that wound infection is universal and the bacterial type varies with geographical location, resident flora of the skin, clothing at the site of wound, time between wound and treatment (Anupurba *et al.*, 2006).

Efforts are being made all over the world to discover agents that can promote healing and thereby reduce the cost of hospitalization and save patients from amputation or other severe complications. The continuous development of antibiotic resistance of pathogenic microorganisms and particularly *Streptococcus pneumoniae* to penicillin, *Staphylococcus aureus* to methicillin, and *Enterococcus spp.* to vancomycin is a major health concern worldwide (Melissa *et al.*, 2005). More than 80% of the world's population now depends on traditional medicine for their ailments, especially for wound management (James and Isaac, 2010).

P. angulata L. belongs to the Solanaceae family and includes about 120 species with herbal characteristics and perennial habits. It is distributed throughout tropical and subtropical regions of the world (José *et al.*, 2003). *P. angulata* has a broad spectrum of biological activity including antibacterial, molluscicidal, antiprotozoal, anticancer, cytotoxic and immunomodulatory activities (Bastos *et al.*, 2005; Hseu *et al.,* 2011). In Ghana, it is called "*totototo*"

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among the Akans. The juice is used in the treatment of earache, jaundice, fever, bladder disease to mention a few. The fruit and other aerial parts are used in the treatment of boils, sores or wounds, constipation and digestive problems (Dokosi, 1998). Zinc oxide has been shown to have therapeutic application in treating a variety of skin conditions in products such as baby powder, barrier creams to treat diaper rashes, calamine cream, anti-dandruff shampoos, and antiseptic ointments (Harding, 2007). Application of oleaginous base as a vehicle in the formulation of zinc oxide for medicinal purpose may be worthwhile. This study was conducted to validate the scientific basis of using *P. angulata* in the treatment of wounds and to verify if zinc oxide ointment in combination with the extract of *P. angulata* will result in enhancement of inhibitory activity of the extract.

MATERIALS AND METHODS

Source of test bacteria

Clinical isolates of *S. aureus* and *P. aeruginosa* were obtained from incision type wounds at the Microbiology Department of the Tamale Teaching Hospital in the Northern Region of Ghana, in the Month of March 2012. Bacterial isolates were stored at a temperature between 2 and 8^{o}C in nutrient broth. Pure cultures of each of the bacterial isolates were obtained by sub-culturing the isolates on Chocolate agar (OXOID, Basingstoke, Hampshire, England).

Plant material

The fruits of *P. angulata* L were collected from Navrongo in the Upper East Region of Ghana in the month of November, 2011 and authenticated by Dr. Walter M. Kpikpi of the Department of Applied Biology in the Faculty of Applied Sciences of the University for Development Studies, Navrongo.

Ethanolic extract of the fruits

The fruits were air dried under shade and then powdered using surface-sterilized mortar and pestle. One hundred and twenty (120 g) grams of the powdered material was macerated in 240 mL of ethanol and refluxed exhaustively. The extract was filtered after 24 hours with the aid of sterile cotton. The filtrate was then concentrated under reduced pressure using a rotary evaporator at 45^{0}C to obtain a yield of 7.2 g of crude extract. This was carried out according to the method described by Tomassini *et al*., (2009).

Preparation of *P. angulata* crude extract-ointment formulation

An ointment base was prepared by melting 2.5 g of white wax in a beaker on a thermostatic water bath. White petroleum jelly BP (47.5 g) was added and warmed until liquefied. The mixture was stirred until it began to congeal. Three batches of the *P. angulata* crude extract - ointment containing 100 mg g^{-1}, 125 mg g^{-1} and 150 mg g^{-1} respectively of the extract were prepared and used in the antibacterial activity studies.

Preparation of zinc oxide - ointment formulation

To verify whether zinc oxide has inhibitory effect on the test microorganisms, a zinc oxide - ointment was formulated with oleaginous emulsion base in batches with concentrations of 100 mg g^{-1}, 125 mg g^{-1} and 150 mg g^{-1} respectively. These concentrations were weighed into separate beakers labeled A, B and C respectively. Molten ointment base was then added to each beaker to make 1000 mg of each batch. Each of the individual batches was then homogenized by trituration to give the zinc oxide-ointment.

Preparation of *Physalis angulata* crude extract

P. angulata crude extract was weighed into separate beakers labeled E, F, G respectively, and dispersed in a buffer of pH 7.2 to derive concentrations of 100 mg mL^{-1}, 125 mg mL^{-1} and 150 mg mL^{-1} respectively. These concentrations were used for the antibacterial studies.

Preparation of *P. angulata* crude extract-zinc oxide ointment formulation

P. angulata crude extract and zinc oxide ointment was formulated together to determine whether they could have synergistic effect on the bacterial isolates. Equal quantities (50, 62.5, and 75 mg) of *P. angulata* crude extract and zinc oxide were weighed into separate beakers labeled J, K and L respectively. The mixtures were then treated with oleaginous

base at varying concentrations of 100 mg g^{-1}, 125 mg g^{-1} and 150 mg g^{-1} respectively.

Agar Diffusion Bioassay

The modified agar well diffusion method described by Perez *et al.* (1990) was employed. Inoculum of each test organism was prepared by emulsifying in 100 mL of sterile peptone water and standardized to get a turbidity of 0.5 McFarland Standard. Within 15 minutes of its preparation, a sterile cotton swab was then dipped into the standardized inoculum suspension. Surplus moisture was removed by rotating the swab several times whilst pressed firmly against the walls of the tube at a level above the peptone water. Agar plates (Mueller Hinton Agar, Oxoid) were then inoculated with the cotton swabs by rotating the swab while rubbing taking care that the whole area is inoculated. The inoculated plates were allowed to dry and five-millimeter (5 mL) diameter wells made on the plate with a sterile cork-borer at wide enough intervals. The wells corresponded with the prepared number of concentrations of each formulation, the positive and negative controls.

For each formulation, 1 mL of each concentration was drawn into a labeled well with a sterile micropipette taking care to avoid spillage onto the surface of the culture plate. A similar volume of the negative control (98% ethanol) and positive control (Chloramphenicol at 100 mg g^{-1}) was also introduced into a well each on the same plate. The plates were left to stand until complete diffusion of the formulations into the medium. The plates were incubated in inverted positions at 37°C for 24 hours after which they were observed for inhibitory activity depicted by zones of inhibition around the wells. Inhibition zone diameters were measured using a ruler and recorded in millimeters (mm). The experiments were repeated three times to check for reproducibility.

Statistical Analysis

Means and standard error of the mean were calculated for the zones of inhibition measured for the three sets of experiments in each case. These means were statistically compared using the one – way ANOVA to determine if they were significantly different at $P < 0.05$.

RESULTS

All the three concentrations of the *P. angulata* crude extract formulation did not show activity against *P. aeruginosa.* They were however active against *S. aureus* with recorded zones of inhibition of 12.8 mm at the concentration of 100 mg g^{-1} which increased to 20.3 mm at a concentration of 150 mg g^{-1} (Table 1). *P. aeruginosa* was resistant to all concentrations of the zinc oxide ointment formulation used while *S. aureus* was only susceptible to the 150 mg g^{-1} concentration with a mean zone of inhibition of 11.8 mm as compared to 45.5 mm recorded for the control (Chloramphenicol) at 100 mg g^{-1} concentration (Table 2).

Table 1: Activity of P. angulata crude extract-ointment preparation against test organisms

PAG extract - ointment preparation	Zone of inhibition (mm)*	
	S. aureus	P. aerugonosa
PAG 100 mg g^{-1}	12.75 ± 1.06	0.00 ± 0.00
PAG 125 mg g^{-1}	16.25 ± 0.35	0.00 ± 0.00
PAG 150 mg g^{-1}	20.25 ± 0.35	0.00 ± 0.00
CLP 100 mg g^{-1}	45.75 ± 0.35	0.00 ± 0.00

PAG = *P. angulata* and CLP = Chloramphenicol, *Values represent means ± SEM of three independent measurements

Table 2: Activity of zinc oxide-ointment formulations against test organisms

Zinc oxide-ointment	Zone of inhibition (mm)*	
	S. aureus	***P. aeruginosa***
ZnO 100 mg g^{-1}	0.00 ± 0.00	0.00 ± 0.00
ZnO 125 mg g^{-1}	0.00 ± 0.00	0.00 ± 0.00
ZnO 150 mg g^{-1}	11.50 ± 0.71	0.00 ± 0.00
CLP 100 mg g^{-1}	45.75 ± 0.35	0.00 ± 0.00

ZnO = zinc oxide and CLP = Chloramphenicol, *Values represent means ± SEM of three independent measurements

Antibacterial activity of *P. angulata* crude extract

Mean diameters of zones of inhibition of *S. aureus* by various unformulated *P. angulata* crude extract increased with concentration. *P. aeruginosa* was, however, not susceptible to the unformulated crude extract of *P. angulata* (Table 3). A maximum mean zone of inhibition diameter of 50.5 mm was attained at a concentration of 150 mg g^{-1} against *S. aureus* as compared to the standard antibiotic (Chloramphenicol) which gave a mean zone of inhibition diameter of 79.8 mm at 100 mg g^{-1} concentration.

Antibacterial activity of *Physalis angulata* crude extract-zinc oxide ointment formulation

The *P. angulata* crude extract-zinc oxide ointment formulation gave the lowest inhibitory activity of all the formulations prepared. While *P. aeruginosa* was resistant to all concentrations, *S. aureus* was only inhibited by the 150 mg g^{-1} concentration of the formulation with a mean zone of inhibition diameter of 7.50 mm (Table 4). This could be probably be due to the fact that the non-concentrated bioactive compound present in the crude extract has been prevented by the oleaginous base from having much contact with the test organisms. Ethanol was used as negative control and indicated no inhibitory activity in all test models.

DISCUSSIONS

According to Bastos *et al.*, (2005), the fruits of *P. angulata* possess steroids known as physalins, physagulins with anolides and flavonoids. The inhibitory effects of *P. angulata* crude extract on the *S. aureus* could be due to these bioactive phytochemical compounds which are known to possess antibacterial properties. Results obtained from this study is similar to the work of Melissa *et al.,* (2005) and Osho *et al.*, (2010) which reported strong activities for methanol/water extracts and ethanolic extracts of *P. angulata* against some Gram-positive and Gram-negative organisms respectively.

Table 3: Activity of unformulated *P. angulata* crude extract against test organisms

Unformulated PAG extract	Zone of inhibition (mm)*	
	S. aureus	***P. aeruginosa***
PAG 100 mg g^{-1}	34.50 ± 0.71	0.00 ± 0.00
PAG 125 mg g^{-1}	40.50 ± 1.41	0.00 ± 0.00
PAG 150 mg g^{-1}	50.50 ± 0.71	0.00 ± 0.00
CLP 100 mg g^{-1}	79.75 ± 0.35	0.00 ± 0.00

PAG = *P. angulata* and CLP = Chloramphenicol, *Values represent means ± SEM of three independent measurements

Table 4: Activity of *P. angulata*–zinc oxide ointment formulation against test organisms

P. angulata –zinc oxide ointment formulation	Zone of inhibition (mm) *	
	S. aureus	*P. aeruginosa*
PAG + ZnO 100 mg g^{-1}	0.00 ± 0.00	0.00 ± 0.00
PAG + ZnO 125 mg g^{-1}	0.00 ± 0.00	0.00 ± 0.00
PAG + ZnO 150 mg g^{-1}	7.50 ± 0.71	0.00 ± 0.00
CLP 100 mg g^{-1}	9.50 ± 4.80	0.00 ± 0.00

PAG = *P. angulata,* ZnO = Zinc oxide and CLP = Chloramphenicol, *Values represent means ± SEM of three independent measurements

Zinc oxide has been reported to have antibacterial activities (Padmavathy and Vijayaraghavan, 2008) and therefore was expected to have shown high inhibitory activity against the test organisms. The reduced activity exhibited by the zinc oxide could partly be due to the effect of oleaginous base which has been reported to have poor drug releasing potential and hence preventing the zinc oxide from exhibiting full efficacy against the microorganisms (Shargel *et al.,* 2009). The antibacterial activity of the formulated extract-ointment was low compared with the unformulated extract. This may be attributed to the effect of oleaginous base or the direct effect of the P. angulata components on the microorganisms.

CONCLUSION

The comparatively high activities shown by both the *Physalis angulata* crude extract-ointment formulation and the unformulated *P. angulata* crude extract against *Staphylococcus aureus* seem to justify the

widespread use of the plant in the treatment of boils, sores and wounds in Ghana. Isolation, purification and formulation of the bioactive compounds could lead to the development of novel drugs from *P. angulata* with maximal therapeutic activity.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Attenuation of Cardiovascular response by ß-blocker esmolol during laryngoscopy and intubation

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Cardiovascular responses to laryngoscopy and intubation have long been recognized and various efforts have been made to attenuate this response. The aim of this study was to evaluate the efficacy and safety of ß-blocker esmolol in attenuating cardiovascular response to laryngoscopy and tracheal intubation in the Ghanaian population. After obtaining institutional ethical approval, 80 patients aged 18 to 65 years from either sex and classified as American Society of Anaesthesiologists (ASA) physical status I (normal healthy patients) or II (Patients with mild systemic disease) undergoing elective surgery under general anaesthesia were selected for the study. Participants were randomly allocated into two groups comprising 40 subjects each. Group I received esmolol 2 mg kg^{-1} I.V. bolus and group II (control) received a placebo 2 minutes prior to laryngoscopy. Changes in heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and rate pressure product (RPP) were measured before induction as baseline, and at minute 1, 3 and 5 minutes respectively after tracheal intubation while they were also observed for any complications. There was a significant attenuation in HR, SBP, DBP, MAP and RPP in the experimental group as compared to the control group ($P < 0.05$) at 1 minute with onward decreases at 3 and 5 minutes respectively after intubation. However attenuation to baseline values at 5 minutes after intubation in the experimental group was significantly higher than that in the control group. Percentage changes in haemodynamic variables in experimental group versus control group at 5 minutes are as follows: HR = -2.90% vs 10.22%; SBP = 0.96% vs 6.21%; DBP = -3.54% vs 4.06%; MAP = -1.56% vs 4.94%; RPP = -1.86% vs 17.25%. Prophylactic therapy with esmolol was found to be safe and effective in attenuating cardiovascular responses to laryngoscopy and tracheal intubation among the Ghanaian population.

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Keywords*:* Cardiovascular response, intubation, attenuation, haemodynamic changes

INTRODUCTION

Laryngoscopy and tracheal intubation have been associated with haemodynamic changes (Bostana and Eroglu, 2012) such as transient hypertension (Manjunath *et al.*, 2008), tachycardia (Moon *et al.*, 2012), arrhythmias (Bae *et al.*, 2007), myocardial ischaemia or infarction (Landesberg *et al.*, 2009). These haemodynamic changes are of little consequence in healthy individuals but may be more severe and life threatening in patients with hypertension, coronary artery diseases and cerebrovascular diseases. Several attempts have been made to attenuate hemodynamic changes which include increase in blood pressure and heart rate in response to laryngoscopy and tracheal intubation. Pharmacological approaches involving the use of lidocaine (Manjunath *et al.*, 2008), remifentanil (Kaygusuz *et al.*, 2007), nitroglycerine (Fassoulaki and Kaniaris, 1983), diltiazem (Mikawa and Ikegaki, 1990), esmolol (Figueredo and Garcia, 2004), buprenorphine (Khan and Kamal, 1989), fentanyl (Bostana and Eroglu, 2012) and a combination of esmolol and nicardipine (Moon *et al.*, 2012) have been utilized

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to attenuate the pressure responses to laryngoscopy and tracheal intubation. Each of these drugs has a unique advantage and disadvantage in blunting the pressor response to intubation.

Reflex changes in the cardiovascular system are most marked after laryngoscopy and intubation and lead to an average increase in blood pressure by 40-50% and 20% increase in heart rate (Savio *et al.*, 2011). An increase in arterial pressure and heart rate following laryngoscopy and intubation can have deleterious effects on the heart as shown by Stoelting (1978). Esmolol is used to control periods of intense sympathetic stimulation (Hassan *et al.*, 1991) and plasma catecholamine release (Derbyshire *et al.*, 1983) in general anaesthesia. There has been no consensus regarding the optimum dose and timing of esmolol delivery (Gupta *et al.*, 2009).

Perioperative myocardial infarction is a leading cause of post-operative morbidity and mortality in normotensive patients due to hypertension and tachycardia (Savio *et al.*, 2011) following laryngoscopy and intubation. Studies from the African continent have shown avoidable anaesthesia mortality rates of 1:3000 in Zimbabwe (McKenzie, 1996), 1:1900 in Zambia (Heywood *et al.*, 1989), 1:500 in Malawi (Hansen *et al.*, 2000) and 1:150 in Togo (Bang'na Maman *et al.*, 2005). These demonstrate a serious and sustained absence of safe anaesthesia for surgery in developing countries. Deaths attributable to anaesthesia could be reduced by controlling the haemodynamic changes that occur during endotracheal intubation. There is increasing evidence that control of the heart rate and blood pressure response to endotracheal intubation is essential to prevent adverse cardiovascular outcomes (Korpinen *et al.*, 1998; Manjunath *et al.*, 2008)

Hypertension is known to occur more frequently in the black population and is associated with a higher incidence of cerebrovascular and renal complications. Strokes have been found to be more common in black hypertensives and hypertension associated end-stage renal failure occurs up to 20 times more commonly in black patients, compared to non-blacks (Gibbs *et al.*, 1999), therefore, the need for assessment in this study cohort.

Efforts are being made to practice safe anaesthesia in Ghana in an attempt to reduce intraoperative complications and mortality during anaesthesia. Esmolol is considered appropriate to attenuate haemodynamic changes in Caucasians during endotracheal intubation as it reduces heart rate as well as blood pressure. Specific racial differences need to be considered before treatment in view of a report that African-Americans respond much less to beta adrenergic receptor blocking drugs than whites (Materson *et al.*, 1993). Beta-blockers tend to be less effective in black hypertensives and thus higher doses are required to control blood pressure (Gibbs *et al.*, 1999). There is paucity of literature on studies to control haemodynamic changes during laryngoscopy and endotracheal intubation among the Ghanaian population. The purpose of this study was, therefore, to determine the efficacy and safety of intravenous esmolol in attenuating haemodynamic response to laryngoscopy and intubation in the Ghanaian population.

PATIENTS AND METHODS

Study site and participants

Eighty (80) adult patients of American Society of Anaesthesiologists (ASA) physical status I (normal healthy patients) or II (Patients with mild systemic disease) undergoing various elective surgeries at the Komfo Anokye Teaching Hospital (KATH) between November 2011 and May 2012 were divided into two groups of 40 patients each. Patients with a history of hypertension, diabetes, cardiac diseases, bronchial asthma and those on beta-blockers were excluded.

Dosing of Esmolol

Bolus dosing of esmolol was evaluated with doses varying between 1 mg kg^{-1} and 4 mg kg^{-1} (Savio *et al.*, 2011). The use of esmolol at 3 mg kg^{-1} (Korpinen *et al.*, 1995) and 4 mg kg^{-1} (Berg, 1998) respectively has been observed to have adverse effects such as unplanned hypotension and bradycardia during induction. Kumar *et al.* (2003) in their study claimed optimal results while using lesser doses of esmolol in Asian population (i.e. 2 mg kg^{-1}).

These findings were the basis for using smaller doses of esmolol in this study in a Ghanaian population.

Dosing procedure

Group I received injection esmolol (2 mg kg^{-1}) and Group II (control group) received injection normal saline as placebo. A preoperative history was taken prior to administration of the drug. Clinical examination and routine investigations such as haemoglobin, haematocrit, total lymphocyte count, differential lymphocyte count, serum electrolytes, blood group/ Rh typing, blood urea nitrogen, serum creatinine, fasting blood sugar, chest radiography and electrocardiogram in all patients. This study was undertaken at the Department of Anaesthesia and Intensive Care at KATH, Kumasi, following institutional approval by the Committee on Human Research Publications and Ethics (CHRPE), School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. The study objectives and procedure were explained to the participants and written informed consent was obtained.

Intravenous access was secured and infusion of Ringer's lactate solution started. The patients were pre-medicated 30 minutes prior to surgery with glycopyrrolate-bromide (0.008 mg kg^{-1}) intramuscularly. Patients were then taken to the operating room after which routine non-invasive monitor Infinity Delta XL Drager was applied and vital signs monitored. Midazolam (0.04 mg kg^{-1}) was administered intravenously over 30 seconds as pre-medication and patients were pre-oxygenated with four to five breaths of 100% oxygen. The patients were induced with thiopentone sodium (6 mg kg^{-1} IV) in incremental doses until loss of eyelash reflex occurred, vecuronium bromide (0.12 mg kg^{-1} IV) was given over 20 seconds, followed by the administration of the study drugs (normal saline or esmolol) two minutes before laryngoscopy and intubation. The study drug was randomly allocated to patients in a double blinded manner.

Patients were ventilated with oxygen and halothane (1%) using intermittent positive pressure ventilation with a fresh gas flow of 6 litres per minute by Bain circuit until intubation. About 2 minutes after IV vecuronium, laryngoscopy was performed with a Macintosh laryngoscope blade and trachea intubated with an appropriate size cuffed endotracheal tube. After confirmation of correct placement of endotracheal tube, anaesthesia was then maintained with oxygen, halothane mixed with vecuronium (0.1 mg kg^{-1}). Heart rate (HR), systolic blood pressure (SBP), Diastolic blood pressure (DBP), Mean Arterial Pressure (MAP), Rate Pressure Product (RPP), Oxygen saturation (SpO_2) and Electrocardiogram (ECG) changes were recorded before induction (Basal) and after tracheal intubation at 1, 3 and 5 minutes for the purpose of this study. Manipulation (e.g. painting and draping) of the area of operation was not allowed until 5 minutes after the study drug has been administered. Injection fentanyl (2 μg kg^{-1}) was given before surgery.

Parameters and statistical analysis

Vital signs were recorded before induction (Basal) and after tracheal intubation at 1, 3 and 5 minutes respectively. Multi-channel monitor (Infinity Delta XL Drager, Draeger Medical Systems, Inc. Telford, PA 18969, USA) was used for recording HR, SBP, DBP, MAP, ECG, SpO_2. RPP was calculated by multiplying heart rate with systolic blood pressure. Patients were also observed for complications like hypotension, hypertension, arrhythmias, hypoxaemia and myocardial ischaemia. Haemodynamic variables were represented by mean ± standard deviation (SD). Means were compared with student *t*-test and p values were calculated. ANOVA with repeated measures was used to compare the changes in HR, SBP, DBP, MAP and RPP values. Bonferroni's multiple comparison tests were used to make intragroup comparisons. In all analyses, $p<0.05$ was considered statistically significant. All statistical analyses were performed using GraphPad prism 5.00 for windows (GraphPad software, San Diego, California, USA, www.Graphpad.com).

RESULTS

A comparison of the demographic profile of the study groups are as shown in Table 1. The male to female ratio of group I was 1:1.5 whereas that of group II was 1:2.6 ($p<0.34$). No significant difference was observed in the mean age for group I pa-

tients (40.8 ± 13.7 years) when compared to those in group II (41.8 ± 12.7 years) (p<0.74). The average weight was 70.1 ± 9.6 kg and 69.7 ± 9.8 kg in groups I and II respectively (p<0.85).The average height in group I was 164.2 ± 5.4 cm and group II was 163.3 ± 4.6 cm (p<0.47).

Table 2 shows a comparison of changes in mean haemodynamic variables in patients within the two groups at baseline and time (1, 3 and 5 minutes) after intubation. In group I, significant increases in the mean estimates for SBP, DBP, MAP, RPP were observed 1 minute after intubation when compared to the mean baseline estimates with the exception of the mean value for pulse which did not show any significant variation from the mean value at baseline. A time dependent decrease in the mean value for the parameter estimates was observed at 3 and 5 minutes respectively from first minute. Significant percentage decreases in the parameter estimates were observed at 5 minutes of intubation for group I patients (Table 3). In group II patients however, significant increases in all the mean estimates for the parameters were observed within the first minute after intubation and a time dependent decrease in the mean values for the parameter estimates was observed up to the fifth minute. A critical look at the percentage changes in the haemodynamic variables showed consistently higher percentage change values in the times after intubation in group II patients compared to group I patients (Table 3). It was evident that vital signs remained attenuated from 3 minutes to 5 minutess after intubation in group I patients, returning to baseline values after

Table 1: Demographic profile of the study groups stratified by treatment

Variables	Group-I (n = 40)	Group-II (n = 40)	p-value
Age (yrs)	40.8 ± 13.7	41.8 ± 12.7	0.743
Weight (kg)	70.1 ± 9.6	69.7 ± 9.8	0.846
Height (cm)	164.2 ± 5.4	163.3 ± 4.6	0.465
Sex			
Males	16(40.0%)	11(27.5%)	0.344
M:F	1:1.5	1:2.6	

Data are presented as means ± SD, ratio and percentages. Group-I (Esmolol treated group), Group –II (Control group).

Table 2: Change in haemodynamic variables in the two study groups after intubation

parameter	Basal	1 minute	3 minutes	5 minutes	p-value
Group I					
Pulse (bpm)	90.8 ± 8.6	92.2 ±9.7	91.4 ±7.5	88.2 ± 8.8*†¥	0.0005
SBP (mmHg)	125.1 ± 8.3	137.5 ± 8.9*	131.9 ± 8.8*†	126.3 ± 8.7†¥	< 0.0001
DBP (mmHg)	81.9 ± 5.0	90.4 ± 5.7*	82.6 ± 5.2†	79.0 ± 5.9*†¥	< 0.0001
MAP (mmHg)	96.3 ± 4.3	106.1 ± 5.0*	99.0 ± 4.1*†	94.8 ± 4.9*†¥	< 0.0001
RPP	11355 ± 1229	12681 ± 1605*†	12062 ± 1352*	11144 ± 1401†¥	< 0.0001
Group II					
Pulse (bpm)	89.0 ± 9.3	116.1 ± 7.6*	109.5 ± 7.4*†	98.1 ± 9.3*†¥	< 0.0001
SBP (mmHg)	123.9 ± 6.5	153.1± 12.8*	145.0 ± 12.9*†	131.6 ± 10.1*†¥	< 0.0001
DBP (mmHg)	83.7 ± 6.7	99.4 ± 5.2*	94.3 ± 5.1*†	87.1 ± 5.5*†¥	< 0.0001
MAP (mmHg)	97.1 ± 5.0	117.3 ± 5.9*	111.2 ± 5.5*†	101.9 ± 4.8*†¥	< 0.0001
RPP	11012 ± 1196	17778 ± 1926*	15861 ± 1690*†	12912 ± 1574*†¥	< 0.0001

Data are presented as means ± standard deviation, and p value. ANOVA with repeated measures was used to compare the changes in HR, SBP, DBP, MAP and RPP values. Bonferroni's multiple comparison tests were used to make intragroup comparisons. Comparison symbols used - * with basal, † with 1min. and with ¥ 3min. Group-I- esmolol, bpm - beat per minute, B.P-Blood Pressure, MAP-Mean Arterial Pressure, mmHg- millimetre of mercury

5 minutes in both groups but attenuations in group I were more significant than those in group II. Patients in group II undergoing laryngoscopy and intubation showed an incidence of 8% ventricular ectopics and 5% dropped beats. However, the use of esmolol did not result in any kind of arrhythmias or hypotension.

Table 3: Percentage changes in haemodynamic variables from baseline and times after intubation in the study population stratified by treatment

Variables	1 min.	3 min.	5 min.
Group I			
Pulse (bpm)	1.50%	0.70%	-2.90%
Systolic (mmHg)	9.90%	5.40%	0.96%
Diastolic (mmHg)	10.40%	0.85%	-3.54%
MAP (mmHg)	10.20%	2.80%	-1.56%
RPP	11.68%	6.23%	-1.86%
Group II			
Pulse (bpm)	30.45%	23.03%	10.22%
Systolic (mmHg)	23.57%	17.03%	6.21%
Diastolic (mmHg)	18.76%	12.66%	4.06%
MAP (mmHg)	20.80%	14.52%	4.94%
RPP	61.44%	44.03%	17.25%

Percentage change was calculated as follows: [(Variable estimate for time after intubation – Basal estimate for variable)/Basal estimate for variable] X 100%, RPP = Rate pressure product

DISCUSSION

This study assessed the effect of esmolol on haemodynamic changes due to tracheal intubation in normotensive black population. Results from the study consistently showed that esmolol blunts unwanted haemodynamic responses to endotracheal intubation with significantly less circulatory responses experienced by patients receiving intravenous esmolol. In the control group, markedly high cardiovascular changes occurred after one minute following laryngoscopy and intubation. Esmolol (2mg kg^{-1}) given two minutes before intubation sufficiently reduced the circulatory responses in this cohort of normotensive black patients. ß- blockers minimize the increase in heart rate and blood pressure by attenuating positive chronotropic and ionotropic effects of the increase in adrenergic activity. Esmolol possesses several properties which makes it a valuable agent to obtund the cardiovascular response. It is a cardio selective agent, has ultra-short duration of action (9 minutes) and has not been reported to have significant drug interaction with commonly used anaesthetic drugs (Savio *et al.*, 2011).

Korpinen *et al.* (1998) reported that the administration of esmolol bolus 1 mg kg^{-1} and an infusion of 200 µg kg^{-1} $minute^{-1}$ IV 2 minutes before laryngoscopy and intubation suppressed the increase in heart rate rather than arterial blood pressures. Bostana and Eroglu (2012) reported that IV esmolol in doses of 1 mg kg^{-1} before intubation was effective in suppressing heart rate and arterial blood pressure in Caucasians. Kumar *et al.,* (2003) have reported optimal results while using higher doses of esmolol (2 mg kg^{-1}) in an Asian population, without any incidence of unplanned hypotension or bradycardia. In this normotensive cohort of black population, esmolol, at a dose of 2 mg kg^{-1} effectively decreased HR, SBP, DBP, MAP and RPP without any incidence of hypotension or bradycardia. This study further observed a reduction in DBP less than that in SBP resulting in a better control of the MAP in the study population. No consensus has however been reached regarding the optimum dose and timing of its delivery (Gupta *et al.*, 2009; Savio *et al.*, 2011) in Caucasian population.

The difference in the results of Korpinen *et al.* (1998) and Bostana and Eroglu (2012) involving esmolol 1 mg kg^{-1} to some extent, can be explained by differences in study designs including variations in patient population, age, racial differences, dose and timing of drug administration in relation to intubation. In addition, techniques used for induction, method of measurement of circulatory responses are contributing towards mixed effect of esmolol in these studies.

Increases in heart rate of patients receiving esmolol in this study was attenuated as compared to the

control group for a maximum duration of 5 minutes after intubation. Several studies have shown that there is increased incidence of myocardial infarction when intraoperative heart rate increases above 110 beats min^{-1} (Stone *et al.*, 1988; Slogoff and Keats, 1989). None of the patients in this study groups showed heart rate >110 beats min^{-1} (Table II).

RPP is a good estimate of myocardial oxygen requirement (Moon *et al.*, 2012). RPP levels close to 20,000 are normally associated with angina and myocardial ischemia (Cokkinos and Voridis, 1976). RPP one minute after intubation remained at 12,681 in the esmolol group thus confirming the cardioprotective effect of esmolol during laryngoscopy and intubation.

CONCLUSION

The use of esmolol for the control of haemodynamic responses to laryngoscopy and intubation has shown promising results in this cohort of black population. This study has established the prophylactic role of 2mg kg^{-1} dose of esmolol in attenuating hemodynamic responses to laryngoscopy and intubation in normotensive patients without any associated complications such as hypotension and bradycardia. However further studies needs to be done in high-risk patients, using longer duration infusions to investigate the safety and efficacy of esmolol in reducing the frequency of myocardial ischaemia after non-cardiac surgery.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Prevalence of smear positive tuberculosis among outpatient attendees, the case of the Tamale Teaching Hospital

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There is paucity of data on the prevalence of pulmonary tuberculosis (PTB) among out-patient attendees from individual Institutions and Health Care Facilities performing sputum smear microscopy in Ghana. This retrospective study analyzed sputum smear microscopy results among pulmonary TB suspected patients presenting to the Tamale Teaching Hospital in the Northern Region of Ghana. Sputum smear microscopy for Acid Fast Bacilli (AFB) results of new suspected pulmonary TB (Diagnosis) patients and their demographic data comprising age and sex recorded from January 2004 to December 2010 were retrieved from the TB Laboratory Register (TB04) of the Bacteriology unit and analyzed. Out of a total of 5,720 registered cases, 4,762 (83.3%) were new patients with suspected pulmonary TB (diagnostic cases). This comprised of 2,766 (58.1%) males and 1,996 (41.9%) females giving a female to male ratio of 1:1.4. Assessment of recorded data for newly suspected pulmonary TB patients rose from a minimum of 165 (9.9%) in 2004 to a maximum of 948 (19.9%) in 2009. Out of a total of the 4,762 recorded new cases, 620 were sputum smear positive yielding positivity rate of 13.0%. The positivity rate on a year-on-year basis was 15.7% (2004), 15.8% (2005), 13.4% (2006), 12.7% (2007), 20.6% (2008), 10.0% (2009) and 6.3% (2010). The median age for recorded smear positive cases was 42 years. Generally the percentage proportion of smear positives in the recorded cases stratified by age showed a steady rise from 0.3% in the <5 year olds and peaked at 16.3% in the 30-35 years age group. A gradual decline in smear positive cases was observed within the 36 – 41 years age group from 10.0% to 4.8% in the 54 – 59 years age group from where a gradual rise was observed up to the >72 years age group. There has been a remarkable improvement in diagnostic requests for suspected TB patients. The decline in positivity rates might have been impacted upon greatly by the national strategy to stop TB which emphasized on active case finding and prompt reporting at the community level, improving diagnostic processes and strengthening the health systems. The rapid urbanization and changes in the social fibre of inhabitants cannot be underestimated in the overall TB control efforts.

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Keywords*:* Microbacteria tuberculosis, microscopy, acid fast bacilli, Ghana

INTRODUCTION

Tuberculosis (TB) is one of the major infectious diseases worldwide and an opportunistic infection in Africa due to the relatively high rates of human immunodeficiency virus (HIV) co infection (Fairlie *et al.*, 2010). It is estimated that about one-third of the world's population are infected with the bacterium and new infections occur at a rate of about one per second. Most infections in humans are asymptomatic or latent infections, however 5-10% of the infected persons develop active respiratory disease (infectious) at some time in their life (Nitesh and Kiran, 2012). Sputum smear positive patients with active respiratory disease transmit the bacilli to other persons via droplets (WHO, 2003). Left undetected and untreated, each person with active TB disease will infect on average between 10 and 15 people every year (WHO, 2010). Early detection and treatment in other to reduce the transmission

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within communities are therefore essential for an effective control of the disease (Borgdorff *et al.*, 2002).

Passive detection where self-referred out-patient attendees at health facilities are evaluated on signs and symptoms suggestive of TB and referred to the laboratory for sputum smear microscopy for Acid Fast Bacilli (AFB) remains the major diagnostic tool of TB diagnosis and management in developing countries (WHO, 1997; Steingart *et al.*, 2006; Addo *et al.*, 2010). Passive detection is and continues to be the main strategy for TB case finding in Ghana. Although Ghana is not ranked among the world's high-burden TB countries, the disease still remains a major cause of morbidity and mortality in the country (WHO, 2009). One of the major challenges facing the National Tuberculosis Control Program (NTP) in Ghana is the reported low case detection due partly to under reporting from health facilities (Addo *et al.*, 2010). In spite of the over 46,000 new TB cases reported annually (WHO, 2006a), which is estimated to rank between 19% and 31% (1990-2009) detection rates, this still falls short of the expected average of 50% in Africa and a Global target of 70% (WHO, 2008). Data from NTP presented at national level are collated from the peripheral health facilities through the Regional Health Directorates; however, there is indication that less than a third of the estimated number of TB cases detected within these health facilities are officially reported each year (Addo *et al.*, 2010).

Moreover there is limited independent data from institutions and health care facilities enumerating TB case detection performance of such institutions to support the data from the NTP. This study was therefore aimed at assessing the prevalence of new smear-positive pulmonary tuberculosis diagnosed among suspected persons presenting at the Tamale Teaching Hospital from January 2004 to December 2010.

MATERIALS AND METHODS

Study design and site

This hospital-based retrospective study was conducted at the Tamale Teaching Hospital (TTH) and comprised of review of available data from January 2004 to December 2010. TTH is a 340 bed complement hospital situated in the Northern Region of Ghana. In addition to offering clinical care to inhabitants of the Tamale metropolis and its surrounding districts, it also serves as a referral hospital to the two Upper Regions (Upper East and Upper West) of Ghana. The hospital runs six clinical departments including the Chest clinic/ward which attends to patients with complicated respiratory tract infections including TB cases visiting the hospital from the metropolis, surrounding districts and catchment areas beyond the region.

Data extraction

Data comprising age, sex and results for Ziehl-Neelsen stained sputum smear microscopy for Acid Fast Bacilli (AFB) of all recorded cases from January 2004 to December 2010 were retrieved from the TB Laboratory Register (TB04) of the Tamale Teaching Hospital Bacteriology Laboratory. From the recorded data information about patients for whom diagnosis has been requested for the first time were retrieved and theses were classified as new suspected TB. Repeat cases and patients requesting follow-up test were excluded from the analysis.

Case definition

A case of pulmonary TB was classified as positive (confirmed case of PTB) if at least one out of the two/three smears from the two/three sputum specimen received was AFB positive and quantified as being scanty, 1+, 2+ and 3+ AFB present. New patients for the purposes of this study were defined as patients who were not on TB treatment .

Data analysis

Data retrieved were entered into Microsoft Excel and analyzed using GraphPad Prism® Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA). Normality of data was tested using Kolmogorov-Smirnov normality test ($\alpha > 0.05$). Descriptive statistics was employed to explain the general distribution of data. Categorical variables were compared using Chi-square test where appropriate. For all statistical comparisons a p-value of <0.05

was considered statistically significant. This study was approved by the Planning and Research unit of the Tamale Teaching Hospital.

RESULTS

A total of 5,720 cases were registered during the period under study in the TB 04 register at the laboratory, out which a total of 4,762 new patients with suspected pulmonary TB (diagnostic) were screened for AFB. More males reported with symptoms of pulmonary TB compared to females for the periods under review. Males comprised 2,766 (58.1%) and females 1,996 (41.9%) giving a female to male ratio of 1:1.4. (Table 1).

Table 2 represents the yearly age variables among the patients from 2004 to 2010. The median ages for newly suspected cases ranged from a least of 40 years in 2006 and 2007 respectively to a maximum of 44.5 in 2010. The study recorded a minimum age of one (1) year and a maximum age of 102 years in the years under review with inter-quartile (IQR) age spanning from 27 years to 70 years.

In total, 620 of the 4,762 suspected cases examined were Acid Fast Bacilli positive representing cumulative positivity rate of 13.0%. When stratified into the respective years, the positivity rates were as follows: 15.7% (74/471) in 2004, 15.8% (66/416) in 2005, 13.4% (66/494) in 2006, 12.7% (78/616) in 2007, 20.6% (183/890) in 2008, 10.0% (95/948) in 2009 and 6.3% (58/927) in 2010. The proportion of suspected cases to smear positives was 7:1, implying that for every seven new suspected pulmonary TB patients screened, there was one smear positive for the period under review. The general trend showed a gradual decline in the proportions of smear positives from 2004 (15.7%) to 2010 (6.3%), with the exception of 2008 which showed a sharp rise in smear positive cases (20.6%) (Figure 1).

For the years under review, dominance in male smear positivity was observed over that of females. Out of the 620 smear positives, 383 (61.8%) were males giving a male to female ratio of 1.0:0.6. A marked rise in the proportions of smear positives in females (46.4%) was observed in 2008(Figure 2).

Table 1: General overview of TB cases examined within the years under review

		New Suspected Cases		
Year	Total Registered Cases	**Male**	**Female**	**Total**
2004	594	306(65.0)	165(35.0)	471
2005	534	277(66.6)	139(33.4)	416
2006	611	310(62.8)	184(37.2)	494
2007	770	348(56.5)	268(43.5)	616
2008	1114	496(55.7)	394(44.3)	890
2009	1117	543(57.3)	405(42.7)	948
2010	1012	486(52.4)	441(47.6)	927
Total	**5720**	**2766**	**1996**	**4762**

Table 2: Age stratification for reviewed cases in suspected TB patients

	Years Under Review						
Age	2004	2005	2006	2007	2008	2009	2010
Minimum year	1	5	2	6	1	1	1
Median year	42	44	40	40	42	44.5	41
Maximum year	100	90	95	92	95	90	102
IQR year	27 – 60	32 – 60	30 – 56	27 – 60	29 – 65	29 – 70	27 – 65

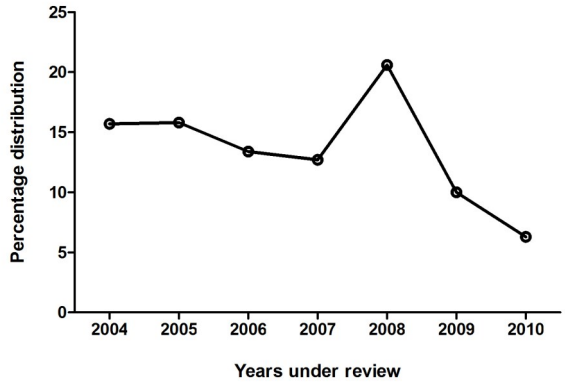

Figure 1: Pulmonary TB sputum smear positive cases over the study period

Figure 3 shows the distribution of cumulative smear positive cases by age groups over the review period.

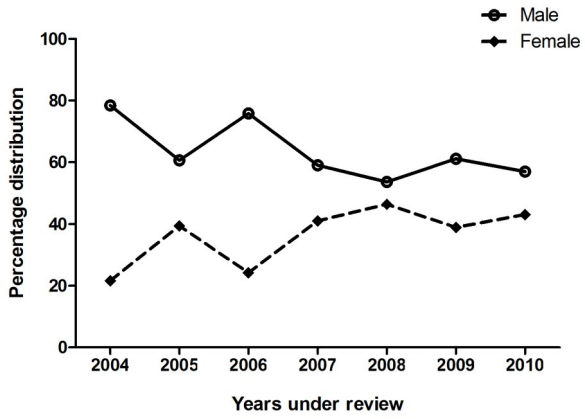

Figure 2: Sex distribution of Pulmonary TB sputum smear positive cases over the study period.

Generally, the percentage proportion of smear positives rose steadily from 0.3% in the <5 year olds and peaked to 16.3% in the 30 – 35 years age group. There was a gradual decline smear positive cases from 10.0% in the 36 – 41 years age group to 4.8% in the 54 – 59 years age group from where a gradual rise was observed up to the >72 years age group.

DISCUSSION

The number of individuals with active TB disease

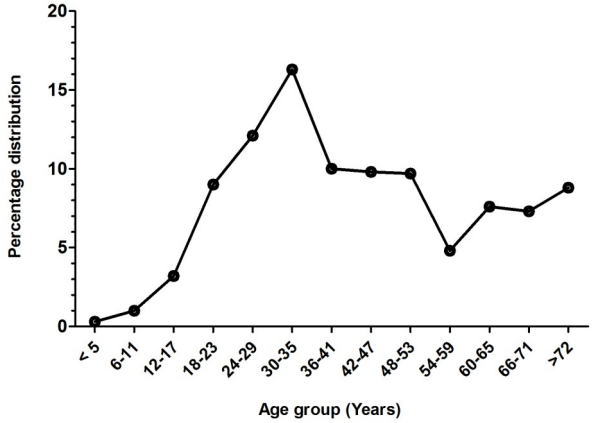

Figure 3: Age group distribution for recorded pulmonary TB sputum smear positive cases over the study period (2004 – 2010).

and proximity with these persons are important risk factors for TB infection. Thus, the Stop TB Strategy adopted by the NTP aims to control TB mainly by cutting the transmission chain which should be achieved through early detection and effective treatment of all people with active TB disease (WHO, 2006b; Lonnroth *et al.*, 2009). This study aimed at reviewing the prevalence of new smear positive TB cases among out patient department (OPD) attendees reporting to the Tamale Teaching Hospital. The study observed improvement in reported case activity among suspected individual patients in a year-on-year basis with steady declines in positivity rates with an exception in case activity for 2008 which recorded a high positivity rate. This sudden spike could be due to the national action plan from NTP in 2008 tasking all health facilities to be actively involved in case finding and this mandated that patients who are high suspects or with cough were sent to the laboratory for sputum smear microscopy. Cases for which sputum smear turns out positive, the case is traced back to the exact home address and all members of the household are encouraged to take sputum smear tests hence the increase in registered cases and new smear positives for that particular year. This notwithstanding, the observation of declines in positivity rates could be suggestive of one of the following: an overall impact with the initiated treat-

ment plan accompanying the Stop TB Strategy adopted by NTP; a decline in TB prevalence among patients within the catchment area of the hospital or the fact that those who are really burdened with TB disease are either not reporting promptly to the hospital to seek treatment or are missed due to institutional challenges affecting case detection activities within the hospital.

The impact of initiated treatment plans and support packages afforded to patients burdened with TB could have led to the effective management of cases and improvements in living and social conditions of the people thereby leading to a reduction in prevalence rates and declines in risk of transmission from infected individuals. Over time, the social and living conditions of indigenes have improved tremendously with improvement in diet and environmental hygiene. The resultant decrease in overcrowding in homes as a result of social development could have impacted greatly on the degree of exposure, reduction in the risk of transmission and susceptibility to infection in exposed persons as related in the studies of Vynnyvk and Fine (1999) and Lonnroth *et al.,* (2009). In order to improve case detection, other studies have advocated for active case search through community and household visits which will not only improve smear positivity rates but as well address the social and gender inequities associated with accessibility of health care services by TB patients (Horie *et al.*, 2007; Yimer *et al.*, 2009; Tadesse *et al.*, 2011).

The observed smear positivity rate from adolescence, into young adulthood could partly be attributed to the propensity of such age class being actively involved in social events where overcrowding is rife and as such being in the relatively high risk group as related in the studies of Lonnroth *et al.,* (2009) and Bekker and Wood (2012). The study further observed a low prevalence of TB in children (<5 years) which finding corroborates other studies that reported low smear positive pulmonary TB cases in children, supporting the fact that children rarely produce bacteriologically positive sputum (Starke, 1993; Osborne, 1995; Raqib *et al.*, 2009). Bacteriological diagnosis of TB in young children has remained a major challenge particularly in resource-limited areas due to diagnostic constraints (Osborne, 1995). In countries, where TB is not endemic, most childhood TB cases are detected through close contact with an infectious index patient, a positive tuberculin skin test (TST) result, and presence of suggestive abnormalities on a chest radiograph. With the exception of contact with an infectious index patient, the aforementioned diagnostic tools are virtually nonexistent in resource-limited countries where childhood TB is closely associated with poverty, overcrowding and malnutrition.

Furthermore, active case detection and contact tracing activities are not routinely done primarily because of deficiencies in home address allocation and as such diagnosis, is solely based on suggestive signs or symptoms and chest radiograph abnormalities (Cruz and Starke, 2007). The positivity rate of sputum smear microscopy and culture in children has been reported to be less than 15% and between 30%–40% respectively (Marais and Pai, 2007). The challenge lies in the acquisition of adequate quality specimens, particularly from smaller children. Even though diagnostic algorithms have been recommended through consensus and expert opinion, these algorithms prolong the child's evaluation over a period of time. The resultant delayed diagnosis might be an important cause of increased TB mortality in children particularly situated in resource-limited countries (Eamranond and Jaramillo, 2001).

The steady rise of positivity rates in the elderly from the 60-65 years age group as observed in the current study agrees well with other studies (Davies, 2007; Schaaf *et al.*, 2010). These studies have shown that the elderly because of age related decline in immunity and risk factors such as poverty, malnutrition and smoking especially in resource-limited areas are most often affected with other chronic diseases either with subtle or atypical clinical manifestations thereby making early diagnosis more difficult and going unrecognized. The diseases are therefore mostly detected in the advanced state (Zevallos and Justman, 2003; Davies, 2007; WHO, 2007; Schaaf *et al.*, 2010) and as such a high index of suspicion is of the essence in order to make an

early diagnosis; and timely initiation of treatment are important in both the very young and the elderly.

In consent with other reports (Murray *et al.*, 1990; Connolly and Nunn, 1996; Hudelson, 1996; WHO, 2004), this study observed high proportion of sputum smear positivity among males than in females. This observed trend agrees well with global picture of tuberculosis cases notified (WHO, 2004). According to the WHO report, more smear-positive males than females infected with tuberculosis are diagnosed every year and notified. Several reasons including biological, epidemiological, social and cultural barriers have been cited for this observed gender-based difference (Jianming *et al.*, 2008; Neyrolles and Quintana-Murci, 2009). Health care accessibility and health seeking behaviours with regards to stigmatization and its social consequences have been noted to be more pronounced in the females than in males and therefore affect their health care seeking behaviours hence the overall seemingly low female-related cases (Diwan *et al.*, 1998; Uplekar *et al.*, 1999; Borgdorff *et al.*, 2000).

Other studies have indicated that men are more likely to be associated with factors and behaviours such as, frequent use of alcohol and illicit drugs that may increase their exposure and influence the rate at which the infection progresses into active disease (Hudelson, 1996). The quality of sputum specimen produced by suspected individuals has also been cited. Indications of poor-quality sputum specimens usually submitted by women has been noted to account for lower smear positivity in women than in men (Khan *et al.*, 2007). This review has provided the baseline information for further studies into the sociological and behavioural factors mediating the gender and age differentials associated with TB infections within the catchment area. Furthermore, research into the transmission dynamics of the disease would also provide insight into whether the high rates of infection among males and the young adults are due to reactivation of latent infection or recent transmission.

CONCLUSIONS

There is an indication of remarkable improvement in the case detection efforts in the hospital which could possibly have resulted in the steady decline of positivity rates. Such gains made can further be enhanced by creating effective help-seeking environments within the communities and the hospital, improving diagnostic and health systems efficiency. Furthermore, to minimize delays in initiating effective chemotherapy, intensified case-finding activities should be directed towards high-risk communities and age-specific groups so as to increase awareness of typical symptoms of TB disease.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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