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ORIGINAL ARTICLE

Bacterial contamination of street vending food in Kumasi, Ghana

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Street vending foods are readily available sources of meals for many people but the biological safety of such food is always in doubt. The aim of this study is to ascertain bacterial isolate and determine total counts of bacterial species responsible for the contamination of the street vending food in Kumasi so as to determine the microbiological safety of such a food. This prospective study was conducted among street vending food at four bus terminals in Kumasi. From November, 2008 to February, 2009, 60 food samples comprising ice-kenkey (15), cocoa drink (15), fufu (5), ready-to-eat red pepper (normally eaten with kenkey) (5), salad (10) and macaroni (10) were purchased and analyzed. The food samples were purchased and transported to the laboratory in sterile plastic bags and analyzed for bacterial contamination. Serial dilution of each food was prepared in buffered peptone water and inoculated onto plate count agar (PCA), MacConkey and blood agar plates. Growths on PCA were counted; those on other agar plates were identified by their colonial morphology, Gram stain, biochemical and sugar fermentation methods. The mean bacterial counts in these foods expressed to $\log_{10}$ CFU/ml were: fufu 6.36±0.47, cocoa drink 6.16±0.5, red pepper 5.92±0.64, ice-kenkey 5.58 ±0.52, macaroni 5.58±0.97 and salad 5.13±0.77. Most of these foods contained higher than acceptable contamination level of <5.0 $\log_{10}$ CFU/ml. The isolates obtained were Coagulate negative *staphylococci* (23.7%), *Bacillus species* (21.5%), *Klebsiella pneumoniae* (18%), *Aeromonas pneumophila* (17.7%), *Enterobacter cloacae* (6.7%), *Staphylococcus aureus* (3.7%), *Escherichia coli* (2.2%) and *Pseudomonas aeruginosa* (2.2%). Most ready-to-eat foods in Kumasi were contaminated with enteric bacteria and other potential food poisoning organisms with bacterial counts higher than the acceptable levels. Food vendors therefore need education on food hygiene.

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Keywords*:* Ready-to-eat foods, Bacterial contamination, Food poisoning

INTRODUCTION

The street food industry plays a very important role in meeting food requirements of commuters and urban dwellers in many cities and towns of developing countries, as it feeds thousands of people daily with a large range of foods that are relatively cheap and easily accessible (Tambekar *et al.,* 2008). However, food borne illnesses of microbial origin are a major health problem associated with street foods (Kaneko *et al.,* 1999; Mensah *et al.,* 2001; Mensah et *al.,* 1997; Mensah *et al.,* 2002). The traditional processing methods that are used in the preparation, inappropriate holding temperature and poor personal hygiene of food handlers are some of the main causes of contamination of ready to eat foods (Barro *et al.,* 2006; Mensah *et al.,* 2002). Also the food are not effectively protected from flies and dust (Bryan *et al.,* 1997; Bryan *et al.,* 1992). In Ghana, street food are mostly prepared and processed manually and sold to the public at various lorry terminals, by the roadside or by itinerant vendors (Mensah *et al.,* 2002).

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In Ghana, diarrhoea has been recognized as one of the major causes of hospital attendance and 16% of deaths in African children younger than five years are directly attributable to diarrhoeal diseases (Bruce *et al.,* 2005). This leads to a major ongoing campaign about hand-washing to reduce the incidence of diarrhoea. Despite the commitment and dedication of the Ghana Food and Drugs Board, improved food safety systems have not been widely implemented which raises more concern about the probable role street vending food play in food poisoning (Kosek *et al.,* 2003; Soyiri *et al.,* 2008). Therefore the aim of this study is to determine the level of bacterial contamination of selected street vending food in Kumasi.

MATERIALS AND METHODS

Study sites

The study was conducted in Kumasi at four bus terminals: Kejetia, Asafo, Race course and Bantama. These bus terminals are most often congested with heavy trucks, minibuses, and taxis. These vehicles are often seen offloading goods and passengers to the bus stops. These passengers are often food vendors who sell at any convenient space along the gutters. Various commuters, shoppers and passers-by can be seen at the bus terminals daily and all constitute a huge market for all kinds of goods. Common items sold include clothes, food stuffs and vegetables and snacks including many kinds of ready-to-eat foods which are sold along the walkways and under sheds and umbrellas. Common street food available here are cakes, potato chips, ice-kenkey, cocoa drink, yoghurt, bread, meat pie and different kinds of locally produced drinks. Stew, macaroni and salads (consisting mainly of cut tomato and onion mixtures and other ingredients such as vinegar, salt and spices), are usually packed in sieves and trays and carried around. As these itinerant vendors walk in between the vehicles they sell to the passengers waiting to be transported to their destinations.

Sample Collection

Sample collection and analysis was from November, 2008 to February, 2009. The street vending foods were purchased randomly at the bus terminals, between mid-day and 1.00 pm when such foods are most patronized, without repetition from the same vendor. Food types analyzed were as follows: ice-kenkey, cocoa drink, fufu, ready -to-eat pepper (normally eaten with kenkey), salad and macaroni. These foods were packaged and needed no further processing before consumed. The samples were purchased and transferred into sterile Whirl-Pak bags (Nasco, USA). They were then placed in a cold-box with ice packs and transported to the microbiology laboratory for analysis the same day. Information about how the food were prepared and packaged for sale were obtained from the vendors and presented in Table 1.

Sample Analysis

Five milliliters (5 ml) of each liquid food were mixed with 45 ml of buffered peptone water and homogenized by manual shaking. Solid foods were diluted by adding five grams of food to 50 ml of buffered peptone water and then shaken vigorously to dislodge adhered bacteria. The liquid phase then forms the stock sample from which dilutions were made to obtain 10^{-1}, 10^{-2}, 10^{-3} up to 10^{-10} dilutions. After mixing each tube with the dilution, 0.1 ml of it was transferred onto a sterile plate count agar (PCA) (Oxoid Ltd, Basingstoke Hants, England) then spread on the agar surface and immediately placed in an incubator. The plates were incubated at 37^{o}C overnight. The remaining stock samples were incubated at 37^{o}C for 4 hours after which they were subcultured onto Blood Agar, MacConkey Agar (Oxoid Ltd, Basingstoke Hants, England) plates and incubated at 37^{o}C overnight.

Viable Bacterial Count

After overnight incubation, growth on the PCA showing 30-300 colonies was counted. Bacterial counts were expressed as the log of colony-forming-units per ml for liquid food or per g for solid food sample analyzed.

Bacterial identification

The MacConkey and Blood agar plates were examined for bacterial growth. Growth characteristics and other colonial morphology such as lactose fermentation, formation of mucoid colonies of the bacteria were carefully recorded. Less than five identical colonies for a particular organism growing on a plate were ignored. When more than five similar colonies were counted on a plate, then five isolated identical colonies on either the blood agar or MacConkey agar plates were picked carefully, one by one and inoculated into buffered peptone water in sterile microtitre wells. Culture from each microtitre well was re-inoculated onto a Nutrient agar (Oxoid Ltd, Basingstoke, Hamspshire, England) to obtain pure growth. Organisms which were identified to be the same from the microtitre wells were grouped as one isolate from the food sample analyzed. Bacterial identification was done using the pure culture on the nutrient agar plates.

Biochemical tests

The first test was the Gram staining and the results were followed by the appropriate biochemical tests (catalase,

coagulase, oxidase, sugar fermentation, indole, citrate utilization, urease production) and motility test.

The catalase test was performed on the Gram positive cocci. This was done my mixing a dense culture with two drops of H_2O_2 and looking for bubbles. Organisms positive (produced bubbles) in the test were considered to be *Staphylococci*, while those negative were *Streptococci*. The *Staphylococci* were further tested with the coagulase test. The coagulase test was performed by mixing a dense suspension of the culture with plasma contained in a small test tube. The set up was incubated overnight, and then observed for fibrin clot. Those positive in the coagulase test were identified to be *Staphylococcus aureus*, and those negative were coagulase negative staphylococci.

The Gram negative organisms were also identified. Those organisms which were non-fermenters of lactose on the MacConkey agar were tested by the oxidase test using tetrametyl-*p*-phenylene-diamaine hydrochloride reagent sticks. The oxidase stick was used to touch the organism on the nutrient agar plate. The development a blue/black colour on the stick in less than 10 seconds was considered positive for oxidase production. *Pseudomonas aeruginosa* is positive in this test, so the oxidase test and other tests (growth characteristics, pigment production and smell) were used to identify the organism.

Citrate utilization employed the Simon's citrate agar (Oxoid Ltd, Basingstoke, Hamspshire, England). The citrate agar was inoculated with growth on the nutrient agar and incubated overnight. *Klebsiellae* are positive in this test, so enabled it to be differentiated from *E. coli*.

Triple sugar iron (TSI) agar (Oxoid Ltd, Basingstoke, Hamspshire, England), was used for the differentiation of the *Enterobacteriaceae*. Using a sterile straight wire the TSI was stabbed deep to the bottom and the surface of the agar slant was streaked with the test organism. By the different three sugar fermentation, gas accumulation and hydrogen sulphide production abilities the enterobacteria were identified.

The indole test was performed by inoculating peptone water (Oxoid Ltd, Basingstoke, Hamspshire, England), and incubating it overnight. The detection of indole was by the addition of Kovac's reagent (Oxoid Ltd, Basingstoke, Hamspshire, England). *E. coli* is positive (forms red ring) in this test, so was used to distinguish it from *Klebsiella*. Motility of the organism was tested by using a sterile loop to pick culture from the microtitre wells were placed on a microscope slide, covered with a cover slip and observed under the microscope for locomotion.

RESULTS

The study was conducted between November 2008 and February 2009, when a total of 60 food samples were analyzed. Bacterial growth was observed in all the food

Table 1: Food type analyzed and its preparation

Food type	Ingredients	How prepared	How served
Salad	Leaves, fresh vegetable	No boiling	Served with spoon or hand
Macaroni	Wheat flour	Extruded wheat flour; Partial	Served with spoon or hand
Fufu	Cassava with plantain, cocoyam or yam	Boiling and pounding in mortar with pestle while turning it over with hand	Served by hand in bowls
Ice kenkey	Mixture of milled kenkey, milk powder and sugar	Mixing, with water milk, sugar; no boiling	Packaged into polythene bag by hand
Cocoa drink	Cocoa powder and sugar	Mixture of cocoa powder and water; No boiling	Packaged into polythene bags by hands
Red pepper	Pepper, onion, salt	Mashing/grinding, no heating	Served with spoon

samples tested. The 15 cocoa drinks had the heaviest level of contamination with 31 isolates, where 10(15) yielded *Aeromonas hydrophila*, 8(15) *Klebsiella pneumoniae*, 6(15) and 7 (15) yielded *Bacillus* species and coagulase negative staphylococci respectively. Other isolates were found in fewer samples of the cocoa drink. A total of 29 isolates were obtained from the 10 macaroni samples, where 8(10) of the macaroni samples had *Klebsiella pneumoniae* isolates. Both *Aeromonas hydrophila* and coagulase negative staphylococci were obtained from six of the macaroni samples. 15 ice-kenkey samples were analyzed with 10(15) yielded coagulase negative staphylococci, 8(15) yielded *Bacillus* species, 4(15) had *Aeromonas hydrophila* in them and 2(15) yielded *S. aureus* and then 1(15) yielded *Klebsiella pneumoniae, E.coli, Citrobacter freundii* and *Enterobacter cloacae*. There were 10 salad samples out of which six had *Klebsiella pneumoniae* and both *Bacillus* species and coagulase negative staphylococci were each isolated from three of the samples. Five samples each of fufu and red pepper were analyzed. All the fufu samples had coagulase negative staphylococci, but only 2(5) of red pepper had it, whilst 4(5) fufu and 3(5) red pepper had *Bacillus* species, but all other isolates were obtained from one sample each of fufu and red pepper. Pseudomonas aeruginosa was isolated from only macaroni and fufu, but not in the other food types. The most prevalent bacteria types were coagulase negative staphylococcus*, Bacillus* species *Aeromonas* species*,* and *Enterobacter* species. These bacteria appeared in every food type analyzed as shown in Table 2.

The isolates obtained and the mean bacterial count in the food items expressed as $\log_{10}$ CFU/g or CFU/ml were as follows: ice-kenkey-5.58, cocoa drink-6.16, macaroni-5.48, salad-5.13, fufu-6.36 and 5.92 for red-pepper are as shown in Table 3. Samples of macaroni, salad and cocoa drink had mean contamination levels of > 5.0 $\log_{10}$ cfu/ml as compared to the acceptable bacterial count in these foods of <5.0 $\log_{10}$ cfu/ml.

DISCUSSION

The results obtained for the various food types have been discussed below. High levels of bacterial contamination at varying degrees were detected in the food types tested. Samples of macaroni, salad, fufu and cocoa drink had levels of contamination higher than the acceptable reference figures of Ghana Standard Board which prescribe values of <5.0 $\log_{10}$cfu/g

Fufu

The mean bacterial count in fufu was 6.36 $\log_{10}$cfu/g which is higher than the national reference value of <5.0 $\log_{10}$cfu/g. This level of contamination is probably due to the mode of preparing this food. The preparation of fufu involved boiling the cassava and plantain, then pounding it in a mortar using a pestle while turning the resulting paste with the bare hands, which were occasionally washed in a container of water. This practice can promote the introduction of microorganism, and the organisms would multiply when the fufu is not eaten immediately. These processes may be responsible for this level of contamination in the fufu. A similar study (Mensah *et al.,* 2002) in Accra, observed similarly high bacteria count of 6.2±1.57 $\log_{10}$cfu/g in fufu and the isolates found were *Citrobacter freundii, Enterobacter cloacae* and *Enterobacter sakazzkii.* In this current study these same bacteria in addition to *Pseudomonas aeruginosa,* coagulase negative staphylococci and *Aeromonas hydrophila* were also isolated. Though, the level of contamination in fufu in this study was high, most of the isolates were coagulase negative staphylococcus*,* which are known to be normal flora on the skin (Koneman *et al.,* 1988) and *Bacillus* species which are ubiquitous organisms (found in soil, skin, water and dust) which can be found in a variety of foods (Bergdoll, 1981).

Ice-kenkey

The level of contamination of ice-kenkey was 5.58±0.52 $\log_{10}$ cfu/ml as compared to the national standard of <5.0 $\log_{10}$ cfu/ml (Adu-Gyamfi and Nketsia-Tabiri, 2007). *Escherichia coli, Staphylococcus aureus and Aeromonas hydrophila* were isolated from this food. The presence of *E. coli* in this food types is an indication of faecal contamination probably at one stage of preparation or from the materials used. *Staphylococcus* contamination on the other hand might have resulted from man's respiratory passages, skin and superficial wounds which are common sources of *Staphylococcus aureus* (Burt *et al.,* 2003). When *Staphylococcus aureus* is allowed to grow in foods, it can produce a toxin that causes illness. Although, cooking destroys the bacteria, the toxin produced by *Staphylococcus aureus* is heat stable and may not be destroyed even by heating, let alone by refrigeration the main process of keeping ice-kenkey prior to consumption. Contamination of ice-kenkey with *Staphylococcus aureus* could lead to food poisoning and this could be attributed first to non-adherence to standard hygienic practices employed during food preparation (Ghana Standard Board, 2003) and second to the type of water used in mixing the food which is often not clean (Muleta, 2001). There is relatively high level of coagulase negative staphylococcus contamination (7.4%) in the food, which was likely introduced during preparation. The manual mixing of this food, promotes contamination skin flora (Koneman *et*

al., 1988). *Bacillus* species were also introduced into the food during its preparation and packaging because the entire process is performed in the open and dirty environment which promotes contamination as observed in

Table 2: Bacterial isolates and their level of contamination in the street vending food in Kumasi

Isolates	Ice-kenkey (n=15)	Cocoa drink (n=15)	Macaroni (n=10)	Salad (n=10)	Red pepper (n=5)	Fufu (n=5)	Total (n=60)
Coag. neg. staphylococci	10(7.4%)	6(4.4%)	6(4.4%)	3(2.2%)	2(1.5%)	5(3.7%)	32(23.7%)
Bacillus sp.	8(5.9%)	7(5.1%)	4(3.0%)	3(2.2%)	3(2.2%)	4(3.0%)	29(21.5%)
Klebsiella sp.	1(0.7%)	8(5.9%)	8(5.9%)	6(4.4%)	2(1.5%)	0(0.0%)	25(18.5%)
Aeromonas sp.	4(3.0%)	10(7.4%)	6(4.4%)	2(2.2%)	1(0.7%)	1(0.7%)	24(17.7%)
Enterobacter sp.	1(0.7%)	4(3.0%)	1(0.7%)	1(0.7%)	1(0.7%)	1(0.7%)	9(6.6%)
Citrobacter sp.	1(0.7.%)	2(1.5%)	1(0.7%)	1(0.7%)	0(0.0%)	0(0.0%)	5(3.7%)
S. aureus	2(1.5%)	1(0.7%)	1(0.7%)	0(0.0%)	1(0.7%)	0(0.0%)	5(3.7%)
E. coli	1(0.7%)	1(0.7%)	0(0.0%)	1(0.7%)	0(0.0%)	0(0.0%)	3(2.2%)
P. aeruginosa	0(0.0%)	0(0.0%)	2(1.5%)	0(0.0%)	0(0.0%)	1(0.7%)	3(2.2%)
Total	28(20.7%)	39(28.8%)	29(21.4%)	17(12.6%)	10(7.4%)	12(8.8%)	135(100%)

Table 3: Bacterial count in street vending food in Kumasi

No.	Ice-kenkey	Cocoa drink	Macaroni	Salad	Fufu	Pepper
1	5.47	5.6	6.56	6.20	5.90	5.00
2	6.32	6.91	5.54	5.20	6.70	6.44
3	4.90	5.84	6.54	5.47	6.90	6.30
4	7.23	6.00	4.00	6.17	6.60	6.60
5	5.46	6.07	4.17	4.00	5.70	3.30
6	5.59	5.86	6.30	4.00	-	-
7	5.46	6.77	6.25	5.47	-	-
8	5.23	6.90	4.17	4.14	-	-
9	5.34	5.77	5.34	5.30	-	-
10	5.49	6.83	6.00	5.34	-	-
11	5.25	6.53	-	-	-	-
12	5.67	5.50	-	-	-	-
13	5.47	5.77	-	-	-	-
14	5.46	5.62	-	-	-	-
15	5.47	6.50	-	-	-	-
Mean	5.58±0.52	6.16±0.5	5.48±0.97	5.13±0.77	6.36±0.47	5.92±0.64

many reports from many parts of the world [(Black *et al.,* 1991; Bryan *et al.,* 1992 ; Burt *et al.,* 2003; Ghosh *et al.,* 2007).

Macaroni

The mean bacterial count in macaroni samples analyzed was 5.48±0.97 $\log_{10}$ cfu/g. Though, this is higher than the national reference value of < 5.0 $\log_{10}$ cfu/g, it was less than a value of 6.0±1.64 $\log_{10}$ cfu/g obtained in Accra (Mensah *et al.,* 2002) and similar to results observed in India (Olukoya *et al.,* 1991; Tambekar *et al.,* 2008).

Klebsiella species*, Pseudomonas* species*, Enterobacter* species *and Staphylococcus* aureus were the isolates obtained from macaroni. This food is often prepared by heating but gets cold by the time it is served because the sellers are not able to keep the food at a good holding temperature and therefore ambient temperatures provide a suitable condition for the growth of the microorganisms (Mensah *et al.,* 2002). *Shigella sonnei* was not isolated from macaroni in this study probably due to the relatively small sample size (10) as compared to 26 samples of macaroni studied in Accra, where *Shigella sonnei* was isolated (Mensah *et al.,* 2002). The contamination of this food was not surprising because after cooking the food, serving was performed with bare hands (Mensah *et al.,* 2002). The vendors sell and dish out food with bare hands and also simultaneously handle currency as they take money from the buyers, a common practice implicated in introducing pathogens into the food (Kubhekar *et al.,* 2001). It was also reported in Manila, Philippines that, the consumption of such food served with bare hands led to cholera outbreak (Barry, 2005).

Salad

The level of contaminant in salads was 5.13 $\log_{10}$ cfu/g which is higher than the national reference value of <5.0 $\log_{10}$ cfu/g [12]. A previous study of this kind was carried out in Accra (Mensah *et al.,* 2002) and a total bacterial count of 6.3 ±0.78 log cfu/g was obtained, but a count of 5.0 log cfu/g was obtained in Johannesburg (Olukoya *et al.,* 1991).

The bacterial pathogens isolated in this study were: *Klebsiella pneumoniae*, *Escherichia coli*, *Citrobacter freundii, Aeromonas hydrophila*, *Bacillus species*, s*taphylococcus* and *Enterobacter cloacae*. Contamination of salad with these bacterial pathogens conformed to previous finding in Ghana (Mensah *et al.,* 2002). *Escherichia coli* are significant diarrhoeal causing organisms usually found in localities of poor sanitary conditions (Umoh and Odam, 1999). It has been associated with "travelers' diarrhoea" and hemorrhagic colitis. Therefore, consumption of this food could be associated to diarrhoeal diseases (Hanoshiro *et al.,* 2004). Furthermore, the presence of these pathogens indicates that, food hygiene and sanitation procedures were lacking during the preparation of this food (Ghana Standard Board, 2003).

This level of contamination was not unexpected with salad because, in Ghana, all types of water are used for watering vegetables, especially those grown in the cities, where there are not many natural bodies of water (Mensah *et al.,* 2002). Untreated manure is also usually used to fertilize many vegetables. The use of untreated manure to fertilize vegetable has led to isolation of *Salmonella* species*, Shigella flexneri* and *Escherichia coli* from lettuce and tomatoes in 2001 in Accra (Mensah *et al.,* 2001). Similar observations were also reported in the Annual Report of Ghana Health Service in 2007, where school children who were fed with rice, stew and salad, developed gastroenteritis attributed to cross-contaminated green vegetables.

Cocoa drink

The mean bacterial count of cocoa drinks analyzed was 6.16 $\log_{10}$ cfu/ml much higher than the national acceptable reference of <5.0 $\log_{10}$ cfu/ml. This level of contamination could be due to the unhygienic production practices (Ghana Standard Board, 2003) as the preparation involves manual mixing of the cocoa powder with sugar and probable non-potable water collected from streams nearby when municipal water supply is interrupted. The isolates obtained from the food were *Aeromonas hydrophila, Klebsiella pneumoniae, Enterobacter cloacae,* and *Escherichia coli* organism are enteric pathogens (Adu-Gyamfi and Nketsia-Tabiri, 2007). Individuals who patronize this food product contaminated with enteropathogenic *Escherichia coli* are likely to develop "traveler's diarrhoea" and hemorrhagic colitis (Umoh and Odam, 1999) therefore the presence of the organisms in street food is also an indication of faecal contamination and constitutes food risk to the patrons. The predominant isolate obtained from this food was *Aeromonas species* which is a bacterium mostly found in water and resides in sinks and drain pipes (Koneman et al., 1988) hence the possible sources of contamination of the cocoa drink. Coagulase negative staphylococci isolated normally found on the human skin.

"Red Pepper" eaten with kenkey.

The mean bacterial count of the Red Pepper sauce analyzed was 5.92 $\log_{10}$ cfu/g, this level of contamination is

higher than the national reference level of <5.0 $\log_{10}$ cfu/g. A previous study in Ghana (Mensah *et al.,* 2002) had a contamination level of 5.1±1.73 $\log_{10}$ cfu/g in this food. Similar findings were obtained, when a wide range of menu items including formula foods, soups and stews were examined in Peru (Kwakye-Akyea, 2007) and where contamination level of 5.9 $\log_{10}$ cfu/g were found. The isolates obtained from the red pepper analyzed in this study were *Klebsiella pneumoniae, Staphylococcus aureus,* coagulase negative staphylococcus and *Enterobacter cloacae, Bacillus* species *and Aeromonas hydrophila.* When *Staphylococcus aureus* is allowed to grow in foods, it can produce a heat stable toxin that causes illness (Koneman *et al.,* 1988), so such organisms in food constitute food poisoning risk to the consumer (Muleta, 2001). Moreover, sauces, such as red pepper normally eaten with kenkey, are made from fresh vegetables (pepper, tomato, and onion) and eaten without heating. All bacteria introduced in at the time of preparation, survive and multiply if held for long periods at ambient temperature (Ghana Standard Board, 2003).

Generally, this study indicates that most ready to eat foods sold on the streets of Kumasi are contaminated with various microbial types. The bacterial isolates were 23% coagulase negative staphylococci*,* 18% *Klebsiella pneumoniae,* 3.7% *S. aureus* and 2.2% *E. coli* were seen in the food samples maybe as a result of either wearing of dirty clothing, improper cleaning of dishes, unhygienic handling and serving practices or all of these. Other contributing factors may be the contaminated hands of vendor, and perhaps lack of knowledge of hygienic practices and safety of food products (Fang *et al.,* 2003). Unhygienic surroundings (where the foods were prepared and sold) like flowing sewage in open gutters, improper waste disposal system and inadequate water supply attracts houseflies or fruit flies, probably further increases food contamination (Chumber *et al.,* 2007). Also of concern is reluctant behaviour of the vendors to wash hands. The itinerant vendors carry small volumes of water along as they sell, but do not change the water and the washing is normally not done with soap. When municipal water supplies fail, the vendors resort probably resort to 'seemingly clean' water from nearby streams for the food production. Also many vegetable growers rely on such stream waters for irrigation of the crops and then washing of the harvested crops before they are sold, making them heavily polluted from the farm. Efforts must be made to wash harvested crops with clean potable water.

CONCLUSION

This study has demonstrated that some of the most popular types of ready-to-eat foods that are sold on the streets of Kumasi are contaminated, and do not meet the required quality and safety levels. Some of the bacteria isolated such as *Staphylococcus aureus* and *Escherichia coli* are potential enteric pathogens and are known to cause gastroenteritis. Street foods therefore pose a health threat to the patron and efforts to reduce level of contamination in the street vending food are recommended. Water for preparing such foods can be boiled, cooled and then used to prepare food like cocoa drink and ice-kenkey. Use of previously boiled water for washing vegetables may help reduce food contamination.

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ORIGINAL ARTICLE

Effect of maternal age on endometrial morphology among Ghanaian infertile women

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As more women choose to delay childbearing, increasing numbers of them face age-related fertility problems. Normal endometrial receptivity is essential for the establishment of any pregnancy and its evaluation is thus considered a basic goal in the assessment of female infertility. It is unclear as to whether women who present to infertility clinics at older age have age-related endometrial retardation or luteal phase defect. This study was designed to investigate the prevalence of luteal phase defect (LPD) among infertile women and its relationship with age. Mid-luteal endometrial biopsies were taken from eighty (80) infertile women attending fertility clinics of Komfo Anokye Teaching Hospital, Magazine Clinic and the Bomso Specialist Hospital in Kumasi metropolis and ten fertile women as control using dilatation and curettage and then processed for light microscopy. The results show that 65.0% of the biopsies of the infertile women were normal in development hence their infertility could be due to other factors. In 35.0% of the biopsies the endometrial development was out-of-phase and therefore suggestive of a defective luteal phase which may lead to a non-receptive endometrium during the implantation window. There was no significant difference when LPD was analyzed according to age suggesting that ageing has no significant effect on endometrial retardation from this study.
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Keywords: Luteal phase defect, Infertility, Ageing, Non-receptivity

INTRODUCTION

Female age is the single most important determinant of spontaneous as well as treatment related conception, with a gradual decline in fertility especially after the age of 35 years (Menken *et al.*, 1986). Demographic studies have shown that more women are delaying childbearing at the present time than previously (Abaidoo *et al.*, 2000; Botting *et al.*, 2000). This trend is expected to cause a corresponding rise in the mean age at which women first present with infertility (Botting *et al.*, 2000). It is unclear as to whether Ghanaian women who present to fertility clinics at an older age have a different diagnostic profile from that in younger women. It has been well established that female fertility declines with age (Fox *et al.*, 1991; Tietze, 1957). This phenomenon has been attributed in-part to the ageing of the ovaries resulting in poor oocyte quality (Abdalla *et al.*, 1991; Navot *et al.*, 1991). Moreover, ovarian follicles from older women contain gametes that have a higher rate of chromosomal abnormality (Richardson *et al.*, 1990; Wramsby *et al.*, 1987).

In some animal species, mainly rat and mouse, marked age-related endometrial changes have been described. In older animals, an increase in collagen has been observed (Craig *et al.*, 1985) along with a reduction in stromal cells (Wilcox *et al.*, 1988) and oestrogen receptors (Han *et al.*, 1989). Furthermore, reductions in oestral periods (Rahima *et al.*, 1978) and the endometrial cells' ability to express a

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decidual reaction *in-vitro* have also been documented (Otha, 1987). These data suggest a decline in endometrial receptivity and might contribute to an explanation of the age-dependent decrease in fertility in females of these species. However, other studies have also suggested that age does not appear to have a significant effect on the morphology or histological responses of the endometrium to steroid stimulation (Lenton *et al*., 1984a; Menken *et al*., 1986; Noci *et al*., 1995; van Noord-Zaadstra *et al*., 1991). Although there have been some studies on female infertility in Ghana, there has not been any study to evaluate the effect on maternal age on endometrial development in Ghana. The present study was therefore designed to study endometrial development among infertile with respect to maternal age in Ghanaian setting.

MATERIALS AND METHODS

Sample collection

Endometrial biopsies were obtained by qualify clinical staff from 80 selected infertile women attending the fertility clinics at the Komfo Anokye Teaching hospital, Magazine clinic and the Bomso Specialist Hospital in Kumasi metropolis between January 2005 to December 2008. Endometrial biopsies were similarly obtained from ten fertile women and used as a control group. These were women who had regular menstrual cycles of between 25 and 29 days with no evidence of menstrual disorders, had not used hormonal contraceptives or intrauterine contraceptive device for at least four months and have had at least one successful pregnancy and had no evidence of pathology associated with their reproductive tract.

All biopsies were timed with reference to the last menstrual cycle. Using a Sharman's curette (Down's Surgical Limited, Sheffield, UK), a single biopsy tissue sample was taken from the fundus of the body of the uterus of each subject between days 18-22 of the menstrual cycle. The samples were then fixed immediately in 10% formalin (Sigma Chemicals Company, UK) and sent to the laboratory for processing. Socio-demographic characteristic of the participant including age, type of infertility, clinical diagnosis, last menstrual period and hormonal therapeutic histories were retrieved from their medical records for subsequent correlative analysis with informed patient consent. The entire protocol for the work was approved by the ethics committee of the School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi.

Endometrial dating

All the biopsies were chronologically dated in relation to the last menstrual period (LMP). All the biopsies were processed using the wax embedding techniques and stained with haematoxylin and eosin (H and E) for light microscopic examination. Detailed examination was performed on each of the biopsies using Carl Ziess standard research microscope (Carl Ziess Inc) so as to date it histologically using the combined traditional dating criteria (Noyes *et al*., 1950) and Li's appraisal (1988). The two day dating was done and expressed as day X ± 1. It is a 2 day reading system for dating the luteal phase of the menstrual cycle based on the cyclic variation in the sequential development of human endometrium in response to the changing levels of oestrogen and progesterone. The following features were used for the dating; a) shape of glands, b) pseudostratification of epithelial cell nuclei and c) the presence and position of vacuoles which were either sub-nuclear vacuolations or supra-nuclear vacuolation. d) The presence of luminal secretions and gland mitosis were also considered. e) In the stroma the presence and amount of stromal oedema, stromal mitosis, pseudodecidual reactions and infiltration of the stroma by leucocytes were also considered. An out of phase biopsy was defined as 2-day lag between the chronological date and the histological date.

Staistical analysis

All categorical variables were analyzed using Chi- Square analysis. In all statistical tests, a value of $P<0.05$ was considered significant. All analysis was performed using Sigma Plot for Windows, Version 11.0, (Systat Software, Erkrath, Germany; www.systat.com).

RESULTS

Generally, the number of women presenting with infertility increases with age as shown in figure 1. From 13 (16.25%) women at age 20-25 years, the number rose to 24 (30.0%) women at 26-30 and then peaked at 31-35 years with 35 (43.75%) women before decreasing to 8 women among the 36-40 years women as shown in figure 1.

Type of infertility and Age

Figure 2 represents age distribution of primary and secondary infertility among the study group. Among the 20-25 years group, 7 (53.8%) presented with primary infertility compared with 6 (46.2%) who presented with secondary infertility. Among the 26-30 year group, the number of secondary infertile women rose to 21 (87.5%) while primary infertility decreased to 3 (12.5%). Whereas there is general decrease in the number of women with primary infertility

with age, there appear to be a normal distribution among the women with secondary infertility.

Maternal age and endometrial development

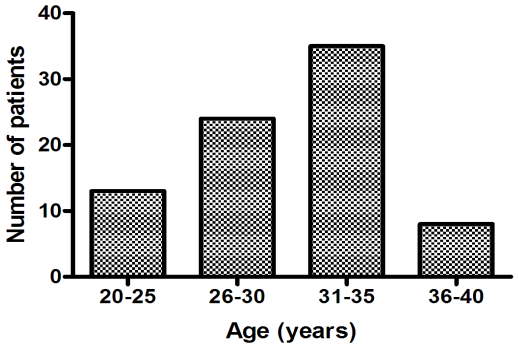

Figure 1: Age distribution of the infertile subjects

Among the 27 women who were in the 20-29 age group, 19 (70.40%) of them showed in-phase endometrial development and 8 (29.60%) out-of phase endometrial development. Similarly, those in the 30-40 year group, 33 (62.30%) were in-phase while 20 (37.70%) had their biopsies showing out-of phase (Table 1). When the difference between the two age groups was analyzed, there were no significant difference between the groups (P=0.4723, χ^2 = 0.5167, OR = 1.439, 95% CI = 0.53 to 3.90).

DISCUSSION

From this study, when the studied population was stratified based on infertility and age, about 70.0% of those who had secondary infertility cases where within the 26-35 years age group. Also, 30.0% and 43.7% of the infertile women were within the 26-30 and 31-35 age group respectively These figures are relatively higher than the earlier report by Menken *et al.*, (1986) in which they indicated a percentage of 9.0% at age 25–29 and 15.0% at age 30–34. The difference could be attributed to the fact that whilst we use infertile subjects, their study was a population base study.

It has been documented that female fecundity is at its peak within 26-35 years and infertility becomes more pronounced after the age of 35 (Gindoff *et al.*, 1986; van Noord-Zaadstra *et al.*, 1991). Thus, for the bulk of the women in this study seeking to resolve their secondary infertility to fall in this optimum fecund age group and voluntarily seek medical attention suggests that this knowledge may not only be scientifically known but it may also be a common knowledge in society as to when women are best suited to have children. Hence the anxieties of these women in the study group to voluntarily seek help more than the others in the other age group because they think their biological clocks are 'ticking away past their prime'. This thought is corroborated by the findings of the Ghana demographic survey, (2003) which indicates that the percentage of age at first birth occurring at age 18 or less had fallen from 25% among the oldest cohort of

Table 1: Incidences of LPD and Maternal Age

Age	In-phase	Out-of-phase
20-29	19 (70.40%)	8 (29.60%)
30-40	33 (62.30%)	20 (37.70%)

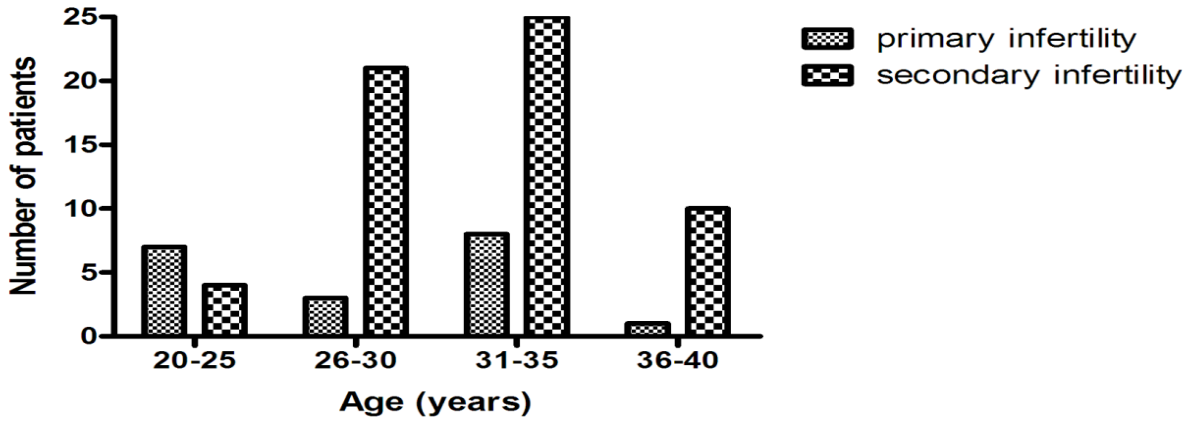

Figure 2: Distribution of primary and secondary infertility in the different age groups

women age 45-49 to 15% among the youngest cohort aged 20-24. The reports associate the reduction in the percentage of women giving birth early to the fact that more young women are postponing marriage and using contraceptives at an earlier age to pursue education or careers. It is thus reasonable to assume that when these life ambitions are fulfilled in the young women and they have settled down expecting to have babies readily they may rush to fertility clinics after a few years of trying. As a result of the above evidence it is important for infertility care givers to carefully counsel patients regarding family planning issues, especially with regards to advancing age and diminishing pregnancy rates. Patients who are in their early to mid-thirties or beyond who are considering pregnancy or have been trying for any length of time without success warrant an early referral for infertility evaluation.

Stratification of the studied population based on age and LPD indicates that about 66.2% (53/80) of the study population where within the 30-40 age group whilst only 33.8% (27/80) were aged 20-29 year (Fig 2). The incidence of LPD among the two age groups did not show any significant difference, giving an indication that age may play a less significant role on the incidence of endometrial retardation in pre-perimenopausal women. Research has shown that the process of aging in humans especially females affects all biological systems and that these changes become apparent at different ages in different systems of the human body and become more obvious when the system is required to function to its maximum potential (Seibel, 1997). The reproductive capacity of the human couple is limited in time by progressive, age -dependent subfertility and eventually by menopause, which imposes absolute sterility (Gindoff *et al*., 1986). It was therefore hypothesized that as the person is ageing there would be a gradual decrease in the response of the endometrium to steroid hormones but this was not the case as the results of the present study has shown. The result of the present study however, is in conformity with others (Lenton *et al*., 1984b; Noci *et al*., 1995; van Noord-Zaadstra *et al*., 1991). These previous studies also suggested that age does not appear to have a significant effect on the morphology or histological responses of the endometrium to steroid stimulation. The endometrial secretory function and endometrial development appear unaffected significantly among the subjects in the present study and thus arguing on the surface against an increased rate of luteal phase defect in cycling older women.

On the other hand, the lack of significant age-related difference is in contrast to the work of Meldrum, (1993) and Sterzik *et al.,* (1988) which suggested that the development of the endometrium is frequently abnormal in older women implying that the endometrial receptivity to implantation may also deteriorate with advancing age. They attributed this reduced receptivity to deficient progesterone secretion by the corpus luteum or the inability of the endometrium to respond to progesterone stimulation. However, in protocols used for oocyte retrieval, the endometrium is exposed to very high and perhaps deleterious hormonal levels, which may explain the difference in results found in stimulated as in Sterzik *et al.,* (1988) and spontaneous cycles such as the one evaluated in the present study. Also, Sterzik *et al.,* (1988) obtained the endometrial biopsies on day 2 after HCG induced ovulation in infertile women and not at the time of implantation, as was done in the present study.

CONCLUSION

Infertility was generally on the increased with advancing age peaking at the age of 30-35 years with secondary infertility being higher among the study population. This study thus confirm the earlier findings which suggest that age and infertility among Ghanaian women is an increasing problem due to general societal trends for women to delay childbearing until later ages. Though prevalence of luteal phase defect among the study subjects was relatively high, the findings show that age was not a predictive factor for luteal phase defect indicating that maternal age may not be associated with increase endometrial retardation.

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ORIGINAL ARTICLE

The impact of seminal zinc and fructose concentration on human sperm characteristic

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This study assessed the association between the estimated fructose and zinc concentration and various seminal characteristics. The study participants include 90 male subjects visiting the Komfo Anokye Teaching Hospital between January and July, 2010 for semen analysis as part of routine fertility investigations prior to treatment. Seminal fructose concentration was significantly lower when the normozoospermic group was compared to the oligozoospermic group ($P < .0001$) and in the normozoospermic group compared to the azoospermic group ($P = 0.0096$). A comparison between the oligospermic group and the azoospermic group gave no statistically significant difference. Fructose correlated positively with volume ($r = 0.36$, $P < 0.0001$) and head defect ($r = 0.07$, $P > 0.05$) and negatively with count ($r = -0.21$, $P < 0.05$). Zinc correlated negatively with volume ($r = -0.09$) and head defect ($r = -0.20$) and positively with motility ($r = 0.18$), count ($r = 0.15$) and tail defect ($r = 0.11$). Seminal fructose and zinc concentrations correlated negatively ($r = -0.26$, $P < 0.05$). The role of seminal fructose concentration does not only lie in the assessment of seminal vesicle dysfunction but in conjunction with other seminal properties could give a useful indication of male reproductive function whilst seminal zinc concentration might not be most appropriate for the assessment of male reproductive dysfunction.
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Keywords*:* Spermatozoa, carbohydrate, infertility, Ghana

INTRODUCTION

The total number of spermatozoa, the total fluid volume contributed by the various accessory glands, the nature of the spermatozoa (i.e. viability, motility and morphology) and the composition of seminal fluid are important for sperm function (Weiske, 1994; WHO, 2010). They have thus been established as good indicators of human male fertility. An understanding of the factors affecting these characteristics is critical to proper understanding of the mechanisms underlying male infertility (Lewis-Jones *et al.*, 1996; WHO, 2010). Knowledge about the impact of seminal zinc and fructose concentration on sperm characteristics is inconsistent and scanty (Lewis-Jones *et al.*, 1996).

Zinc plays an important role in normal testicular development, spermatogenesis and sperm motility (Lin and Cheng, 1996; Wong *et al.*, 2001). It is a cofactor for a number of metalloenzymes in man. Deficiency of zinc in the reproductive system causes hypogonadism and gonadal hypofunction (Sandstead *et al.*, 1967). Kvist, (1980) and Steven *et al.,* (1982) have reported that zinc in seminal plasma is involved in nuclear chromatin decondensation and acrosin activity. Zinc deficiency in the nucleus may destabilize the quaternary structure of chromatin, a feature important for the fertilizing capacity

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of the spermatozoa. Kvist *et al.,* (1987) and Prasad (1991) conducted an experiment in adult males which revealed that Leydig cell synthesis of testosterone was dependent on adequate dietary zinc. It has also been suggested that zinc is necessary for the conversion of testosterone into its biologically active form 5α-dihydrotestosterone (DHT) via the role of 5α-reductase enzyme (Netter *et al*., 1981) and that depletion of dietary zinc decreases semen volume and serum testosterone levels (Hunt *et al*., 1992). Zinc content in seminal plasma is predominantly secreted by the prostate gland and may reflect prostatic function. However, the association between zinc contents in seminal plasma and other spermatic parameters in both fertile and infertile men is yet to be firmly established.

Apart from zinc, another factor which is essential for spermatozoa metabolism and motility is fructose which serves as an energy source for spermatozoa. It is produced in humans mainly by the seminal vesicles with some contribution from the ampulla of the ductus deferens (Schoenfeld *et al*., 1979). Absence of fructose in patients with low volume ejaculate is indicative of ejaculatory duct obstruction, seminal vesicle dysfunction or hypoplasia (Aumuller and Riva, 1992).

The differences in opinion concerning the concentrations of fructose and zinc in seminal plasma justify further studies into their dynamics *vis a vis* male infertility. The purpose of this study was therefore to determine the association between the estimated fructose and zinc concentration and various seminal characteristics in men referred for semen analysis as part of routine fertility investigations.

MATERIALS AND METHODS

Sample preparation

Zinc and fructose levels in seminal plasma of 90 Ghanaian males (age range 27 – 47 years) referred for semen analysis at Komfo Anokye Teaching Hospital (KATH), Kumasi Ghana, between January and June, 2010, as part of their routine fertility investigations prior to treatment, were used for the study. Semen samples were collected in wide mouthed sterile plastic containers by masturbation after a minimum of 4 days sexual abstinence. Semen analysis (volume, pH, sperm concentration, total count, motility and morphology) of all the samples was performed after liquefaction according to the World Health Organization criteria (1992). On the basis of the assessed parameters, sperm concentration and sperm motility were considered as the important parameters. The study group was assigned into three (3) cohorts based on normal ejaculate (40 million spermatozoa per ejaculate) (normozoospermia), sperm concentration < 20 million mL^{-1} (oligozoospermia) and complete absence of spermatozoa in the ejaculate (azoospermia).

Zinc and fructose assessment

After the semen analysis, samples were centrifuged at 1000 x *g* for 5 min and zinc and fructose concentrations assayed from the supernatant (i.e. seminal plasma). Zinc concentration was assessed using atomic absorption spectrophotometry (Mann, 1964). Fructose content in seminal plasma was determined by the resorcinol method where fructose reacts with resorcinol in concentrated hydrochloric acid (HCl) solution to form a red compound measured at a wavelength of 490 nm against blanks (Mann, 1964).

Statistical analysis

Mean values were compared by the student t-test (unpaired). Categorical variables were compared using the chi-square analysis and correlation determined with the Pearson's correlation coefficient test statistic. All statistical analysis were performed using GraphPad prism version 5.00 for windows (GraphPad software, San Diego California USA, www.graphpad.com).

RESULTS

General seminal properties

The age and general seminal properties of the study population stratified into normozoospermic, oligozoospermic and azoospermic populations are shown in Table 1. Forty one percent (41.1%) of the population were normozoospermic, 51.1% were oligozoospermic and 7.8% azoospermic. No significant difference was observed when the mean liquefaction time in males with normozoospermia (55.50 ± 4.41 min) was compared to males with oligozoospermia (51.52 ± 3.69 min). However, a comparison of the mean liquefaction times in both males with normozoospermia and oligozoospermia to males with azoospermia (19.50 ± 4.50 min) showed a significant difference ($P < 0.001$).

About 45.7% of the males with oligozoospermia had seminal pH less than 7.2 compared to 21.6% of males with normozoospermia ($P < 0.05$). The mean count (60.41 ± 5.72 millions mL^{-1}) and the mean motility (67.08 ± 4.13%) in males with normozoospermia were significantly higher than that in males with oligozoospermia (6.36 ± 0.65 millions mL^{-1} and 31.76 ± 2.71% respectively) ($P < 0.0001$). About 21.7% of males with oligozoospermia had seminal head defects compared to 5.4% in males with normozoospermia ($P < 0.05$) but no significant difference in tail defect was observed when

Table 1 - General characteristics of the study population stratified by count

Variables	Normospermic 37 (41.1%)	Oligospermic 46 (51.1%)	Azoospermic 7 (7.8%)
Age (years)	36.57 ± 1.30	37.46 ± 0.98	36.43 ± 1.81
Abstinence (days)	8.35 ± 1.30	6.67 ± 0.62	13.14 ± 4.72‡‡
Liquefaction (min)	55.50 ± 4.41	51.52 ± 3.69	19.50 ± 4.50##‡‡
Motility (%)	67.08 ± 4.13	31.76 ± 2.71***	0.00 ± 0.00
asthenozoospermia (< 50%)	7 (18.9)	32 (69.6)***	0 (0.0)
normal (≥ 50%)	30 (81.1)	14 (30.4)	0 (0.0)
Volume (ml)	2.99 ± 0.22	3.37 ± 0.24	2.96 ± 0.36
less than 2.0 ml	7 (18.9)	7 (15.2)	1 (14.3)
Count (millions ml^{-1})	60.41 ± 5.72	6.36 ± 0.65***	0.00 ± 0.00
Total count (per ejaculate)	190.60 ± 27.32	20.23 ± 2.62***	0.00 ± 0.00
pH	7.61 ± 0.08	7.53 ± 0.09	7.50 ± 0.27
less than 7.2	8 (21.6)	21 (45.7)*	4 (57.1)
> 7.8	9 (24.3)	15 (32.6)	2 (28.6)
Haematospermia (per HPF)	0	3	0
Pyospermia (per HPF)	5	4	3
Head defect	1 (5.4)	10 (21.7)*	0 (0.0)
Tail defect	35 (94.6)	43 (93.5)	0 (0.0)

Results are presented as mean ± SD. *p < 0.05, **p < 0.001, ***p < 0.0001 defines the level of significance when normospermic was compared to oligospermic (unpaired t-test); #p < 0.05, ##p < 0.001, ###p < 0.0001 defines the level of significance when normospermic was compared to azoospermic (unpaired t-test); ‡p < 0.05, ‡‡p < 0.001, ‡‡‡p < 0.0001 defines the level of significance when oligospermic was compared to azoospermic (unpaired t-test). Categorical variables were compared with Chi-square test statistic.

the proportion in males with normozoospermia was compared to that of males with oligozoospermia.

Zinc concentration and seminal parameters

Even though the mean seminal zinc concentration in males with normozoospermia (101.20 ± 2.24 mg dL^{-1}) was slightly higher than that in males with oligozoospermia (97.75 ± 2.17 mg dL^{-1}) and azoospermia (94.62 ± 6.74 mg dL^{-1}) these differences did not reach a significant level (Figure 1B). Seminal zinc concentration correlated positively with motility (r = 0.18), count (r = 0.15), total count (r = 0.16) and tail defect (r = 0.11) and negatively with head defect (r = -0.20) without any statistical significance (Table 2).

Fructose concentration and seminal parameters

The mean fructose concentration in males with normozoospermia was significantly lower compared to males with oligozoospermia (P < 0.0001) and males with azoospermia (P = 0.0096). There was however no statistically significant difference in the mean fructose concentration in males with oligozoospermia compared to males with azoospermia (Figure 1A). Fructose showed a significant negative correlation (r = -0.21) with count. Negative correlations were also observed with motility (r = -0.04), total count (r = -0.05) and tail defect (r = -0.18) with no statistical significance. A positively non-significant correlation was observed between fructose and head defect (Table 2). Seminal fructose concentration correlated negatively, in a statistically significant way, with zinc concentration (r = -0.26) (Table 2).

DISCUSSION

The WHO criteria for normal semen includes a volume of 2.0 ml or more, sperm concentration of 20 million mL^{-1} or more, sperm motility of 50% or more with forward movement and sperm morphology of 30% or more of normal forms (World Health Organization, 1992). The mean seminal volume of the three study groups classified by count was higher than the documented 2.0 mL and is in agreement with other study

(Promdee and Pongsritasana, 2005). This could be due to the fact that in using at least one abnormal parameter to classify the samples, other parameters e.g. volume could be normal. This study observed positive but not significant correlations between zinc content of seminal plasma and motility, total count and sperm concentration. Stanwell-Smith (Stanwell-Smith *et al.*, 1983), found a positive correlation between sperm concentration and zinc level in fertile men but not in infertile men and (Wong *et al.*, 2000) reported increased proportion of spermatozoa with progressive motility after oral zinc supplementation which corroborates very well with the correlation results observed in this study. The relationship between seminal zinc content and acrosin and nuclear chromatin activity

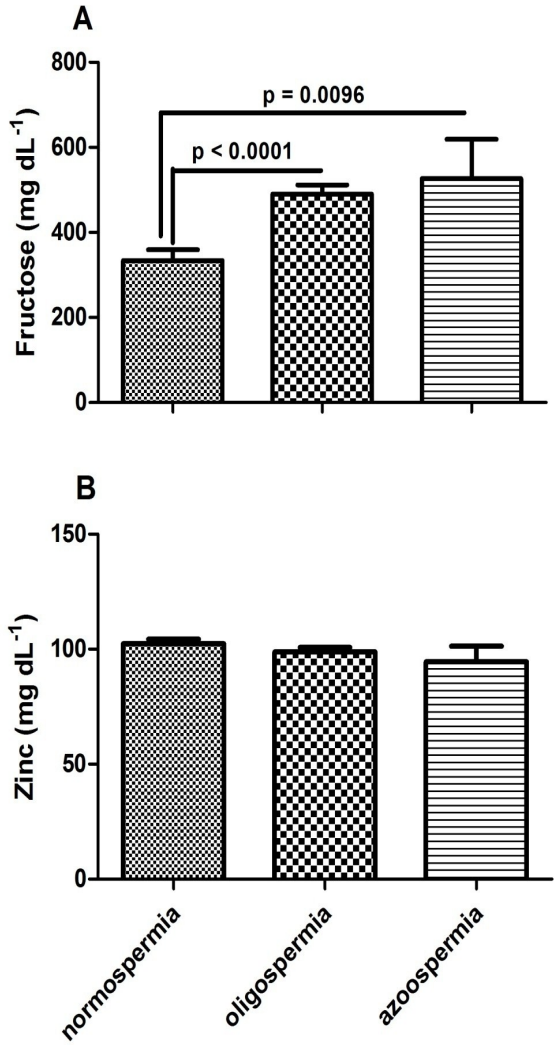

Figure 1 - Seminal fructose (A) and zinc (B) concentrations in the three study categories

Table 2: Pearson product correlation coefficient among the study variables

Variables	AB	Motility	Volume	Count	TC	Zinc	pH	LQ	Immotile	HD	TD	Fructose
Age (years)	0.07	-0.29*	-0.07	-0.06	-0.09	-0.08	0.11	0.05	0.19	0.22*	-0.04	-0.05
AB (days)		-0.20*	0.17	0.08	0.16	0.06	0.07	0.09	-0.07	0.09	-0.23*	0.01
Motility (%)			0.01	0.66***	0.56***	0.18	-0.02	0.05	-0.54***	-0.23*	0.47***	-0.04
Volume (ml)				-0.01	0.27*	-0.09	0.1	-0.03	0.01	0.12	-0.05	0.36***
Count (mil mL-1)					0.89***	0.15	0.06	0.03	-0.41***	-0.12	0.18	-0.21*
TC (ejaculate-1)						0.16	0.12	-0.01	-0.36**	-0.02	0.07	-0.05
Zinc (mg dL-1)							-0.07	0.06	-0.05	-0.2	0.11	-0.26*
pH								0.40***	0	0.11	-0.22*	0.13
LQ (min)									0.05	-0.04	0.03	0.13
Immotile (%)										0.24*	0.18	-0.07
HD											-0.33**	0.07
TD												-0.18

****Correlation is significant at 0.05 level (2-tailed), **Correlation is significant at 0.001 level (2-tailed) and ***Correlation is significant at 0.0001 level (2-tailed). TC = Total count, LQ* = Liquefaction, AB = Abstinence, HD = Head Defect, TD = Tail Defect**

as related in the studies of Kvist (1980) and Steven *et al.,* (1982) was clearly observed in the negative correlation between seminal zinc content and seminal head defect observed in this study. Zinc deficiency might therefore affect acrosin and chromatin activity thereby leading to increased head defects which further reduces the fertilizing capacity of spermatozoa. Wong *et al.,* (2001) demonstrated that zinc content in fertile men were not different from those of infertile men. Abou-Shakra *et al.,* (1989) reported that zinc content in men grouped by sperm concentration was not different from each other. Zinc content in the three study categories grouped by sperm concentration did not show any significant difference although values in normozoospermic males were higher than that in oligozoospermic males which was also higher than that in azoospermic males. This is in agreement with the work of (Wong *et al*., 2001).

Determination of seminal fructose concentration has been used in the examination of obstructive azoospermia and inflammation of male accessory glands (Carpino *et al*., 1997; Manivannan *et al*., 2005). Inflammation may lead to atrophy of the seminal vesicles and low seminal fructose concentration. When ejaculatory ducts are blocked, fructose concentration in seminal plasma usually decreases and may become undetectable (Coppens, 1997). Additionally, seminal plasma fructose concentration determination is useful for auxiliary diagnosis of obstructive and non-obstructive azoospermia. Seminal fructose concentration in non-obstructive azoospermia is usually higher than or equal to that in males of normal fertility (Buckett & Lewis -Jones, 2002). However, fructose concentration in seminal plasma of patients with obstructive azoospermia is usually absent or significantly lower than that in men of normal fertility (Manivannan *et al*., 2005). Absence of seminal fructose has also been found in patients with congenital vas deferens-seminal vesicle developmental defect (Kise *et al*., 2000; Kumar *et al*., 2005).

Significantly higher seminal fructose concentrations were observed in azoospermic patients in this study compared to that in oligozoospermic and normozoospermic patients which rules out the possibility of obstructive azoospermia or inflammation of the male accessory glands in azoospermic patients in this study as related to the studies of Buckett and Lewis-Jones, (2002), Manivannan *et al.,* (2005) and Coppens, (1997). This finding confirms an abnormality in the normal function of the seminal vesicles other than obstruction or inflammation which may require further investigation. Montagnon *et al.,* (1990) reported that fructose concentration in seminal plasma is one of the most important markers of seminal vesicular function and that when seminal vesicular function is decreased, semen coagulation, sperm motility, stability of sperm chromatin and semen immune-protection are affected. A definite ratio between the fructose level and the number of spermatozoa in the ejaculate has also been documented; as such, an increase in the number of spermatozoa is generally accompanied by significant fall of fructose in the semen (Biswas *et al*., 1978; Rajalakhshmi *et al*., 1989).

Negative correlations were observed between fructose content and sperm concentration, total count and motility. Fructose concentration has been noted to be essential for spermatozoa metabolism and motility as an energy source (Schoenfeld *et al*., 1979). The reduced fructose concentration in normozoospermic males compared to oligozoospermic males in this study could be attributed directly to the significantly higher mean motility and sperm counts in normozoospermic males which would have used up fructose as an energy source. This finding is in line with that of Lu *et al.,* (2007) who reported a significantly positive correlation between motile sperm concentration and decrease in fructose concentration and further demonstrated that motile sperm in vitro could unceasingly consume fructose. Biswas *et al.,* (1978) and Schirren *et al.,* (1979) also reported decreased fructose concentrations with increasing sperm density and motility. A further finding of a negative correlation between fructose concentration and tail defect further buttresses the need for fructose as an energy source for effective motility of spermatozoa as related in the study of Schoenfeld *et al.,* (1979). Fructose showed a negative correlation with seminal head defect showing that fructose in semen has very little to do with the maintenance of the acrosome and nuclear chromatin activities.

A significant inverse relationship was also observed between zinc and fructose concentrations emphasizing the fact that for effective seminal fluid activity, zinc and fructose concentrations should always be inversely related to each other.

CONCLUSIONS

Seminal zinc plasma levels may not be an appropriate indicator of male reproductive dysfunction and the role of seminal fructose concentration does not only lie in the assessment of seminal vesicle dysfunction but in conjunction with other seminal properties could give a useful indication of male reproductive function.

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ORIGINAL ARTICLE

The Anthelmintic Activity of *Vernonia Amygdalina* (Asteraceae) and *Alstonia Boonei* De Wild (Apocynaceae)

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Intestinal worms affect a host of individuals resulting in malnutrition, stunted growth, intellectual retardation and cognitive deficits. The aim of this study is to investigate the antihelminthic activity of *Alstonia boonei* De Wild (Apocynaceae) and *Vernonia amygdalina* (Asteraceae) using earthworms (*Lumbricus terretris*). The worms were directly exposed to 50, 100, and 200 mg/ml of aqueous and ethanolic bark extracts of *Alstonia boonei* and leaf extract of *Vernonia amygdalina* and piperazine citrate in a petri dish and in an organ bath. The control group was exposed to distilled water. The time of paralysis and death were determined within a period of 6 h in the petri dish method whiles spontaneous movements of the worms before and after drug administration were recorded on a slow moving kymograph drum in the organ bath method. All doses of the aqueous and ethanolic extracts significantly ($P \leq 0.001$) reduced the time of paralysis and time of death compared to the vehicle treated group. The time of paralysis and time of death in the tissue bath method corresponded to that obtained by direct exposure. The extracts exhibited anthelmintic activity and thus could be an inexpensive and readily available source of anthelmintic treatment.
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Keywords*:* Piperazine citrate, Time of paralysis, *Lumbricus terretris,* Tannins

INTRODUCTION

Anthelmintics are drugs that act locally to expel worms from the gastro-intestinal tract or systemically to eradicate adult helminths or developmental stages that invade organs and tissues (Devi *et al.,* 2009). These medicines are used in human and other animal populations to destroy parasites that live in the body. According to World Health Organisation statistics, more than two billion people harbour parasitic worm infections (Khurana, 2010). In areas of high prevalence, simultaneous infection with more than one type of helminths is common.

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One of the problems with antihelmintics is that many of them have been used for a long time and over time parasites have developed drug resistance (Sarojini *et al.*, 2011). Most of the existing anthelmintics e.g. levamisole produce side effects such as abdominal pain, loss of appetite, nausea, vomiting, headache and diarrhea (Goodman and Gilman, 2001). Much emphasis has been placed on phytomedicine for some time now due to their outstanding advantages over synthetic drugs. Among these advantages are; least side effects, low cost and least drug resistance. Thus phytomedicine has become a good alternative to synthetic anthelmintics (Pawan, 2009). However, most phytomedicine still depends on "trial and error" basis or based on conventional wisdom. A variety of *in vivo* and *in vitro* methods has been employed to evaluate and validate anthelmintic properties of plant remedies (Pawan, 2009).

Two plants namely *Alstonia boonei* De Wild *(Apocynaceae)* commonly known as Pattern wood or stool wood and *Vernonia amygdalina (Asteraceae)* commonly known as bitter leaf are known to possess anthelmintic activity (Ghana Herbal Pharmacopoeia, 2007). *Alstonia boonei* is distributed throughout the tropics and the rain forest of West and Central Africa (Olajide, 2000). A bark extract is widely used to treat malaria, typhoid fever, gonorrhea, yaws, asthma and dysentery, and is also applied to sores, ulcers, snakebites, rheumatic pain and toothache, and as a galactagogue. A maceration of the bark is taken to treat jaundice, cough and sore throat, and is applied externally to treat skin conditions like eczema, ringworm and acne (Palla, 2005).

Vernonia amygdalina is indigenous to tropical Africa and is found wild or cultivated all over Sub-Saharan Africa (Bosch *et al*., 2005). Bitter leaf, commonly called, is a highly appreciated vegetable in West and Central Africa and can be consumed in various dishes. In traditional medicine; Leaf decoctions are used to treat fever, malaria, diarrhoea, dysentery, hepatitis and cough, as a laxative and as a fertility inducer. It is also used as a medicine for scabies, headache and stomach-ache. Root extracts are also used as treatment against malaria and gastrointestinal disorders. One of the most common medicinal uses of *V. amygdalina* is as a treatment against intestinal worms including nematode (Fomum, 2004). Leaf and root barks extracts showed antimalarial activity against drug-sensitive *plasmodium berghei* (Abosi and Raseroka, 2003).

Development of resistance to most of the commercially available anthelmintics became a severe problem worldwide (Waller *et al*., 2004). Moreover, these drugs are unaffordable to the resource-poor individuals in developing countries, inaccessible or inadequately available as the majority of the population lives in the rural areas where these medicinal plants are readily available (Hammond, *et al*., 1997). These factors paved the way for herbal remedies as alternative anthelmintics (Fajmi, *et al.,* 2005).

Although some work has been done on the antihelmintic activity of *Vernonia amygdalina* and *Alstonia boonei* most of them were *in vivo* (Siamba *et al*., 2007; Alawa *et al*., 2010). Therefore to verify further the antihelmintic activity of these plants and instill more confidence in their use as antihelminthics, this *in vitro* protocol is being used on the common earthworm (Lumbricus terrestris).

The physiological resemblance of earthworms (*Lumbricus terrestris*) to intestinal roundworm, *Ascaris lumbricoides* (Nirma *et al*, 2007; Ashok, 2010) make this model suitable for antihelmintics studies in humans. The aim of the study therefore is to investigate the antihelminthic activity of *A. boonei* De Wild (Apocynaceae) and *V. amygdalina* (Asteraceae) using earthworms (*Lumbricus terrestris*) to affirm their use as alternatives to anthelmintic therapy.

MATERIALS AND METHODS

Plant collection

The bark of *Alstonia boonei* and the leaves of *Vernonia amygdalina* were collected from the botanical gardens of the Kwame Nkrumah University of Science and Technology (KNUST), and authenticated at the Department of Pharmacognosy, KNUST, and dried for extraction.

Preparation of Plant Extracts

Aqueous Extracts

The stem bark of *Alstonia boonei* was washed, sun dried and ground to a coarse powder. A 1,250 g quantity of the powdered drug was put into a glass container with 3000 ml of water. The mixture was boiled for 30 minutes and cooled. Filtration was done and the marc pressed. The filtrate was dried in an oven at 40^{o}C. A solid mass weighing 40 g was obtained (percentage yield: 3.2%). This was labeled aqueous *Alstonei boonei* bark extract (AQ ABE) for use in this study. The same procedure was used in the preparation of the *Vernonia amygdalina* leaf extract. A 625 g quantity of the powdered leaves yielded 100 g of dry residue (percentage yield: 16%) labeled aqueous *Vernonia amygdalina* leaf extract (AQ VLE) for use in this study.

Ethanolic Extracts

A 1000 g quantity of *Alstonia boonei* bark powder was packed into a percolator with its discharge port packed with cotton. Sufficient quantity of 70% ethanol was added to cover the drug and left for about 24 hours. The liquid was drained slowly from the bottom of the percolator (about 20 drops per minute) into a flask. The marc was pressed and this liquid was added to the percolate in the flask which was then concentrated in the Buchi Rotor Evaporator (Rotavapor R-210, Switzerland) and dried in the Gallenkamp hot air oven (Oven 300 plus series, England) at 40^{o}C. A dry mass of 56.8 g was obtained (representing a yield of 5.68%) and labeled as ethanolic *Alstonei boonei* bark extract (ET ABE) for use in this study. The same procedure was followed in the preparation of the *Vernonia amygdalina* leaf extract in which 300 g of powdered leaves was used. A dry mass of 21.1 g was obtained (representing a yield of 7.03%) and labeled ethanolic *Vernonia amygdalina* leaf extract (ET VLE) for use in this study.

Collection of Worms
Earthworms *(Lumbricus terrestris)* of lengths 6-12 cm were obtained from the damp, cool, and covered area of the gardens of the Faculty of Horticulture (KNUST). The worms were transferred into a glass bottle with some quantity of the soil from which they were taken. The worms were identified and authenticated at the Department of Biological Science, KNUST, Kumasi, Ghana.

Experimental design
Phytochemical screening
Phytochemical screening was conducted on AQ ABE, AQ VLE, ET ABE and ET VLE to ascertain the presence of phytochemicals as described by Wagner and Bladt, (1996) and Harborne, (1998). The tannin content was determined according to the method of Glasl (1983) using pyrogallol (99.5% HPLC) as reference compound.

Gross Motility and Mortality Studies
This method was carried out as described by Ajaiyeoba *et al.,* (2001) and Iqbal *et al.,* (2001) with some modifications. Fifty (50) ml quantities of suspensions (concentrations: 50 mg/ml, 100 mg/ml and 200 mg/ml) prepared from weighed quantities of AQ ABE, AQ VLE, ET ABE and ET VLE were poured into labelled petri-dishes. Five worms were put into each petri-dish and compared with that of piperazine citrate (50, 100, and 200 mg/kg); the reference anthelmintic and the control (distilled water). Time for paralysis and death were determined as described in Table 1. The experiment was terminated after 6 hours.

Tissue-bath Studies
The tissue-bath experiment was designed as described by Goodwin, 1958 with some modifications by researchers. Worms were placed in prepared fine nylon stockings (18 cm long and 0.6-0.8 cm wide) and suspended in a 40 ml capacity glass tube of length 40 cm and width 6 cm containing modified Tyrode solution maintained at 37 ^{o}C in a Havard research apparatus (Harvard Apparatus Ltd, Kent, UK). The open end of the stockings was closed behind the worm with the use of a rubber band. The band was attached to a sinker made from a piece of heavy metal. The closed anterior end was tied to a piece of thread (which lowered the worm into the tube containing Tyrode) and attached to the frontal writing lever of Havard apparatus. Oxygen was bubbled through the tyrode solution. With sufficient counterweight on the lever, the worm was kept upright in the tube. Enough time was allowed for spontaneous movement of the worm in the bath to be stable (seen as a uniform baseline recording on the Harvard kymograph) before administration of 20 ml of 200 mg/ml of AQ ABE, AQ VLE, ET ABE and ET VLE or 5 ml of 150 mg/ml piperazine citrate (doses were selected from preceding experiment). In the control, the worms were suspended in modified Tyrode solution. Time for paralysis and death (seen as a decrease in spontaneous movement and no movement respectively) of the worm as recorded on a slow moving Harvard kymograph drum was noted. Termination time for the experiment was 3 hours. The procedure was repeated five times for each treatment group.

Table 1: A guide to ascertain paralysis and death of experimental worms

Parameter	Description
Paralysis	Marked decrease in vigorous wriggling movement of the worm indicates paralysis
	If the worm revived in physiological solution (Tyrode)
Death	Evoked pin prick response: Slow movement of the worm after being pricked with pin indicates paralysis. No movement indicates death.
	Confirmed by dipping the worm in warm water at 50 ^{o}C and shaken vigorously. No response indicates death

Statistical Analysis
Data is presented as mean ± SD (N=5). Analysis of the effects between doses in treatment groups and the control and between the aqueous and ethanolic extracts was conducted by two-way ANOVA followed by Bonferroni's post hoc test. GraphPad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses. $P \leq 0.05$ was considered statistically significant in all analysis.

RESULTS

Preliminary Phytochemical Screening
Results from phytochemical screening indicate the presences of alkaloids, tannins, and glycosides in all extracts (Table 2).

Antihelmintic Activity
After 6 hours in the petri-dish, worms in the control group were still active (no paralysis); there were no deaths. Piperazine citrate and all the extracts however caused paralysis in the worms between 3.68 ± 0.90 to 59.9 ± 8.3 minutes of exposure which were very signifi-

cant ($P \leq 0.001$) compared to the control (Table 3). The paralyzing effect was dose-dependent. Again, piperazine citrate and the extracts dose-dependently and very significantly ($P \leq 0.001$) caused death in the worms between 17.4 ± 2.3 and 158.4 ± 16.4 minutes in comparison to the control (Table 4).

Table 2: Results from phytochemical screening of the aqueous and ethanolic extracts of *A. boonei* De Wild (Apocynaceae) and *V. amygdalina* (Asteraceae)

Constituent	AQ ABE	AQ VLE	ET ABE	ET VLE
Alkaloids	+	+	+	+
Tannins	+	+	+	+
Flavonoids	-	+	-	+
Glycosides	+	+	+	+
Steroids	-	-	-	-

While worms in the control group saw no paralysis or death when suspended in the tissue bath, again piperazine citrate and all the extracts significantly caused paralysis of the worms between 8.73 ± 2.38 to 16.52 ± 4.63 minutes of exposure and death between 21.5 ± 3.10 to 27.8 ± 8.62 minutes of exposure (Table 5).

DISCUSSION

In this study, anthelmintic assay was performed on adult earthworms (*Lumbricus terrestris*) due to its physiological resemblance with the intestinal roundworm parasite of human beings (Thorn *et al.,* 1977; Vigar, 1984). The experimental results indicate that the extracts of *Vernonia amygdalina* and *Alstonia boonei* have potent anthelmintic activity (the short duration of action is worth noting).

From phytochemical screening all the extracts have alkaloids, tannins, and glycosides which have been associated with antihelminthic activity (Sarojini *et al.,* 2011). Alkaloids in the aerial parts of *Cissampelos capensis* (Menispermaceae) and *Macleaya microcarpa* (Maxim) Fedde are known to be responsible for their anthelminthic activity (Ayers *et al.,* 2007; Wang *et al.,* 2010). Tannins are known to produce anthelmintic activity by binding to glycoprotein on the cuticle of the parasite. They hinder energy production in helminth parasites by uncoupling

Table 3: The time (min) taken for paralysis of the earthworms (*Lumbricus terrestris*) on exposure to AQ ABE, AQ VLE, ET ABE, ET VLE and piperazine citrate at doses of 50, 100, and 200 mg/kg in a petri-dish

Dose (mg/ml)	Control	AQ ABE	AQ VLE	ET ABE	ET VLE	Piperazine citrate
Vehicle	$> 360 \pm 0.00$					
50		43.50 ± 7.67 ***	59.94 ± 8.25 ***	34.89 ± 2.48 ***	33.18 ± 12.41 ***	9.62 ± 1.32***
100		28.22 ± 2.63 ***	9.61 ± 2.10 ***	10.03 ± 0.86 ***	8.72 ± 2.99 ***	5.75 ± 1.66 ***
200		12.23 ± 0.67 ***	4.05 ± 1.06 ***	4.78 ± 0.39***	3.56 ± 0.37 ***	3.68 ± 0.90 ***

Values are means ± SD. (N=5). *** $P \leq 0.001$; compared to control group (Two-way ANOVA followed by Bonferroni's *post hoc* test).

Table 4: The time (min) taken for death of the earthworms (*Lumbricus terrestris*) on exposure to AQ ABE, AQ VLE, ET ABE, ET VLE and piperazine citrate at doses of 50, 100, and 200 mg/kg in a petri-dish

Dose (mg/ml)	Control	AQ ABE	AQ VLE	ET ABE	ET VLE	Piperazine citrate
Vehicle	> 360 ± 0.00					
50		158.4 ± 16.42 ***	76.65 ± 12.73 ***	120.5 ± 4.3 ***	37.46 ± 13.55 ***	37.72 ± 6.2***
100		39.22 ± 4.70 ***	19.64 ± 5.27 ***	17.47 ± 1.96 ***	11.82 ± 3.45 ***	22.82 ± 4.36 ***
200		21.77 ± 2.08 ***	8.14 ± 2.15 ***	9.0 ± 0.59***	4.48 ± 0.39 ***	17.37 ± 2.27 ***

Values are means ± SD. (N=5). *** $P \leq 0.001$; compared to control group (Two-way ANOVA followed by Bonferroni's *post hoc* test).

Table 5: The time (min) taken for paralysis, and death of the earthworms (*Lumbricus terrestris*) on exposure to AQ ABE, AQ VLE, ET ABE, ET VLE and piperazine citrate at a dose of 100 mg/kg in the tissue bath.

Treatment groups	Time for Paralysis (min)	Time for Death (min)
Control	> 360 ± 0.00	> 360 ± 0.00
AQ ABE	16.52 ± 4.63 ***	27.8 ± 8.62 ***
AQ VLE	14.18 ± 2.84 ***	25.16 ± 6.09 ***
ET ABE	15.88 ± 4.47 ***	21.4 ± 5.00 ***
ET VLE	13.91 ± 2.92 ***	24.32 ± 4.88 ***
Piperazine citrate	8.73 ± 2.38 ***	21.5 ± 3.10 ***

Values are means ± SD. (N=5). *** $P \leq 0.001$; compared to control group (Two-way ANOVA followed by Bonferroni's *post hoc* test). Termination time for the experiment was 6 hours.

oxidative phosphorylation (Martin, 1997).

Piperazine a known anthelmintic is GABA mimetic. By increasing chloride ion conductance of worm muscle membrane, it produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis (Martin, 1985; Sutar *et al.,* 2010). Comparing anthelmintic activity of *Vernonia amygdalina* and *Alstonia boonei* to piperazine, the extracts may contain constituents that could probably have weak GABA-mimetic effect similar to piperazine citrate.

CONCLUSIONS

Antihelmintic effects of the extracts could ease the economic burden on antihelmintic therapy. The bark extract of *Alstonia boonei* and the leaf extract of *Vernonia amygdalina* have antihelmintic activity. These findings may partly explain some of the folklore use of these plants in the treatment of worm infestations.

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ORIGINAL ARTICLE

Oxidative Stress among Ghanaian Patients presenting with Chronic Kidney Disease

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Dyslipidaemia and lipid peroxidation are known risk factors for chronic kidney disease (CKD). This study assessed the lipid profile and oxidative stress/lipid peroxidation in CKD patient, using the oxidative stress marker, Malondialdehyde (MDA) and antioxidants; Vitamins A and C, Catalase and Uric Acid. The study population included 146 individuals with mean age 50.18 ± 1.14 with various CKD, s and who were undialysed. Another 80 healthy subjects without kidney pathology but of similar age and sex distribution were used as controls. With the exception of HDL-C, which showed no significant difference when CKD patients were compared with controls (1.35±0.0 5 vs 1.61±0.20, p= 0.2114, total cholesterol (TC) (4.54±0.13 vs 5.63±0.13, p=0.0274), low density lipoprotein cholesterol (LDL-C) (106.30 ± 4.00 vs 126.30± 5.57, p=0.0134), and triglycerides (TG) (1.52± 0.08 vs 1.84±0.09, p=0.0086) increased significantly in the CKD patients. Serum MDA increased significantly (1.22 ± 0.10 vs 2.66 ± 0.07, p=0.0001) in the CKD patients as compared to the controls and increased with the severity of the condition. Vitamin A (9.76±3.03 vs 16.1±5.21, p=0.0012), Catalase (57.49±1.18 vs 71.98±2.91, p=0.0001) and Uric Acid (266.68±11.00 vs 333.90±10.02, p=<0.0001) increased significantly in the CKD subjects compared to controls, whilst vitamin C (0.54±0.02 vs. 0.34±0.05, p=0.0001) decreased significantly among the CKD subjects. Dyslipidaemia and increased oxidative stress with abnormal antioxidant levels are common in CKD patients. Therapeutic regimens aimed at strengthening the antioxidant defenses besides normalizing lipid concentrations would protect CKD patients against oxidative stress and any related complications.
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Keywords*:* Chronic kidney disease; dyslipidaemia; lipid peroxidation; MDA; catalase

INTRODUCTION

The prevalence of chronic kidney disease (CKD) has reached epidemic proportions globally. (Coresh *et al.*, 2007; Di Angelantonio *et al.*, 2007; Flessner *et al.*, 2009). Nationally representative samples from USA and Taiwan report a prevalence of 12-13%, (Wen *et al.*, 2008) though most of these individuals may not be aware of their condition. In Ghana, data on the prevalence of CKD has been varied over the years. Bamgboye in 2006 put the prevalence at 1.6% per million people (Bamgboye, 2006). However, Addo *et al.,* (2009) put the prevalence among Ghanaian hypertensives at 4%. In a recent publication, Osafo et al., (2011) put the prevalence at 46.9% among hypertensives in Ghana.

The most severe forms of CKD are characterized by kidney failure and patients need renal replacement therapy (haemodialysis, peritoneal dialysis or renal transplantation); however more patients may be affected by less severe forms of CKD. Together with obesity or smok-

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ing, CKD contributes to around a tenth of all deaths in Taiwan (Wen *et al*., 2008) than any other cause in the general population.

CKD leads to numerous complications as the condition progresses. Notable among them are cardiovascular, cerebrovascular and peripheral vascular diseases. Cardiovascular complications are the main cause of death and are about 20 folds higher in CKD patients than any other cause in general population (Oda and Keane, 1998). These complications are down to several metabolic and endocrinal disturbances among which dyslipidaemia is a regular feature. Abnormalities in lipid profiles are noticed immediately renal function begins to decline (GFR≤60ml/min/1.73m) though the type and severity vary among patients (Oda and Keane, 1998; Wanner, 2000). Similarly, CKD patients are also at risk of increased oxidative stress characterized by low antioxidant systems and an upsurge in prooxidant activity (Locatelli *et al*., 2003). In the course of the process, polyunsaturated fatty acids (PUFA), located in cell membrane are oxidized in vivo to produce aldehydes of different chain length like malondialdehyde (MDA). This product of lipid peroxidation can structurally change DNA, RNA, body protein and other biomolecules (Daschner *et al*., 1996). Lipid abnormalities and enhanced oxidative stress in CKD patients are involved in disease progression and accelerates the process of atherosclerosis resulting in cardiovascular complications. This study, sought to assess the lipid profile and oxidative stress/lipid peroxidation in patients presenting with CKD by determining relevant oxidative stress markers (MDA) and antioxidant levels (vitamins A and C, catalase and uric acid).

MATERIALS AND METHODS

Study area and subjects

This study was carried out at the Komfo Anokye Teaching Hospital (KATH), Kumasi and Tamale Teaching Hospital (TTH) between August 2007 and September 2009. One hundred and forty six (146) patients comprising of eighty (80) females and sixty-six (66) males within age range 14-80 years were signed on as cases. Patients with clinically diagnosed CKD including those yet to start dialysis were randomly selected for the study. Patients on any form of dialysis were excluded from the study. The aetiology of the CKD ranged from diabetic nephropathy, 90(61.6%) patients; chronic glomerulonephritis, 12(8.2%) patients; adult polycystic kidney disease, 1(0.7%) patient; hypertensive nephropathy, 10(6.8%) patients and chronic kidney disease of unknown aetiology, 33(22.6%) patients. Eighty (80) healthy volunteers of similar age and sex distribution were studied as controls. The participation of the respondents who are all indigenes of Ghana was voluntary and informed consent was obtained from each of them. The study was approved by the School of Medical Sciences and KATH Committee on Human Research, Publication and Ethics (CHRPE).

Sample Collection

Venous blood samples were collected after an overnight fast (12 – 14 hours). About 5 ml of venous blood was collected and dispensed into vacutainer® plain tubes. After centrifugation at 500 g for 15 min, the serum was stored at - 80°C until assayed.

Biochemical assays

Serum biochemistry was performed with ATAC® 8000 Random Access Chemistry System (Elan Diagnostics, Smithfield, RI, USA). Parameters that were determined include; blood urea nitrogen (BUN), creatinine (CRT), total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) which was calculated using the Friedwalds formula (Friedewald *et al*., 1972).

The methods adopted by the automated instrument for the determination of the above parameters were predetermined by the reagent manufacturer - JAS™ diagnostics, Inc. (JAS Diagnostics, Inc. Miami Florida, USA). Total cholesterol and HDL-C determination was according to the method described by Trinder (Trinder, 1969). Triglycerides determination employed a modified Trinder method (Trinder, 1969; Barham and Trinder, 1972). Low density lipoprotein cholesterol (LDL-C) determination: LDL-C (mmol/l) was calculated according to Friedwald's formula in accordance to the manufacturer's instructions i.e. LDL-C = TC- TG/2-HDL-C.

Measurement of Oxidative Stress

Parameters measured included; Malondialdehyde (MDA) (µmol/l), Vitamin C (Vit C) (mg/ml), Catalase (CAT) (units/ml) and Vitamin A (Vit A) (µg/ml).

Malondialdehyde (MDA)

Malondialdehyde (MDA) levels were determined by the MDA thiobarbituric acid (TBA) test which is the colorimetric reaction of MDA and TBA in acid solution. MDA, a secondary product of lipid peroxidation, reacts with thiobarbituric acid (TBA) to generate a red-coloured product, which was detected spectrophotometrically at 535 nm. The protocol used in this study was the Kamal *et al.,* (1989) modification of the Shlafer and

Shepard (1984) protocol. The absorbance of the mixture was measured at 535 nm with a spectrophotometer (Biomate 3S UV Visible spectrophotometer, Thermoelectron Inc USA) and the results were expressed as μmol/l, using the extinction coefficient of 1.56×10^5 L $mmol^{-1}$ cm^{-1}.

Vitamin C
Vitamin C was determined by the method of Omaye *et al.,* (1979) at most 3 hours after sample collection. Ascorbic acid in plasma is oxidized by Cu (II) to form dehydroascorbic acid, which reacts with acidic 2,4- dinitrophenylhydrazine to form a red dihydrazone which is measured at 520 nm with a spectrophotometer.

Catalase
Catalase was assayed by the method of Takahara *et al.*, (1960). To 1.2 ml of 50 mM phosphate buffer (pH 7.0), 0.2 ml of plasma was added and the enzyme reaction was started by the addition of 1.0 ml of 30 mM H_2O_2 solution. The decrease in absorbance was measured at 240 nm at 30 second intervals for 3 minutes with an ultra violet-visible spectrophotometer. The enzyme blank was run simultaneously with 1.0 ml of distilled water instead of hydrogen peroxide. The enzyme activity was expressed as units/ml.

Vitamin A
Plasma- retinol (vitamin A) was determined by reverse phase high performance liquid chromatography (HPLC) (Zaman *et al.*, 1993).

Proteinuria
Early morning urine was collected in plastic containers from the respondents and urine protein was determined using the dip-stick qualitative method (CYBOW™ DFI Co Ltd, Gimhae-City, Republic of Korea).

Anthropometric variables
Anthropometric measurements included height to the nearest centimetre without shoes and weight to nearest 0.1 kg in light clothing. Subjects were weighed on a bathroom scale (Zhongshan Camry Electronic Co. Ltd, Guangdong, China) and their height measured with a wall -mounted ruler. BMI was calculated by dividing weight (kg) by height squared (m^2).

Estimation of GFR
The 4-variable Modification of diet in renal disease (4v-MDRD) equation (Levey *et al.*, 1999) was used to estimate the GFR of both subjects and controls using serum creatinine.

$$4v-MDRD = 186 \times SCr^{-1.154} \times age^{-0.203}$$

The equation is multiply by 1.212 if black and 0.742 if female

The GFR results from the equations was used to divide the study population into five categories corresponding with the five stages of CKD in the kidney disease outcome quality initiative (K/DOQI) CKD classification (National Kidney Foundation, 2002).

The staging classified GFR ≥ 90 ml/min/1.73 m^2 as stage 1; 60-89 ml/min/1.73 m^2 as stage 2; 30-59 ml/min/1.73 m^2 as stage 3; 15-29 ml/min/1.73 m^2 as stage 4; and < 15 ml/min/1.73 m^2 as stage 5.

Blood pressure
Blood pressure was measured by trained personnel using a mercury sphygmomanometer and stethoscope. Measurements were taken from the left upper arm after subjects had been sitting for >5 min in accordance with the recommendation of the American Heart Association (Kirkendall *et al.*, 1967). Duplicate measurements were taken with a 5 min interval between measurements and the mean value was recorded to the nearest 2.0 mmHg.

Statistical analysis
The results are expressed as Means ± SEM. Unpaired t-test was used to compare mean values of continuous variables and χ^2 was used to compare discontinuous variables. Correlation was assessed by the Pearson's method. A level of $p<0.05$ was considered as statistically significant. GraphPad Prism version 5.00 for windows was used for the statistical analysis (GraphPad software, San Diego California USA, www.graphpad.com).

RESULTS

Demographic and clinical characteristics of the study population are shown in Table 1. The mean age of the 146 participants involved in this study was 50.18 ± 1.14 ($p=0.0720$) with 45.2% of the participants being males. Apart from estimated GFR (eGFR) and haemoglobin (HGB) which were significantly decreased as compared to the control, lipid fractions (LDL-C, TC, TG) (except HDL-C), fasting blood glucose (FBG), blood pressure (SBP and DBP), creatinine (CRT), blood urea nitrogen (BUN) and proteinuria (PRT) were significantly increased in the CKD subjects compared to the control group. Marker of oxidative stress (MDA) increased significantly in the CKD patients compared to the controls. Apart from Vit C which decreased significantly in the

CKD group compared to the controls, the other antioxidants (Vit A, CAT and uric acid) showed a significant

Table 1. Demographic, clinical and biochemical characteristics of study population.

Parameters	Control (n=80)	CKD (n=146)	P value
Age (yrs)	46.35 ± 1.96	50.18 ± 1.14	0.0720
BMI (kg m^{-2})	24.66 ± 0.80	24.44 ± 0.44	0.8021
SBP (mmHg)	120.7 0 ± 1.82	140.40 ± 3.84	0.0001
DBP (mmHg)	70.42 ± 1.25	90.32 ± 2.61	0.0001
PRT (g/l)	0.04 ± 0.02	1.17 ± 0.26	0.0001
Biochemical assays			
CRT (µmol/l)	105.90 ± 3.96	268.00 ± 25.60	0.0001
BUN (mmol/l)	3.51 ± 0.17	15.45 ± 2.80	0.0001
FBG (mmol/l)	5.31 ± 0.17	8.75 ± 0.33	0.0001
TC (mmol/l)	4.54 ± 0.13	5.63 ± 0.13	0.0274
TG (mmol/l)	1.52 ± 0.08	1.84 ± 0.09	0.0086
HDL-C (mmol/l)	1.35 ± 0.05	1.61 ± 0.20	0.2114
LDL-C (mmol/l)	2.75 ± 0.10	3.30 ± 0.14	0.0134
eGFR (ml/min/173 m2)	92.40 ± 5.67	57.61 ± 4.15	0.0001
Oxidative stress markers			
VIT C (mg/ml)	0.54 ± 0.02	0.34 ± 0.05	0.0001
VIT A (µmol/l)	9.76 ± 3.03	16.17 ± 5.21	0.0012
MDA (µmol/l)	1.22 ± 0.10	2.66 ± 0.07	0.0001
Uric acid (µmol/l)	266.68 ± 11.00	333.90 ± 10.02	<0.0001
CAT (units/ml)	57.49 ± 1.18	71.98 ± 2.91	0.0001

BMI=Body mass index, SBP=Systolic blood pressure, DBP=Diastolic blood pressure, PRT=Proteinuria, TC=Cholesterol, HDL-C=High density lipoprotein, TG=Triglyceride, LDL-C= Low density lipoprotein, CRT=Creatinine, BUN=Blood urea nitrogen, FBG=fasting blood glucose, eGFR=Glomerular filtration rate, CAT=Catalase.

increase in CKD patients compared to controls as shown in Table 1.

Table 2 represents demographic, clinical and biochemical parameters during various stages of CKD. Apart from eGFR which decreased significantly among the CKD subjects as the condition progressed from stage 1 to 5; CRT, BUN, PRT, SBP and DBP, increased significantly at some stages of CKD. With the exception of LDL and HDL, the other lipid fractions (TC and TG) increased significantly as the condition progressed with TG increasing significantly at stage 5. The marker of oxidative stress (MDA) increased significantly with the severity of CKD, whereas with the exception of Vit C which decreased as the condition progressed, the antioxidant (Vit A, CAT, Uric acid) increased with the severity of the condition.

From the Pearson correlation analysis in Table 3, there is a positive correlation between blood pressure (SBP and DBP) and the various oxidative stress parameters (MDA, Vit C, uric acid and Catalase) among the CKD group. Furthermore, with the exception of uric acid, Vit A and CAT which showed generally a positive correlation with eGFR, the other antioxidant (Vit C) showed a negative but significant correlation with eGFR. Again there was significant negative correlation between FBG and MDA among CKD subjects. Furthermore, there was a significant negative correlation between eGFR and FBG and between CRT and eGFR among subjects with CKD.

Figure 1 represents levels of plasma MDA (A), catalase activity (B), uric acid (C), and albumin (D) in controls and CKD patients. Apart from albumin (1D) which

Table 2. Demographic, blood pressure and biochemical parameters during various stages of chronic kidney disease

Parameters	CKD STAGE						
	Controls (n=80)	1 (n=25)	2 (n=35)	3 (n=37)	4 (n=25)	5 (n=24)	P Value
Anthropometric data							
Age (yrs)	46.35 ± 1.96	48.08 ± 3.72	50.45 ± 2.20	52.76 ± 2.13	50.12 ± 2.31	47.67 ± 2.50	0.3793
Sex (Males)	30 (37.5%)	10 (40.0%)	13 (37.1%)	15 (40.5%)	9 (36.0%)	10 (41.6%)	0.0149
BMI (kg/m2)	24.66 ± 0.80	25.97 ± 0.92	25.60 ± 1.54	24.60 ± 0.98	24.11 ± 0.81	23.56 ± 0.75	0.7815
SBP (mmHg)	120.70 ± 1.82	129.1 ± 3.38*	126.00 ± 3.70	134.3 ± 3.82*	135.6 ± 4.36	136.00 ± 4.36*	0.005
DBP (mmHg)	70.21 ± 1.26	79.2 ± 2.70***	78.92 ± 1.85*	79.43 ± 1.87*	84.20 ± 2.80	90.50 ± 4.94***	0.0001
PRT (g/l)	0.04 ± 0.02	0.29 ± 0.06	0.82 ± 0.26	1.13 ± 0.29	1.27 ± 0.38	1.49 ± 0.40	0.0001
Biochemical assay							
CRT (µmol/l)	105.90 ± 3.96	62.19 ± 3.26	110.30 ± 2.25	160.00 ± 3.73	302.90 ±12.22**	835.50 ± 79.42**	0.0001
BUN (mmol/l)	3.51 ± 0.17	4.91 ± 0.64	4.48 ± 0.48	10.85 ± 1.95	15.40 ± 5.33	46.49 ± 6.37***	0.0001
FBG (mmol/l)	5.31 ± 0.17	8.19 ± 0.81	8.55 ± 0.85	8.95 ± 0.70	10.66 ± 0.93*	7.28 ± 0.71	0.0001
TC (mmol/l)	4.54 ± 0.13	5.56 ± 0.28	5.65 ± 0.25	5.75 ± 0.39	5.98 ± 0.24	6.55 ± 0.26	0.0172
TG (mmol/l)	1.52 ± 0.08	1.05 ± 0.26	1.07 ± 0.02	1.53 ± 0.37	1.83 ± 0.25	2.69 ± 0.46**	0.0013
HDL (mmol/l)	1.35 ± 0.05	1.73 ± 0.28	1.09 ± 0.16	1.76 ± 0.25	1.32 ± 0.16	1.23 ± 0.10	0.0660
LDL (mmol/l)	2.74 ± 0.10	3.45 ± 0.53	3.90 ± 0.38	4.11 ± 1.00	2.77 ± 0.28	2.45. ± 0.50	0.1423
Oxidative markers							
Uric acid (µmol/l)	266.68 ± 11.00	255.70 ± 17.83	266.20 ± 17.12	277.70 ± 19.83	324.50 ± 29.5	309.70 ± 21.41	0.7363
MDA (µmol/l)	1.22 ± 0.10	2.41 ± 0.15*	2.45 ± 0.13**	2.59 ± 0.17**	2.67 ± 0.15***	2.87 ± 0.18***	0.0001
CAT(units/ml)	57.49 ± 1.18	58.58 ± 2.61	59.25 ± 4.77	60.00 ± 5.00	71.63 ± 6.88	79.72 ± 8.45***	0.0001
VIT C (mg/ml)	0.54 ± 0.02	0.54 ± 0.07	0.34 ± 0.03**	0.30 ± 0.04**	0.33 ± 0.06**	0.21 ± 0.02***	0.0001
VIT A (µmol/l)	9.76 ± 3.03	8.79 ± 1.04	9.94 ± 2.20	19.42 ± 2.10**	8.72 ± 1.08	33.98 ± 1.22***	0.0001
e GFR(ml/min/173m2)	92.40 ± 5.67	150.50 ± 9.22**	67.47 ± 1.34	44.34 ± 1.35***	21.50 ± 0.98***	8.45 ± 0.67***	0.0001

=p<0.05; **p< 0.01; ***p<0.001. BMI = Body mass index, SBP=Systolic blood pressure, DBP= Diastolic blood pressure, PRT = Proteinuria, TC= Total Cholesterol, HDL-C=High density lipoprotein cholesterol, TG= Triglyceride, LDL-C= Low density lipoprotein cholesterol; CRT = Creatinine, BUN= Blood urea nitrogen, GFR=Glomerular filtration rate, FBG= fasting blood glucose, MDA= Malondialdehyde, Vit C=Vitamin C, Vit A=Vitamin A, CAT= Catalase. Stage 1 = eGFR ≥90 mL/min/1.73m²; stage 2 = eGFR 60-89 mL/min/1.73m²; stage 3 = eGFR 30-59 mL/min/1.73m²; stage 4 = eGFR 16-29 mL/min/1.73m²; stage 5 = eGFR<15 mL/min/1.73m² or dialysis..

Table 3. Pearson correlation coefficients of clinical variables and biochemical parameters for CKD subjects

Variables	DPB	TC	TG	HDL	LDL	CRT	eGFR	FBG	MDA	Vit C	Vit A	CAT	UA
SBP	0.76***	-0.23	-0.19	-0.37**	-0.14	0.05	-0.05	-0.23	0.16	0.13	0.16	0.07	0.21
DBP		-0.21	-0.14	-0.18	-0.17	0.03	0.05	-0.30*	0.19	0.09	0.19	0.16	0.15
TC			0.02	0.32*	0.96***	-0.24	0.19	0.17	-0.15	-0.08	-0.1	-0.16	0.11
TG				-0.43**	-0.17	0.22	-0.10	-0.06	-0.21	0.04	-0.04	-0.01	-0.27
HDL					0.28*	-0.03	-0.06	0.06	0.01	-0.26	-0.17	-0.08	0.02
LDL						-0.24	0.17	0.20	-0.08	-0.05	-0.10	-0.12	0.19
CRT							-0.70***	-0.28*	0.17	-0.29*	0.14	0.32*	-0.13
eGFR								0.34*	-0.31*	0.05	-0.25*	0.08	-0.05
FBG									-0.11	-0.30*	0.03	-0.12	-0.07
MDA										-0.31*	0.11	0.22	0.22
Vit C											-0.29*	0.59***	-0.03
Vit A												0.22	-0.08
CAT													-0.14

****.Correlation is significant at the 0.05 level (2-tailed), **.Correlation is significant at the 0.01 level (2-tailed), ***.Correlation is significant at the 0.001 level (2-tailed). eGFR= Estimated Glomerular filtration rate, UA= Uric acid, MDA= Malondialdehyde, CAT= Catalase.***

showed a significant decrease in the CKD subjects compared to the controls, MDA (1A), uric acid (1C) and catalase (1B) generally increased significantly in the CKD subjects compared to the controls.

DISCUSSION

Renal disease, in early as well as advanced stages, is associated with abnormalities in lipoprotein metabolism. These abnormalities have been reported to be characterized by low plasma concentrations of HDL-C, high concentrations of TG, LDL-C particles, and TC (de Sain-van der Velden *et al.*, 1998; Vaziri, 2006) as confirmed by this study except for HDL-C which was not significantly different from the controls. However, the level of HDL in CKD is inconsistent as some researchers have shown that it can either decrease (Vaziri, 2006) or remain unchanged (Shah *et al.*, 1994).

The alteration in lipid metabolism has been proposed to accelerate the progression of CKD through various mechanisms. First, reabsorption of fatty acids, phospholipids, and cholesterol contained in the filtered proteins (albumin and lipoproteins) by tubular epithelial cells can stimulate tubulointerstitial inflammation, foam cell formation, and tissue injury (Magil, 1999). Secondly, accumulation of lipoproteins in glomerular mesangium can promote matrix production and glomerulosclerosis (Wheeler and Chana, 1993). In this context, native and oxidized lipoproteins, particularly LDL, stimulate production of matrix proteins by cultured mesangial cells and promote generation of proinflammatory cytokines, which can lead to recruitment and activation of circulating and resident macrophages (Rovin and Tan, 1993).

Furthermore, dyslipidaemia is an important risk factor for the development of cardiovascular events in CKD patients and it requires intervention like the use of statins to avoid or minimize the sequel of these complications (Kasiske, 2003). The effects of dyslipidaemia on the kidney are mainly observed in those with other risk factors for renal disease progression such as hypertension, diabetes and proteinuria (Keane 2000).

Oxidative stress status among the CKD patients was evaluated by measuring plasma lipid peroxidation end product MDA, whereas antioxidant vitamins (vitamin A and C), uric acid and catalase evaluated the antioxidant status. The increase in MDA found in this study is in agreement with the results of other studies (Rutkowski *et al.*, 2006) which support the observation that MDA is increased in CKD patients compared to controls. This gives an indication of the state of oxidative stress to

which the patients are subjected. Furthermore, levels of MDA increased with the progression of disease from stage 1 to stage 5. This is in accordance with the study of Yilmaz *et al.,* (2006). Lipid peroxidation products may contribute to endothelial injury and may be involved in intensive oxidative modifications of LDL (Esterbauer *et al.*, 1992) and in the development of atherosclerosis (Basha and Sowers, 1996).

Moreover, MDA altered LDL-C (Ox LDL) leads to cholesterol ester accumulation within human monocyte macrophages and it has been hypothesized that modification of native LDL may be a prerequisite for the accumulation of cholesteryl esters within the cells of atherosclerotic lesion (Fogelman *et al.*, 1980). Secondly, increased lipid peroxidation causes endothelial dysfunction through the breakdown of nitric oxide (Taddei *et al.*, 1998). Consequently, the endothelial cells lose their ability to protect the vessel wall and become atheroscle-

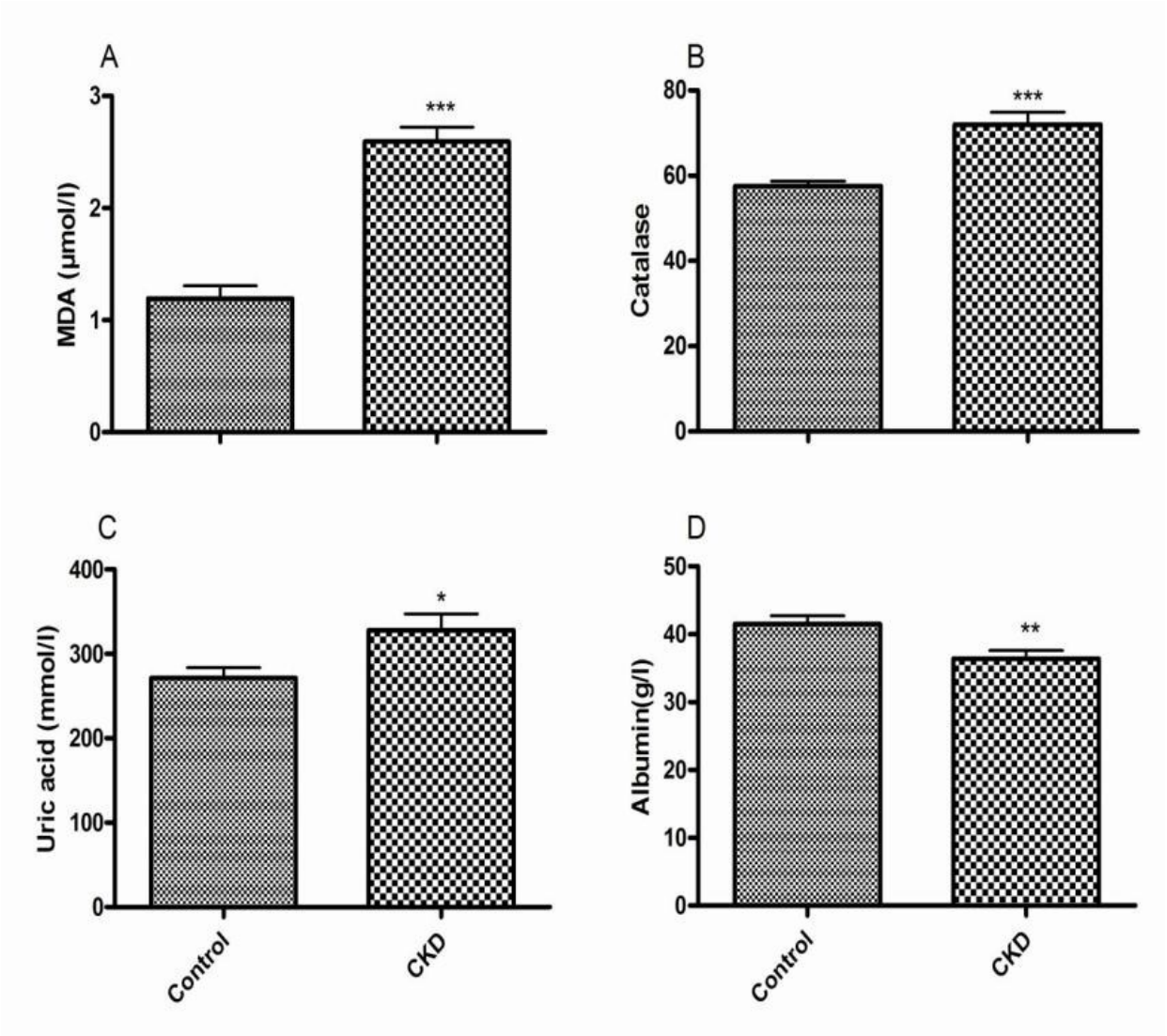

Figure 1. Levels of plasma MDA (A), catalase activity (B), uric acid (C), and albumin (D) in controls and CKD patients. Results are means ± SEM.. Values significantly different from controls *=p<0.05, **=p<0.01, ***p<0.001

rotic promoters (Lüscher and Vanhoutte, 1990). Thus lipid peroxidation marked by an increased MDA level may also contribute to the high incidence of premature atherosclerosis in CKD patients.

Catalase is a haemoprotein catalyzing the reduction of hydrogen peroxides and protects against the formation of highly reactive hydroxyls. The plasma concentrations of catalase increased significantly in the CKD patients compared with the controls, and increased with the severity of the condition. This observation is however contrary to the findings made in other studies elsewhere (Chen *et al.*, 1997) and may thus require further scientific enquiry.

Ascorbic acid represents one of the most prominent antioxidants, exerting beneficial effects by an inhibition of lipid peroxidation and by reducing endothelial dysfunction (Deicher and Horl, 2003). The significant decrease in the plasma concentration of Vitamin C observed in this study is consistent with the observations made in other studies (Bakaev *et al.*, 1999). Although in CKD deficiency of vitamin C can be observed, its administration in these patients requires caution.

The elevated plasma retinol (Vitamin A), an antioxidant fat-soluble vitamin, observed among the CKD patients in this study is consistent with the findings of Hala *et al.,* (2000). The high level of plasma retinol in CKD may be due to the increased levels of RBP (retinol binding protein), reduced vitamin excretion and decreased conversion of retinol to retinoic acid in the whole blood (Gerlach and Zile, 1990). In renal dysfunction both the excretory and tubular catabolism of RBP are reduced which results in the accumulation of these proteins in the blood (Smith and Goodman, 1976). In this study there was a negative correlation between eGFR and plasma retinol. Ayatse, (1991) however observed a positive correlation between serum creatinine and plasma retinol.

Uric acid is generated in the human body by the degradation of purines. It has been found that uric acid is a powerful antioxidant and is a scavenger of singlet oxygen and other radicals (Grootveld and Halliwell, 1987). In this study, the significantly higher plasma uric acid concentration that was found compared to the control, is probably a consequence of the failure of the excretory function of the kidneys as well as increased protein catabolism as part of the hypercatabolic state in CKD (Davison *et al.*, 1999). Hyperuricaemia is often present and associated with an increased prevalence of gouty arthritis and tophi in CKD. It also acts as an independent factor in the progression of renal disease and cardiovascular risk factor as well. High uric acid levels induce vasoconstriction, inflammation and intrarenal generation of angiotensin II, all of which promote hypertension (Johnson *et al.*, 2005).

CONCLUSION

It has been established by this study that dyslipidaemia and increased lipid peroxidation (oxidative stress) with abnormal antioxidant levels are common in CKD patients. Based on the findings in this study, it may seem reasonable to propose that therapeutic regimens aimed at strengthening the antioxidant defenses as well as normalizing lipid concentrations would be useful in protecting CKD patients against lipid peroxidation (oxidative stress) and any related complications.

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ORIGINAL ARTICLE

Asymptomatic Bacteriuria among Pregnant Women Attending Antenatal Clinic at the University Hospital, Kumasi, Ghana

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The apparent decline in immunity of pregnant women appears to promote the growth of both commensal and non-commensal microorganisms. The objective of the study was to determine the prevalence of asymptomatic bacteriuria in pregnant women visiting the University hospital, Kumasi. This prospective hospital-based study was carried out between April-June 2009. A total of 200 pregnant women were recruited for this study. The ages of the women ranged from 15 to 46 years. About 5-10mls of clean catch urine was cultured on Cysteine Lactose Electrolyte Deficient (CLED) agar aerobically at 37^{o}C . Isolates were identified to the species level using standard protocol. Antibiotic sensitivity test were carried out using the Kirby-Bauer disc diffusion method. Of the 200 women examined, 19 had significant bacteriuria representing a prevalence of 9.5% in the study population. Pregnant women in their second trimester from the study had the highest prevalence of significant bacteriuria (52.6%) with age ranges between 30-34 years having the highest prevalence (36.8%). Nulliparous women were 35 (17.5%) with 3 (8.6%) testing positive for bacteriuria and 165 (82.5%) were multiparous with 16 (9.7%) testing positive for bacteriuria. E. coli (36.8%) was the common bacteria isolate from this study. From this study, asymptomatic bacteriuria is common among antenatal women in the population studied. It is therefore recommended that periodic testing of pregnant women is advocated and those found to be infected need to be treated to avoid complications.
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Keywords*:* Asymptomatic bacteriuria, pregnancy, urine, Prevalence, Parity

INTRODUCTION

Urinary Tract Infections (UTIs) is an infection caused by the presence and growth of microorganisms anywhere in the urinary tract. It is perhaps the single most common bacterial infection of mankind (Morgan and McKenzie, 1993; Ebie *et al.*, 2001a). UTI is evident when there are 10^5 or more of microorganisms or of a single strain of bacterium per millilitre in midstream urine samples (Davidson *et al.*, 1989; Bloomberg *et al.*, 2005). The presence of bacteria without symptoms is termed asymptomatic bacteriuria. Common pathogens associated with UTI include *E. coli* and *Klebsiella* species although the distribution of pathogens that cause UTI is changing (Ojiegbe and Nworie, 2000).

Pregnant women are at increased risk for UTIs with incidence rates being as high as 8% in the United States (Delzell and Lefevre, 2000). Asymptomatic bacteriuria.in pregnancy has been attributed to increase urinary stasis, ureteric relaxation and other anatomical changes. These pathological conditions begin in week 6 and peak during weeks 22 to 24 and this prevent easy passage of urine

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(Delzell and Lefevre, 2000). Women with asymptomatic bacteriuria during pregnancy are more likely to deliver premature or low-birth-weight infants (Ronald, 1987; McGregor *et al*., 1990; Schults *et al*., 1991). These pregnant women also have a 20 to 30-fold increased risk of developing pyelonephritis (Zhanel *et al*., 1990; Mittendorf *et al*., 1992; Gratacos *et al*., 1994) compared with women without bacteriuria. Other conditions including transient renal failure, acute respiratory distress syndrome, sepsis, shock and haematological abnormalities occur in cases where asymptomatic bacteriuria is untreated or inadequately treated. However, variations have been noted to exist in the incidence of bacteriuria and subsequent UTI in different countries and this has been attributed to differences in definition, methods of screening and associated risk factors such as age, parity and pregnancy.

There are several ways to diagnose UTI, but urine culture still remains the most reliable tool for its diagnosis. Urine culture has shown *Escherichia coli* to be the most common bacterial isolate of UTI during pregnancy (Stein and Funfstuck, 2000; Ebie *et al*., 2001b; Bloomberg *et al*., 2005; Obiogbolu *et al*., 2009). Other studies have also reported *Klebsiella spp* (Omonigho *et al*., 2001) and *Staphylococcus aureus* (Ugbogu *et al*., 2010) as the commonest isolates.

This hospital-based prospective study was carried out to determine the prevalence of asymptomatic bacteriuria in pregnant women visiting the University hospital, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi. The aim of the study was to evaluate the possible effects of maternal age, gestational age and parity on the prevalence of asymptomatic bacteriuria and to find the predominant causative agents associated with bacteriuria and subsequent UTI.

MATERIALS AND METHODS

Study site
This prospective hospital-based study was conducted in the antenatal clinic at the Kwame Nkrumah University of Science and Technology hospital, Kumasi during the periods of April 2009 to July 2009.

Study population
In all, a total of 200 asymptomatic pregnant women with ages ranging from 15 to 46 years were randomly selected on an every other day basis within the periods of 8:00am to 12:00 noon. Pregnant women who were on antibiotic treatment two weeks prior to their initial visit, those who exhibited clinical signs and symptoms of urinary tract infection (UTI) and those at 38 weeks gestation or more were excluded from the study. Information on parity and stage of pregnancy was extracted from the antenatal folders of the pregnant women. Other information was obtained using a well structured questionnaire. All the pregnant women recruited into the study over the period agreed to a written informed consent.

Sampling and bacteriological analysis
Subjects were properly educated to collect clean catch midstream urine samples into wide-mouthed sterile capped containers after proper cleansing of the external genitalia. Urine samples were labelled and immediately sent to the laboratory in cold boxes and cultured on appropriate media.

Culture process
Urine samples were cultured using a standard loop calibrated to hold 0.01 ml of urine onto blood agar and Cysteine Lactose Electrolyte Deficient (CLED) agar. Inoculated plates were incubated at 37^{o}C aerobically overnight. After overnight incubation the plates were read and the growths were identified based on the growth characteristics on the inoculated media.

Colony counts
Colonies were counted on CLED and multiplied by the loop volume. A bacterial count of $1x10^5$ per ml was considered significant for UTI and counts of 10^2 – 10^4 per ml were considered as suspected/doubtful bacteriuria while counts less than 10^2 per ml were considered no significant bacterial growth.

Bacterial identification
Growths on the culture media were identified using their growth characteristics, Gram stain and biochemical and sugar fermentation tests. The biochemical tests used were: Indole test for lactose fermenting bacteria particularly Escherichia coli and Klebsiella and Coagulase test to differentiate *Staphylococcus aureus* from *Staphylococcus*.

Sensitivity tests
Antimicrobial susceptibility test were performed using Kirby-Bauer disc diffusion test. The isolates from this study were tested against the following antibiotics: Ampicillin (10µg), Cefuroxime (30µg), Cotrimoxazole (25µg), Gentamicin (10µg), Tetracycline (30µg), Nalidixic acid (30µg), Nitroforantoin (300µg) and pipemidic acid (20µg). Zone diameter was measured by Clinical Laboratory Standard Institute (CSLI).

RESULTS

Out of the 200 asymptomatic pregnant women sampled, 19 (9.5%) had colony counts of 10^5 colonies/mL or more (positive) with the highest prevalence of bacteriuria being observed in pregnant women within the 30 – 34 years age group (36.8%). This was followed by women within the 25 – 29 years (26.3%), then the 35 – 39 years age group (15.8%), 20 – 24 years age group (10.5%), 15 – 19 and 40 – 44 years age group respectively (5.3%) and the 45 – 49 years age group which recorded no case of bacteriuria. The prevalence of asymptomatic bacteriuria amongst the study participants stratified by age group and colony count from urine culture samples is shown in Table 1. Forty-eight (48) representing 24.0% of pregnant women had bacterial colony counts between 10^2 - 10^4 colonies/mL with the highest prevalence of 25.0% being observed in women within the 25 – 29 and 30 – 34 years age groups respectively. This was followed by the 35 – 39 years age group (20.8%), 20 – 24 years age group (12.5%), 15 – 19 years age group (10.4%) and 40 – 44 years age group. A total of 133 (51.5%) pregnant women had no bacteria growth in their urine samples.

Out of the 200 asymptomatic pregnant women sampled, 73 (36.5%) were within the first trimester of pregnancy with 4 (5.5%) testing positive for significant bacteriuria; 65 (32.5%) were in their second trimester of pregnancy and 10 (15.4%) tested positive for significant bacteriuria and 62 (31.0%) in their third trimester of pregnancy with 5 (8.1%) testing positive for significant bacteriuria. A total of 181 (90.5%) pregnant women comprising 69 (94.5%) in their first trimester, 55 (84.6%) in their second trimester and 57 (91.9%) in their third trimester showed no bacterial growth in their urine samples. A comparison of the occurrences of number of women with significant bacteria showed pregnant women in the second trimester having marginally significant likelihood of testing positive for significant ($\chi^2 = 3.70$; $p = 0.054$) bacteriuria when compared to pregnant women in the first trimester. A comparison of first trimester pregnant women with significant bacteriuria to third trimester pregnant women and then second trimester to third trimester showed significant differences (Table 2).

Table 3 shows the prevalence of asymptomatic bacteriuria in pregnant women sampled for the study based on parity. The pregnant women were classified as nulliparous and multiparous. Out of the 200, 35 (17.5%) were nulliparous with 3 (8.6%) testing positive for bacteriuria and 165 (82.5%) were multiparous and 16 (9.7%) of them tested positive for bacteriuria. A comparison of the frequencies of occurrences of pregnant women with significant bacteriuria when classified as nulliparous and multiparous showed no significant difference ($\chi^2 = 0.043$; $p = 0.8366$).

The prevalence of bacteria isolated from the 19 positive cases is shown in Figure 1. The prevalence of *Escherichia coli* was 36.8% (7/19) which ranked as the most prevalent isolated organism followed by *Klebsiella* spp. (26.3%), Staphylococcus aureus (21.1%) and other coliforms (15.8%).

DISCUSSION

The prevalence of asymptomatic bacteriuria among the pregnant women in this study was 9.5%. Varying prevalence rates of asymptomatic bacteriuria in pregnant women have been reported with Hazhir (2007) reporting

Table 1: Prevalence of asymptomatic bacteriuria in the study population based on age distribution

	CFU (colonies/mL)			
	10^5	10^2-10^4	$<10^2$	
Age group	Positive (%)	Suspected (%)	Negative (%)	Total
15 – 19	1 (5.3)	5 (10.4)	12 (9.0)	18 (9.0)
20 – 24	2 (10.5)	6 (12.5)	25 (18.8)	33 (16.5)
25 – 29	5 (26.3)	12 (25.0)	29(21.8)	46 (23.0)
30 – 34	7(36.8)	12 (25.0)	41 (30.7)	60 (30.0)
35 – 39	3 (15.8)	10 (20.8)	24(18.1)	37 (18.5)
40 – 44	1 (5.3)	3 (6.3)	1 (0.8)	5 (2.5)
45 – 49	0 (0.0)	0 (0.0)	1 (0.8)	1 (0.5)
Total	**19(9.5)**	**48 (24.0)**	**133 (51.5)**	**200**

Data are presented as proportions; CFU = Colony forming unit

Table 2: Prevalence of asymptomatic bacteriuria in the study population based on trimester

Trimester	No. of pregnant women	No. with significant bacteriuria	No. without significant bacteriuria
1	73 (36.5)	4 (5.5)	69 (94.5)
2	65 (32.5)	10 (15.4)[a]	55 (84.6)
3	62 (31.0)	5 (8.1)	57 (91.9)
Total	**200**	**19(9.5)**	**181 (90.5)**

Data are presented as proportions; [a](P = 0.054, χ^2 = 3.70) indicates the level of significance when Trimester one was compared to Trimester 2 (Chi-square test)

Table 3: Prevalence of asymptomatic bacteriuria in the study population based on parity

Parity	No. of pregnant women	No. with significant bacteriuria	No. without significant bacteriuria
Nulliparous	35 (17.5)	3 (8.6)	32 (91.4)
Multiparous*	165 (82.5)	16 (9.7)	149 (90.3)
Total	**200**	**19(9.5)**	**181(90.5)**

Multiparous = as used here was defined as a pregnant woman with at least an existing child; χ^2 = 0.043; P value = 0.8366

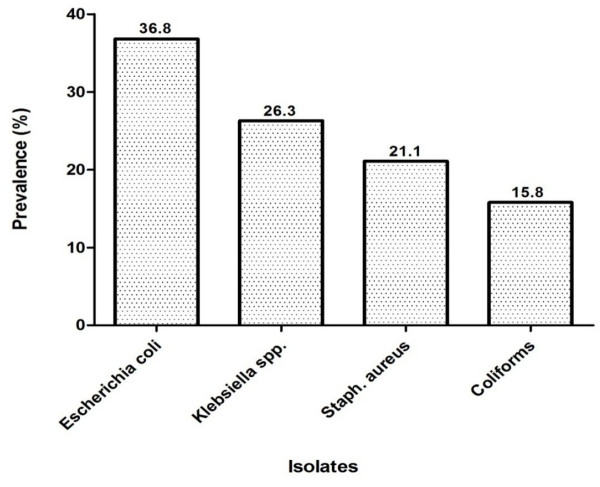

Figure 1: Prevalence of bacterial species isolated from cases of significant bacteriuria in pregnant women

a prevalence rate of 6.1%, Turpin *et al* (2007) reported a prevalence of 7.3%, Hernandez *et al* (2007) reported a prevalence of 8.4% and Tadesse (2007) reported a prevalence of 9.8%. Prevalence rates as low as 3.3% (Moghadas and Irajian, 2009) and 3.7% (Mobasheri *et al*., 2002) have been reported and rates as high as 22.2% (Famurewa, 1992) and 23.9% (Olusanya *et al*., 1993) have also been reported in separate studies. Bint and Hill (1994) in a study on bacteriuria in pregnancy reported that the prevalence of asymptomatic bacteriuria in pregnancy ranges from 4% to 7% depending on the population being studied.

The results of this study compares well with that of Tadesse (2007) but in relation to other determined rates affirms the findings which ascribed variations in prevalence to population characteristics such as age, parity, socio-economic status, sexual activity (multiple sexual partners) and health care during pregnancy (Wong and Stamm, 1983; Strom *et al*., 1987; Andriole and Patterson, 1991; Olusanya *et al*., 1993). There is therefore an impli-

cation that about 9.5% of pregnant women from this study are at risk of developing acute episodes and complications of UTI during pregnancy if they are not properly treated.

In analyzing the study respondents by age, the highest prevalence of asymptomatic bacteriuria was observed in pregnant women within 30 – 34 years age group followed by the 25 – 29 years age group and then the 35 – 39 years age group. Turpin *et al.,* (2007) reported a high prevalence of asymptomatic bacteriuria in pregnant women aged 35 – 39 years. Alghalibi *et al.,* (2007) reported a higher prevalence of UTI in pregnant women aged 21 – 25 years in their study. The observed trend of bacteriuria in this study and reports from other studies shows the age range of 25 – 39 years serving as a risk group for developing UTI in pregnant women.

By parity, pregnant women with at least an existing child (multiparous) had high prevalence of asymptomatic bacteriuria when compared to nulliparous women although the differences between the percentage occurrences were not statistically significant (p=0.251). These findings are however contrary to that of El Sheikh *et al.,* (1999) who in their study on characteristics of bacteriuria in pregnant women attending a teaching hospital in Sudan reported a decreased incidence of bacteriuria with age of patients and a significantly high bacteriuria in primigravidae than multigravidae. This finding, they suggested, to be attributable to the trauma caused by the movement of the penis in the vagina hence the increased prevalence of bacteriuria in young women and primigravidae as related in the study of Olusanya *et al.* (1993). The reason/s for the observed increase in bacteriuria among older pregnant women in this study can however not be readily explained from this study in the light of the absence of clinical data on signs and symptoms of UTI, history of sexual frequency and time of marriage.

This study further observed that pregnant women in the second trimester of pregnancy had the highest prevalence of asymptomatic bacteriuria followed by pregnant women in the third trimester of pregnancy which is in consonance with the findings of Alghalibi *et al.,*(2007) who in a study on bacterial urinary tract infection among pregnant women in Yemen reported the second and third trimesters of pregnancy as being associated with the highest prevalence of UTI. It is however contrary to the findings of Turpin *et al.,* (2007) who reported a high percentage of asymptomatic bacteriuria in the first and early second trimesters of pregnancy and attributed it to pregnant women reporting at the antenatal clinic for booking during these periods. This impression can however not be said to be same from the findings of this study in that it is evident that pregnant women report for booking within the first and second trimesters of pregnancy and continue with their scheduled antenatal visits until delivery thereby making it possible for this study to enrol most pregnant women within the third trimester of pregnancy.

The most common bacterial isolates from midstream urine samples of asymptomatic pregnant women enrolled in this study were *Escherichia coli* (36.8%) followed by *Klebsiella species* (26.3%). Rahman *et al.,* (1990) and Ahmed and Rashid, (1996) in separate studies also reported *E. coli* as being the commonest pathogen responsible for bacteriuria which is consistent with the findings of this study. Delzell and Lefevre, (2000), Colgan *et al.,* (2006), Turpin *et al.,* (2007), Hernandez *et al.,* (2007) and Hazhir (2007) have all reported *E. coli* as the dominant bacterial agent causing asymptomatic UTI. Mohammad *et al.,* (2002) suggested that the high risk of acquiring *E. coli* UTI is because of the anatomical and the functional changes that occur during pregnancy and the fact that *E. coli* is the most common micro-organism in the vaginal and rectal area. Shanson, (1989) and Delzell and Lefevre, (2000) reported that this significant finding could be due to the fact urinary stasis is common in pregnancy and since most *E. coli* strains and other bacteria prefer that environment, they are able to persist and cause UTI. Akram *et al.,* (2007) reported that the anatomical proximity of the anal and urogenital opening in females makes it possible for faecal contamination of the urinary tract from commensals of the bowel of which *E. coli* is a typical example.

The antibiotic sensitivity patterns from this study showed that most of the *E. coli* isolated were sensitive to nitrofurantoin and gentamicin. The choice of antibiotic should however be based on urine culture, stage of gestation, clinical data and the characteristics of the antibiotic. Although aggressive antibiotic treatment may be necessary to reduce the risk of pyelonephritis and other complications of asymptomatic bacteriuria in pregnancy, this should be done with caution as it known that urinary pathogens are becoming resistant to commonly used antibiotics which could be attributed to wide spread and indiscriminate use of the drugs (Okonko *et al.*, 2009).

CONCLUSION

This study showed that approximately 10% of the pregnant women recruited had asymptomatic bacteriuria. It is therefore imperative that pregnant women are

screened for bacteriuria periodically in every trimester of the gestational period. Talks on personal hygiene and cleanliness around the urogenital and anal area to prevent faecal contamination of the urinary tract should be emphasized during antenatal visits.

COMPETING INTERESTS

We declare that we have no competing interests.

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