PACIFIC JOURNAL OF MEDICAL SCIENCES

VOLUME 11, No. 2, AUGUST 2013

PACIFIC JOURNAL OF MEDICAL SCIENCES

(Formerly Medical Sciences Bulletin)

ISSN: 2072 – 1625 Volume 11, No. 2, August 2013

A multidisciplinary journal for publication of medical and biomedical research findings on issues pertinent to improving family health and related issues of public health

INFORMATION

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CASE REPORT

GASTROPROTECTIVE EFFECTS OF AQUEOUS EXTRACT OF UNRIPE CARICA PAPAYA FRUIT IN RATS

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Running title: Pawpaw fruit and gastroprotection.

ABSTRACT:

Aqueous extract of unripe Carica papaya fruit (AEUCPF) was investigated for its anti-ulcer, mucus secretion, anti-acid secretory and pepsin binding effects in rats. Ethanol/HCl and Indomethacin were used to induce ulcers while acid and mucus secretion was measured in ulcerated and treated animals. The animals were divided into five groups for each of the anti-ulcer studies and each group was made up of five animals each. These groups included a control and reference groups administered saline and cimetidine (Kg/Kg b.w), while the remaining three groups were administered with 2.5, 3.5 and 4.5ml/Kg of the decoction of the unripe fruits. The extract, cimetidine and saline were all administered orally twice daily for ten days while necrotizing agents were administered (p.o) once daily from day 8 through day 10. The results showed that higher doses of the extract significantly ($p<0.05$) reduced the ulcer index from 3.6± 0.24 (control) – to 0.70 ± 0.37 (4.5ml/Kg) in the ethanol induced ulcer. The extract also produced similar effects in the indomethacin induced ulcer and in both cases the gastric acidity was significantly reduced. The extract did not increase mucus secretion but it bind substantially with pepsin. In conclusion this study has shown that AEUCPF has beneficial effects on the normal function of the stomach. It has the capacity to ameliorate gastric ulcer as suggested by local traditional medical practitioners.

KEYWORDS: Carica papaya; Extract; Gastric acid; Pepsin; Ulcer; Rats.

(Submitted March 2013; Accepted June 2013)

INTRODUCTION:

The stomach is the most distensible and one of the vital parts of the gastrointestinal tract [1]. It is involved in digestion of various foods which it receives from the oesophagus. In addition to food, the stomach is exposed to many potentially injurious agents such as acids, pepsin, bacterial products and drugs [2,3]. There is a continuous effort to find good synthetic or phytochemical agents that will offer gastroprotective effects. Thus many plants and plants derived products have been screened for their anti-ulcer effects. These include: Persea americana, Landolphia owarriensis, Ananas ananassoides, Garcinia kola seeds, a biflavonoid kolaviron isolated from G. Kola seeds, licorice, etc [3-7].

Carica papaya (L) commonly called pawpaw is a large tree like plant with stem growing from 5 to 10 metres tall. It has spirally arranged leaves which are confined to the top of the trunk. It produces fruits which are mainly oval in shape with light green colour in the unripe state but which may turn yellow when it ripens. The unripe fruit can be cooked as parts of salads, jellies and stews while the ripe fruits are usually eaten raw without the skin or seed [8-9]. The plant is employed in the treatment of several ailments by traditional medical practitioners with such uses including but not limited to the treatment of the following: sore throat, asthma, sickle cell anaemia, wound, ulcers, boils, malaria, fever, pain, tonsillitis, indigestion, dyspepsia, jaundice and cancer [8-11]. The unripe fruit have been reported to have anti-sickling, laxative, arbortifacient and diuretic properties [11] while the intake of the extract of unripe fruit of the plant has been linked with an anti-ulcer effect [12].

Ezike et al, [12] had investigated the probable beneficial effect of unripe papaya fruit on the treatment of gastric ulcer by administering extract of unripe papaya fruit however; the focus of the present study is to investigate the use of aqueous extract of the unripe fruit on gastric mucosa irritation, mucus secretion and gastric acidity. Therefore this study was designed to specifically mimic the exact practice of the traditional medical practitioners and to see if this practice is effective. Thus we investigated if the aqueous extract of unripe and mature fruit of C. papaya has therapeutic effects in animal models of ulcer and the probable mechanism for such effects.

MATERIALS AND METHODS:

Plant material and preparation of decoction:

The unripe fruit of C. papaya fruits were collected from fruit gardens in Ilorin metropolis and the mini campus of University of Ilorin. The plant had been previously [9] identified at Forestry Research Institute of Nigeria (FRIN) with a voucher specimen number FHI 106933. The unripe fruits (with total weight of 2.9 Kg) were washed with distil water and sliced into small cubed shaped pieces each weighing 50g each. The slices of each of the three fruits were soaked

in 2.5 litres of distil water for 96 hours after which the resulting solution was sieved and immediately used for pharmacological studies.

Animals

Male Wistar rats weighing 180±10.1g were used for these studies. The animals were bred in the animal house of the Faculty of Basic Medical Sciences, University of Ilorin and fed on standard mouse cubes (exotic Feeds, Ilorin, Nigeria). They were kept in clean cages with optimum temperature of about 250C, humidity of 60-65% and 12 hours light/dark cycle. Animals were provided with water ad libitum. The research was conducted in accordance with the ethical rule for animal experimentation, approved by Ethical Committee, College of Health Sciences University of Ilorin.

The animals were divided into five groups with each group comprising of five animals each. Group A (control) was administered saline (10ml/Kg), group E was administered 11.5g/Kg of cimetidine. The animals in groups B-D were administered 2.5, 3.5 and 4.5ml/Kg of the decoction of unripe papaya fruit extract twice daily for ten days. Ethical approval was obtained from the Ethic Committee of the Department of Physiology, University of Ilorin in accordance with the University of Ilorin guidelines on the care and use of laboratory animals.

Anti-ulcer studies

HCl/Ethanol induced ulcer: Ulceration was induced in experimental animals by the administration of 1 ml of necrotizing solution (150 mm of HCl in 60% ethanol) in accordance with the method used by Mizui and Douteuchi [13]. Animals were orally administered saline, cimetidine or decoction of unripe fruit (2.5, 3.5 or 4.5mg/Kg) of C. papaya twice daily for ten days. However the administration of the necrotizing agent started on the eighth day once daily for three days. The animals were sacrificed 2hrs after the administration of the test substances and saline. The stomachs of the animals were dissected out and an incision was made at the greater curvature in order to collect gastric contents and observation of gastric mucosa for the presence of gastric ulceration. Ulceration was confirmed by using a hand held lens (x10) and the ulcer scores were determined using the arbitrary scale used by Singh et al, [14] as in previous studies [9]. A score of 0 was assigned to no visible lesion; 0.5 for hyperaemia; 1 for one or two slight lesions, 2 for severe lesions; 3 for very severe lesions and 4 for mucosal that is full of many lesions. The ulcer index was also calculated as the means of ulcer scores.

Indomethacin induced ulcer: Ulceration was induced in these groups of animals by administration of 20 mg/Kg of indomethacin as necrotizing agent. Animal grouping and drug administration were as in the HCl-ethanol induced ulcer above. The same ulcer scoring method was also used.

Determination of gastric acidity

Samples of gastric contents from each rats used for antiulcer studies were collected and

centrifuged (2000 rpm)for 10 min. after which 1 ml of the supernatant was analysed for hydrogen ion concentration by titration against 0.1 M NaOH to a pH of 7.0 using phenolphthalein as an indicator.

Mucus secretion

Measurement of mucus production

Gastric mucus production was assessed in rats that were administered HCl/ethanol necrotizing agent immediately after the determination of the ulcer scores of the animals as described previously [7]. Briefly, the mucus layer of the stomach of each rat was scraped using a glass slide into a glass tube containing 1 ml of water whose weight was predetermined. The final weight of the container and the mucus was determined using a digital electronic balance and the difference between the final weight and the predetermined weight was taken as the weight of the mucus.

Pepsin binding activity

Pepsin binding activity of AEUCPF was determined as previously reported, [6,15] 50 ml of the aqueous extract was added to 1 mL of pepsin solution (2 mg/mL) in a test tube followed by the addition of 4 ml of 0.2 N HCl buffered with 1 ml of 0.2 N sodium citrate solution. Thereafter, 1 ml of bovine serum albumin (5 mg/mL) was added to treat the excess pepsin except the control test tubes. All reagents were kept at a temperature 37°C for 30 minutes prior to incubation and at the same temperature for 30 minutes after incubation. The remaining protein in each tube was treated with 1.0 ml of Biuret reagent and 5 ml 0.2 N NaOH solution. The absorbencies were read at 546 nm and the result was expressed as percentage binding of pepsin.

Phytochemical analysis

Preliminary phytochemical analysis of the extract was carried out using standard procedures for alkaloids, reducing sugars, tannins, flavonoids, saponins, steroids, and anthraquinones [16-18].

Statistical analysis

All values are expressed as mean ± standard error of the means (SEM). Statistical significance was determined using the Student's t-test. Values with $P < 0.05$ compared with the control group were considered as being significantly different.

RESULTS:

Anti-ulcer studies

The results of the anti-ulcer studies showed that gastric mucosa lesions were significantly ($p< 0.05$) reduced by all the doses of AEUCPF. The ulcer score was reduced by the 4.5ml/Kg from 3.6± 0.24 (control) – 0.70±0.37 in the HCl/Ethanol induced lesion (Table 1.). Likewise 4.5ml/Kg of AEUCPF significantly ($p< 0.05$) reduced the ulcer score from 3.8± 0.2 (control) - 0.9 ± 0.33 in the indomethacin induced gastric lesion (Table 2.). Fig. 1 shows the percentage protection of the mucosal by AEUCPF in the two models of ulcerogenesis.

Table 1: Effects of aqueous extract of unripe fruit of Carica papaya on HCl/Ethanol induced ulcer in rats

Groups	Dose (mL/Kg)	Ulcer index	Total Gastric acidity (µEq/mL)
Control (saline)	-	3.60 ± 0.24	62.2 ± 8.1
C. papaya	2.5	1.70 ± 0.44*	32.3 ± 7.8*
C. papaya	3.5	1.10 ± 0.51*	25.8 ± 1.2*
C. papaya	4.5	0.70 ± 0.37*	24.6 ± 2.5*
Cimetidine	11.5 (mg/Kg)	0.60 ± 0.19*	24.3 ± 7.2*

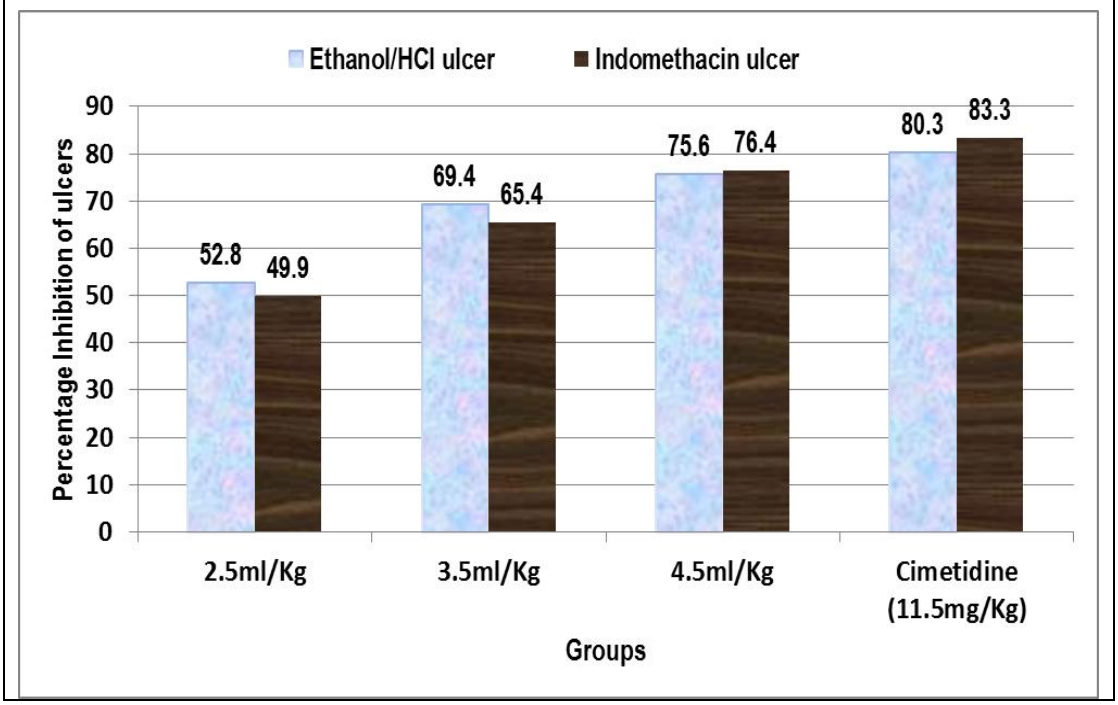
[a]Each value is mean ± S.E.M. for 5 rats. *P < 0.05 compared with control.

Table 2: Effects of aqueous extract of unripe fruit of Carica papaya on indomethacin induced ulcer in rats

Groups	Dose (ml/Kg)	Ulcer index	Mucus secretion	pH	Total Gastric acidity (µEq/mL)
Control (saline)	-	3.81 ± 0.20	11.10±5.51	2.28± 0.10	66.0 ± 2.9
C. papaya	2.5	1.91 ± 0.51*	12.51± 4.57	2.21± 0.19	24.2 ± 2.1*
C. papaya	3.5	1.32 ± 0.54*	9.69± 5.0	2.42± 0.19	12.4 ± 1.1*
C. papaya	4.5	0.90 ± 0.33*	11.3± 6.40	2.59± 0.09	22.1 ± 1.4*
Cimetidine	11.5 (mg/Kg)	0.75 ± 0.19*	54.21± 10.70*	2.68± 0.14	22.4 ± 0.7*

[a]Each value is mean ± S.E.M. for 5 rats.*P < 0.05 compared with control..

Fig 1: Percentage inhibition of ulcers by aqueous extract of unripe fruit of Carica papaya. Each value is the mean of 5 rats.



Gastric acidity, Pepsin binding and mucus production

Tables 1 and 2 show the results of the effects of administration of AEUCPF on gastric acid secretion in the HCl/ethanol and indomethacin induced lesions respectively. The acidity was significantly ($p < 0.05$) reduced by all the doses of AEUCPF.

AEUCPF produced 103.25% binding with pepsin. However, the decoction did not produce any significant changes in the mucus production in stomach of animals.

Phytochemical Analysis

The results of the phytochemical screening showed that the extract contains alkaloids, flavonoids, polyphenols, anthraquinones, reducing sugars, saponins and steroids

DISCUSSION:

It is indeed amazing why certain plants are used for the treatment of specific ailments. C. papaya is such a useful plant for traditional medical practitioners. The desire to unravel the usefulness of this plant has led us to previously investigate the anti-inflammatory, analgesic and anti-ulcer effects of the leaves based on ethnopharmacological information [9].

The present study investigated the gastroprotective effects of aqueous extract of unripe C. papaya fruit (AEUCPF) based on its use locally for the treatment of ulcer which included the administration of 96 hours soaked unripe fruit solution to treat ulcer patient for a period of ten days in the first instance. The doses chosen in the study were carefully calibrated to resemble the common doses used by the traditional practitioners where 3.5 ml/kg approximated the doses used twice per day and the 2.5 and 4.5ml/Kg were alternate doses used for comparison with the standard dosage of 3.5 ml/Kg. The two methods used for producing gastric lesion (HCl/Ethanol and indomethacin) are validated models [19-22].

The findings showed that administration of AEUPCF for ten days produced a dose dependent anti-ulcer effects in the HCl/ethanol induced ulcers with the minimum dose producing a percentage inhibition of 52.8% which is relatively high (Fig.1). The highest dose (4.5ml/Kg) of AEUPCF produced comparable anti-ulcer effects with the standard drug cimetidine (11.5mg/Kg). In the indomethacin induced gastric lesion AEUPCF also produced similar dose dependent pattern of anti-ulcer effects with highest dose of the extract producing effects that is comparable with that of cimetidine. The minimum dose also inhibited ulcerogenesis by 50% which is a value similar to what the equivalent dose produced in the HCl/Ethanol induced ulcer model. This showed that the results can probably be replicated in most models of ulcer caused by effects of diverse ulcerogens.

Prior treatment of the animals with AEUPCF before the administration of necrotizing agent was able to strengthen the gastric mucosa against the activities of necrotizing agents. Generally, necrotizing agents may produce gastric lesion by a combination of many factors which includes but not limited to the following; inhibition of prostaglandins (PGE2 and PGI2) synthesis especially with indomethacin a non-steroidal anti-inflammatory agents [23-25], promotion of acid-pepsin aggression on gastric mucosal [26-28] decrease in gastric mucosal barrier/resistance [26] and an increase in lipid peroxidation [26, 29], or the direct increase of gastric acid secretion. Observation in this study showed that AEUPCF use some of these mechanisms to inhibit gastric lesion hence its effectiveness in binding pepsin and reduction of gastric acidity. These help in strengthening mucosal barrier and reducing the direct effects of gastric acid on the mucosa. It can also counteract the effects of NSAIDS on prostaglandins synthesis. Mucus production seems not to be parts of AEUPCF mechanism of protecting gastric mucosal from insults of necrotizing agents as there was no significant changes in the mucus secretion in treated rats compared with the control.

The results of the phytochemical analysis also showed that the AEUPCF might be exerting it effects via its contents of flavonoids, alkaloids, anthocyanides or saponins. Flavonoids have been specifically linked with gastroprotective activities [4,30]. Likewise some alkaloids and saponins have been implicated as active principles responsible for gastroprotective activities of some plants such as Pyrenacantha staudii and Zizyphus sativa.[31-33]. The findings from the phytochemical analysis agree with that of Ezike et al,[12]. They used aqueous and methanol extract of unripe fruit of the plant but the dosages and preparation of the plant material was different from what was used in this study. While they used a single dose of 300 mg/Kg for both aqueous and methanol extract, we used three doses of aqueous decoction which was freshly prepared to simulate the practice by traditional medical practitioners and local users. Oduola et al, [8] also administered a decoction of the unripe fruit for the treatment of sickle cell disease. The ulcer models by Ezike et al, [12] and in this study were nearly the same with modifications in the dose of ulcerogen in indomethacin induced ulcer; however, they used absolute ethanol as the second ulcerogen while we employed HCl/Ethanol in our studies. Nevertheless our method of extraction is relatively easier for would be users of this plant product if it is to be consumed raw and Ezike et al, [12] recognized that water extraction is the preferred method by traditional practitioners. Therefore, the present study has thrown more light into the beneficial effect of the use of unripe and mature fruit of C. papaya.

CONCLUSION:

In conclusion, the overall finding of this study is that aqueous extract of unripe fruit of Carica papaya possess antiulcer properties which may be due to its ability to inhibit gastric acid secretion and reduction in pepsin activity and availability. It is a promising material for treatment of gastric mucosal injury and therefore further studies on this plant are encouraged.

ACKNOWLEDGEMENT:

Authors are grateful to Mrs F.E. Olawale-Bello for technical assistance.

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HOUSE TYPES AND DEMOGRAPHIC RISK FACTORS FOR SUSTAINED ENDEMIC FILARIASIS IN SOUTH-EASTERN NIGERIA

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ABSTRACT:

This study was aimed at assessing both the quality of housing in the coastal and upland areas of Eastern Nigeria using a predetermined three-category house type approach, as well as the distance of the respective houses from major vector breeding sites, in relation to the prevalence of microfilaraemia. From each community, all individuals who were more than one year old or resident in the area for at least a year were screened. The target population was 1000 persons each for coastal and upland areas. Houses in the areas were categorized into three main types. Type I: mud houses built with thatched roofs, small windows and yawning eaves that hold no barrier for mosquitoes; Type II: mud houses built with zinc roofing sheets; Type III: modern-style houses built and plastered with cement and having large doors and windows. A total of 855 houses were screened, out of which 191 (22.3%) were Type I; while 430 (50.3%) and 234 (27.4%) were Type II and Type III respectively. An average of three and four persons per house was recorded for the Upland and Coastal populations respectively. Familial clustering was the settlement pattern in the former, while houses were adjoined one to another in the latter. In the Upland area, 10.0% of the houses had at least one Wuchereria bancrofti microfilaraemia (Wbmf) positive individual, and this comprised of 8.7% harbouring one mf positive individual each, and 1.3% housing two Wbmf positive individuals each. In total, 52% of all positive microfilaraemia cases were from Type I while 39% and 9% of positive microfilaraemia cases were from Type II and Type III respectively. Microfilaraemia was significantly higher among those that lived in poorest quality house type, and in proximity to major vector breeding sites such as streams.

KEYWORDS: Human settlement, filariasis, house type, breeding sites, Nigeria

(Submitted March 2013 Accepted June 2013)

INTRODUCTION:

Bancroftian filariasis is a mosquito-transmitted parasitic nematode infection, which still ranks high globally among causes of debilitating morbidity in man [1]. Three decades ago about 20.6% of the world's population was at risk from lymphatic filariasis and the prevalence of infection was put at 9.2% [1]. It is one of the diseases of concern in the resource limited countries, particularly in sub-Saharan Africa, and is endemic in parts of Nigeria [2-6], where its public health implications have been of much concern to some scientists [6].

Although filariasis is preventable, the transmission and epidemiological quotients of the filarial infection appear to be sustained over a century in eastern Nigeria despite general improvements in basic hygiene [5]. This scenario therefore underlines the need to explore and understand other potential risk factors associated with Wuchereria bancrofti infection in the area, for further improvement in the control efforts. One of such possible risk factors is the house quality, which have strongly associated malaria transmission (vectored also by mosquito) with house types of residents in endemic areas [7-10]. Although malaria is caused by a parasitic protozoan Plasmodium, the fact that it is also transmitted by blood-sucking female mosquitoes, as is the case with Filariasis, suggests there could be similarities in vector ecology of these parasitic infections.

This study was aimed at assessing the quality of houses in the coastal and upland areas of Eastern Nigeria using a predetermined three-category house type approach, the distance of the respective houses from major vector breeding sites, and also to determine the proportion of positive microfilaraemics resident in each house type category.

SUBJECTS AND METHODS:

A total of eight communities were selected for the study in South-Eastern Nigeria. Two of the communities (Umuowaibu and Ndiorji) were chosen from the filariasis endemic Upland area, and are located near Okigwe in Imo State, from the Igbo speaking (a majority tribe) area of the country. Six communities (Oduoha, Rumuoro, Ahai, Rumuada, Okporoworo, and Rumuakani) were chosen from the filariasis endemic coastal rainforest area, and are from the Ikwerre speaking area near Port Harcourt in Rivers State, which is further south by the Atlantic Ocean.

Local authorities were consulted and properly briefed about the study, and these included the traditional heads (the Ezes of the Ndiigbo and the Nyewalis of the Ikwerre), the leaders of the respective Town Unions, and the officials of the two Local Government Areas (LGAs), Okigwe in Imo State and Emohua in Rivers State. Their consent was obtained, and their cooperation solicited in the mobilization of their subjects, before commencement of data collection. During the parasitological and clinical surveys, health personnel from the LGAs formed part of the team

to ensure compliance with approved safety and ethical stipulations.

All individuals in the selected communities who were more than one year of age were included in the study population, which comprised of natives and non-natives who had resided there for at least one year. The target population for each of the two geographical entities was 1000 persons. Houses were given identification numbers, and their appropriate locations noted; in addition markets, religious places, major roads and some track roads, as well as water bodies (such as rivers, streams, et cetera) in the respective communities were noted. Each consenting individual admitted into the study, was given a card bearing a personal identification number, and their residential number.

The houses were categorized into three main types:

House type I: Mud houses built with locally-made thatch roofs, normally with small windows, enormously yawning eaves that hold no barrier for mosquitoes.

House type II: Mud houses built with zinc roofing sheets. These houses were similar to Type I but may have ceiling that constitute barrier for mosquitoes gaining entrance through the ceiling.

House type III: Modern-style houses built and plastered with cement, having standard doors, windows, and good ceiling.

For the parasitological survey, about 50 µl blood was collected, after finger-prick, on a grease-free microscopic slide for a thick smear. It was stained with Giemsa using the standard methods of Uttah et al. [11].

Ethical approval was obtained from the Ministry of Health in the Okigwe and Emohua Local Government Areas for the studies in the Upland and Coastal rainforest areas respectively. The Ethical Committee of University of Port Harcourt (where the laboratory work was carried out) approved the protocols.

The Epi-Info version 6 .0 was used in entering data from parasitological survey, and SPSS for windows (1995 version) was used for data analysis. The geometric mean intensity (GMI) of microfilaraemia was calculated as antilog ($\sum \log (x+1)/n$), with x being the number of mf per ml of blood in microfilaraemic individuals and n was the number of microfilaraemic individuals examined.

The odds ratio (OR) of becoming microfilaraemic in the three categories of house Types (I, II, III), and in the Upland and Coastal rainforest areas was calculated. The geospatial distribution of houses with positive microfilaraemic cases in relation to proximity to major breeding sites was also assessed.

RESULTS:

When results from both the Upland and Coastal rainforest areas are pooled together, about 50% of all houses were of the house Type II category; house Types III and I accounted for 28% and 22% of the other houses respectively. Analysis of the data indicated that 52% of all wbmf positive

cases were living in House type I, 39% were living in House type II and 9% were living in House type III.

A total of 381 houses were recorded in the two Upland communities: 216 in Umuowaibu and 165 in Ndiorji, giving an average of three persons per house. A familial settlement pattern was evident as houses were in clusters according to familial relationships. Each cluster was separated from another by arable farmlands of varying sizes. About 53.5% of the houses were of the type II. Types I and II houses had kitchen just adjacent to the room belonging to the 'woman of the house' in the same building. At night, livestock, mostly fowls, goats and sheep, were kept in the kitchen. Most of the types 1 and II houses had just two living rooms each. Type II houses comprised 49% of all houses in the Upland area, while house type III and house type I made up 26% and 25% respectively.

In the coastal rainforest communities a total of 474 houses were recorded, giving an average of four persons per house. Ahai had the most number of houses (25.1%), while Okporoworo had the least (11.8%). Ahai had the least number of persons per house (3). The settlement pattern was urban with houses adjoining one another. The houses in the coastal area were relatively of better quality than those in the upland area in terms of number of rooms and modernization. However, all the three types of houses were represented. Type II houses comprised 51% of all houses in the Coastal rainforest area, while type III and type I houses made up 30% and 19% respectively.

The Prevalence and risk of Wbmf in relation to house types:

The overall 'House-prevalence' (proportion of houses with residing positive microfilaraemic persons) of Wbmf in both areas combined was 14.7% (Table 1). Wbmf Prevalence among type I occupants (34.6%) was significantly higher than among type II (11.4%) and type III occupants (4.7%, χ2-test; $p < 0.05$ for both) respectively. The risk of developing Wbmf was significantly higher among type I occupants than among occupants of other House types (OR 5.32, 95% CI 1.272 to 2.070). The risk of contracting Wbmf was reduced by 29% among type II occupants (OR 0.71, 95% CI 0.727 to 0.041), and by 78% among type III occupants (OR 0.22, 95% CI -2.167 to -0.889).

In the Upland area, the 'House-prevalence' for type I houses was 22.8%, which was significantly higher than 7.4% for type II and 2.4% for type III houses (χ2-test; $p< 0.05$). The risk of contracting Wbmf was high among type I occupants (OR 4.73, 95% CI 0.864 to 2.244), but reduced by 51% among type II (OR 0.53, 95% CI -1.319 to 0.049), and by 83% among type III occupants (OR 0.17, 95% CI -3.217 to -0.327).

In the Coastal rainforest area, the 'House-prevalence' for type I houses was 45.5%, which was significantly higher than 15.0% for type II and 6.0% for type III houses (χ2-test; $P < 0.05$ for all tests). The risk of developing Wbmf was high for the Type I houses (OR 6.43, 95% CI 1.354 to 2.368), but was reduced by 36% among type II

occupants (OR 0.64, 95% CI -0.919 to 0.027), and by 80% among type III occupants (OR 0.20, 95% CI -0.889 to -2.329).

Comparative assessment of risk of infection between upland and coastal settlements:

The result indicates that Wbmf prevalence was higher in the coastal settlements than in the upland areas in all categories of house types (χ2-test; $P < 0.05$). The risk of contracting Wbmf in the Coastal rainforest area was as twice high as in the upland area (OR 2.06, 95% CI 0.310 to 1.134, χ2-test; $p < 0.05$). Living in the upland area conferred a level of protection, reducing the risk of developing Wbmf by 51%.

Number of Wbmf positive cases per house in relation to house types:

In all, 12.9% of houses had one Wbmf positive case each, 1.8% had two Wbmf positive cases each, while 0.1% had three Wbmf positive cases each. There was no observation of four or more Wbmf positive cases per house. In the upland area, 8.7% of the houses harboured one Wbmf positive case each, 1.3% harboured two Wbmf positive cases each, but there was no case of three mf positive cases per house. In the Coastal area, 16.2% of the houses harboured one Wbmf positive case per house, which was significantly higher than recorded in the Upland area (χ2-test; $p < 0.05$). A prevalence of 2.1% for two positive cases of Wbmf per house was recorded, which was comparable to what was recorded in the Upland area (χ2-test; $p < 0.05$). Three positive cases of Wbmf per house were recorded in 0.2% of houses.

When all Wbmf positive cases from both the Upland and Coastal areas are pooled together, one-positive-case per house constituted about 87.3% (86.87% in Upland area; 87.5% in Coastal area), double-positive-cases per house made up 11.9% (13.2% in Upland area; 11.4% in Coastal area), while triple-positive-cases constituted 0.8% (0% in Upland area; 01.1% in Coastal area).

Spatial clustering of positive cases of Wbmf in relation to major vector breeding sites:

Partitioning of the upland and coastal areas into proximal and distant sections in relation to major breeding sites, mainly streams and rivers showed significantly higher prevalence of Wbmf in the proximal sections in both areas (χ2-test; $p < 0.05$) (Table 2). The Odds ratio to be infected with Wbmf in the areas proximal to vector breeding sites was high (OR 3.51, 95% CI 0.84 to 1.674), with a risk reduction of 72% in distant areas.

The Odds to be Wbmf infected was thrice as high in the proximal section as in the distant section in the upland areas (OR 3.13, 95% CI 0.388 to 1.894). Similar result was obtained in the coastal area except that the odds were four times as high in the proximal section as in the distant section (OR 4.05, 95% CI 0.891 to 1.907).

There was positive relationship between geographical locations of houses with positive cases of microfilaraemia. Houses that were proximal to major vector breeding sites, such as rivers/streams, produced most cases of positive microfilaraemia, and consequently houses that were farthest from the major vector breeding sites, presented least cases of microfilaraemia.

Table 1: Prevalence of filarial infection in relation to house quality in south-eastern Nigeria

Suburb	House type I		House type II		House type III		Total	
	Exam.	Inf. (%)	Exam.	Inf. (%)	Exam.	Inf. (%)	Exam.	Inf. (%)
Okigwe	92	21 (22.8)	204	15 (7.4)	85	2 (2.4)	381	38 (10.0)
Port Harcourt	99	45 (45.5)	226	34 (15.0)	149	9 (6.0)	474	88 (18.6)
Total	191	66 (34.6)	430	49 (11.4)	234	11 (4.7)	855	126 (14.7)

Legend: Exam: Number examined; Inf.: Number infected;

Table 2: Prevalence of Wbmf in relation to proximity of houses to vector breeding sites in south-eastern Nigeria

Area	Proximal	Distant	χ^2-test
Upland prevalence (%)	14.7 (28/190)	5.2 (10/191)	$p < 0.05$
Coastal prevalence (%)	29.9 (63/211)	9.5 (25/263)	$p < 0.05$
Total prevalence (%)	22.7 (91/401)	7.7 (35/454)	$p < 0.05$

DISCUSSION:

Shelter is essential to man for resting, recuperation and sustenance. Unfortunately, some adult vectors such as mosquitoes do rest in human shelters from where they bite the inhabitants. In some cases, favourable condition for completing their life cycle is provided by human shelter. Vector-borne diseases transmissions are effected in houses [12], and are directly influenced by housing pattern [13-14]. Several factors relating to human shelter do influence transmission of vector-borne infections. Results from this study show that the location of human shelter in relation to its distance from major vector breeding sites could increase or decrease the chances of contracting microfilaraemia. Living in distant places from rivers and streams which are major vector breeding sites in South-eastern Nigeria could confer some degree of protective advantage against contracting bancroftian filariasis, reducing the risk of contracting microfilaraemia, when

compared to living very close to the vector breeding sites. This is corroborated by findings elsewhere that Filariasis prevalence was significant among those living in close proximity to irrigated agriculture, the vector breeding site [15].

Results in the present study indicate that the prevalence of Filariasis was significantly affected by the house type in both the Upland and Coastal rainforest communities. Type I and Type II houses that are poor quality houses recorded significantly higher prevalence of Filariasis in both areas, compared to the Type III houses. This is congruent with findings reported elsewhere [16]. Significantly higher wbmf rates were found in huts/thatched house type, which are equivalent to type I in this study, than in better quality house types [16]. Higher densities of mosquitoes are generally found in poorly constructed houses such as Type I and Type II, than in well-constructed houses, the Type III [17]. House construction also plays important role in the vector resting preferences as poorly constructed houses, especially those made of mud, represented by Type I, avail more darker places for mosquito vectors [16,18]. Different housing structures have been found to post significant differences in vector density, infection rate, infectivity rate, and microfilaria prevalence [18]. The density of mosquitoes and transmission of filarial is highly correlated with the type of house construction standards [19]. Highest infection and infectivity rates were recorded from the poorly-constructed group of houses [20]. It was observed in Ghana that people living in poorly constructed and unscreened houses in slum areas of Accra were more exposed to mosquito biting than those living in more modern and salubrious areas, and this was attributed to house quality [21]. Health authorities in Awash Valley, Ethiopia, had difficulty in controlling malaria, another mosquito-borne parasitic infection, malaria. This was attributed to some factors, which included re-plastering of houses [22]. House types, standard, house density, and other factors such as the location can have profound effect on the prevalence of vector-borne diseases [16, 23]. Living in the poorest type of houses increases the risk of malaria 2.5 folds when compared with better houses constructed with complete bricks, plastered walls and tiled roofs, [7-10]. It is found that house structure with cross ventilation, white painted walls, meshed doors, and windows are most likely to reduce mosquito-resting places and filariasis transmission, as a large reduction of indoor biting could have a significant effect on reducing morbidity [18]. This indicates that there is a need to modify housing structures, to reduce the man-mosquito contact [24], which has a direct impact on the vector density and transmission dynamics of filariasis [18]. Another dimension to this is that poorly constructed houses are owned by low income people who normally live in less hygienic conditions and thus are more prone to the infection [19]. This may explain why low income people are more at risk to lymphatic Filariasis and the disease burden is relatively higher in this demographic group [25].

Another factor that could explain the relatively higher wbmf prevalence in Coastal rainforest than in the Upland area is possibly the higher average number of house inmates in the coastal area than the Upland area. Human population flocculation does affect vector efficiency in endemic areas as overcrowding increases the number of infective bites given by a single infected vector in its lifetime as less time would be needed for host seeking. This could be very significant in increasing transmission, and implies that less efficient vectors could become significant in a situation where all other factors favoured high transmission [26]. More persons per room at a particular time also attract more mosquitoes because they produce and release more carbon dioxide blooms [16].

The implication of the high frequency of infective bites per person is that for control of the parasitic infection to be achieved, there must first be drastic reductions in mosquito populations for there to be any effect at all [26]. In high density human populations, large numbers of positive cases of W. bancrofti infection are likely to be produced even when the biting rate of a vector is relatively low. However, each individual in such high density human population will have a relatively low risk of being bitten by the vector [27]. The variation in transmission of filariasis in a particular geographical zone depends on differences in the human and vector populations and on their degree of interaction [28]. If the human population exceeds a certain threshold determined by vector population, intensity of transmission is limited. This is because it would give a much lower fly-to-man ratio and this should result in a significant dilution of transmission with a much lower force of infection and less severe disease. On the other hand, a major fall in the human population density will intensify transmission and aggravate the severity of disease. For instance high onchocercal blindness rate of over 5% are generally found on small, isolated communities [29].

In conclusion, the important risk factors of Wbmf in the Upland and Coastal rainforest areas of South-eastern Nigeria are House Type 1 (mud houses built with thatched roofs with enormously yawning eaves), and proximity to vector breeding sites. Living in the Upland area tends to confer significant reduction in the risk of developing bancroftian filariasis in endemic south-eastern Nigeria.

ACKNOWLEDGEMENTS:

The officers in Ministry of Health in both the Okigwe Local Government Area and the Emohua Local Government Area are appreciated for their assistance in mobilizing the communities.

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PREVALENCE OF UNDESCENDED TESTES (CRYPTORCHIDISM) FROM BIRTH TO SIX MONTHS IN BENIN CITY, NIGERIA

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ABSTRACT:

Although an undescended testes (UDT) is the most common developmental anomaly of the urogenital organ in males, they have often been regarded as mild malformation and as a consequence have been poorly reported despite its association with increased risk of infertility and testicular cancer in later life. In Nigeria, data on its prevalence is scarce. The objective of the study was to determine the prevalence rates of undescended testes at birth, 3 months and 6 months of age respectively. A prospective hospital-based cohort study was conducted to determine the prevalence rates of UDT at birth, 3 months and 6 months of age. The infants were examined at birth for UDT, using standardized technique. The infants who were identified at birth to have undescended testes were re-examined at the ages of 3 and 6 months respectively. To minimize inter-observer error, one physician examined all the infants. The gestational age, birth weight and birth position of each the neonates were recorded. The maternal age, parity, educational attainment and occupation of the parents were noted. The season in which each of the infants was born was recorded. At birth, 2.8% (39 of 1,394) of live-born male infants had undescended testes (UDT). This prevalence rate declined to 1.2% at the age of 3 months and 0.6% at the age of 6 months with an overall spontaneous testicular descent rate of 79.5% (31 of 39 cases). The prevalence rates were significantly elevated for low birth weight and preterm infants. The frequency of spontaneous descent of the testes was higher in both low birth weight and preterm infants compared to normal birth weight and full-term infants respectively. Comparing twin and non-twin infants, the prevalence of UDT was 3.8% (2 of 52) versus 2.8% (37 of 1,342); $p>0.05$. At birth, low birth weight and preterm infants had a significantly higher prevalence than their full-term as well as preterm counterparts with preterm infants having a greater tendency to achieve spontaneous descent of the testicles than full-term infants.

KEY WORDS: Undescended testes, cryptorchidism, testicular ascent, prevalence, Nigeria.
(Submitted April 2013 Accepted June 2013)

INTRODUCTION:

At birth, undesended testes (UDT) or cryptorchidism is the most common developmental anomaly associated with the male external genitalia but it has often been regarded as mild malformation [1]. Despite the association of UDT with increased risk of infertility and testicular cancer in later life, it has not received sufficient attention [1,2]. Little has been published on the incidence and prevalence of UDT in Nigeria [3]. In addition, the presence of UDT at birth could point to the existence of serious endocrine or genetic disorder [4]. For instance, UDT has been linked to testosterone deficiency, complete androgen insensitivity syndrome, mixed gonadal dysgenesis and Klinefelter syndrome [4].

The process of testicular descent occurs in two phases (intra-abdominal, 8-15 weeks of gestation and inguino-scrotal, 28 weeks of gestation to birth) and is regulated by an interaction between hormonal and mechanical factors, such as testosterone, dihydrotestosterone, mullerian-inhibiting factor, the gubernaculums, intra-abdominal pressure, and the genitofemoral nerve [2,5]. Endocrine disruption of the function of the developmental genes, such as insulin-like factor 3 (INSL3) and homeobox (HOX) family have been linked to occurrence of cryptorchidism [5]. Usually, testicular descent is completed by the 35th week of gestation [5]. Therefore, most testes that are undescended at birth in preterm babies, descend spontaneously before the age of three months [6]. Although a variety of classification of UDT exists, the most useful is based on whether the testes are palpable or non-palpable on clinical examination [7]. Palpable undescended testes are either retractile, ectopic or truly undescended within the canal [7]. Non-palpable testes may be truly undescended (intra-canalicular or intra-abdominal) or are absent [7]. The consequences of UDT include infertility, testicular cancer, associated hernia, torsion of the cryptorchid testis, and the possible psychologic effects of an empty scrotum [2].

The prevalence of UDT at birth varies from 1.6% to 9.0% among male infants [7,8]. Different prevalence rates have been reported from different countries. For instance, at birth, 2.4 -9.0% in Denmark [9,10], 2.4% in Finland [9], 3.7% in USA [11], 5.9% in United Kingdom [12], 4.8% in Malaysia [13] and 5.7% in Lithuania [14]. However, many of these studies did not state the case definition and the examination techniques employed in their study [9,10,14]. It is well established that in epidemiological studies standardized diagnostic criteria and examination techniques are required to obtain reliable data [1,15]. Approximately, two-third of infants with UDT at

birth will have descent of the testicles into the scrotum by the age of 6 months [6]. In most cases, the UDT descend spontaneously during the first 3 months of life with the incidence of UDT dropping from 1.5% to 0.8% between the ages of 3 and 12 months [8,,11,14]. If the testes have not descended by the age of six months, spontaneous descent occurs only very rarely, indicating that the strategy of watchful waiting after the age of six months is no longer recommended [11,16]. It is estimated that 6% to 7% of siblings of boys with UDT may have similar problem with testicular position and descent, indicating that the presence of UDT in one infant could serve as a window for identifying other male siblings with similar clinical conditions [9,14]. Estimates indicate that the heritability of cryptorchidism in first degree relatives is 0.67 [16]. In Nigeria, data on the prevalence and incidence of cryptorchidism is scarce. Although in a recent study in Nigeria the prevalence of UDT was 0.82% among primary school children, the question of its prevalence at birth still remains unanswered [3]. A research of the literature did not reveal a Nigerian study on the prevalence and incidence UDT from birth to the first six month of life. This lack of published data prompted us to conduct this study.

The purpose of the present study was to determine the prevalence rates of UDT at birth, at 3 months and 6 months of age.

PATIENTS AND METHODS:

In this prospective hospital-based cohort study, a total of 1,394 consecutive live-born male neonates delivered between 1st January, 2010 and 31st December, 2011 at St Philomena Catholic Hospital (SPCH), Benin City were examined at birth for testicular position, using standard techniques [15]. The examination took place in the postnatal ward at room temperature with the neonate lying in supine position. The testicular examination of the infant involved a two-handed technique. The examining hand is gently swept along the inguinal canal, starting at the superior-lateral extent of inguinal canal. A true undescended or inguinal testicle will be felt to “pop” under the examiner’s fingers during this maneuver. A retractile testicle will be felt by the opposite hand as it is manipulated into the scrotum. The ectopic testicle will immediately spring out of the scrotum when it is released whereas the retractile testis will remain in the scrotum. The position of the testis was recorded after its manipulation to the most distal position along the normal pathway of anatomical descent without forced traction. In this study, the position of the each testis was categorized into two major group as normal (if they were either normal scrotal or normal retractile) or undescended.

The undescended group was sub-classified into prescrotal (if they were high scrotal or suprascrotal), inguinal or non-palpable testes

[17]. Where a testis cannot be palpated in the inguinal canal or the scrotum or in ectopic sites such as the femoral region or perineum, evaluation for non-palpable testis was initiated, including inviting the urologist. The laterality of the UDT was recorded (right, left, or bilateral). All the examination for testicular position was performed by one specialist paediatrician to minimize inter-observer errors. Infants who were identified at birth as having UDT were re-examined at the ages of 3 and 6 months. The birth weight and birth order of each of the neonates was recorded. The gestational age was assessed using as an appropriate antenatal ultrasonography report performed between 18 and 20 weeks of gestation, data on last menstrual period or Dubowitz score [18] postnatally. Infants born before 37 completed weeks of gestation were considered preterm. A small-for-gestational age infant was defined as that whose birth weight for gestational age was below the 10th percentile. Any testis that could not be manipulated into a stable scrotal position was considered undescended. The maternal age and parity were also recorded. The socio-economic status of the parents was determined using the classification suggested by Ogunlesi et al [19].This was determined by combining the highest educational attainment, occupation and income of the parents (based on the mean income of each educational qualification and occupation). In this Social Classification System, classes I and II represent high social class, class III represents middle social class while classes IV and V represent low social class. In this way, the women were categorized into high, middle and low socio-economic groups. The deliveries were recorded according to the month and year of delivery to allow for assessment of influence of season. The season was categorized into two; dry season (November to April) and wet season (May to October). The infants were examined for the presence of other congenital malformation of the genitalia, particularly hydrocoele and hypospadias.

Ethical clearance and approval for the study were obtained from the appropriate hospital authority. Permission to examine the infants was obtained from the parents after explaining the purpose and the potential benefits of the study to them. The data was analyzed using SPSS for Windows version 11.0 (Chicago, IL USA). Where applicable and appropriate, descriptive statistics such as frequencies, means, odd ratios, standard deviations, confidence intervals, percentages were used to describe all the variables.

RESULTS:

During the two-year study period there were 2,688 deliveries, resulting in 2,741 live-born babies (1,394 males and 1,347 females); giving an overall male-to-female ratio of 1.03:1. Of the 2,688 women who delivered, 53 (2.0%) had twins with gender-pair distribution of 20(37.7%) different-gender, 17 (32.1%) female same-

gender and the remaining 16 (30.2%) male same-gender pairs.

Fifty two (3.7%) of the 1,394 males were derived from the twin deliveries. Among a total of 1,394 male live-born infants, 39(2.80%; 95% Confidence Interval, CI= 2.75-2.85) had undescended testes (UDT) or cryptorchidism at birth. The testes were palpable in 89.7% (35 of 39 cases) and non-palpable in 10.3% (4 of 39 cases). At the age of 3 months, the testes remained undescended in 17(43.6%) of the 39 babies identified at birth. In eight (20.5%) of the 39 cases, the undescended testes persisted till the age of six months.

Table 1 shows that 56.4% (22 of 39 cases) of the infants with UDT at birth had descent of the testicles into the scrotum by the age of 3 months while an additional 23.1%(9 of 39 of cases) had descent of the testicles into the scrotum at the age of 6 months, giving an overall spontaneous descent rate of 79.5% (31 of 39 cases).

Table 1: Prevalence of undescended testes (cryptorchidism) at birth, 3 months and 6 months of age

Age	Study population	Number with UDT	Prevalence (%)	95% CI
At birth	1,394	39	2.80	2.75-2.85
At 3 months	1,394	17	1.22	1.16-1.24
At 6 months	1,394	8	0.57	0.55-0.59

UDT = Undescended testes

Table 2: Prevalence of undescended testes (cryptorchidism) at birth according to birth weight and gestational age

Variable	Number (%)	Prevalence: N (%)	Odd ratio (95% CI)
Birth weight < 2500g	190 (13.6)	14 (7.4)	0.08
Birth weight ≥2500g	1204 (86.4)	25 (2.1)	(0.04-0.14)
Gestational age <37 weeks	166 (11.9)	11 (6.6)	2.90
Gestational age ≥37 weeks	1228 (88.1)	29 (2.4)	(1.44-5.99)
Small-for-gestational age	77 (5.5)	3 (3.9)	1.40
Appropriate-for-gestational age	1317 (94.5)	36 (2.7)	(0.43-4.79)

Table 3: Distribution of testicular descent at 3 and 6 months of age among infants with undescended testes (cryptorchidism) detected at birth

Variable	Testicular descent at 3 months of age (n=22) No (%)	Testicular descent at 6 months of age (n=31) No (%)
Birth weight < 2500g	14(63.6)	19(61.3)
Birth weight ≥2500g	8(36.4)	12(38.7)
Gestational age < 37 weeks	18(81.8)	22(71.0)
Gestational age ≥37 weeks	4(18.2)	9(29.0)
Small-for-gestational age	1(4.5)	2(6.5)
Appropriate-for-gestational age	21(95.5)	29(93.5)

As shown in Table 2, low birth weight (birth weight < 2500g) and prematurity (gestational age < 37 weeks) were the significant risk factors associated with undescended testes. Comparing twin and non-twin infants, the prevalence of UDT was 3.85% (2 of 52) versus 2.76% (37 of 1,342); Z-statistic = 0.372, p>0.05. The frequency of low birth weight (birth weight < 2,500g) was 46.2% (24 of 52) in twins and 8.0% (107 of 1,342) in singletons. The frequency of preterm delivery was 51.9% (27 of 52) in twins and 11.9% (160 of 1,342) in singletons.

As depicted in Table 3, the frequency of spontaneous descent of the testes was higher in both low birth weight and preterm infants compared to normal birth weight and full-term infants respectively.

The overall mean maternal age and parity in this general obstetric population was 27.8±4.6 years and 1.5±1.2 respectively. When the mean maternal age of infants with UDT was compared with that of infants without UDT, it was 28.2±4.4 years versus 28.6±4.7 years t=0.780 p>0.05. Comparing the mean maternal parity of infants with UDT and infants without UDT, it was 1.5±1.4 versus 1.4±1.6 t= 0.438 p>0.05. Among the 1,394 male live-born infants, 701(50.3%) were born during the dry season while the remaining 693(49.7%) were born during the wet season. Of the 39 infants with UDT at birth, 21(53.8%) were born during the dry season while the remaining 18(46.2%) were born during the wet season. Comparing the prevalence of UDT during the dry and wet seasons, it was 3.0% (21 of 701 infants) versus 2.6 % (18 of 693 infants) X^2 =0.203 p>0.05. The distribution of socioeconomic status (SES) of this general obstetric population was as follows: high SES 20.0% (538 of 2,688 mothers), middle SES 25.0% (672 of 2,688 mothers) and low SES 45.0% (1,433 of 2,688 mothers) respectively. The prevalence of delivery of infants with UDT according to SES was as follows: high 1.7 % (9 of 538 mothers),

middle 1.9% (13 of 672 mothers), and low 1.2% (17 of 1,433 mothers).

Of the 35 infants with palpable UDT, 18(51.4%) were prescrotal (high scrotal or suprascrotal), 17(48.6%) were inguinal (variously located along the inguinal canal), and none was ectopic. In 65.7% (23 of 35 unilateral cases) it was on the right side while in 34.3% (12 of 35 unilateral cases) it was on the left side. Only in one (2.6%) of the 39 cases of UDT was it bilateral and non-palpable with a normal renal ultrasound scan result. The patient with bilateral non-palpable testes was a low birth weight infant and did not have clinical features suggestive of Prune belly syndrome, ambiguous genitalia or Prader-Willi syndrome and was referred to the Paediatric Surgeon/Urologist for further investigation.

DISCUSSION:

The prevalence (2.8%) of UDT, at birth, being reported here is comparable to 2.4% separately reported from Finland and Denmark [9,10] but lower than the 3.7%, 4.8%, 5.7% and 5.9% reported from the USA, United Kingdom , Lithuania and Malaysia respectively [11-14]. This finding is not surprising as it reflects the known variability in prevalence rates from different countries. This country-to-country variability in prevalence has been attributed to genetic factors as well as environmental factors like the endocrine disruptors and lifestyle (cigarette smoking and alcohol consumption during pregnancy) [7,20]. On the other hand, the case definition, the population characteristics and the examination technique used in a given study may influence the observed prevalence in that study [21], making comparison of prevalence rates between studies difficult.

At the age of three months, the prevalence of UDT in the present study was 1.2% which was lower than the 1.9% and 2.4% found in Denmark and in the United Kingdom respectively [9,12] but slightly higher than 1.0% found separately in Finland and USA [10,9]. In another study in Oxford by the John Radcliffe Hospital Study Group (1984-1988) in which they examined a cohort of 7,500 consecutive newborn boys the prevalence at birth was 4.1% but dropped to 1.6% at the age of three months [22]. As in other studies [10,11], majority of the spontaneous descent of the testis in infants with UDT occurred in the first three months of life. This drop in prevalence of UDT by the age of 3 months has been attributed to short-term postnatal endogenous testosterone secretion [23].

In the present study, the prevalence of UDT dropped further to 0.6% at 6-month assessment. Considering the dynamics of testicular descent, the significance of the prevalence at 6-month assessment is that the figure might represent the true birth prevalence of cryptorchidism. A similar view was also expressed by Abdullah et al [24] who reviewed the prevalence of cryptorchidism and

hypospadias in northern England, using data derived from northern region hospital episodes statistics (HES) (1993-2000). This view is based on the knowledge that after the age of 6 months spontaneous testicular descent is unlikely [4].Comparison with previous studies was not possible because they did not report on 6-month assessment [5,7,8]. However, in a study in southern Nigeria, the prevalence of cryptorchidism among primary school children aged 5 to 13 years was 0.82% [3]. This prevalence is higher than the 0.6% observed at the age of 6 months in the present study. The observed difference is intriguing in that it might, at least indirectly, represent the prevalence of testicular ascent (acquired cryptorchidism) among children in southern Nigeria. There is absolutely no published data on prevalence of testicular ascent in Nigeria. Testicular ascent (acquired cryptorchidism) is a well-documented phenomenon [15]. Our data indicated that the UDT persisted in 20.5% of cases after 6 months. This finding is comparable to 23.5% reported by Thong et al [11]. The explanation for the persistence of UDT in some cases may be found in the report of Ferlin et al [25]. In that study, they reported that boys with persistent cryptorchidism had a 17-fold greater odd of having a genetic alteration such as Klinefelter syndrome or mutation in the INSL3 receptor genes. However, such a conclusion cannot be derived from the present study as it was not designed to assess the reason for persistence of cryptorchidism beyond the age of 6 months.

Data from the present study revealed that the prevalence of UDT was significantly elevated for low birth weight (LBW) and preterm infants. This finding is in consonance with the report of previous studies [8,9] . One possible explanation in the case of preterm infants is that the infant may not have achieved full descent of the testes before delivery. Testicular descent is usually completed at 35 weeks of gestation [6]. A similar argument holds true for LBW infants as many of them were preterm. In addition, Abdullah et al [24] in their report suggested that fetal androgen dysfunction might play a role in the aetiologic link between LBW and the occurrence of cryptorchidism. Although twin gestation is associated with increased frequency of delivery of LBW and preterm infants, the risk of UDT does not appear to be higher in twins compared to singletons. A study in Denmark has reported a similar finding [8]. The reason for the lack of significant bearing between twins and frequency of UDT despite the established higher incidence of LBW and preterm infants in twin gestations is not clear. The social class, maternal age, parity, mode of delivery and the season in which a baby is born had no bearing with the frequency of occurrence of UDT in the present study. This finding is largely similar that reported in previous studies [8,10,12]. In contrast, Berkowitz et al [26] reported a positive correlation between the mode of delivery (Caeserean section) and the season in which a baby was born with frequency of UDT. In that

study, the frequency of UDT was found to be higher between September and November as well as between March and May. The reason for this opposite finding between our study and that of Berkowitz et al [26] is not clear.

In the present study, some laterality in occurrence of UDT was demonstrated. The frequency of right-sided UDT was higher than left-sided UDT. This observation is in consonance with the report of previous studies [27]. In only 2.6% of cases were UDT bilateral in the present study. This is lower than the 8.5% prevalence rate of bilateral UDT reported by Boisen et al [9]. The difference may be a reflection of the higher frequency of abnormal testicular function reported among Danish men [9]. Such an increased frequency of abnormal testicular function has not been reported among Nigerian men. There is no readily available explanation for the observed laterality in UDT in the present study. The only infant with bilateral UDT had a non-palpable type, indicating the need to exclude bilateral anorchia. Bilateral anorchia is characterized by elevated Lutenizing hormone and Follicle stimulating hormone, absence of detectable Mullerian inhibiting substance and inhibin B. A rise in serum testosterone level following βHCG stimulation reflects the presence of functioning testicular tissue. However, we were unable to differentiate between bilateral cryptorchidism and bilateral anorchia because of poor laboratory facility for measuring these biochemical parameters. This scenario depicts one of the challenges experienced in the practice of paediatric endocrinology in a developing country. However, in keeping with the current consensus statement on management of UDT, the parents of all infants with UDT were informed of the findings, while those infants whose testes remained undescended at the age of six months were referred to the Paediatric Surgeon/Urologist. For those infants with UDT at birth who achieved spontaneous descent of the testicles by the 6-month assessment (or were classified as retractile testes), the parents were advised that annual follow-up will be needed throughout childhood because there is a significant risk for re-ascent [7].

One limitation of the present study is that it is based on data from only one hospital. A multicentre study in future will ensure a larger and a more representative study sample, allowing for generalization of the conclusion as it relates to the country (Nigeria). This is important, given that different prevalence rates have been reported from different countries [10-14]. On the other hand, different prevalence rates have also been observed within the same country [1,27], making the present study relevant because it provided a prevalence rate from one of the regions of Nigeria. Hopefully, the data from the present study will encourage clinicians to examine and document in details the external genitalia findings during a routine newborn physical examination, thereby improving the standard of clinical practice in

Nigeria. UDT should normally be diagnosed during the routine newborn examination as its recognition at this time is an important step in preventing adverse consequences [15].

CONCLUSION:

In conclusion, at birth, low birth weight and preterm infants had a significantly higher prevalence than their full-term as well as preterm counterparts with preterm infants having a greater tendency to achieve spontaneous descent of the testicles than full-term infants.

ACKNOWLEDGEMENT:

We would like to thank members of staff of the records department of SPCH who ensured that all the subjects in this study were correctly directed to us by painstakingly following the list and appointment dates.

There was no conflict of interest as we did not receive any fund or benefit to conduct this study.

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ASSESSMENT OF THE FULL BLOOD COUNT PARAMETERS OF HIV/AIDS PATIENTS IN HEDURU CLINIC, PAPUA NEW GUINEA

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ABSTRACT

Abnormal haematology parameters in patients with HIV / AIDS are common. Anaemia is a frequent complication among these patients and it is usually associated with serious complications. Neutropenia and eosinophilia are common in the advanced stages of AIDS. It is therefore important to determine the haematology parameters in HIV /AIDS patients. This prospective study was carried out between July and September 2008. A total of 113 case notes of HIV / AIDS patients attending the Heduru Clinic were randomly selected. Their certified full blood count electronic results were obtained from the Haematology laboratory in Port Moresby General Hospital. Detailed re-examination of the fixed stained peripheral blood film of each patient was carried out using high-powered microscope. Gender distribution of the 113 case notes indicated 46 (41%) males and 67 (59%) females. Analysis of the data indicated high frequency of anaemia among the patients. Microcytic hypochromic anaemia was prevalence among 47.8% of the patients, macroscopic hypochromic anaemia 29.2% and normochromic normocytic anaemia 23.0%. Blood film showed presence of anisocytosis and poikilocytosis. Although only one patient had leucocytosis, leucopenia was prevalent among 20.4% of the patients; of these mild leucopenia was more frequent than moderate leucopenia. A total of 51 (45.1%) patients had Eosinophilia; of these 76.5% had mild eosinophilia, 17.6% moderate eosinophilia and 5.9% marked eosinophilia. Thrombocytopenia was observed in 21.2% and 0.9% with induced pseudothrombocytopenia. It is hoped that these findings will serve as base line for more detailed studies, and support the need to strongly advocate for routine monitoring of full blood count haematological parameters of HIV/AIDS patients in Papua New Guinean.

KEYWORDS: HIV/AIDS, Anaemia; Lymphocytopenia, Eosinophilia, Thrombocytopenia, Neutropenia, Monocytosis

(Submitted November 2012 Accepted June 2013)

INTRODUCTION

The full blood count (FBC) refers to the different types of cells in the blood [1]. FBC is one of the important laboratory investigations in HIV/AIDS patients because of the reported high prevalence (30 to 40%) of anaemia, leucopenia and thrombocytopenia [2]. In anaemia of chronic diseases caused by HIV/AIDS there are reported variations in the parameters and functions of erythrocytes, leucocytes and thrombocytes [3]. Some researchers indicated that microcytic and macrocytic hypochromic anaemia [4] are linked with the HIV and the managements for HIV/AIDS [5,6]. Papua New Guinea (PNG) the prevalence of anaemia, lymphocytopenia, eosinophilia, thrombocytopenia, neutropenia and monocytosis among HIV/AIDS patients attending the weekly HIV Heduru clinic in Port Moresby General Hospital (PMGH) have not been fully investigated.

The major aim of this study was to access the prevalence of cytopenia and cytophilia in HIV/AIDS patients attending Heduru clinic in PMGH.

The objectives were to use the case notes at Heduru Clinic and FBC parameters electronic results in the PMGH Haematology laboratory to determine if microcytic hypochromic anaemia, macroscopic hypochromic anaemia, normochromic normocytic anaemia, leucopenia, lymphocytopenia, eosinophilia, thrombocytopenia, neutropenia and monocytosis are prevalent in HIV/AIDS patients in PNG.

SUBJECTS AND METHODS:

This was a retrospective study carried out between July and September in 2008. The study site was the Heduru Clinic, which is the major sexually transmitted disease clinic in PMGH the major general and referral hospital in the National Capital District (NCD) PNG.

A total of 113 randomly selected case notes of HIV/AIDS patients in Heduru Clinic and their corresponding FBC parameters electronic results in the PMGH Haematology laboratory were obtained. The fixed stained peripheral blood film for each patient was obtained and re-examined in detail with high-power microscopice for red blood cell morphology, leucocyte morphology and platelet morphology. The re-examination of each slide was carried out by two qualified laboratory scientists. The independent findings in each slide were compared before accepting the final result.

Ethical clearance and permission for this study was obtained from the School of Medicine and Health Sciences (SMHS), University of Papua New Guinea (UPNG) ethics and research grant committee and the authorities in PMGH.

RESULTS

The age range of the 113 patients was 10 to 60 years. Gender distribution of the patients indicated 67 (59.3%) females and 46 (40.7%)

males. Among the female patients 37.3% were in the 20 to 29 years age group and 40.3% in the 30 to 39 years age group. For the male patents 21.7% were in the 20 to 29 years age group and 41.3% in the 30 to 39 years age group. Using the World Health Organization (WHO) cut-offs for anaemia [7], indicated a prevalence rate of 31.0% among all the patients; of these 28.6% had severe anaemia. Gender distribution of the 35 anaemic patients indicated 67.7% females and 34.3% males. Among the female patients 51.1% had mild anaemia and 17.4% had severe anaemia; For the male patients 41.7% had mild anaemia and 50.0% had severe anaemia.

Blood film from the 113 patients showed variation in in size (anisocytosis) and shape (poikilocytosis) that are typical of different anaemia. Microcytes, target cells, stomatocytes and elongated cell such as ovalocytes were more frequent than the macrocyte, target cells and stomatocytes. In addition red cell morphology was variable with majority microcytic hypochromic (47.8%), followed by macrocytic hypochromic (29.2%) and normochromic normocytic (23.0%).

Total leucocyte counts were graded as normal (4-11 x 109/L), mild leucopenia (3-3.9 x 109/L), moderate leucopenia (2-2.9 x 109/L), severe leucopenia (<1.9 x 109/L) and leucocytosis (>11 x 109/L). Although only one patient had leucocytosis, 20.4% had leucopenia. Mild leucopenia was common than moderate leucopenia. Immature granulocytes were identified in two patients.

Neutropenia, defined as less than 2.5 x109/L of neutrophil absolute value occurred in 34.5% of the patients and neutrophilia (more than 7.5 x 109/L) was observed in only 0.9% of the patients. Hypersigmented neutrophils occurred in the macrocytic hypochromic anaemia.

Lymphocytopenia, defined as less than 1.5 x109/L of lymphocyte absolute value occurred in 67.30% and lymphocytosis in 1.7% of the patients. Atypical lymphocytes occurred in 3.5% of the patients.

Monocytosis, defined as more than 0.8 x109/L of monocyte absolute value occurred in 5.3% of the patients.

Eosinophilia, defined as more than 0.44 x109/L of eosinophil absolute value was prevalence in 45.1%) of the patients; of these patients 74.5% had mild eosinophilia, 19.6% with moderate eosinophilia and 5.9% with marked eosinophilia.

Platelet counts were graded as normal (150-400 x 109/L), mild thrombocytopenia (100-149 x 109/L), moderate thrombocytopenia (50-99 x 109/L), severe thrombocytopenia (< 50 x 109/L) and thrombocytosis (>400 x 109/L).

Normal platelet count was observed in 77.0%, and thrombocytopenia in 21.2% of the patients. Mild thrombocytopenia (100-149 x 103/µl) was common (79.2%) than moderate thrombocytopenia (50-99 x 103/µl) (20.8%). Six had abnormal distribution of platelets that appeared anisothrombocytes morphologically. Mild to moderate thrombocytosis was observed in 3.0% of the female patients.

DISCUSSION:

HIV/AIDS is still a global health crisis with majority of the patients in the developing countries [8,9]. Although haematological abnormalities are common manifestations of HIV/AIDS patients, and may have considerable impact on their well-being, treatment and care, very few studies on haematological parameters have been undertaken in PNG. Our findings are supported by prior investigations of adult patients with neutropenia [1,10-12], thrombocytopenia [6,13-14] and lymphopenia [4,15]. The data indicated that HIV/AIDS was common among the so called early adulthood (age group 20 to 40 years) rather than the premature adulthood (age group 10 to 19 years). The gender distribution observed in this study is similar for other sexually transmitted infections, for example gonorrhoea patients attending the same clinic.

In this study anaemia was prevalent among the patients. Majority of the patients had mild anaemia with haemoglobin 8-14 g/dl for men and 8-12 g/dl for women rather than severe anaemia defined as haemoglobin less than 8 g/dl for both males and females.

The severity of anaemia in the present study ranges from microcytic hypochromic anaemia, macrocytic hypochromic anaemia and normochromic hypochromic anaemia. The findings are supported by other studies reported for adult patients with anaemia [17-19,20].

Blood film from the 113 patients in this study showed anisocytosis and poikilocytosis found in different anaemia. Microcytes, target cells, stomatocytes and elongated cell especially ovalocytes were more frequent than the macrocytes, target cells and stomatocytes. These findings are found in iron deficiency, anaemia of chronic disease and other nutritional deficiency including folate or vitamin B12 deficiency [1,3,21]. Ovalocytes has been reported to be common among individuals in Melanesian and Asian countries and protects against malaria [22].

Mild leucopenia was common in among the patients. Differential counts absolute values revealed lymphocytopenia (67.3%) and eosinophilia (45.1%) were more common followed by neutropenia (34.5%) and monocytosis (5.3%), these findings are similar to those reported by other researchers [10-11,18-19,23-24]. Hypersigmented neutrophils are an early sign of megaloblastosis associated with nutrition deficiency such as folic acid or vitamin B12. The high level of neutropenia observed in this study is common in HIV /AIDS patients; this is similar to findings reported by other researchers [10-11,23-24, 25]. Anaemia and neutropenia in female HIV/AIDS patients were strongly associated with lower CD4 cell counts [23], in this study the CD4 count was not available to indicate its association to decrease level of neutrophil absolute value. However, the normal functions of neutrophils will be affected as a result of underlying opportunistic bacterial infections. Neutropenia, due to bone-marrow suppression caused by anti-HIV drugs and the virus infecting haemopoietic progenitor cells have been reported [3,18]. Antigranulocyte antibodies

have been described in HIV/AIDS patients [26], and neutropenia observed in these patients may be attributed to decreased production of granulocyte colony-stimulating factor [27]. Although only one patient was found with neutrophilia, in some cases the presence of neutrophilia was most probably due to stress and drugs [3].Stress and drugs triggers the haemopoietic granulocyte series to proliferate, differentiate and into mature neutrophils have been reported [27]. Protozoa and helminthes opportunistic infections are commonly associated to HIV/AIDS [20]. Therefore it is not surprising to observe a high number of eosinophil counts in the present study. Monocytosis is associated with chronic infection such as tuberculosis and efforts should be made in microbiological monitoring of ART for wide variety of bacterial and fungal common opportunistic pathogens in the HIV/ AIDS patients [3,28]. Primary infection of HIV associated with heterophil-negative mononucleosis can be seen in high titer viraemia. Thrombocytopenia was found in 21.2% of the patients; this finding is similar to other studies elsewhere [20, 29]. Platelet number and morphology findings revealed mild thrombocytopenia was common than moderate thrombocytopenia. Some of the thrombocytopenic patients had abnormal platelet distribution and when examined morphologically were diagnosed with anisothrombocytes. Abnormal platelet distribution may be associated with splenomegaly and liver disease [1], which is not surprising for some HIV/AIDS patients. Thrombocytopenia in splenomegaly is actually platelet pooling apart from normal 30 percent of platelet in the spleen. Anisothrombocytes is found in Wiskott-Aldrich syndrome and its presence in HIV/AIDS patients is uncommon. Few of the patients in the present study had very mild thrombocytosis suggesting a reactive thrombocytosis rather than essential thrombocytosis myeloproliferative disorders [3].

It has been reported [15] that HIV/AIDS patients on HAART were the category with mild to moderate to even severe thrombocytopenia. Mild thrombocytopenia may be due to HIV infection of megakaryocytes that express a functional CD4 molecule [15]. In the present study mild thrombocytopenia was found in patients with microcytic hypochromic anaemia.

CONCLUSION:

Anaemia, leucopenia, eosinophilia, thrombocytopenia, neutropenia and monocytosis were prevalent among HIV/AIDS patients in the present study. Anaemia was prevalent in the patients, especially those with microcytic hypochromic anaemia, megaloblastic anaemia and normochromic normocytic anaemia. Although neutropenia and monocytosis are associated with opportunistic and chronic infections, mild thrombocytopenia was more common than moderate thrombocytopenia.

As anaemia, leucopenia and eosinophilia are the most common FBC abnormalities observed in the present study it is important to routinely assess these parameters for timely and adequate clinical management of paitents in Heduru clinic in PMGH.

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USING ENZYME IMMUNOASSAY TO ASSESS THE PREVALENCE OF HELICOBACTER PYLORI IgG IN SALIVA AND BLOOD PLASMA

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(This project was funded by Colgate-Palmolive PNG Ltd)

ABSTRACT:

Helicobacter pylori (H. pylori) are gram negative bacteria that are strongly associated with gastro-duodenal disorders and some extra-intestinal manifestations, such as chronic bacterial infection. Dental plaque has been implicated as possible reservoir for H. pylori in individuals with and without gastric or duodenal disorders. Early detection and management of H. pylori can effectively reduce the prevalence of their pathologic effects and frequency of transmission. Non-invasive and inexpensive methods for detection of H. pylori can be used for screening of those at risk in the population. The major objective of this study was to assess the prevalence of H. pylori in saliva and plasma samples of residents in the National Capital District, Papua New Guinea. Subjects for this prospective cross-section study were selected from patients and their relatives attending dental clinics in Port Moresby General Hospital and St John's Hospital Gerehu. Saliva and blood samples were collected from randomly selected subjects after obtaining their signed informed consent. Solid phase Enzyme-Immuno-Assay (EIA) commercial kits were used for the estimation of IgG antibodies against H. pylori in human saliva and plasma. The guidelines and cut-off index indicated by the manufacture of the EIA kits were used for interpretation of the data. The results indicated that of the 204 saliva samples 183 (89.7%) were negative, 15 (7.4%) were equivocal and 6 (2.9%) were positive for H. pylori IgG. Subjects in the 40 – 49yrs age group had the highest positive (2.0%) prevalence for H. pylori IgG. Results obtained for 44 plasma samples collected, indicated that 19 (43.2%) were negative, 11 (25.0%) were equivocal and 14 (31.8%) were positive for H. pylori IgG. Comparison of the data indicated statistically significant difference ($p = 0.01$) between the results obtained for the plasma and corresponding saliva samples. A statistically significant positive linear correlation was obtained between the H. pylori IgG in saliva and plasma samples (Spearman $\rho = 0.514$, $p = 0.01$). The results indicated higher sensitivity of EIA in detecting H. pylori IgG in plasma compared to saliva samples.

KEYWORDS: H. pylori, Saliva, Plasma, IgG, EIA

(Submitted December 2012, Accepted June 2013)

INTRODUCTION:

Helicobacter pylori (H. pylori) are strongly associated with gastro-duodenal diseases, including chronic active gastritis, peptic and duodenal ulcers, gastric cancer, distal gastric adenocarcinoma, and gastric mucosal lymphoproliferative disease [1- 5]. H. pylori can also cause extra-intestinal manifestations; they have been identified in some patients with chronic bacterial infection without any clinical signs or symptoms [6]. Dental plaque (DP) has been implicated as possible reservoir for H. pylori in individuals with and without gastric or duodenal disorders [7, 8]. The prevalence of H. pylori infection worldwide is very high, with the highest rates reported among children and adults in the developing countries [6-11].

The exact modes of transmission of H. pylori are not clearly understood, but some researchers have suggested person-to-person via saliva, fecal-oral, oral-oral and gastro-oral routes [6 -12]. Different methods are available for the diagnosis of H. pylori [4, 11, 15, 16]. The invasive methods involve obtaining biopsies by endoscopy for culture and histology, the serology and Rapid Urease Test (RUT). These tests are not suitable for large scale screening or epidemiological studies in resource limited countries [4, 11, 15, 16]. The non-invasive tests include the detection of H. pylori antibodies or antigens in urine, saliva or feces samples using Enzyme Immunoassay (EIA) technique [11, 15, 16]. This technique is relatively simple, reproducible and inexpensive; it can be used for large scale screening and in epidemiological studies.

Recent studies have indicated saliva as a non-invasive sample for detection of antibodies (IgA and IgG) to H. pylori [4, 11, 14, 15]. Collection and testing of salivary specimen is non-invasive, painless, convenient, and fast and carries no risk of needle stick injury [11]. Assay for the detection of H. pylori antibodies in saliva is a useful and noninvasive way to identify infection, permit selective use of endoscopy, and monitor the response to antimicrobial therapy [17]. In addition, the use of EIA technique for early detection of H. pylori infection can reduce the prevalence of transmission and pathologic effects of the bacteria, especially among the population in resource limited countries like Papua New Guinea (PNG).

There are no published data on the prevalence of H. pylori infection among the population in PNG. The aim of this study was to assess the prevalence of H. pylori among residents in the National Capital District (NCD) in PNG using saliva and serum samples.

SUBJECTS AND METHODS:

This prospective cross-sectional study was carried out in the NCD, which is the incorporated area around Port Moresby, the capital of PNG. The dental clinics in the School of Medicine and Health Sciences (SMHS) in University of Papua New Guinea (UPNG), Port Moresby General Hospital (PMGH) and St John's hospital in Gerehu a major suburb in NCD were the selected study sites. These sites were selected mainly because of the difficulty in obtaining blood samples from health individuals in NCD. Calculation of sample size was based on a design effect of one, relative precision of 10%, assumed prevalence rate of 25%, with confidence interval of 95% and non-response rate of 20%. All individuals, both patients and relatives that visited the clinics during the study period were enrolled in the study. However, individuals with gastric disorders, those on antibiotic medications, beetle-nut chewers, and those not residing in NCD were excluded from the study. Final selection of subjects was by simple random sampling.

About 3.0 – 5.0ml of saliva was collected in clean plastic vial, which was then put in a cool-box kept at 4 – 8^{o}C in the field. About 3.0ml of blood was collected in Heparinized vacutainer, which was put in a cool-box kept at 4 – 8^{o}C in the field. Both samples were transported to the Micronutrient Laboratory (MNL) in SMHS, UPNG.

The blood samples were centrifuged and aliquots of plasma stored in dark vials. The vials containing the untreated saliva and plasma samples were kept frozen at – 70^{o}C till required for analysis. Demographic data of each consented subject was obtained using a pretested self-designed questionnaire.

All reagents used in the assay were of analytical grade and were components of the IBL commercial Enzyme-Immunoassay (EIA) Kits. Before analysis, unlike the saliva samples, each plasma sample was diluted 101 times as recommended by the manufacturer. Samples were analyzed using Solid Phase EIA for qualitative and quantitative determination of IgG antibodies against H. pylori in human serum and plasma [18]. The same EIA Kits were used after modification of the protocol for assay of the saliva samples as approved by the manufacturer [18]. Automated 96-wells Microplate Washer and Reader were used for processing and analysis of the Microplates.

Ethical clearance and permission for the study were obtained from SMHS Ethics and Research Grant committee, CEO PMGH, and CEO of the St John's Hospital Gerehu. Both saliva and blood samples were collected from subjects only after obtaining their signed informed consent.

The Statistical Package for Social Sciences (SPSS) version 13 for Windows was used for Statistical analysis of the data. The guidelines (Table 1) indicated by the manufacturer were used for interpretation of the data [18].

Table 1: Guideline for interpretation of the data [18]

Cut-off Index (U/mL)	Interpretation
< 0.8	Negative
0.8 – 1.2	Equivocal (Borderline)
>1.2	Positive

RESULTS:

A total of 216 subjects were randomly selected from the over 1000 that visited the clinics during the duration of this study. However, informed consent was obtained from 204 subjects (consent rate 94.4%). This gave a non-response rate of 5.6%, which was lower than the predicted non-response rate of 20% used for calculating the sample size. The mean age of all the consented subjects was 30.5 ± 13.7 years. Saliva sample was collected from each of the 204 consented subjects. Analysis of the data using the cut-off index indicated that 183 (89.7%) saliva samples were negative, 15 (7.4%) were equivocal and 6 (2.9%) were positive for H. pylori IgG. There was no significant difference in the results obtained for the dental and non-dental patients. Thus the data was pooled for all further analysis.

The data was separated and analyzed according to age groups of the subjects. The results are presented in Table 2. Those in the 40 – 49 years of age group had the highest positive (2.0%) prevalence. Equivocal was highest (2.9%) among those in the 20 – 29 years of age group followed by 1.5% of those in the 30 – 39yrs and 40 – 49 years of age groups. Gender distribution of the 204 subjects indicated 83 (40.7%) males and 121 (59.3%) females. The mean age for the males was 32.1 ± 14.3 years and for the females 29.4 ± 12.0 years. The prevalence of H. pylori IgG in the saliva samples of the male and female subjects is presented in Table 3. Both male and female subjects had low positive prevalence of H. pylori IgG in their saliva samples. There was no statistically significant difference between the results for the male and female subjects.

Of the 204 consented subjects a total of 50 were randomly selected and requested to donate blood for the analysis of H. pylori IgG in plasma. Signed informed consent for the collection of blood was obtained from 44

subjects (consent rate 88.0%). The mean age of the consented subjects was 31.7 ± 10.2 years. The results of the H. pylori IgG in the 44 plasma samples indicated that 19 (43.2%) were negative, 11 (25.0%) were equivocal and 14 (31.8%) were positive.

Table 2: Distribution of the 204 subjects according to age groups and prevalence of H. pylori IgG in saliva samples

Age Range (years)	Negative % (n)	Equivocal % (n)	Positive % (n)
< 10	2.0% (4)	0.5% (1)	0
10 – 19	17.2% (35)	0.5% (1)	0
20 – 29	30.9% (63)	2.9% (6)	0
30 – 39	18.6% (38)	1.5% (3)	0
40 – 49	12.7% (26)	1.5% (3)	2.0% (4)
50 – 60	6.4% (13)	0.5% (1)	0.5% (1)
>60	2.0% (4)	0	0.5% (1)
Total	89.7% (183)	7.4% (15)	2.9% (6)

Table 3: Distribution of male and female subjects according to the cut-off index and prevalence of H. pylori IgG in saliva

Interpretation	Males (n = 83)	Females (n = 121)
Negative	91.6% (76)	88.4% (107)
Equivocal	6.0% (5)	8.3%) (10)
Positive	2.4% (2)	3.3% (4)

Gender distribution of the 44 plasma samples indicated 19 (43.2%) males and 25 (56.8%) females. The mean ages of the male and female subjects were 32.2 ± 14.3 years and 31.3 ± 12.0 years respectively. The interpretation of the results indicating prevalence of H. pylori IgG in the plasma of the male and female subjects is presented in Table 4. There was no statistically significant ($p>0.05$) difference between the results for the male and female subjects. Comparison of the results for H. pylori IgG in the saliva and plasma samples of the 44 subjects indicated that both data were not normally distributed as indicated in the box-plot in Fig. 1. The data spread was greater for the plasma compared to the saliva samples. The results obtained for prevalence of H. pylori IgG in the saliva and plasma samples of the 44 subjects are presented in Table 5.

Table 4: Distribution of male and female subjects according to prevalence of H. pylori IgG in their plasma

Interpretation	Males (n = 19)	Females (n = 25)
Negative	36.8% (7)	48.0% (12)
Equivocal	31.6% (6)	20.0% (5)
Positive	31.6% (6)	32.0% (8)

Fig. 1: Box-plots of the results for H. pylori IgG in saliva and plasma of the 44 subjects

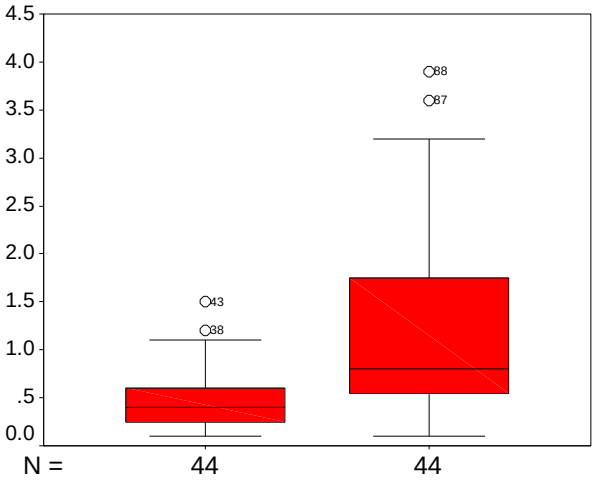
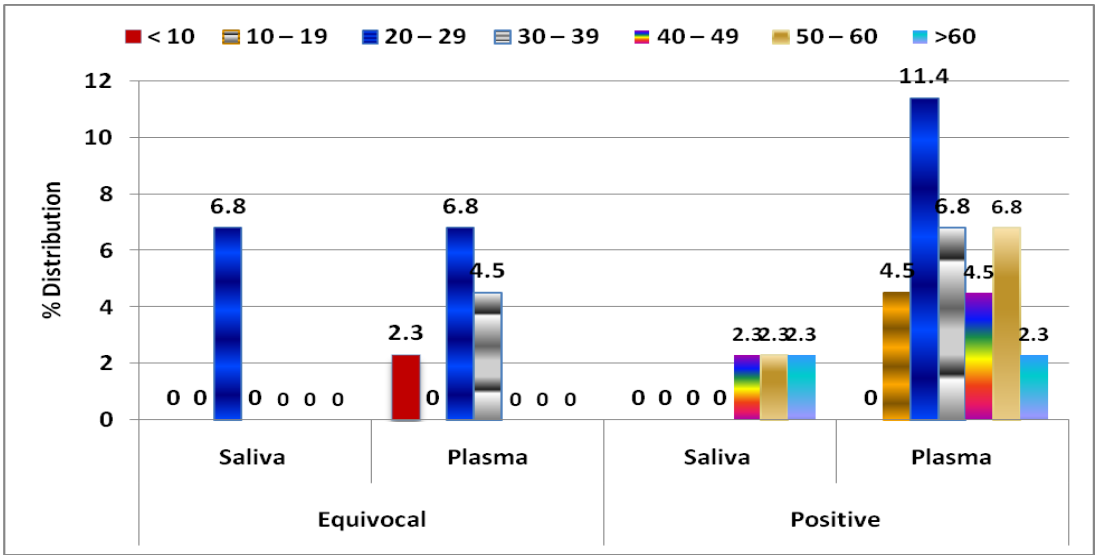


Table 5: Distribution of the subjects according to the prevalence of H. pylori IgG in their saliva and plasma samples

Interpretation	Saliva (n = 44)	Plasma (n = 44)
Negative	84.1% (37)	43.2% (19)
Equivocal	11.4% (5)	25.0% (11)
Positive	4.5% (2)	31.8% (14)

Analysis of the data using the Mann-Whitney and Wilcoxon Signed Rank tests indicated statistically significant difference (p = 0.01) between the saliva and plasma results. This indicates higher sensitivity of EIA in detecting H. pylori IgG in plasma samples compared to saliva samples. However, statistically significant positive linear correlation was obtained between the H. pylori IgG in saliva and plasma samples (Spearman ρ = 0.514, p = 0.01). The H. pylori IgG data in the saliva and plasma samples of the 44 subjects was distributed according to age groups. Fig. 2 shows the percent distribution of the equivocal and positive H. pylori IgG results for saliva and plasma samples in the various age groups. The prevalence of H. pylori IgG was highest (11.4%) in the plasma of subjects in the 20 – 29 years of age group, compared to the zero in their saliva samples. Compared to the saliva samples, H. pylori IgG was also prevalent in the plasma of subjects in the 10 to 19 years of age group (4.5%) and the 30 to 39 years of age group (6.8%).

Fig. 2: Percent distribution of subjects according to age groups and H. pylori IgG in saliva and plasma samples (Equivocal and Positive distributions are shown)



DISCUSSION:

The 5.6% non-response rate of the subjects during the collection of saliva samples in the present study was lower than the predicted non-response rate of 20% used for calculating the sample size. This high response rate supports the observation by other researchers that non-invasive methods are less problematic for collection of biological samples from subjects [10, 11].

It is true that this procedure offers definite advantages, such as, avoiding the risk of needle-stick injury, ease of sample collection and better comfort of consented subjects.

In the present study H. pylori was prevalent in 2.9% of all the subjects. This was higher than the 1.6% prevalence reported in the saliva of dentate patients, but lower than the 3.5% prevalence reported in saliva of edentulous patients [7]. The prevalence of H. pylori IgG was highest in the saliva of subjects in the 40 – 49 years of age group compared to the other age groups. These findings were different from those of other authors, who reported highest prevalence among patients in the 25 – 35 years and over 60 years of age groups [19, 20]. These findings indicated that prevalence of H. pylori IgG does not increase with age, especially in patients without gastric disorders [19, 20]. Some authors have reported significantly lower prevalence of H. pylori infection in females compared to males, while others reported higher prevalence among males compared to females [19, 20]. In the present study there was no difference in the prevalence of H. pylori IgG in the saliva of male and female subjects.

The high prevalence (31.8%) of H. pylori IgG in the plasma samples of the 44 subjects compared to 4.5% prevalence in their saliva samples was comparable with other similar studies [17, 21], but different from others [10 – 12]. Some studies indicated that the concentration of H. pylori IgG in oral fluid is between 500 – 1500 times lower than in plasma or serum [21]. In our study the mean amount of H. pylori IgG in the saliva samples was 497 times lower than the mean amount in the plasma samples. However, the sensitivity and specificity of the EIA kits used for the assay of H. pylori IgG in the saliva samples were not determined. This represents one of the limitations in our present study.

The prevalence of H. pylori IgG was highest in the plasma of subjects in the 20 – 29 years of age group compared to the other age groups. These findings further indicate that in patients without gastric disorders the prevalence of H. pylori IgG may not be age dependent [19, 20].

There was no statistically significant difference between the prevalence of H. pylori IgG in the plasma samples of the male and female subjects. This finding was similar to the result obtained in the saliva samples for the male and female subjects.

The statistically significant positive linear correlation between the levels of salivary and plasma H. pylori IgG antibodies, tend to indicate that the EIA test for saliva can be considered as a viable alternative when there are problems in obtaining plasma samples.

CONCLUSION:

H. pylori IgG was prevalent in the saliva of 2.9% of all the subjects. Prevalence of H. pylori IgG in plasma was significantly higher than the prevalence in saliva. There were no significant differences in the gender distribution of both salivary and plasma H. pylori IgG. The statistically significant positive linear correlation between the levels of salivary and plasma H. pylori IgG antibodies, tend to indicate that the EIA test for saliva can be considered as a viable alternative when plasma samples cannot be obtained.

ACKNOWLEDGEMENTS:

We thank Colgate- Palmolive PNG LTD for the research grant used in this project. We acknowledge the support of all the nursing staff and patients in PMG Dental Clinic and St John's Hospital Gerehu. We also acknowledge the support of Prof. Victor J. Temple, Ms Paula P. Riman, Dr. E. Falealunga-Ovia, Billy Architects, Samson Grant, Elias Nara, Jennie Bautau-Grant and Theresa Dunamb.

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ANTI-INFLAMMATORY ACTIVITY OF COCOS NUCIFERA WATER AND OIL

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ABSTRACT

Cocos nucifera is one of the most valuable plants to man and was used as a primary source of food, drinks and even medication. It has been used as a traditional medicine by many cultures. The rats were placed in Group A, B, C, D, E and F of five each. The distal 2 – 3 cm portion of the rats tails were immersed in hot water maintained at 55±1^{O}C for 30 seconds and removed. The rats were placed back in their different cages. Packed cell volume (PCV) decreased in all the groups except for group C which was at equilibrium with the negative control, but was not statistically different from the controls. Total white blood cell (TWBC) decreased significantly when compared with the negative and was statistically significant in group A when compared with Ibuprofen (standard drug), and negative control; group B was also statistically significant when compared with the negative control. Platelets increased significantly in the groups in the entire treatment groups when compared with the controls. This study showed that *Cocos nucifera* possesses potential anti-inflammatory activities, thus, confirms the folklore use of the plant in the treatment of ailments associated with pain and inflammation.

KEY WORDS: Cocos nucifera, Anti-inflammatory, Treatment, Platelets, WBC, RBC

Submitted June 2013, Accepted July 2013

INTRODUCTION:

Herbal products are gaining growing attention due to less toxicity and high efficacy against free radical mediated diseases [1]. Flourishing for many centuries [2], at present, approximately 25% of drugs in modern pharmacopoeia are derived from plants. Cocos nucifera (C. nucifera), belongs to the family of Palmae in Araecaeae order and it is originated from Malaysia, Polynesia and Southern Asia and is now prolific in South America, India and the Pacific Island [3]. C. nucifera have long been used in traditional medicine for treatment of metabolic disorders [4]. Literature data revealed that C. nucifera endocarp is a rich resource of phenolic and flavanoid compounds which are responsible for diverse biological activities in medicinal plants beneficial to human health and disease prevention [5-13]. Is one of the most valuable plants to man and was used as a primary source of food, drinks and even medication. It has been used as a traditional medicine by many cultures [14, 15].

The fruit of C. nucifera contains the coconut water (juice) and coconut meat (kernel), famous in the Malays folklore medicine for its ability to relieve fever [16]. In addition, Alleyne et. al. [17] have also reported on the C. nucifera juice extract ability to control hypertension in a clinical trial carried out at the University of the West Indies, St Augustine, Trinidad and Tobago, West Indies.

Previous studies have also demonstrated that the aqueous extract of husk fiber of C. nucifera possessed peripherally- and centrally-mediated antinociceptive and antioxidant [18], antimicrobial and antiviral [19] and leishmanicidal [20] activities.

MATERIALS AND METHOD:

Extraction of Cocos nucifera oil and Water

The fresh endosperm of the coconut meats were cut into pieces and milled using crusher mill. The resulting mass was mixed with lukewarm water and shaft was filtered using a cotton cloth. The product was gently heated to dry all traces of moisture and filtered through a cotton cloth to obtain the oil. Fresh C. nucifera used for this study were dehusked and broken carefully, and the liquid endosperm used for experiments.

Thirty five (35) albino rats with average weight of 222 kg which were purchased from Oba in Enugu Ezike Nsukka, Enugu state, were used for the research. The animals were grouped into seven (7) of five each. They were fed with growers marsh produced by grand cereals and oil mills limited, Bukuru Jos, Plateau State Nigeria, obtained from Chrys Ventures Limited Owerri, Imo state.

The albino rats were weighed after gaining maximum of two weeks acclimatization. The animals were subjected to heat to induce inflammation on their tails.

Tail Immersion and Administration: The rats in Group A, B, C, D, E and F of five each, distal 2 – 3 cm portion of their tails were immersed in hot water maintained at 55±1^{O}C for 30 seconds and removed. The rats were placed back in their different cages.

The animals were immersed and administered the following orally:

Group A: Tail immersed + 2ml/kg of C. ucifera oil;
Group B: Tail immersed + 1ml/kg of C. nucifera oil;
Group C: Tail immersed + 4ml/kg of C. nucifera water;
Group D: Tail immersed + 3ml/kg of C. nucifera water;
Group E: Tail immersed+ 0.1g/kg of Ibuprofen;
Group F: Tail immersed + water (Positive Control)
Group G: water (Negative Control)

The dosages were given once a day in the respective groups for 4 days. At the end of the treatment, all the rats were sacrificed, the tail was dissected for histological examination and blood was taken for haematological analysis.

Haematological analysis: Blood samples were collected for analysis of Packed Cell Volume (PCV), Total White Blood Cell Count (TWBC) and platelet count.

RESULTS:

Pack cell volume (PCV) decreased in all the groups except for group C which was at equilibrium with the negative control, but was not statistically different from the controls. Total white blood cell (TWBC) decreased significantly when compared with the negative and was statistically significant in group A when compared with Ibuprofen (standard drug), and negative control; group B was also statistically significant when compared with the negative control. Platelets increased significantly in the groups in the entire treatment groups when compared with the controls (Table 1).

Each value represents the mean ± standard deviation (n = 5); values are statistically different from Ibuprofen (a), Positive control (b) and Negative control (c) at $p< 0.05$ one-way analysis of variance (ANOVA) + Tukey – Kramer Multiple Comparison Test.

DISCUSSION:

The haematological results for Cocos nucifera oil revealed that there was shortage of blood; the Packed cell volume (PCV) decreased but was not statistically different from the controls, while total white blood cell (TWBC) decreased and was statistically significant in group A when compared with Ibuprofen (standard drug), positive and negative control and B when compared with the negative control. Platelets increased significantly in group A, when compared with Ibuprofen (standard drug),

positive and negative controls and decreased significantly in group B and was significant when compared with Ibuprofen (standard drug) and positive controls (Table 1). The increase in platelets indicates quick responds to the site of injury.

Table 1: Haematological parameter of rats administered with *C. nucifera oil and water*

	PCV (%)	**TWBC**	**PLT**
A (Oil 2mls)	37.20 ± 50	17.60 ± 0ac	493 ± 10abc
B (Oil 1ml)	35.00 ± 30	12.70 ± 0c	413 ± 30ab
C (W 4ml)	42.00 ± 26	19.3 ± 0ac	996 ± 0abc
D (W 3ml)	39.00 ± 24	16.6 ± 0c	498 ± 0abc
E (Ib 10mg/kg)	36.00 ± 40	13.60 ± 0	461 ± 40
F (Post CNT)	36.00 ± 40	15.20± 0	563 ± 23
G (Neg CNT)	42.00 ± 10	21.50 ± 40	432 ± 20

In C. nucifera water PCV decreased in group C, Ibuprofen and positive control groups but was not statistically different from the negative control. The slight decrease in PCV may be connected to damage tissue.

The TWBC decreased and was statistically significant in group A when compared with Ibuprofen (standard drug), and negative control and B when compared with the negative control. The result of the TWBC corroborates that of PCV. Platelets increased significantly in group C and D, when compared with Ibuprofen (standard drug), and positive and negative controls (Table 1). This agrees with the research by Nurden et al., [21] who states that "Platelets help prevent blood loss at sites of vascular injury. They adhere, aggregate and form a procoagulant surface favouring thrombin generation and fibrin formation. In addition, platelets express and release substances that promote tissue repair"

CONCLUSION:

This study showed that Cocos nucifera possesses potential anti-inflammatory activities, thus, confirms the folklore use of the plant in the treatment of ailments associated with pain and inflammation. It is recommended that further studies using isolated constituents of the C. nucifera oil and water should be carried out to further substantiate its anti-inflammatory properties.

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ANTIMICROBIAL ACTIVITY OF ENDOPHYTES IN SIX MEDICINAL PLANTS COLLECTED IN THE CENTRAL PROVINCE, PAPUA NEW GUINEA

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ABSTRACT:

Endophytic microorganisms are recognized as potential source of novel chemical molecules that might be useful in the treatment of infectious diseases. In this study, six medicinal plants (*Morinda citrifolia*, *Plumeria rubra*, *Artocarpus altilis*, *Musa nana*, *Sansevieria trifasciata* and *Saccharum officinarum*) traditionally. They are used for treatment or management of symptoms such as cough, fever and skin diseases, were investigated for the antimicrobial activity of metabolites produced by endophytic microorganisms. Seven endophytes were isolated using the mycological media, potato dextrose agar (PDA). Three of the endophytes were identified as fungi from the *Aspergillus* species, three as actinomycetes and one as a gram negative bacterium. All endophytic isolates were subjected to anti-bacterial, anti-tuberculosis (TB), anti-fungal, anti-HIV, and cytotoxicity assays using micro-broth dilution assay technique in sterile 96-well plates. All isolates displayed antimicrobial activity inhibiting at least one of the test pathogens at the concentration of 100µg/ml. Of the 7 endophytic isolates, 6 isolates were active against TB, 7 were active against the bacterial test pathogens, and 2 were active against fungi while none were active against HIV. From the 7 isolates, 3 were non-toxic to mammalian cells at a concentration of 100µg/ml.

KEYWORDS: Antimicrobial activity, endophytes, *Morinda citrifolia, Plumeria rubra, Artocarpus altilis*, *Musa nana*, *Sansevieria trifasciata* and *Saccharum officinarum*.

Submitted June 2013, Accepted August 2013

INTRODUCTION:

Nearly all plant species host endophytes, but few of these microorganisms have been characterized [1]. Antibiotics, antimycotics, immunosuppressant, and anticancer compounds are only a few examples of what endophytes have been found to produce in the recent past [2]. Thus, the prospects of finding new drugs that may be effective candidates for treating newly emerging diseases in humans, plants, and animals are great. A recent study noted that endophytes from tropical regions produced significantly more bioactive secondary metabolites than those from temperate parts of the world [3].

Natural products from endophytic microbes have been observed to inhibit or kill a wide variety of harmful disease-causing agents including, but not limited to, phytopathogens, as well as bacteria, fungi, viruses, and protozoans that affect humans and animals [4]. Studies done by Strobel and colleagues have demonstrated endophytic microbes possessing antibacterial, antifungal, anti-tuberculosis, antiviral and anticancer agents [5, 6]. To name a few that have provided modern medicine with valuable new cures include penicillin from the fungus *Penicillium notatum*, bacitracin from the bacterium *Bacillus subtilis* and taxol, an important chemotherapeutic agent, is synthesized by an endophyte of the Pacific Yew tree [6]. A newly described species of *Pestalotiopsis*, an endophytic fungus *Pestalotiopsis jesteri*, from the Sepik River area of Papua New Guinea (PNG) produces jesterone and hydroxy-jesterone which exhibit antifungal activity against a variety of plant-pathogenic fungi [7]. An additional secondary metabolite identified as ambuic acid, is an antifungal agent which has been recently described from several isolates of *P. microspora* found as representative isolates in many of the world's rainforests including PNG [8]. PNG is not only located in the tropical region but rich in biodiversity and harboring a variety of medicinal plants. These may be a potential source of drug discovery from endophytes. The current study was carried out to determine the antimicrobial activity of secondary metabolites biosynthesized by endophytes obtained from some medicinal plants in the Central Province.

METHODOLOGY:

Collection of Plant Samples and Isolation of Endophytes

Six plants (*Morinda citrifolia, Plumeria rubra, Artocarpus altilis, Musa nana, Sansevieria trifasciata* and *Saccharum officinarum*) were selected based on their use in (Table 1) traditional medicine for the treatment of cough, fever and various infections [9, 10]. Plant parts such as leaves, stem, and petioles were collected in the field, stored in sterile plastic bags, and subsequently processed for isolation of endophytes.

Table 1: Medicinal plants used in the study and their traditional uses

Plant species	Family	Plant material	Medicinal use	Reference
Morinda citrifolia	Rubiaceae	Leaves & petiole	Cough & TB	[9]
Plumeria rubra	Apocynaceae	Leaves	Scabies and stings from insect bites	[10]
Artocarpus altilis	Moraceae	Leaves	Cough & tonsillitis	[10]
Musa nana	Musaceae	Stem	Fever	[10]
Sansevieria trifasciata	Agavaceae	Leaves	Ringworm & fungal disease	[10]
Saccharum officinarum	Poaceae	Leaves	Sore throat	[10]

The collected samples were washed thoroughly with sterile distilled water and air dried. The materials were then surface sterilized by immersing them sequentially in 70% ethanol for 3min and 0.5% NaOCl for 1min and rinsed thoroughly with sterile distilled water [11]. The excess water was dried under laminar airflow chamber. Using a sterile scalpel outer tissues were removed and inner tissues of 0.5cm2 size were dissected and placed on petri-plates containing Potato Dextrose Agar (PDA) media [12, 13]. The plates were then incubated at 25±2°C until growth appeared. The explants were observed once a day for the growth of endophytic microorganisms. Growths from the plated explants were immediately transferred into PDA slant and maintained at 4°C. The endophytic isolates were identified using staining techniques based on their morphological and reproductive characters using standard identification manuals [14, 15]. Isolates that possessed typical characteristics of fungal growths were identified using lacto phenol cotton blue stain while those having bacterial characteristics were identified using gram staining. All the isolates were maintained in Potato dextrose agar slant and sterile water. The experimental procedures were carried out in the Molecular Biomedicines and Biodiscovery Laboratory (MBBL) in the School of Medicines and Health Sciences (SMHS), University of Papua New Guinea (UPNG).

Lignin Degradation Assay via Poly-R agar clearance (Poly-R agar)

Lignin modifying enzyme (LME) Basal Medium (LBM) was prepared by supplementing with 0.02 % w/v polymeric dye Poly-R 478 (Poly-R; Sigma) and 1.6 % w/v agar and autoclaved. Then 1.0 ml of a separately sterilized 20 % w/v aqueous glucose solution was added to each 100 ml of growth medium prepared and aseptically transferred to Petri dishes. After inoculation of test fungus on the Petri dishes, they were incubated at 25 oC in darkness and

examined daily for 10 days. The production of LME was recorded as clearance of violet colored medium [16].

Cultivation and Extraction

The endophytes were cultivated in Potato dextrose broth (Difco) by placing agar blocks of actively growing pure culture (3mm in diameter) in 250ml Erlenmeyer flasks containing 100ml of the medium [12]. The flasks were incubated at 25±1 ° C for 3 weeks with periodical shaking at 150 rpm. After the incubation period, the cultures were taken out and filtered through sterile cheesecloth to remove the mycelia mats for fungal isolates. The endophytic metabolites were extracted by sequential solvent extraction procedure using hexane, dichloromethane, ethyl acetate and methanol as solvents. Equal volumes of the filtrate and the solvents was taken in a separating funnel and shaken vigorously for 10 min. The solution was then allowed to stand, and the solvent was collected. The organic solvents were evaporated and the resultant residue was dried in vacuum evaporator to yield the crude extracts. The crude extracts were then dissolved in Dimethyl sulphoxide (DMSO) at 100µg/ml for antimicrobial bioassay [17].

Evaluation of Antimicrobial Activity

Assays that were carried out to determine antimicrobial activity of endophytic crude extracts included anti-bacterial assay, anti-fungal assay, anti-tuberculosis (TB) assay and an anti-HIV assay. Antimicrobial assays were carried out using micro broth dilution assay technique in sterile 96- microtiter plate [18]. Four common human pathogens were used in the present study. The test pathogens included a Gram-positive bacteria - *Bacillus subtilis* (Bs), two Gram-negative bacteria - *Escherichia coli* (Ec) and *Francisella novicida* (Fn), a fungal pathogen - *Candida albicans* (Ca) and a TB pathogen - *Mycobacterium tuberculosis*. All the test pathogens were obtained from College of Pharmacy, Department of Pharmacology University of Utah, USA.

Antibacterial and antifungal assay

Bacterial and fungal strains were assayed according to CLSI/NCCLS method M07-A5 in Mueller-Hinton II broth [19]. The micro-broth dilution assay technique was carried out using 96-microtitre plates. The positive control and endophytic extracts were dissolved in DMSO to produce stock solutions at 100 µg/ml. Ampicillin was used as the control for *E. coli*, gentamycin was used as the for control *B. subtilis*, kanamycin was used as the control for *F. novicida* and econazole was used as the control for *C. albicans* DMSO was used as a negative control. Initially, the human pathogen cultures were dispensed in 200 µL of Muella Hinton II Broth medium into a 96-well culture plates at 100,000 cells per well. Then 1.0 µL of the control and 1.0 µL of the extract were added in duplicate wells. After 24 hours incubation at 37oC, absorbance at 570 nm was measured using a Biorad Model 450 microtiter plate reader (Biorad, Hercules, CA). All data

were corrected against media-only blank wells. The percent inhibition was derived as the fraction of the sum of the test wells over the sum of control wells subtracted from unity and multiplied by 100. This was calculated using the formula below:

$$\% \text{ inhibition} = \frac{\text{Absorbance of the control} - \text{Absorbance of the test sample}}{\text{Absorbance of the control}} \times 100$$

Anti-tuberculosis (MTT) Assay

Inhibition of M. tuberculosis H37Ra growth was quantified using a colorimetric 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay modified from previously published methods [20, 21]. Rifampacin and plant extracts were dissolved in DMSO to produce stock solutions at 100µg/ml. DMSO was used as a negative control. M. tuberculosis cultures were dispensed in 200 µL of ADC (albumin-dextrose complex) enriched 7H9 medium into a 96-well culture plates at 100,000 cells per well. One µL of DMSO (control) or DMSO containing drug or extract was added in triplicate wells. After four days incubation at 37oC, 11 µL of sterile MTT [5 mg/mL in PBS (phosphate buffered saline)] was added and incubated overnight. Viable M. tuberculosis metabolizes the MTT to an insoluble purple formazan product that was solubilized by the addition of 50 µL of a solubilization solution [5% SDS (sodium dodecyl sulfate) w/v, 50% DMF (dimethylformamide) v/v, 45% H2O v/v]. Absorbance was read at 570 nm and all data were corrected against media-only blank wells. The percentage inhibition was calculated using the formula as shown in the antibacterial and antifungal assay.

Cytotoxicity Assay

CEM-TART (human T- cell lymphoblastic leukemia) cells were maintained in Rosewell Park Memorial Institution (RPMI) culture medium supplemented with 20% fetal bovine serum and standard concentrations of penicillin and streptomycin (10,000 U and µg/liter respectively). Doxorubicin (1 µg/mL) was used as the positive control. On the first day cells were grown in suspension and seeded in a 96-well plate (2 x 104 cells/well). After 24 hours the medium was exchanged [22]. Solutions of the crude extracts were prepared in DMSO and the final concentrations (100µg/ml) were achieved by direct dilution into the cell medium. The samples or the vehicle were added and incubated for a period of 48 hours. After this interval, 50 µL of a MTT solution (5 mg/mL in PBS) were added to each well and the cells were incubated for an additional 3 hours. Subsequently, the medium was removed, cells were washed two times with PBS, and 300 µL of isopropanol–HCL (0.04mol/L) were added. Cell viability was determined by absorbance measurements at 570 nm. The color intensity

is correlated with the number of healthy living cells [23]. Cell survival was calculated using the formula:

$$\% \text{ survival} = \frac{100 \times (\text{abs. of treated cells} - \text{abs. of culture medium})}{\text{abs. of untreated cells} - \text{abs. of culture medium}}$$

where "abs." means absorbance

Anti-HIV Assay

The cytoprotection assay used in this study was developed by Kiser and co-workers [24]. In brief, CEM-TART and the 1A2 subline cells were maintained in RPMI culture medium supplemented with 20% fetal bovine serum and standard concentrations of penicillin and streptomycin (10,000 U and µg/liter respectively). When grown at, 5% CO2, and maintained at a concentration between 5 x l04/ml and 2 x l06/ml, the cells doubled approximately every 24 hours. For infection with virus, 2 x106 CEM-TART cells were pelleted by centrifugation and resuspended in 0.5 ml of virus stock (freshly thawed). After 1 hr incubation at 37°C the cells were pelleted and resuspended at 2 x l05/ml for growth. Virus stock was prepared by centrifugation of a virus-producing culture of CEM-TART cells, 7 to 9 days after infection, and simply freezing aliquots of virus-containing supernatant. Following overnight incubation, the cells were counted and dispensed into a 96 well micro titer culture dish at 20,000 cells/well in 1.5 ml culture medium. Serial dilutions of test extracts were added to this (concentrations spanning 5 logs from 50ug/ml to 0.05µg/ml). Cultures were examined microscopically daily, and quantified every other day to generate cell growth inhibition curves. Cells were counted by removing 10 µl of cells and mixing with an equal volume of MTT dye.

Cultures are treated in quadruplicate with azidothymidine (AZT) as a positive control. Significant cytoprotection is evident at AZT concentrations of 0.5 to 5 µg/ml. Concentrations of AZT above 10 µg/ml are cytotoxic. This modified assay uses MTT exclusion as an alternative means of quantifying CEMTART and CEM-1A2 cell growth. The T-cell leukemia subline 1A2 is more sensitive than the TART line to cell killing by HIV infection and therefore it was selected as the primary cell line for this assay [24, 25]. When the total cell population was quantified daily over 4 days, it was found that the number of cells in non-infected and infected cultures decreased after approximately 10 days. The

DMSO concentration was less than or equal to 1% culture volume. Only HIV-IM Δtat rev strain was utilized. Absorbance was read at 570 nm and all data were corrected against media-only blank wells. The percentage inhibition was calculated using the formula as shown antibacterial and antifungal assay.

RESULTS:

Identification of organisms

The actinomycetes species were isolated from the petiole and of *M. citrifolia*, and the leaves of *M. citrifolia* and *P. rubra*. The *Aspergillus* species were isolated from the leaves of *A. altilis, S. trifasciata* and *S. officinarum* while the Gram – bacteria was isolated from the stem of M. nana.

Enzymatic Activity of Lignin Degradation Assay

Only fungal organisms were subjected to the lignin degradation assay because they are known to be good degraders of lignin. All fungal isolates indicated negative reaction thus no production of lignin degrading enzymes was observed. In the present study, the most active crude extract against the bacterial pathogen *E. coli* was the ethyl acetate extract from the *Aspergillus* sp. isolated from the leaf of *S. officinarum* having a total inhibition of 73% which was compared to the positive control, ampicillin which had a total inhibition of 100% (table 2).

B. subtilis were highly susceptible to all of the crude extracts, especially the methanol extracts. The most active crude extract was from the methanol extract of an actinomycete isolated from the leaf of *M. citrifolia* having a total inhibition of 118% which was compared to the positive control, gentamycin which had a total inhibition of 104% (table 2). *F. novicida* was also sensitive to most of the extracts. The most active crude extracts was from the methanol extract of a gram negative bacterium isolated from the stem of *M. nana* having a total inhibition of 91% compared to the positive control, kanamycin which had a total inhibition of 100 % (table 2).

One fungal pathogen was tested for sensitivity to the endophytic crude extracts. The most active crude extract was from the dichloromethane extract of an *Aspergillus* sp. isolated from the leaf of *A. altilis* having a total inhibition of 91%, which compared to the positive control, econazole that had a total inhibition of 88.16% (table 2). The anti-tuberculosis assay demonstrated that the most active endophytic crude extract was from the methanol extract of a gram negative bacterium isolated from the stem of M. nana with a total inhibition of 91% compared to the positive control, rifampacin, which had a total inhibition of 100.66% (table 2).

Antimicrobial Assay

Table 2: Antimicrobial activity data of the titled controls and endophytic crude extracts at 100µg/ml by the broth dilution assay technique after 24 hours.

Percentage inhibition (%)

	Bacteria												Fungi				Mycobacterium				Virus			
Controls	**E.c**				**B.s**				**F.n**				**C.a**				**MTB**				**HIV**			
Negative Control	No Percentage Inhibition																							
Ampicillin	100				—				—				—				—				—			
Gentamycin	—				104				—				—				—				—			
Kanamycin	—				—				100				—				—				—			
Econazole	—				—				—				88				—				—			
Rifampacin	—				—				—				—				100				—			
Azidothymidine	—				—				—				—				—				77			
Crude extract	**H**	**D**	**E**	**M**	**H**	**D**	**E**	**M**	**H**	**D**	**E**	**M**	**H**	**D**	**E**	**M**	**H**	**D**	**E**	**M**	**H**	**D**	**E**	**M**
Mcp	12	13	4	15	23	21	30	97	75	61	32	68	4	84	54	20	75	61	32	68	36	20	35	16
Mcl	4	2	9	1	18	36	2	16	45	81	60	66	7	13	18	17	45	81	60	66	30	22	31	14
Prl	4	21	14	9	83	48	1	107	39	83	49	67	16	15	17	19	39	83	49	67	16	31	15	35
Aal	14	15	20	5	24	12	1	114	40	39	12	90	2	91	5	17	40	39	12	90	30	16	27	7
Mns	5	29	8	11	22	113	2	95	68	91	77	91	14	13	17	1	68	39	77	91	57	61	41	53
Stl	25	43	20	4	1	104	1	116	67	79	74	83	12	18	17	11	67	39	74	83	58	50	48	9
Sol	51	28	73	16	114	113	87	113	68	13	45	74	13	18	48	15	68	13	45	74	51	45	54	3

E.c-*Escherichia coli*, B.s-*Bacillus subtilis*, F.n - *Francisella novicida* , C.a- *Candida albicans,* MTB – *Mycobacterium tuberculosis*, HIV - Human Immune Deficiency Virus
H – hexane, D – dichloromethane, E – ethyl acetate, M – methanol
Mcp – *Morinda citrifolia* (petiole), Mcl - *Morinda citrifolia* (leaf), Prl – *Plumeria rubra* (leaf), Aal – *Artocarpus altilis* (leaf), Mns – *Musa nana* (stem). Stl - *Sansevieria trifasciata* (leaf), Sol - *Saccharum officinarum* (leaf)
% inhibition activity - A $\geq$ 70, 30 $\leq$ Q < 70, I < 30 [A – active, Q – questionable, I – inactive]

Table 3: Cytotoxicity activity data of endophytic crude extracts at 100 µg/ml by the broth dilution assay technique after 24 hours

	Percentage survival (%)			
Control		**Human T cells**		
Concentration	**1µg/Ml**	**0.1µg/mL**	**0.01 µg/mL**	**0.001µg/mL**
Negative Control		No Percentage Inhibition		
Doxorubicin	1.78	7.1	22.4	99.9
Crude extract	**Hexane**	**Dichloromethane**	**Ethyl acetate**	**Methanol**
Mcp	30	2	5	3
Mcl	71	75	55	98
Prl	110	62	7	31
Aal	71	60	1	5
Mns	4	57	1	15
Stl	3	34	8	2
Sol	40	2	1	85

Mcp – *Morinda citrifolia* (petiole), Mcl - *Morinda citrifolia* (leaf), Prl – *Plumeria rubra* (leaf), Aal – *Artocarpus altilis* (leaf), Mns – *Musa nana* (stem). Stl - *Sansevieria trifasciata* (leaf), Sol - *Saccharum officinarum* (leaf)
% survival activity - Active < 30% Survival, Questionable 30 < 70% Survival, Inactive > 70% Survival

None of the endophytic crude extracts tested were HIV active, thus all inhibited less than 50 % of AZT in HIV infected cells (table 2). The positive control used to compare the potency of the crude endophytic extracts was AZT.

The cytotoxicity results indicate extracts of three isolates have limited toxicity to human T cells. The highest number of Human T cells survived in the crude extract of the hexane extract from an Actinomycete isolated from the leaf of *P. rubra* having a percentage survival of 110% (over 100% because the artifact of clearing of medium) followed by methanol extract of an Actinomycete isolated from the leaf of *M. citrifolia* having a percentage survival of 98 % and having a percentage survival of human T cells at 85 % was the methanol extract of an aspergillus's sp. isolated from the leaf of *S. officinarum* (table 3). The effectiveness of the endophytic crude extracts was compare to four different concentrations of doxorubicin and their number of surviving human T cells as indicated in table 3.

DISCUSSION:

Medicinal plants are reported to harbor bioactive endophytes. These microorganisms are currently considered as a source of novel secondary metabolites for medical, agricultural and/or industrial exploitation. Endophytes are thought to preclude bioactive compounds because they are occupying literally millions of

unique biological niches [1]. Very few metabolites from endophytes are reported in literature. Much work needs to be done in this field.

Six potential medicinal plants used locally by communities in the Central Province were selected for endophytic studies (Table 1). All the plant species were found colonized with endophytic microorganisms (fungi and bacteria). The endophytes were isolated using mycological media potato dextrose agar (PDA). Altogether 7 endophytic microorganisms were isolated from the six medicinal plants, out of which 3 were identified as fungi from the *Aspergillus* species, 3 as Actinomycetes and 1 as a gram negative bacteria.

The lignin degradation assay indicated a negative reaction by all fungal isolates thus there was no production of lignin degrading enzymes. Decolonization of the polymeric dye Poly-R 478 by fungi has been positively correlated with production of the lignin degrading enzymes including laccase [16]. This simple test gives clear results since decolonization of the violet dye can be easily observed.

In the present study, ethyl acetate extract from the *Aspergillus* sp. isolated from the leaf of *S. officinarum* exhibited a total inhibition of 73% of *E. coli* in which appeared to be the most active extract as showed in table 2. From literature, there are no findings of endophytic fungus isolated from *S. officinarum* with antibacterial properties [26].

B. subtilis was highly susceptible to all of the crude extracts, especially the methanol extracts. According to literature, methanol is known to be a far more consistent extraction of antimicrobial substances from compared to other solvents such as water, ethyl acetate, dichloromethane, butanol, etc. [27]. The most active crude extract was from the methanol extract of an Actinomycete isolated from the leaf of *M. citrifolia* having a total inhibition of 118% of *B. subtilis* (table 2). From literature, endophytes isolated from *M. citrifolia* had demonstrated broad antimicrobial activity such as having antibacterial and antifungal properties [28].

The most active crude extract against *F. novicida* was from the methanol extract of a gram negative bacterium isolated from the stem of *M. nana* demonstrating a total inhibition of 91% as seen in table 2. From literature there are no reported findings of endophytes isolated from *M. nana* demonstrating antibacterial properties. The most active crude extract was the dichloromethane extract of an *Aspergillus* sp. isolated from the leaf of *A. altilis* that appeared to have a total percentage inhibition of 91% against *C. albicans* as observed in table 2. The total percentage inhibition of econozole by *C. albicans* was lower compared to the crude extract as indicated in the results in table 2. Endophytes

have been isolated from *A. altilis* however no antifungal activity has been indicated but there had been demonstration of antitumor activity [29].

According to the present study, the anti-tuberculosis assay demonstrated that the most active endophytic crude extract was from the methanol extract of a gram negative bacterium isolated from the stem of *M. nana* having a total inhibition of 91% by the *M. tuberculosis* (table 2). Interestingly, there is no reported study of endophytes being isolated from M. nana.

None of the endophytic crude extracts tested were active against HIV; all inhibited less than 50 % of AZT in HIV infected cells. The cytotoxicity results indicate extracts of three isolates have minimal toxicity to human T cells. These were the hexane extract from an Actinomycete isolated from the leaf of *P. rubra*, the methanol extract of an Actinomycete isolated from the leaf of *M. citrifolia*, and the methanol extract of an *Aspergillus* sp. isolated from the leaf of *S. officinarum* (table 3).

Thus three endophytic extracts that appeared non -toxic to mammalian or human T cells also demonstrated antimicrobial and anti-TB properties. These are candidates that need to be further tested and developed for compound potential pharmaceuticals. The other crude endophytic extracts that were considered more toxic to Human T cells could be considered as anticancer agents and therefore can be subjected to further anticancer assays to quantify the existing results.

CONCLUSION:

Results obtained in this study indicate that endophytic microorganisms isolated from PNG medicinal plants produced some crude extracts that possess antimicrobial potentials especially against pathogenic bacteria and fungi. It was also observed that some endophytic microorganisms produced cytotoxic extracts that could be considered as anticancer agents. The crude extracts of the three isolates that were not toxic to mammalian cells should be subjected for chemical analysis for compound purification and characterization. Our data supports the general scientific opinion that endophytic microorganisms of medicinal plants are potential sources of bioactive compounds.

ACKNOWLEDGEMENT:

The authors wish to acknowledge financial assistance received from the ICBG/University of Utah/UPNG project towards this research work (ICBG 5UO1T006671). The authors are also grateful to Micheal Koch and Chris Pond from the University of Utah in assisting with the Pharmacological component of this project.

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STATUS OF IODINE NUTRITION AMONG SCHOOL-AGE CHILDREN (6 – 12 YEARS) IN MOROBE AND EASTERN HIGHLANDS PROVINCES, PAPUA NEW GUINEA

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ABSTRACT:
Insufficient intake of iodine or consumption of foods containing goitrogens can decrease thyroid function leading to multiple physical and mental disorders known collectively as iodine deficiency disorders. Successful implementation of the universal salt iodization strategy, which is the main intervention strategy for the control and elimination of iodine deficiency, requires constant monitoring. Urinary iodine concentration is the recommended biochemical indicator for assessing the iodine status of a population. The present studies were prompted by the apparent lack of published data on the status of iodine nutrition among school-age children in Morobe and Eastern Highlands provinces in Papua New Guinea (PNG). The aims of these studies were to determine the urinary iodine concentration in school-age children (6 – 12 years) as a way of assessing the impact of the salt iodization programs in Morobe and Eastern Highlands Provinces in PNG. These prospective school based cross-sectional studies were carried out in Aseki-Menyamya district Morobe province and Gouno, Mt. Michael Local-Level Government area in Lufa district Eastern Highlands province, PNG. Simple random sampling was used to select primary schools in each district. The iodine content in salt samples was measured using the single wavelength semi-automated WYD Iodine Checker Photometer. Urinary iodine concentration (UIC) was estimated using the Sandell-Kolthoff reaction. In Lufa district, the mean per capita discretionary consumption of salt was 4.7 ± 2.1g per day with a range of 2.1 – 9.6g; the mean iodine content in salt samples from the households was 17.8 ± 4.5ppm; the iodine content was below 15ppm in 23.8% of all the salt samples. For the children in Aseki-Menyamya district, the median UIC was 149.5µg/L, Interquartile Range (IQR) was 70.0 – 300µg/L; the UIC was below 100.0µg/L in 32.9% of the children and 17.9% had UIC below 50µg/L. For children in Gouno Lufa district, the median UIC was 50.0µg/L, IQR was 23.9 – 76.0µg/L, 87.9% had UIC below 100.0µg/L, and 49.2% had UIC below 50.0µg/L. The results indicate that iodine deficiency should be considered a significant public health problem among the school-age children in Gouno Lufa district. Our findings indicate the urgent need for efficient, sustainable, systematic and functional monitoring system to strengthen and improve on the implementation of the USI strategy in both districts.

KEYWORDS: Iodized salt, Urinary Iodine, Iodine deficiency, Menyamya, Lufa, PNG.

[Submitted February 2013, Accepted June 2013]

INTRODUCTION:

Bioavailability of adequate amount of iodine is one of the prerequisites for biosynthesis of the thyroid hormones, which are essential for normal metabolism, growth and development [1 – 2]. Insufficient intake of iodine or consumption of foods containing goitrogens can decrease thyroid function leading to multiple physical and mental disorders known collectively as iodine deficiency disorders (IDD) [1 – 3]. The expression and extent of these disorders are dependent on the severity and timing of iodine deficiency [1, 3].

Iodine deficiency (ID) is regarded, as the single most common cause of preventable mental impairment in communities with suboptimal intake of iodine [1 – 3]. Currently, the prevalent forms of IDD in most resource limited countries are the more subtle degrees of mental impairment that occur in apparently healthy children with low intake of iodine [1 – 5]. The manifestations include poor performance in school and in psychometric tests, reduced intellectual ability and impaired motor functions. Among the several strategies for reducing the prevalence of IDD, universal salt iodization (USI), a policy of iodizing all salt for human consumption, is recommended for the control and elimination of IDD in affected populations [1, 2, 5 – 7]. Effective implementation of the USI strategy requires continuous monitoring of the process, impact and sustainable indicators in the affected communities [1, 2, 5 – 7]. The recommended principal impact indicator of USI is the assessment of Urinary Iodine Concentration (UIC) among school-age children in the target population [1, 3, 7].

In an attempt to control IDD, and to comply with the international goal of USI, the Government of Papua New Guinea (PNG) amended the pure food standards (PFS) by promulgating the salt legislation in June 1995, banning the importation and sale of non-iodized salt in the country [8]. According to the amended PFS, all salt imported into the country must be iodized with Potassium Iodate, and the iodine content should not be less than 30ppm; in addition, all salt must be packaged in waterproof containers to minimize the loss of iodine [8, 12, 15]. The PNG National Executive Council (NEC) endorsed the implementation of the amended PFS as the PNG Food Sanitation Regulation (PNGFSR) in February 2007 [15].

One of the major issues in some resource limited countries, like PNG, is the long-term sustainability of salt iodization programs, which require constant monitoring of the iodine status of the population [1, 2, 7]. Lack of monitoring can lead to regression in achievements of IDD control programs. In

addition, poorly monitored programs can cause excessive intake of dietary iodine, which may be associated with risks of adverse health consequences, such as, Iodine-Induced Hyperthyroidism (IIH) [1,7,9]. Some recent reports [10, 11] indicated growing evidence that iodine deficiency may be reappearing in some countries where it was previously under control. This statement further underscores the need for continuous monitoring and evaluation of the iodine status of populations that have been at risk of IDD in the past. Thus, the need for evaluating the implementation of the USI strategy in PNG cannot be overemphasized.

According to Amoa et al [12] endemic goiter was prevalent in specific isolated areas in PNG, despite the availability of iodized salt. In Menyamya district Morobe province the incidence of goiter in 1997 was 14% among the schoolchildren surveyed [12]. Results of a study that assessed the UIC among school-age children (6 – 12 years) in Hella district Southern Highlands Province (SHP) PNG indicated that, despite the apparent success of the salt iodization program, there was high prevalence of iodine deficiency among the male and female children [13, 14]. Reports from the PNG National Nutrition Survey carried out in 2005 (PNG NNS 2005) indicated adequate status of iodine nutrition among non-pregnant women of child-bearing age in the four regions of PNG [15]. However, the PNG NNS 2005 did not provide data on the iodine status in the various Provinces and Districts in PNG [15]. Recent data indicated that despite some success in the implementation of the USI strategy in PNG, there is prevalence of mild to moderate status of iodine deficiency among school-age children, pregnant women, lactating women and women of childbearing age in some areas in PNG [13, 16 – 18]. A search of the literature indicates scanty information on the salt iodization program for control of IDD in Morobe [12, 15] and Eastern Highlands [16] provinces. In addition, no published data is available on the assessment of the principal impact indicator of USI in both provinces. The present studies were prompted by the apparent lack of published data on the status of iodine nutrition among school-age children in Morobe and Eastern Highlands provinces in PNG. Thus, the aims of the current studies were to determine the urinary iodine concentrations (UIC) in school-age children (6 – 12 years) as a way of assessing the impact of the salt iodization programs in Morobe and Eastern Highlands Provinces in PNG.

SUBJECTS AND METHODS:

These prospective school-based cross-sectional studies were carried out in Aseki-Menyamya district Morobe province and Gouno, Mt. Michael Local-Level

Government (LLG) area in Lufa district Eastern Highlands province (EHP), PNG. EHP is one of the five Highlands Provinces [19]. It shares provincial boundaries with Morobe, Madang, Gulf and Chimbu provinces. Menyamya and Lufa districts are located at altitudes of about 1100 meters and 1800 meters respectively [19].

The study population consisted of Schoolchildren in the age group 6 – 12 years. Simple random sampling was used to select the primary schools in each district. The total enrolments for each of the randomly selected primary schools, including the ages of children in each of the grades were listed. All children below 6 years of age and above 12 years of age were identified and excluded from the studies.

Calculation of sample size was based on a design effect of three, a relative precision of 10% and confidence level (CL) of 95%. As there was very limited information on likely prevalence rates of IDD in Aseki-Menyamya and Gouno Lufa districts, an assumed prevalence rate of 25% was used for each province. With a predicted non-response rate of 10%, the sample sizes of 200 and 150 school-age children were obtained for Aseki-Menyamya and Gouno Lufa districts respectively. These numbers were higher than the 50 recommended by the WHO/UNICEF/ICCIDD expert committee for school-based studies on the prevalence of IDD in affected populations [1].

For a given school, each of the children in the 6 – 12 years age group was assigned a computer-generated random number. The required number of children from each school was then selected by simple random sampling using the randomly generated number list. In Gouno Lufa district two cohorts of children were selected using separate randomly generated number lists. Those in the first cohort, 42 children, were involved in the assessment of the discretionary use of iodized salt including the availability of iodized salt in the households. This study was not carried out in Aseki-Menyamya district because of logistical reasons. Each of the 42 selected households in Gouno Lufa district was visited twice. Signed informed consent was obtained from the head of the household during the first visit; in addition, the total number of residents that eat food prepared in the household was recorded. One teaspoon (about 5.0g) of the salt available in the household was collected and stored in an airtight zip-lock plastic bag for analysis. A pre-weighed (250.0g) package of iodized salt was given to the head of the household to use for food preparation and consumption as usual. Each household was revisited three days later. The salt remaining in the package was reweighed to the nearest 0.1g. The total amount of salt

consumed was calculated and considered as the discretionary salt intake.

Salt samples were purchased from randomly selected trade and retail shops in Aseki- Menyamya and Gouno Lufa districts for analysis of their iodine content. A self-designed, pre-tested questionnaire was used to assess the knowledge and use of iodized salt in households in both districts. The head of each selected household was requested to complete the questionnaire.

On the spot urine sample was collected from each of the consented schoolchildren in the selected schools in Aseki-Menyamya district and the second cohort of children in Gouno Lufa district. Each urine sample was stored in properly labeled plastic tube with tight fitting stopper that was further sealed with special plastic band. The urine and salt samples were then appropriately packed separately and transported by airfreight to the Micronutrient Research Laboratory (MRL) in the School of Medicine and Health Sciences (SMHS) University of Papua New Guinea (UPNG). The samples were stored in separate refrigerators till required for analyses.

The iodine content in the salt samples from the households and stores was measured using the WYD Iodine Checker [20]. The Sandell-Kolthoff reaction was used for the assay of urinary iodine (UI) after digesting the urine with Ammonium Persulfate in water–bath at 100°C [1]. Internal bench quality control (QC) characterization of the assay methods for UI and salt samples were by the Levy-Jennings Charts and the Westgard Rules. External QC monitoring of the UI assay procedure was by "Ensuring the Quality of Urinary Iodine Procedures" (EQUIP), which is the External Quality Assurance Program (QAP) of the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA.

Microsoft Excel Data Pack 2007 and the Statistical Package for Social Sciences (SPSS) software (version 15) were used for statistical analyses of the data. Kolmogorov-Smirnov test was used to assess normality of the data. Mann Whitney U test was used for differences between two groups; Kruskal-Wallis and Friedman were used for comparison of all groups. Analysis of variance (ANOVA) was also used to compare differences between groups. Scheffe test was used for post-hoc analysis. $P < 0.05$ was considered as statistically significant.

The data were interpreted using the current WHO/UNICEF/ICCIDD criteria [1 – 3, 7, 9]. Appropriate implementation of the USI strategy was assumed if at least 90.0% of households were using salt with iodine content of 15.0ppm or more. Iodine deficiency was considered as a public health problem in the target population if the Median UIC was below 100.0µg/L and more

than 20% of the children have UIC below 50.0µg/L. Specific cut-off points for UIC were used for classifying the Status of Iodine Nutrition into different degrees of public health significance [1 – 3, 7, 9]. Further interpretation of the iodine content in salt samples was carried out using the 30ppm cutoff value proposed by the PNG Food Sanitation Regulation (PNGFSR) [8, 15]. Ethical clearance and permission for these studies were obtained from the Ethics and Research Grant committee in the SMHS UPNG. Permissions were obtained from the appropriate authorities in Menyamya and Lufa districts, and also from the Headmasters / Headmistresses in the various primary schools. Parental consent and approval were obtained from parents / Guardians of the selected children. The verbal approval of each child with parental consent was also obtained at the time of urine collection.

RESULTS:

The mean per capita discretionary consumption of salt in Lufa district was 4.7 ± 2.1g per day (Mean ± Standard Deviation), with a median of 4.2g and range of 2.1 – 9.6g. Salt was available in all the 42 households visited. The mean iodine content in salt samples from the households was 17.8 ± 4.5ppm, median was 18.5ppm and range was 7.4 – 24.4ppm. Although the iodine content was below 15ppm in 23.8% of all the salt samples, 97.6% of the salt samples had iodine content below 30ppm. Only four brands of salt were available in Gouno, Lufa district. The iodine content was greater than 15ppm in all the four brands, but two (50%) of the four brands had iodine content below 30ppm.

A total of 12 salt packages, made up of three “traditional” salt and nine different brands of salt, were purchased from randomly selected stores in Aseki-Menyamya district. The iodine content in each of the three traditional salt samples was below 15ppm; the values were 9.9ppm, 11.4ppm and 14.0ppm. The iodine content was greater than 15ppm in all the nine brands of salt. However, the iodine content in two (22.2%) of the nine brands of salt was below 30ppm. The mean iodine content in all the nine brands of salt was 49.8 ± 17.5ppm, the median was 56.2ppm and the range was 28.9 – 77.7ppm.

A total of 124 and 42 questionnaires were distributed in Aseki-Menyamya and Gouno Lufa districts respectively. All the respondents in Aseki-Menyamya completed their questionnaires, compared to 90.5% respondents in Gouno Lufa district. Analysis of the questionnaires indicated that salt was used regularly by 99.2% and 100% households in Aseki-Menyamya and Gouno Lufa districts respectively. A total of 87.0% of all the respondents in Gouno Lufa had never heard about iodized salt compared to

53.6% in Asemi-Menyamya that were told about iodized salt by friends and relatives. Among respondents in Aseki-Menyamya, 65.0% were sure of using iodized salt at home compared to 96.0% in Gouno Lufa that were not sure of using iodized salt at home. A total of 85.5% of all the respondents in Aseki-Menyamya did not know about the importance of iodized salt compared to 92.0% in Gouno Lufa. Salt was purchased from supermarkets and local markets by 60.2% and 39.8.0% of respondents in Aseki-Menyamya compared to 82.0% and 18.0% of respondents in Gouno Lufa respectively. No particular brand or type of salt was popular among respondents in Aseki-Menyamya and Gouno Lufa because the price of the available salt was the determining factor for them. However, 15% of respondents in Aseki-Menyamya use traditional salt for preparation of some local dishes. Salt was kept in closed plastic containers by 82.9% of respondents in Aseki-Menyamya compared to 74.0% in Gouno Lufa that kept salt in open plastic containers. When asked about frequency of consumption of sea-food, among respondents in Aseki-Menyamya 56.5% had never eaten sea-food, 30.6% said once in a while and 12.9% did not remember. Among respondents in Gouno Lufa 55.0% said once in a while and 45.0% said never.

A total of 222 and 135 schoolchildren in Aseki-Menyamya and in Gouno Lufa district respectively were enrolled for the studies. Informed consents to participate in the study were obtained from 207 and 132 parents in Aseki-Menyamya and Gouno Lufa respectively. Thus, the response rates were 93.2% and 97.8% in Aseki-Menyamya and Gouno Lufa districts. The Kolmogorov-Smirnov test for normality indicated that the frequency distribution curves of the UIC for all the children in Aseki-Menyamya and Gouno Lufa were not normally distributed. Thus, non-parametric statistics was used for analysis of the UIC data.

The summary statistics of the UIC for the children are presented in Table 1. For all the children in Aseki-Menyamya the median UIC was 149.5µg/L and Interquartile Range (IQR) was 70.0 – 300µg/L. The UIC was below 100.0µg/L in 32.9% of all the children and 17.9% had UIC below 50µg/L. For the children in Gouno Lufa the median UIC was 50.0µg/L, IQR was 23.9 – 76.0µg/L, UIC was below 100.0µg/L in 87.9% of them and 49.2% had UIC below 50.0µg/L. Using the Mann-Whitney and Wilcoxon tests, the UIC for children in Aseki-Menyamya was significantly higher ($p < 0.01$) than the UIC for those in Gouno Lufa; similar results were obtained using the Kruskal Wallis and Chi-Square tests ($p < 0.01$).

Distribution of the children according to the range of UIC and status of iodine nutrition is

presented in Table 2. Severe, Moderate and Mild status of iodine nutrition were prevalent in 8.2%, 9.7% and 15.0% of all the children in Aseki-Menyamya respectively, compared to 17.4%, 31.8% and 38.6% of all the children in Gouno Lufa respectively. According to current WHO/ICCIDD/WHO criteria [1, 9], severe to mild status of iodine nutrition was prevalent in 32.9% and 87.8% of the children in Aseki-Menyamya and Gouno Lufa districts respectively. Thus, iodine deficiency should be considered as public health problem among schoolchildren, age 6 – 12 years, in both districts. The situation however, was more severe in Gouno Lufa district compared to Aseki-Menyamya district at the time of these studies.

No statistically significant correlation was obtained when the UIC for children in Aseki-Menyamya and Gouno Lufa were compared with responses in the respective questionnaires.

Table 1: Summary statistics of Urinary Iodine concentration (UIC) (µg/L) for school-age children (6 – 12 years)

Parameters	**Aseki-Menyamya**	**Gouno Lufa**
N	207	132
Median (µg/L)	149.5	50.0
Interquartile range (IQR) (µg/L)	70.0 – 300.0	23.9 – 76.0
Mean (µg/L)	207.6	57.3
Standard Deviation	177.2	40.1
95% Confidence Interval (p = 0.05)	183.3 – 231.9	50.4 – 64.2
% (n) of children with UIC below 100µg/L	32.9 (68)	87.9 (116)
% (n) of children with UIC below 50µg/L	17.9 (37)	49.2 (65)

Table 2: Distribution of the children according to range of urinary iodine concentration (UIC) (µg/L) and status of iodine nutrition

Range of UIC (µg/L)	Status of Iodine Nutrition	Percent (n) distribution of children	
		Aseki-Menyamya (n = 207)	Gouno Lufa (n = 132)
< 20	Severe	8.2% (17)	17.4% (23)
20 – 49	Moderate	9.7% (20)	31.8% (42)
50 – 99	Mild	15.0% (31)	38.6% (51)
100 – 199	Optimal	29.0% (60)	11.4% (15)
200 – 299	Risk of IIH*	12.6% (26)	0.8% (1)
≥300	Risk of adverse health	25.6% (53)	0

*IIH: Iodine Induced Hyperthyroidism

For detailed analysis of the UIC data the children were separated according to age groups. The summary statistics of the UIC for children in the various age groups in Aseki-Menyamya and Gouno Lufa are presented in Table 3.

No statistically significant differences were indicated when the UIC for the children in the various age groups in Aseki-Menyamya were compared using the Mann-Whitney and Wilcoxon tests ($p > 0.05$) and the Kruskal Wallis tests ($p > 0.05$).

Similar results were obtained when the UIC for children in the various age groups in Gouno Lufa were compared. However, the UIC for children in the various age groups in Aseki-Menyamya were significant higher ($p< 0.05$) than the UIC for their counterparts in Gouno Lufa.

Unlike the children in Aseki-Menyamya, the median UIC for the children in all the age groups in Gouno Lufa were below 100.0µg/L (Fig. 1), indicating suboptimal status of iodine nutrition for all of them. Table 4 shows the percent distribution of the children in the various age groups according to the range of UIC and status of iodine nutrition. In Gouno Lufa, prevalence of severe status of iodine nutrition was highest among the children in the youngest age group (6years); followed by moderate status that was highest among children in the 7years age group and mild status highest among children in the 10years age group. Severe to mild status of iodine nutrition was prevalent among children in all the age groups in Gouno Lufa compared to Aseki-Menyamya.

For further analysis, the UIC data was separated according to gender. The gender distribution indicated 108 (52.2%) male and 99 (47.8%) female children in Aseki-Menyamya district; 76 (57.6%) male and 56 (42.4%) female children in Gouno Lufa district. The summary statistics of the UIC for the male and female children are presented in Table 5.

The median UIC for the male and female children in Aseki-Menyamya district were 145.8ug/L and 168.0µg/L respectively. The IQR for the male children was 65.8 – 261.0µg/L and for the female children 81.5 – 350.0µg/L. No statistically significant difference ($p > 0.05$) was indicated when the UIC for the male and female children was compared. The UIC was below 100µg/L among 33.3% of the male and 32.3% of the female children. Among the male children the UIC was below 50.0µg/L in 19.4%, compared to 16.2% of the female children.

Table 3: Summary statistics of UI concentrations (µg/L) for the children in the various age groups

	Districts	Age groups						
Parameters		6yrs	7yrs	8yrs	9yrs	10yrs	11yrs	12yrs
N	Menyamya	31	49	42	20	25	16	24
	Lufa	19	23	10	22	20	14	24
Median (µg/L)	Menyamya	160.0	146.0	135.0	90.0	170.5	189.5	232.6
	Lufa	32.0	43.5	34.5	52.0	54.5	44.0	55.8
IQR (µg/L)	Menyamya	68.0 – 295.0	50.3 – 311.3	67.0 – 263.1	44.4 – 246.8	95.5 – 256.3	137.3 – 425.0	102.6 – 436.3
	Lufa	18.5 – 71.0	29.0 – 71.5	21.8 – 64.8	21.9 – 67.9	35.6 – 79.6	21.4 – 85.5	38.8 – 91.3
Percentage of children with UIC below 100µg/L	Menyamya	32.3%	36.7%	31.0%	60.0%	24.0%	18.8%	25.0%
	Lufa	94.7%	82.6%	90.0%	90.9%	95.0%	85.7%	79.2%
Percentage of children with UIC below 50µg/L	Menyamya	16.1%	24.5%	16.7%	25.0%	8.0%	18.8%	12.5%
	Lufa	52.6%	60.9%	60.0%	40.9%	40.0%	57.1%	41.7%

Table 4: Distribution (%) of children in the various age groups according to the range of UI concentration (µg/L) and status of iodine nutrition

			Percentage of children in various Age groups						
Range of UIC (µg/L)	Status of Iodine Nutrition	Districts	6yrs	7yrs	8yrs	9yrs	10yrs	11yrs	12yrs
< 20	Severe	Menyamya	9.7	10.2	2.4	10.0	8.0	12.5	8.3
		Lufa	26.3	17.4	20.0	18.2	10.0	21.4	12.5
20 – 49	Moderate	Menyamya	6.5	14.3	14.3	15.0	0	6.3	4.2
		Lufa	26.3	43.5	40.0	22.7	30.0	35.7	29.2
50 – 99	Mild	Menyamya	16.1	12.2	14.3	35.0	16.0	0	12.5
		Lufa	42.1	21.7	30.0	50.0	55.0	28.6	37.5
100 – 199	Optimal	Menyamya	29.0	22.4	40.4	15.0	40.0	37.5	16.7
		Lufa	5.3	17.4	10.0	4.5	5.0	24.3	20.8
200 – 299	Risk of IIH	Menyamya	16.1	14.3	11.9	5.0	12.0	6.3	16.7
		Lufa	0	0	0	4.5	0	0	0
≥300	Risk of adverse health	Menyamya	22.6	26.5	16.7	20.0	24.0	37.5	41.7
		Lufa	0	0	0	0	0	0	0

Fig. 1: Median UIC (µg/L) of children in the various age groups in Aseki-Menyamya and Gouno Lufa districts

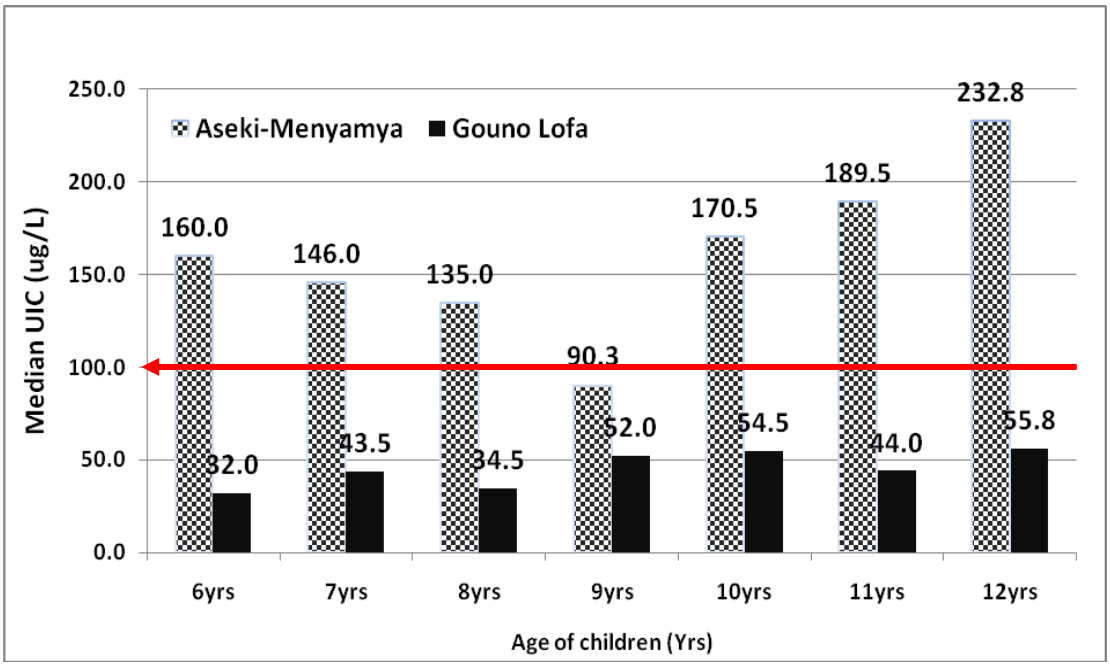

Table 5: Summary statistics of the UIC (ug/L) of the male and female children

Parameters	**Aseki-Menyamya**		**Gouno Lufa**	
	Male	**Female**	**Male**	**Female**
N	108	99	76	56
Median (µg/L)	145.8	168.0	51.3	46.3
Interquartile Range (µg/L)	65.8 – 261.0	81.5 – 350.0	21.6 – 82.1	29.1 – 69.5
Mean (µg/L)	187.4	229.7	59.0	54.9
Std dev	156.0	196.3	41.3	38.6
95% Confidence Interval (p=0.05)	157.7 – 217.2	190.5 – 268.8	49.6 – 68.5	44.6 – 65.3
% (n) of children with UIC below 100µg/L	33.3% (36)	32.3% (32)	85.5% (65)	91.1% (51)
% (n) of children with UIC below 50µg/L	19.4% (21)	16.2% (16)	44.7% (34)	55.4% (31)

Table 6: Percent distribution (n) of the male and female children according to the range of UIC (µg/L) and status of iodine nutrition

Range of UIC (µg/L)	Status of Iodine Nutrition	Aseki-Menyamya		Gouno Lufa	
		Male (n = 108)	Female (n = 99)	Male (n = 76)	Female (n = 56)
<20	Severe	7.4% (8)	9.1% (9)	21.1% (16)	12.2% (7)
20 – 49	Moderate	12.0% (13)	7.1% (7)	23.7% (18)	42.9% (24)
50 – 99	Mild	13.9% (15)	16.2% (16)	40.8% (31)	35.7% (20)
100 – 199	Optimal	30.6% (33)	27.3% (27)	14.5% (11)	7.1% (4)
200 – 299	Risk of IIH	14.8% (16)	10.1% (10)	0	1.8% (1)
≥300	Risk of adverse health	21.3% (23)	30.3% (30)	0	0

For Gouno Lufa district, the median UIC for the male and female children were 51.3µg/L and 46.3µg/L respectively. The IQR for the male children was 21.6 – 82.1µg/L, and for the female children 29.1 – 69.5µg/L. There was no statistically significant difference ($p > 0.05$) between the UIC of the male and female children. A total of 85.5% male and 91.1% female children had UIC below 100µg/L. The UIC was below 50µg/L in 44.7% of the male and 55.4% of the female children.

Statistically significant difference ($p < 0.01$) was obtained when the UIC for the male children in Aseki-Menyamya was compared with that of the male children in Gouno Lufa. Similar result was obtained between the female children.

Distribution of the male and female children according to the range of UIC and status of iodine nutrition is presented in Table 6. For Aseki-Menyamya, 7.4% of the male children were in severe status of iodine nutrition compared to 9.1% of the female children. Moderate status of iodine nutrition was prevalent among 12.0% of the male and 7.1% of the female children. Mild status was prevalent among 13.9% and 16.2% of the male and female children respectively.

For children in Gouno Lufa, although severe status of iodine nutrition was higher (21.1%) among the male children compared to the female children (12.5%), moderate status was higher among the female children (42.9%) compared to the male children (23.7%). The prevalence of severe to mild status of iodine nutrition was, however higher among the female children (91.1%) compared to the male children (85.5%); this difference was not statistically significant ($p > 0.05$).

DISCUSSION:

According to WHO/UNICEF/ICCIDD and other expert committees (1 – 5, 9 – 11) schoolchildren in the 6 – 12 years age group are the useful targets for assessment of the status of iodine nutrition in a population because of their high vulnerability to iodine deficiency and easy accessibility in the community. The school-based approach was used in this study also because of the high enrolment and attendance of both male and female children in primary schools in Morobe province and EHP [19].

The iodine content was greater than 15ppm in the nine brands and the four brands of salt from Aseki-Menyamya and Gouno Lufa districts respectively; this indicates partial implementation of the USI strategy in both districts. However, both districts defaulted in the implementation of the PNGFSR, because 22.2% and 50% of the brands of salt in Aseki-Menyamya and Gouno Lufa districts respectively were below 30ppm. This indicates the need for effective monitoring of the implementation of the PNGFSR in both districts. The low iodine content in the traditional salts (the ash obtained by burning shrubs and canes), strongly suggests the need to discourage their use as the household salt in Aseki-Menyamya district.

The 4.7 ± 2.1g mean per capita per day discretionary consumption of salt obtained in the present study for Gouno Lufa district was within the 3 to 20g range per capita salt intake per day reported for other countries [1, 21, 22]. The result was lower than the 10.0g per capita per day salt intake used in formulating the PNG standards for iodine content in salt indicated in the PNGFSR [8, 12, 15]. It was also lower than the 6.2 to 7.8g reported for Lae City [12] and some areas in Central Province in PNG [14], but higher than the 2.6g reported for Hella region in SHP PNG [13, 14]. The availability of salt in all the selected households strongly supports the use of salt as the major vehicle for iodine supplementation in Gouno Lufa district EHP, PNG.

The mean iodine content of 43.7 ± 24.8ppm for the four brands of salt from the trade-stores was significantly higher ($p < 0.05$) than the mean iodine content (17.8 ± 4.5ppm) in the salt samples from households in Gouno Lufa district. This significant difference may be due to the effects of humidity and poor storage of salt in the households, because 74% of the respondents in Gouno Lufa kept their salt in open plastic containers. Similar findings have been reported by Jooste et al [23] and Sankar et al [24]. Our result indicates that, according to the WHO/UNICEF/ICCIDD criteria [1], the salt samples in 23.8% of the households in Gouno Lufa district were not

adequately iodized. Using the criteria in the PNGFSR, the salt samples in 97.6% of households in Gouno Lufa district were not adequately iodized. These findings may indicate inadequate knowledge about the storage and use of iodized salt which is already available in the community. Thus, it is important that program planners carry out intensive nutrition education, information and awareness campaign to advocate for appropriate storage and use of iodized salt in Gouno Lufa district, EHP PNG.

The effective implementation of the USI strategy requires systematic monitoring of UIC, which is the key biochemical indicator recommended for assessing the impact of iodine deficiency control programs [1, 3]. The non-response rates of 6.3% and 2.2% obtained for Aseki-Menyamya and Gouno Lufa districts respectively were lower than the 10% non-response rates used in the calculation of the sample sizes. In both studies the number of male children was slightly higher than that of female children; no specific reasons can be given for these differences. The decision to participate in the study was voluntary and required the approval of the parents or guardians of the school children. Unlike in Gouno Lufa, in Aseki-Menyamya the younger children (age range 6 – 8yrs) were more willing to participate in the study compared to the older (9 – 12yrs) children.

Optimal status of iodine nutrition in a population is achieved when the median UIC is in the 100 – 200µg/L range among children in the 6 – 12 years age group [1 – 5, 7, 9]. The median UIC for the children in Aseki-Menyamya was 149.5µg/L compared to 50.0µg/L for the children in Gouno Lufa. This indicates optimal status of iodine nutrition among the children in Aseki-Menyamya and suboptimal status of iodine nutrition among the children in Gouno Lufa. This difference was further confirmed by the less that 20% of children in Aseki-Menyamya (17.9%) with UIC below 50.0ug/L compared to 49.2% of children in Gouno Lufa. Thus, according to the current WHO/ICCIDD/UNICEF criteria [1 – 5, 7, 9] iodine deficiency was not at the level of public health significance among children in Aseki-Menyamya district. However, severe status of iodine nutrition was prevalent in 8.2% of the children and mild to moderate status of iodine nutrition was prevalent among 24.7% of the children. This should be of concern to program planners in Aseki-Menyamya district and the PNG National Department of Health.

The UIC result for Gouno Lufa indicated that iodine deficiency was potential public health problem among the children; with 17.4% in sever status and 70.4% with mild to moderate status of iodine nutrition. The situation in Gouno Lufa district should be of great concern to the authorities and the

program planners at all levels of implementation, in the district, province, region and the National Department of Health in PNG.

In Aseki-Menyamya the median UIC for all the children (149.5µg/L) and for the male (145.8µg/L) and female (168.0µg/L) children were higher than the median UIC values obtained for children in Hella Region SHP PNG (48.0µg/L) [13], but lower that the values obtained in Honduras (287.0µg/L), Nicaragua (259.0µg/L), El Salvador (251.0µg/L), Chile (565.0µg/L), Ecuador (590.0µg/L), Brazil (1013.0µg/L) and Mexico (1150.0µg/L) [25].

The median UIC for all the children in Gouno Lufa (50.0µg/L) was higher than the median UIC (48.0µg/L) reported for schoolchildren in Hella Region SHP PNG [13]. The median UIC for the male children (51.3µg/L) in Gouno Lufa was lower than the value reported for male children (67.0µg/L) in Hella SHP PNG; however the value for the female children in Gouno Lufa (46.3µg/L) was similar to the value (44.0µg/L) for the female children in Hella SHP PNG [13]. The high prevalence of severe to mild status of iodine nutrition among the children in Gouno Lufa district may be due to suboptimal discretionary intake of iodine. This is because the mean per capita intake of 4.7g of salt with mean iodine content of 17.8ppm is equivalent to a calculated mean discretionary intake of 83.7µg iodine per day. Assuming that 20% of the iodine in salt is lost during storage and food preparation [1], the calculated discretionary intake of iodine per capita becomes 66.9µg per day. This amount of iodine is lower than the 90 to 120µg daily intake of iodine recommended for children [1]. This low intake of iodine should be of concern to program planners in the Gouno Lufa district and the EHP, because of the severe consequence of iodine deficiency on the children, which may be having deleterious effect upon intelligence, and also on the vulnerable groups in the community, especially pregnant and lactating mothers [1, 4, 5, 7, 9 – 11, 21, 22].

Our results indicated that none of the children in Gouno Lufa district had UIC above 300µg/L compared to those in Aseki-Menyamya with UIC greater than 300.0µg/L in 25.6% of all the children, 21.3% of the male and 30.3% of the female children. This indicates excessive intake of iodine and risk of adverse health among some of the children in Aseki-Menyamya. Excessive intake of iodine has been reported in many countries, particularly when salt iodization is excessive and poorly monitored [1 – 5, 19]. Intake of iodine in excess of 1000µg per day, with similar amount excreted in the urine can be tolerated by some individuals with little or no apparent problems; however regular consumption of large amount of excess iodine can be potentially harmful to

susceptible individuals [1, 19]. According to some experts [1 – 5], in some community the problems caused by excessive intake of iodine may be minor compared to those that can be caused by inadequate intake leading to iodine deficiency. Thus, the general concept, "it is better to consume more iodine per day than to consume less", particularly among the vulnerable groups (children, pregnant or lactating women) in the community [1]. It is important therefore for program planners to improve and strengthen the monitoring of USI and the PNGFSR in both districts, to ensure their efficiency, sustainability and functionality.

CONCLUSION:

The availability of salt in all the selected households in Gouno Lufa district strongly supports the use of salt as the vehicle for iodine supplementation in EHP. However, the discretionary intake of iodine in the households was lower than the recommended intake for children. Thus, there is need, for aggressive awareness campaign, intensive nutrition education and information emphasizing the significance of proper storage of iodized salt, and for using appropriate amount of adequately iodized salt; greater attention should however, be given to Gouno Lufa district EHP.

Although iodine deficiency was not at the level of public health significance among the schoolchildren in Aseki-Menyamya district, mild to moderate status of iodine nutrition was prevalent among 24.7% of them. Iodine deficiency was a significant public health problem among schoolchildren in Gouno Lufa district, because of the high prevalence of severe (17.4%) and mild to moderate (70.4%) status of iodine nutrition. This should be of concern to program planners, because of the severe consequence of iodine deficiency on the schoolchildren, which may be having deleterious effect upon their intelligence.

Our findings indicate the urgent need for reassessing the implementation of USI strategy and the PNGFSR especially in Gouno Lufa district; and for efficient, sustainable, systematic and functional monitoring systems to strengthen and improve on the implementation of the iodine deficiency control programs in Aseki-Menyamya and Gouno Lufa districts in PNG.

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CASE REPORT

RADIATION CARIES IN IRRADIATED PATIENT OF NASOPHARYNGEAL CARCINOMA – A CASE REPORT

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Running title: Radiation caries

ABSTRACT:

Radiotherapy (RT) plays an important role in the management of head and neck cancer, especially oropharyngeal and nasopharyngeal cancer. It is also associated with several undesired side effects such as radiation caries which is a common, yet serious, complication. We are presenting a case of radiation caries in 55 year old male patient who had undergone radiotherapy for nasopharygeal carcinoma.

KEYWORDS: nasopharyngeal carcinoma, radiotherapy, radiation caries

Submitted April 2013, Accepted July 2013

INTRODUCTION:

Worldwide, oral carcinoma is one of the most prevalent cancers and is one of the 10 most common causes of death. Of the more than one million new cancers diagnosed annually in the United States, cancers of the oral cavity and oropharynx account for approximately 3% [1]. Of these, 40% will receive curative benefit from surgery, radiation, chemotherapy, or a combination modality [2]. In dealing with patients with cancer of the head and neck a team approach is required for effective management. When RT is indicated, it is imperative that health of the oral cavity be assessed initially as well as throughout therapy.

Nasopharyngeal carcinoma is a relative rare malignancy of Indian subcontinent. Patients with malignant tumours of the nasopharynx have bewildering arrays of signs and symptoms [3]. Nasopharyngeal carcinoma is radiosensitive, the primary treatment modality external beam radiotherapy. The usual radiation dose delivered to the nasopharynx ranges from 66 to 70 Gray (GY) and approximately 60 GY to neck. This dosage is usually given 1.8 to 2.0 GY daily fractions through two lateral opposing fields with or without an anterior field [4].

Although RT plays an important role in the management of patients with head and neck cancer, it is also associated with several undesired reactions [2]. The RT field of exposure frequently includes the salivary glands, oral mucosa, and jaws, thus, leading to various side effects including hyposalivation, xerostomia, radiation caries, mucositis, and taste loss [5,6,7]. Radiation caries is a rampant form of dental decay that may occur in individuals who receive a course of radiotherapy that includes exposure of the salivary glands. The carious lesions result from changes in the salivary glands and saliva, including reduced flow, decreased pH, reduced buffering capacity, and increased viscosity [8]. Because of the reduced or absent cleansing action of normal saliva, debris accumulates quickly. Irradiation of the teeth by itself does not influence the course of radiation caries [8]. A systematic review of dental disease in patients undergoing cancer therapy was conducted out of which, sixty-four published papers between 1990 and 2008 were reviewed where the overall prevalence of dental caries was found to be 28.1%. The overall decay missing filled teeth (DMFT) indices for patients who were post-antineoplastic therapy was 9.19 [9].

CASE REPORT:

A 55 year old male patient came to the Department of Oral Medicine and radiology with the complaint of decayed tooth in the upper and lower left and right back tooth region since one year. His past medical history revealed that he has been under treatment for undifferentiated nasopharyngeal carcinoma of neck. Surgery included a local tumor resection with radical neck dissection. Radiotherapy was accomplished within 100 days postoperatively. He was irradiated with 60 Gy, at single doses of 2 Gy. Radiotherapy parameters, such as radiation dose and technique used, were recorded. The patient had no muscle tenderness or facial asymmetry and denied any symptoms of temporomandibular joint disorder or myofacial pain dysfunction.

On Intra oral Examination, blanching of the buccal mucosa with physiological melanin pigmentation noticed. The salivary flow appeared to be within normal limits. On hard tissue examination there was multiple root stumps in both maxillary and mandibular teeth with blackish discoloration (Figs 1 & 2).

The Provisional diagnosis of radiation caries was considered. Patient was referred to the department of oral and maxillofacial surgery for total extraction followed by prosthetic rehabilitation with the complete denture.

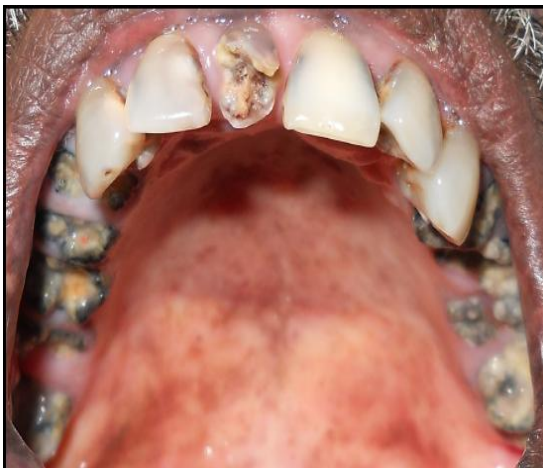

Figure 1: shows blackish discoloured multiple root stumps in the posterior maxilla and right central incisor with class V caries in maxillary anteriors

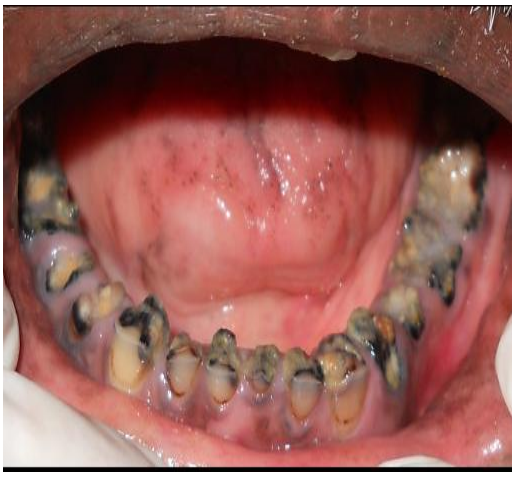

Figure 2: shows multiple black discoloured root stumps in mandible.

DISCUSSION:

The location of the primary tumor or lymph node metastases dictates the inclusion of the oral cavity, salivary glands, and jaws in the radiation treatment portals for patients who have head and neck cancer [7]. In the management of patients with head and neck cancer, RT plays a very important role but it is also associated with several undesired effects. The clinical sequelae of the radiation treatment include mucositis, hyposalivation, loss of taste, osteoradionecrosis, radiation caries, and trismus. These sequelae may be dose-limiting and have a tremendous effect on the patient's quality of life [1, 7]. In irradiated patients there is increased risk for the development of a rapid, rampant carious process known as radiation caries. Usually it affects atypical areas of teeth, such as the lingual surface, incisal edges, and cusp tips and it tends to develop four weeks after completion of RT [5].

Radiation caries is mainly an indirect effect of irradiation induced changes in salivary gland tissue that result in hyposalivation, altered salivary composition, a shift in oral flora toward cariogenic bacteria (S. mutans, Lactobacillus species), and dietary changes. For this reason, prevention of hyposalivation will invariably

contribute to the prevention of radiation caries [7]. In the case presented here, hyposalivation might be the possible reason for initiation of the radiation caries as the patient gave the history **of** xerostomia during and after the course of RT. Few studies have shown that some patients do not clinically appear to be xerostomic after radiation therapy, but may experience a change in the quality of their saliva, leading to rapid dental demineralization. Even a 25 percent decrease in saliva may result in dental breakdown [10]. This may be the other probable cause behind the development of radiation caries in our patient.

In the early days of radiotherapy, extraction of the teeth prior to irradiation was proposed. Advocates for oral hygiene regimens and restorative procedures met with limited success in caries prevention in those days. Since then, comprehensive preventive measures have been recommended for head and neck cancer patients before, during, and after radiotherapy [7]. The level of radiation-induced caries due to xerostomia can be limited by optimal concepts of oral hygiene. In case of a lack of oral hygiene, an indication for dental extraction occurs [11]. In the case reported here, due to lack of proper dental care before, during and after radiotherapy, extraction was the only option left as the treatment due to rapid progression of the radiation caries.

Clinically, three different patterns of radiation caries have been identified. The most common pattern [Type 1] affects the cervical aspect of the teeth and extends along the cementoenamel junction. A circumferential injury develops and crown amputation often occurs. The second pattern [Type 2] presents with areas of demineralization on all dental surfaces. Generalized erosions and worn occlusal and incisal surfaces are not uncommon. The third and least common pattern [Type 3] presents as color changes in the dentin [5,8]. The crown becomes dark brown/black and occlusal and incisal wear may be seen [5, 8]. In the present case it was type 3 radiation caries.

It is now generally accepted that almost complete caries prevention can be achieved by the daily use of fluoride in conjunction with strict oral hygiene in irradiated patients [12,13,14]. Interdental techniques such as flossing, along with plaque-disclosing agents, can also be beneficial [13,15]. Carious lesions have to be restored before radiotherapy is initiated. Dietary instructions about noncariogenic foods should be given. The preventive caries program consisting of daily oral hygiene and daily topical 1.0% NaF gel application by means of custom made fluoride carriers. In a study done by Horiot et al. [16] with 935 head and neck cancer patients with more than 10-year experience, concluded that this fluoride protocol was a highly reliable method for the prevention of radiation caries, and that the use of a toothpaste with a high

fluoride content (3.0% NaF) twice a day was a good alternative, provided its pre-requisites (higher level of compliance) were well-understood by both clinician and patient [16]. In addition, fluoride mouthwashes have been used with considerable success [17,18].

The restoration of carious teeth in patients who have undergone cervicofacial radiotherapy can be extremely demanding on both patients and dentists. An increased prominence of cariogenic microorganisms leads to the rapid circumferential progress of cervical lesions [19]. As it is very difficult to gain access to the cervical lesions, the excavation of caries might be incomplete, the cavity preparation margins can be difficult to define and the preparations might provide little mechanical retention for the restorations. Selection of the most appropriate restorative material is also difficult under these circumstances, with the more-viscous aesthetic conventional glass ionomer cements appearing to offer a reasonable compromise in terms of desirable handling, adhesive, anticariogenic and physical properties [20].

Although radiation caries is a multifactorial condition, its main risk factor in head and neck cancer patients is RT-induced reduction of salivary flow. Thus, the ideal approach to prevent radiation caries would be to avoid radiation-induced hyposalivation caused by damage to the salivary glands. This could be achieved with exclusion of the major and minor salivary glands from the irradiation field [5]. In this context the integration of intensity-modulated radiotherapy (IMRT) techniques into broad routine will be of great benefit to patients [21]. Despite a multifactorial etiology, radiation caries is primarily a consequence of hyposalivation. Therefore, radiation caries would ideally be prevented by sparing salivary glands from radiation. In cases where this is not possible, prevention is achieved with comprehensive dental care before, during, and after RT.

CONCLUSION:

Radiation caries is a one of the undesired effect of RT. So the dentists should be aware about the consequences of RT in head and neck region and about the various preventive measures with optimal treatment when needed. In this context, motivation of patients, adequate plaque control, stimulation of salivary flow, fluoride use, and nutritional orientation are essential to reduce the incidence of radiation caries and ultimately improve the quality of life of in irradiated patients.

Source of support: Nil, No conflict of interest

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