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MORPHOMETRIC ASSESSMENT OF THE EFFECT OF CARICA PAPAYA BARK EXTRACT ON TESTES OF SPRAGUE-DAWLEY RATS

*Taiwo O. KUSEMIJU, Oshiozokhai E. YAMA, Abraham A. OSINUBI, Abayomi O. OKANLAWON

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ABSTRACT:
Carica Papaya (CP) plant (paw paw) is largely used for its curative benefit and now being exploited as an anti-fertility agent. This study aimed at quantifying the effects of aqueous extract of the bark of CP on the testes of adult Sprague–Dawley (S-D) rats. Ninety adult 6-8 weeks old male S-D rats were divided into nine groups [1DW(4wk), 1CP50(4wk), 1CP100(4wk), 2DW(8wk), 2CP50(8wk), 2CP100(8wk), 3DW(16wk), 3CP50(16wk) and 3CP100(16wk)] of 10 rats per group. Rats in groups 1DW(4wk), 2DW(8wk), and 3DW(16wk) served as control and were treated with distilled water (DW) for 4, 8 and 16 weeks respectively. Rats in groups 1CP50(4wk), 2CP50(8wk) and 2CP50(16wk) were fed 50 mg/ml/day CP, while those in groups 1CP100(4wk), 2CP100(8wk) and 2CP100(16wk) were fed 100 mg/ml/day CP. Rats in groups 2CP50(16wk) and 2CP100(16wk) compared to those in 3DW(16wk) were observed for possible reversibility after 8 weeks of withdrawal of the CP extract. Rats were sacrificed after the appropriate duration and testicular histological sections prepared for histometric analysis. Stereological parameters estimated were; tubular diameter, cross sectional area of seminiferous tubules, volume density, number of profiles per unit area, absolute volume of seminiferous tubules and testicular interstitium, numerical density, length density and star volume of the seminiferous tubules. The result showed dose and duration dependent decrease in mean testicular volume, tubular diameter, cross sectional area and star volume of tubules. A converse increase in the length density, numerical density, number of profiles per unit area and volume density of tubules was also observed. Alteration in the histomorphometric data indicates that the CP bark extract can cause impairment in spermatogenesis.

Key words: Carica papaya, Rats, Testes, Histomorphometric, Spermatogenesis

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INTRODUCTION:
The primary intent in biological research whether by default or design, is to compare structure with function. The findings grants explanation to why organizational levels are made the way they are and the reason for their varied functions. Conscious attempts have been made in the past to understanding the 3-dimensional (3-D) morphology of the rats’ testis and the organizational arrangements [1]. The findings relating its structure to function have remained vague [1]. A clearer understanding is provided when more accurate cytometric quantification is employed. It counts and measures structures using formidable but simple stereological tools [2].

A great deal of first-order stereological information exists for the numbers, sizes and component densities of the various tissue compartments of the testes under normal and experimental conditions [3-6]. These methods are based on random intersections between a geometric probe and the object of interest. Several studies have estimated length of biological objects, including length of seminiferous tubules and capillaries in cerebral cortex [7, 8]. In many biological applications, however, tissue landmarks are difficult to recognize following randomization around one or more axis as required for vertical uniform random (VUR) slices and isotropic uniform random (IUR) plane sections. A recent method that avoids this caveat uses virtual isotropic planes to probe linear objects on arbitrary thick sections [9].

Hitherto, there has been a paucity of complementary quantitative information concerning the 3-D arrangement of testicular compartments. However, with the advent of quantitative methods for exploring second-order stereology, it is now possible to examine associations in various spatial and directional distributions [10-12]. The stereological tools allow quantitative 3-D estimates to be obtained from 2-D slices histological sections of a tissue sample. In a recent biological application on rat testis the analysis of stereological parameters provided quantitative description of differences in 3-D arrangements of testicular components [13]. While direct observations are made from 2-D sections, 3-D information is obtained through mathematically proven relationships [10].

In this study, design-based stereological methods were used to analyze testicular tissues of Sprague-Dawley (S-D) rats treated with Carica papaya (CP) bark extract. The findings are expected to contribute considerably in understanding the functional and pathological morphologies in the testes.

MATERIALS AND METHODS:
Ninety adult male S-D rats weighing between 180-250 g were used. They were procured from a breeding stock, maintained in the
Animal house of the College of Medicine, University of Lagos (CMUL) Nigeria. They were allowed to acclimatize for 2 weeks in the animal control room Department of Anatomy CMUL, with an ambient temperature maintained between 26-28°C. They were permitted free access to water and food *ad libitum*.

**Plant Source and Extract Pharmacognosy:**
The bark of the CP plant was obtained from a forest in Lagos Nigeria in the month of September. It was authenticated in the Department of Botany, University of Lagos, Nigeria where the voucher specimen was deposited (ascension number LUH 2151). Aqueous extraction of the bark was carried out in the Pharmacognosy Department, Faculty of Pharmacy University of Lagos, Nigeria. Briefly, the completely oven-dried (40°C for 4 days) bark (350g) was crushed and powdered. The powder was placed in distilled water and allowed to boil, simmering for one hour. The water extract was dialyzed and the internal solution lyophilized. The residue obtained (4.05g, 1.16% yield) were stored at 4°C before use. When required, the residues were dissolved in distilled water and the desired pharmacological concentrations administered based on the animal’s individual body weight.

**Experimental protocol:** The rats were randomized into 9 groups (10 rats per group) identified as: 1DW(4WK), 1CP50(4WK), 1CP100(4WK), 2DW(8WK), 2CP50(8WK), 2CP100(8WK), 3DW(16WK), 3CP50(16WK) and 3CP100(16WK) respectively. The groups consist of 3 treatment intermissions: 4, 8 and 16 weeks. The rats in groups 1DW(4WK), 2DW(8WK) and 3DW(16WK) (control groups) were used to compared events in the other groups.

- **Groups:** 1DW(4WK), 1CP50(4WK) and 1CP100(4WK) were treated with 5.0 ml distilled water (DW), 50 and 100 mg/ml/kg/day CP bark extracts orally for 4 weeks.
- **Groups:** 2DW(8WK), 2CP50(8WK) and 2CP100(8WK) were treated with 5.0 ml DW, 50 and 100 mg/ml/kg/day CP bark extracts orally for 8 weeks.
- **Groups:** 3DW(16WK), 2CP50(16WK) and 2CP100(16WK) were treated with 5.0 ml DW, 50 and 100 mg/ml/kg/day CP bark extracts orally for 8 weeks. The bark extract of CP discontinued and treated subsequently with 5.0 ml DW alone for another 8 weeks.

At the end of the 4th, 8th and 16th week experimental periods, the rats in the respective groups were sacrificed. The experimental intervals were guided by the period taken to complete a spermatogenic cycle in rat [15].

**Preparation of tissues for Histological Study:** Rats were sacrificed according to the methods described previously by Osinubi *et al* [23]. The rats were made unconscious by cerebral dislocation; this was followed by ventral laparotomy to gain access to the testes via the abdomen; the testes were excised, weighed and fixed in Bouin’s fluid and...
processed for morphometric studies. After 48 hours the organs were removed from Bouin's fluid and further fixed in fresh Bouin's fluid for another 72 hours [23]. Fixed testes were dehydrated through graded series of ethanol, cleared in chloroform, and infiltrated and embedded in molten paraffin wax. Before embedding, sections were orientated perpendicular to the long axis of the testes and designated as vertical sections. Serial sections obtained from the solid block of tissue attached to a wooden holder that was fixed to a manually operated microtome and sections were cut at 5.0µm. Selected sections were mounted and subsequently stained by Haematoxylin and Eosin staining techniques for light microscopic examination [23].

**Stereological measurements:** The dissected testes were separated out from the adherent tissue and weighed (TW) to the nearest mg on an electronic balance [5, 6]. The testicular volume (TV) estimated by water displacement method [5, 6]. Seven sections per testis were selected by systematic sampling method that ensured fair distribution between the polar and equatorial region of each testis [1, 2]. This allowed unbiased numerical estimation of morphometric parameters. The diameter of seminiferous tubules (D) with profiles that were round or nearly round were measured for each animal and the mean determined by taking average of two diameters at right angles, D₁ and D₂. They were taken only when D₁/D₂ ≥ 0.85 this is to abolish different degrees of profile irregularities or tissue contraction. The cross sectional areas (AC) of the seminiferous tubules were determined from the formula AC = D² X π/4; i.e. multiplying the mean of the squared diameters of the seminiferous tubules (D²) by a constant (π/4) where π is equivalent to 3.142. The number of profiles of seminiferous tubules per unit area (NA) was determined using the unbiased counting frame proposed by Gundersen [14]. Using the frame, we counted all profiles completely inside and any part inside the frame provided they do not touch or intercept the forbidden line (full-drawn line) or exclusion edges or their extension. Point counting methods were utilized to obtain the volume density (Vv), which is the volume of the seminiferous tubules/unit area of testis [15]. The number of profiles per unit volume (NV) was determined by using the modified Flioderus equation: NV = NA / (D+T) [16]. Where NA is the number of profiles per unit area and D is the diameter and T is the average thickness of the section. The length density (LV) was determined using the equation 2xNA [16]. The star volume (V*) was calculated from the equation Vv = π/3 x mean lo³ [17, 18]. The star volume of the seminiferous tubules provides a direct and unbiased estimate of volume which has a strict mathematical definition i.e. the volume of all parts of a 3-dimension space which are visible on every direction from a given point within it.
**Statistical analysis:** Data were expressed as mean ± standard deviation (SD). Analyses of variance (ANOVA) with Scheffer's post-hoc test were used to analyze the significance of difference and a probability of $p < 0.05$ was considered significant. This was done using the SPSS software package.

**RESULTS:**

**Histological analysis:** The administration of CP bark extract to S-D rats for 4 and 8 weeks showed significant alteration in the histology of the testis (Figures 1 and 2). The seminiferous tubules of control rats contained germ cells up to the level of spermatozoa (Figure 1a, 2a, and 3a). In the samples from rats treated with low dose for 4 weeks, the seminiferous tubules showed focal areas with marked hypospermiation and coagulative necrosis of the seminiferous tubules (Figure 1b) while the high dose showed an extensive necrosis of the seminiferous tubules and damage to the germ cells (Figure 1c). There was also destruction to the basement membrane, focal area of disorganization and sloughing. The low and high doses given for 8 weeks (Figure 2b and 2c) showed a more extensive damage with the nuclei of the cells not seen and very scanty Leydig cells. The reversibility after a period (8 weeks) of discontinuation of extract treatment showed some degree of recovery compared to their control counterpart (Figure 3).

**Morphometric correlation:** The diameters of the seminiferous tubules revealed differences between controls and CP treated rats. Some tubules presented a distorted shape which made it difficult to take the measurement. Consequently only circular profiles were considered in each group, as suggested by Cruz-Orive and Gual-Arnau [19]. The possibility that some tissue shrinkage occurred as a result of the fixation cannot be discounted. However all tissues were treated under the same condition of fixation, embedding and sectioning. It is believed therefore that the differences were most likely due to the different treatments used in the study.

**Tubular Diameter:** There was a dose-dependent and duration-dependent reduction in the tubular diameter of the experimental rats from 215.6 ± 1.66 v. 183.6 ± 8.0 v. 152.9 ± 27.4 for the 4 weeks group and from 198.0 ± 6.50 v. 173.2 ± 9.7 v. 147.3 ± 16.5 for the 8 weeks group compared to the control. The reversal also showed a dose-dependent decrease in the tubular diameter from 188.8 ± 9.4 v. 160.3 ± 9.0 v. 145.9 ± 5.3 compared with the control (Table 1).
Figure 1: Cross section of testis of (a) control (5 ml of distilled water), (b) Low dose (50 mg/ml/kg/day) and (c) High dose (100 mg/ml/kg/day) at 4 weeks; stained with Haematoxylin and Eosin; Magnification x40

Figure 2: Cross section of testis of (a) control (5 ml of distilled water), (b) Low dose (50 mg/ml/kg/day) and (c) High dose (100 mg/ml/kg/day) at 8 weeks; stained with Haematoxylin and Eosin; Magnification x40

Figure 3: Cross section of testis of (a) control (5 ml of distilled water), (b) Low dose (50 mg/ml/kg/day) and (c) High dose (100 mg/ml/kg/day) at 8 weeks, left for another 8 weeks to assess reversibility; stained with Haematoxylin and Eosin; Magnification x40
Table 1: Effects of the extract of *Carica papaya* bark on the testicular diameter, weight, volume and cross sectional area of the seminiferous tubules of Sprague-Dawley rats for 4 wks, 8 weeks and reversal groups

<table>
<thead>
<tr>
<th>4 Weeks duration</th>
<th>DOSES</th>
<th>TD (µm)</th>
<th>TW (g)</th>
<th>TV (ml)</th>
<th>$A_C$ ($x10^3$ µm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>215.0± 1.6a</td>
<td>1.20± 0.55</td>
<td>1.0 ± 0.10</td>
<td>36.51± 6.56</td>
<td></td>
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<tr>
<td>Low dose</td>
<td>183.6± 8.0</td>
<td>1.23± 0.67</td>
<td>0.9 ± 0.49</td>
<td>26.47± 4.62</td>
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<tr>
<td>High dose</td>
<td>152.9± 27.4</td>
<td>1.25± 0.40</td>
<td>0.8 ± 0.35</td>
<td>17.04± 4.66</td>
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<table>
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<th>8 Weeks duration</th>
<th>DOSES</th>
<th>TD (µm)</th>
<th>TW (g)</th>
<th>TV (ml)</th>
<th>$A_C$ ($x10^3$ µm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>198.0± 6.5</td>
<td>2.0± 0.95</td>
<td>1.0 ± 0.10b</td>
<td>30.79± 8.34</td>
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</tr>
<tr>
<td>Low dose</td>
<td>173.2± 9.7</td>
<td>1.9± 0.70</td>
<td>0.7 ± 0.24</td>
<td>23.56± 7.40</td>
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<tr>
<td>High dose</td>
<td>147.0± 16.5</td>
<td>1.8± 0.80</td>
<td>0.7 ± 0.24</td>
<td>15.06± 4.22</td>
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<th>16 Weeks duration (Reversal)</th>
<th>DOSES</th>
<th>TD (µm)</th>
<th>TW (g)</th>
<th>TV (ml)</th>
<th>$A_C$ ($x10^3$ µm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>188.8± 9.4</td>
<td>2.0± 0.65</td>
<td>0.9 ± 0.49</td>
<td>20.03± 2.42</td>
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</tr>
<tr>
<td>Low dose</td>
<td>160.3± 9.0</td>
<td>1.8± 0.50</td>
<td>0.9 ± 0.49</td>
<td>18.42± 1.80</td>
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<tr>
<td>High dose</td>
<td>145.9± 5.3</td>
<td>1.3± 0.45</td>
<td>0.7 ± 0.24b</td>
<td>16.53± 1.20</td>
<td></td>
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</tbody>
</table>

Control: 5.0 ml of distilled water; Low dose: 50 mg/ml/kg/day of *Carica papaya*; High dose: 100 mg/ml/kg/day of *Carica papaya*; a = mean ± S.D; b = $p < 0.05$; n = 10; TD = Tubular diameter; TW = Testicular weight; TV = Testicular volume; $A_C$ = Cross sectional area.

**Testicular Volume:** Although there was a dose-dependent reduction in the testicular volume of the experimental rats from 1.0 ± 0.1 v. 0.9 ± 0.49 v. 0.8 ± 0.35 for the 4 weeks group and from 1.0 ± 0.1 v. 0.7 ± 0.24 v. 0.7 ± 0.24 for the 8 weeks group compared with the control; the reductions were not significantly different. For the reversal group, the testicular volume was also not significantly different (Table 1).

**Cross sectional area of the seminiferous tubules:** There was a dose and duration dependant reduction of the cross sectional area of the seminiferous tubules of the experimental rats from 36.51 ± 6.56 v. 26.47 ± 4.62 v. 17.04 ± 4.66 for 4 weeks group and from 30.79 ± 8.34 v. 23.56 ± 7.40 v. 15.06 ± 4.22 for the 8 weeks group. There was a decrease from 20.03 ± 2.0 v. 18.42 ± 1.8 v. 16.53 ± 1.2 with the reversal group compared with the control (Table 1).

**Number of profiles of seminiferous tubules per unit area of testis:** The experimental rats showed an increase in the number of profiles per unit area from 12.1 ± 2.03 v. 13.0 ± 2.63 v. 13.4 ± 3.61 for the 4 weeks group and from 14.4 ± 3.39 v. 15.9 ± 4.38 v. 19.9 ± 6.23 for the 8 weeks group compared with the controls.
The increase was dose and duration dependent. The reversal group also showed an increase from 18.2 ± 3.08 v. 24.9 ± 6.80 v. 30.1 ± 11.8 (Table 2).

**Table 2:** Effects of the extract of *Carica papaya* bark on the number of profiles per unit area, numerical density, length density and the star volume of the seminiferous tubules of Sprague-Dawley rats for 4 wks, 8wks and reversal groups

<table>
<thead>
<tr>
<th>DOSES</th>
<th>4 Weeks duration</th>
<th>8 Weeks duration</th>
<th>16 Weeks duration (Reversal)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N/A (x10 µm²)</td>
<td>NV (x10⁻¹⁰ µm²)</td>
<td>LV (x10⁻⁶ µm)</td>
</tr>
<tr>
<td>Control</td>
<td>12.1± 2.03</td>
<td>55.0± 9.38</td>
<td>24.2± 2.03</td>
</tr>
<tr>
<td>Low dose</td>
<td>13.0± 2.63</td>
<td>68.9± 10.4</td>
<td>26.0± 2.63</td>
</tr>
<tr>
<td>High dose</td>
<td>13.4± 3.61</td>
<td>97.2± 7.57</td>
<td>26.8± 3.61</td>
</tr>
<tr>
<td>Control</td>
<td>14.4± 3.39</td>
<td>70.9± 8.53</td>
<td>28.8± 3.39</td>
</tr>
<tr>
<td>Low dose</td>
<td>15.9± 4.38</td>
<td>89.3± 8.24</td>
<td>31.8± 4.38</td>
</tr>
<tr>
<td>High dose</td>
<td>19.9± 6.23</td>
<td>130.7±16.43</td>
<td>39.8± 6.32</td>
</tr>
<tr>
<td>Control</td>
<td>18.2± 3.08</td>
<td>18.5± 2.05</td>
<td>30.4± 27.0</td>
</tr>
<tr>
<td>Low dose</td>
<td>24.9± 6.80</td>
<td>18.5± 1.30</td>
<td>49.8± 8.86</td>
</tr>
<tr>
<td>High dose</td>
<td>30.1± 11.8</td>
<td>19.9± 1.20</td>
<td>60.2± 23.2</td>
</tr>
</tbody>
</table>

Control: 5.0 ml of distilled water; Low dose: 50 mg/ml/kg/day of *Carica papaya*; High dose: 100 mg/ml/kg/day of *Carica papaya*; a = mean ± S.D; b = p < 0.5; n = 10; NA = Number of profiles per unit area; NV = Numerical density; LV = Length density; SV = Star volume.

**Numerical density of the seminiferous tubules:** There was an increase in the numerical density of the seminiferous tubules of the Sprague-Dawley rats. The 4 weeks group increased from 55.0 ± 9.38 v. 68.9 ± 10.4 v. 97.2 ± 7.57. While the 8 weeks group increased from 70.9 ± 8.53 v. 89.3 ± 8.24 v. 130.7 ± 16.43. The increase was found to be dose and duration dependent. In the reversal group, there was increase from 18.5 ± 2.05 to 18.6 ± 1.30 to 19.9 ± 1.2 compared with the control (Table 2).

**Length density of the seminiferous tubules:** There was an increase in the length density of the seminiferous tubules of the Sprague Dawley rats for the experimental groups. For the 4 weeks group the increase was from 24.2 ± 2.03 v. 26.0 ± 2.63 v. 26.8 ± 3.61 and for the 8 weeks group was from 28.8 ± 3.39 v. 31.8 ± 4.38 v. 39.8 ± 6.23. This was found to be dose
and duration dependent. The reversal group also showed an increase in the length density from 30.4 ± 27.0 v. 49.8 ± 8.86 v. 60.2 ± 23.2 (Table 2).

**Volume density of seminiferous tubules:**
There was an increase in the volume density of the seminiferous tubules of the experimental rats from 72.6 ± 2.1% v. 76.8 ± 2.5% v. 87.6 ± 1.6% for the 4 weeks group, from 71.4 ± 0.6 v.78.6 ± 3.4 v. 81.2 ± 0.7 for the 8 weeks group and a decrease observed for the reversal group from 68.1 ± 1.7 v. 64.3 ± 1.5 v. 62.5 ± 2.3 (Table 3).

**Table 3:** Effect of the extract of *Carica papaya* bark on the volume density of seminiferous tubules and interstitium of male Sprague-Dawley rats for 4wks, 8 wks and reversal groups

<table>
<thead>
<tr>
<th>PERCENTAGE (%) VOLUME DENSITY</th>
<th>Dose (4 Weeks)</th>
<th>Dose (8 Weeks)</th>
<th>16 Weeks reversal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DOSES</strong></td>
<td><strong>Seminiferous tubule</strong></td>
<td><strong>Tubular interstitium</strong></td>
<td><strong>Seminiferous tubule</strong></td>
</tr>
<tr>
<td>Control</td>
<td>72.6 ± 2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.4 ± 1.2</td>
<td>71.4 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Low dose</td>
<td>76.8 ± 2.5</td>
<td>23.6 ± 1.5</td>
<td>78.6 ± 3.2</td>
</tr>
<tr>
<td>High dose</td>
<td>87.6 ± 1.6</td>
<td>12.4 ± 1.6</td>
<td>81.2 ± 0.7</td>
</tr>
</tbody>
</table>

Control: 5.0 ml of distilled water; Low dose: 50 mg/ml/kg/day of *Carica papaya*; High dose: 100 mg/ml/kg/day of *Carica papaya*; a = mean ± S.D; b =  p < 0.5; n = 10.
Volume density of the testicular interstitium: There was a decrease in the volume density of the interstitium from 27.4 ± 1.2 v. 23.2 ± 1.5 v. 12.4 ± 1.6 for the 4 weeks group and from 28.6 ± 2.8 v. 21.4 ± 3.2 v. 18.8 ± 1.5 for the 8 weeks group. For the reversal group, there was an increase from 31.9 ± 1.8 of control to 35.7 ± 2.5 v. 37.5 ± 3.6 for the low and high dose treatment (Table 3).

Star volume of the seminiferous tubules: There was a reduction from 10.50 ± 0.22 v. 6.48 ± 0.19 v. 2.46 ± 0.17 in the star volume of the experimental rats for 4 weeks and from 8.13 ± 0.21 v. 5.44 ± 0.15 v. 3.35 ± 0.12 for 8 weeks group compared with the control. The reversal group showed an increase from 12.76 ± 4.3 v. 23.48 ± 4.8 v. 32.06 ± 3.5 compared with the control (Table 2).

DISCUSSION:
The morphometric analysis of the effect of CP bark extract on the seminiferous tubules is in concert with the histological result observed on the testis. The study demonstrated a dose and duration dependent decrease in the mean testicular volume, tubular diameter, cross sectional area and star volume of tubules; but an increase in the length density, numerical density, number of profiles per unit area and volume density of tubules.

A reduction in seminiferous tubule star volume can be interpreted in several ways. For instance, it might signify that certain seminiferous tubules are smaller (thinner or shorter) or that all tubules are smaller (i.e. each tubule is simply scaled down in size) or that the coiled pattern is different. There is morphometric evidence that the total length of tubule is reduced by administration of CP bark aqueous extracts and this seems to affect many tubules. This impoverished linear growth could produce the drop in star volume seen in the present study. However, the possibility cannot be excluded that all tubules are reduced in size. In assessing the scale of tubular reduction, it would be imprudent to draw firm conclusion by regarding star volume as summative units of space because they are, in reality locally defined, point-sampled regions of arbitrary space. Therefore, dividing total volumes by star volumes merely indicates the theoretical numbers of star volume which can be contained in total volume. However, such numbers do give an idea of the impact of tubular shrinkage on tubular number and size. The number rose between 4 and 8 weeks. Tubular star volume could be imagined as the volume through which spermatogenic cells travel to reach the rete testis, if its progress in all possible direction was rectilinear. This volume would depends not only on the total volume of tubular space which itself varies due to episodic flow but also the number of Sertoli cells which project into it. The star volume of tubules is therefore determined by length, diameter, curvature and incidence of Sertoli-
Sertoli cell bridges. Star volume is a volume-weighted mean average and therefore highly sensitive to seminiferous tubular size distribution [20]. However gross volume is a result of parameters which are independent of tubular size distribution. Thus the data may suggest that only the gross volume summarizes the data on total testicular volume while star volume indicates that by the 4th week following administration of CP bark there are many significant small seminiferous tubules. Thus the size decrease and tubular cellular changes suggest that the small tubules represent tubular reduction in size.

The use of star volume to determine the size of seminiferous tubules has benefits and disadvantages. Although providing a useful way of realizing the concept of inter-Sertoli-Sertoli cell spaces and the adluminal compartment, star volume is a statistically noisy variable. The Noisiness of these estimates stem from the fact that the adluminal compartment is a point-sampled local region of arbitrary space; therefore the star volume depends on a variety of factors including the number, size, and arrangement of Sertoli cells in the seminiferous tubules. The rational for measuring star volume is that this variable is influenced more directly by differences in total tubular area. By focusing on the relationship between tubular surface area and volume the relevance to issues associated with transport in the tubules is more obvious. Unfortunately like volume-weighted mean volume, the star volume is a noisy variable. Present results suggest that it may be even nosier and so its application in future studies will need to balance this precision against the potential benefits of being able to characterize tubular size more comprehensively. Its usefulness lies in providing a direct and unbiased estimate of volume which has a strict mathematical definition. For the tubules the star volume will be less than the total volume. The number of seminiferous tubules might also alter. A 25% decrease in tubular volume would correspond to a roughly 10% reduction in linear dimensions if all overall size altered isomorphically.

Results obtained in the present study showed that tubular length actually decrease between 40 to 80% suggesting that there was an overall decrease in tubular length. This might also reflect the stunted growth of tubules. This suggestion might represent a general reduction in the proportion of parenchymal tissues which is supported by volumetric analyses.

In terms of tubular function, volume and surface area are influential in determining production and transport of spermatogenic cells whilst the total volume influences supply. However other factors are clearly important. These include number of spermatogenic cells in the basal compartment, the Sertoli-Sertoli cell barrier which determines the number of cells in the adluminal compartment. It is not
possible to predict the likely consequences of these changed geometric relationships on tubular sperm production or flow because the present morphometric data form only part of the complete picture. The increase in the volume density of the seminiferous tubules despite a reduction in the diameter of the tubules only suggest that there was a significant reduction in the interstitium due to the destruction of the Leydig cells. These cells are responsible for the production of testosterone. The size of the testicular interstitium normally correlates positively with the number of Leydig cells, which in turn correlates positively with the germ and testicular level of testosterone [21, 22].

For the reversal group, the parameters remained reversed for the 4 weeks group and remained the same for the 8 weeks group showing an evidence of substantial recovery using a lower dose of the aqueous extract of the bark.

CONCLUSION:
The administration of the CP bark aqueous extract reduces the total length of the tubules thereby producing a drop in the star volume. The effect is more pronounced for the high dose than in the low dose. This suggests that the extract could impair spermatogenesis thereby acting as a potential contraceptive.

REFERENCES:


HIV/AIDS STIGMA: MAIN BARRIER TO VCT AND OTHER HEALTH SERVICES IN FOUR
SUBURBAN VILLAGES IN NATIONAL CAPITAL DISTRICT, PAPUA NEW GUINEA

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ABSTRACT:

The HIV/AIDS stigma, fear and discrimination have been linked with poor participation in Voluntary Counselling and Testing (VCT) programs. Cultural factors, attitudes and behaviours strongly influence the spread of HIV/AIDS and these have been extensively studied in several sub-Saharan African countries. Similar studies in Papua New Guinea are scanty. This study investigates the extents of HIV/AIDS-Stigma, fear, discrimination and other psychological factors as barriers to the access of VCT and other services in four suburban villages in the National Capital District, Papua New Guinea. The study sites were Baruni, Hanuabada, Pari and Kilakila villages. A semi-structured questionnaire comprising of closed and open ended questions was administered to respondents selected randomly. Groups were selected for focus group discussions. Gender stratification was used to ensure that views of both men and women were equally represented. A total of 333 respondents comprising of 166 (49.8%) males, and 167 (50.2 %) females participated in the survey. Self-stigmatization as a major barrier to VCT was indicated by 90.7% of all the respondents. Gender based differences were not statistically significant. Discrimination by relatives and friends was indicated by 74.8% respondents; 79.0% of respondents would discriminate HIV/AIDS-infected people. A total of 42.0% respondents indicated the possibility of rejection from close relationships. The likelihood of dismissal from workplace was indicated by 68.5% of respondents. Lack of support from health providers (67.0%) and fear of HIV test result (61.6%) were other barriers to VCT indicated by respondents. This study recommends the need to heighten awareness of VCT and to eliminate stigma and fear in order to control the HIV/AIDS pandemic.

Key words: HIV/AIDS, stigma, voluntary counseling, testing, VCT, Papua New Guinea.

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INTRODUCTION:
The Joint United Nations Program on HIV/AIDS (UNAIDS) defines HIV-related stigma & discrimination as, the process of devaluation of people either living with or associated with HIV & AIDS [1]. Discrimination follows stigma and is the unfair and unjust treatment of an individual based on his or her real or perceived HIV status [1]. Withdrawal which is a self isolation and / or detachment from the immediate community due to fear of been stigmatized or perceived self stigmatization may also follow. Similarly rejection of people infected with, or perceived by others to be infected or associated with the HIV/AIDS virus is likely.

Rating stigma among different epidemics at Hong Kong University it was found that people strongly expressed negative feelings about individuals with HIV/AIDS and tuberculosis (TB), with Severe Acute Respiratory Syndrome (SARS), a lesser-known threat, at a lower level [2]. They also found that respondents feared HIV/AIDS the most, followed by TB and SARS, in that order [2]. According to the UN Secretary General, Ban Ki Moon [3] "Stigma remains the single most important barrier to public action; It is a main reason why too many people are afraid to see a doctor to determine whether they have the disease, or to seek treatment if so. It helps make AIDS the silent killer, because people fear the social disgrace of speaking about it, or taking easily available precautions. Stigma is a chief reason why the AIDS epidemic continues to devastate societies around the world." HIV/AIDS - stigma and discrimination have the greatest impact on all aspects of a country’s HIV response. The greater the level of stigma, the further HIV spreads [4]. According to Valdiserri [5], “To underestimate the insidious power of stigma is to risk the very success of effective HIV prevention and care programs”.

In Papua New Guinea (PNG), HIV rates have continued to rise almost exponentially to about 32,005 cases since the first report in 1987 [6]. Through various HIV- awareness projects, the National AIDS Council Secretariat (NACS) has been working relentlessly in partnership with many organizations such as: the WHO [7], Australian Agency for International Development (AusAID) [8], UNICEF [9], The Joint United Nations Program on HIV/AIDS (UNAIDS) [10], National Institutions such as the Institute of Medical Research (IMR), the University of Papua New Guinea (UPNG), Non Government Organizations (NGOs) and Faith Organizations within and outside the country to reverse this trend. The concerted efforts have been in response to the Millennium Development Goal 6 (MDG 6) on the combat of HIV/AIDS, Malaria and other diseases, that specifically aims to halt and begin to reverse the global HIV epidemic by year 2015 [11]. To
reduce HIV and realise the MDG 6, people need to know their sero-status through voluntary counseling and testing (VCT). In PNG, some studies on the awareness and attitudes towards HIV among pregnant women were carried out at the Port Moresby Antenatal Clinic in 2003 [12]. This study revealed a large number of patients who knew that transmission of HIV was by sexual contacts (97%), and through Mother to Child Transmission (MTCT) 96%. However, misconceptions that could lead to stigma existed with patients who believed that HIV could be transmitted by mosquitoes (36%), and with those (17%) who thought care for AIDS patients was a risk [12]. Fear of stigma and discrimination had also been reported as a primary barrier to accessing VCT of HIV and other health related services in PNG [13]. A recent preliminary study on the Impact of HIV/AIDS - Stigma and discrimination on the access to VCT in two suburban areas of the National Capital District (NCD), PNG revealed the existence of high levels of stigma (88.2 – 95.8 %), discrimination (85-91.8%) and fear (74.2%) [14]. The findings from the study did not fully reflect the Motu and Koita ethnic groups which together constitute a major proportion of the NCD population. These ethnic groups possess strongly established cultural norms and the majority of them are Christians. Nevertheless, the reported rapid spread of HIV/AIDS among the population constitutes one of the recognized threats to the current Motu and Koitabu generation [15].

The aim of this study was to investigate the extent of HIV/AIDS-Stigma, discrimination, fear and other psychological factors as barriers to the access of VCT and other services in four suburban villages in the NCD, with focus on the Motu-Koita population.

SUBJECTS AND METHODS:
The Motu and Koitabu are a group of people indigenous to areas in and around NCD. They number about 30,000 of the 250,000 NCD population [15]. The study sites included nine Motu-Koitabu villages: Baruni, Tatana, Elevala, Poreporena (Hanuabada) Lahara & Laurabada, Taurama including Tutu and Daugolata, Pari, Kilakila/Mahuru, Korobosea and Vabukori. Two villages from each of the ethnically stratified Motu or Koita groups were randomly selected for sampling. These were Pari & Hanuabada (Motu), and Baruni & Kila Kila (Koita) villages. Both males and females within the age range 15 – 24 years were eligible to participate in this study. A standard questionnaire and interviews were administered to volunteers from the four villages. Pretested, semi-structured questionnaire comprising of closed and open ended questions was used. The three major languages, English, Pidgin and Motu were used in the questionnaire. Information request in the questionnaire included gender, age, occupation, education level, language(s) spoken, current knowledge of the respondent about HIV/AIDS including knowledge on
preventive lifestyle skills and behavioural change in the NCD. It also included questions about the existence of HIV/AIDS related fear, discrimination due to a casual contact with HIV/AIDS infected person. Qualitative questions included in the questionnaire were aimed at exposing fear and discrimination or perceived discrimination that could occur when close relatives and / or people of the immediate community and work place would know about the HIV/AIDS status of individuals. The assessment of negative attitudes of respondents towards persons with HIV/AIDS was used to measure stigma and discrimination. Questions related to what respondents knew about VCT services, and whether they would be willing to make use of the services were also included. The associations between levels of stigma, discrimination versus knowledge of VCT services and VCT utilization was examined through appropriate tabulations and chi square analysis. Before the study was carried out, its objectives were explained to the Chairman of Motu Koitabu, to Counsellors, elders and religious leaders of the selected villages. They in turn, explained the objectives and benefits of the research, to the residents, and sought their cooperation. From each of the four villages, two research assistants (a female and a male) were recruited and trained in order to acquire strong familiarity with the objectives of the study, the research questions and the methods. Selection of research assistants, who also served as interviewers, from the study villages, was aimed at facilitating community ownership of the project and its outcomes. The training emphasized the need to maintain confidentiality, neutrality and high ethical conducts during and after the study period. Interviews were carried out in secluded areas to ensure confidentiality. The interviewers always introduced themselves and explained the objectives and benefits of the study to the consented respondents.

The sample size of 400 subjects of the Motu Koita population was estimated based on the year 2000 census and its extrapolation to the year 2009. The Motu-Koitabu ethnic groups’ population of 30,000 people mainly in the suburban areas of Port Moresby constituted about 10% of the NCD population [15, 17]. Given a growth rate of about 3.6%, this population was estimated to have increased to about 44,300 in the year 2009. Simple random sampling was used to select four villages (2 Motu and 2 Koita) from stratified Motu and Koita groups of 5 and 4 villages respectively. Male and female residents in the age group 15 – 24 years were randomly selected from each of the four villages. Table 1 shows the population, number of households, estimated number of subjects extrapolated from the year 2000 census, the actual number of subjects in the four villages, the number of focus groups in each village.
Exclusion criteria included the following, all residents outside the 15-24 year age group, non Motu-Koita immigrants to the villages, Motuan living in a Koitabuan village and Koitabuan living in a Motuan village.

During the interviews, four focus groups (FG 1-4) each comprising 7 - 9 respondents were carefully identified. The selection process was as follows: Every 12th female and every 12th male respondents in the various sections indicated in the questionnaire were recruited to form the four focus groups. The groups were separated by villages and gender as indicated in Table 2. Focus group meetings were convened in the School of Medicine and Health Sciences (SMHS), UPNG about two weeks after completion of the interviews. A separate questionnaire was designed and used for the focus group discussions. Questions were posed; answers and related discussions were audio-recorded, while a rapporteur took notes. Ethical clearance and permission for this study were obtained from the SMHS, UPNG ethical committee, the PNG National AIDS Council (NACS), appropriate Motu-Koitabu authorities and each respondent.

Collected data were coded, collated, classified, matched with key research questions and analysed with the SPSS software.

<table>
<thead>
<tr>
<th>Villages</th>
<th>Population</th>
<th>No of households</th>
<th>Estimated sample size of subjects</th>
<th>Actual sample size of subjects</th>
<th>Focus groups recruits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baruni</td>
<td>1629</td>
<td>210</td>
<td>90</td>
<td>88</td>
<td>6</td>
</tr>
<tr>
<td>Kila Kila</td>
<td>327</td>
<td>46</td>
<td>20</td>
<td>31</td>
<td>12</td>
</tr>
<tr>
<td>Hanuabada</td>
<td>2628</td>
<td>233</td>
<td>150</td>
<td>100</td>
<td>12</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Villages</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pari &amp; Baruni</td>
<td>Focus Group 1 (9)</td>
<td>Focus Group 2 (9)</td>
</tr>
<tr>
<td>Hanuabada &amp; Kila Kila</td>
<td>Focus Group 3 (7)</td>
<td>Focus Group 4 (7)</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION:
A total of 342 questionnaires were administered to respondents in the four study villages. Of these questionnaires 333 were properly completed while 9 questionnaires were deficient in many sections and hence excluded from the analysis. Thus the response rate was considered as 97.4%. The 333 respondents were made up of 166 (49.8%) males and 167 (51.2%) females. The basic characteristics of the respondents are presented in Table 3. Of the 333 respondents, a total of 15 (4.5%), which include 6 (3.6%) females and 9 (5.4%) males did not have the minimum primary school education. Respondents with primary and secondary education were the majority tallying jointly to totals of 130 (39.0%) and 166 (49.9%) respectively. Only 18 (5.4%) of all the respondents had completed either college or university education. Unemployment was prevalent among 101 (30.3%) of all the respondents, which include 43 (25.9%) males and 58 (34.7%) females.

<table>
<thead>
<tr>
<th>Table 3: Basic characteristics of respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics</td>
</tr>
<tr>
<td>Education Status</td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>Primary</td>
</tr>
<tr>
<td>Secondary</td>
</tr>
<tr>
<td>College or University</td>
</tr>
<tr>
<td>Unanswered</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Occupation</td>
</tr>
<tr>
<td>Student</td>
</tr>
<tr>
<td>Employed</td>
</tr>
<tr>
<td>Self employed</td>
</tr>
<tr>
<td>Unemployed</td>
</tr>
<tr>
<td>Unanswered</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

(Figures in parenthesis are percentages)
Table 4:
Distribution of respondents according to responses to questions on stigma and discrimination

<table>
<thead>
<tr>
<th>Questions</th>
<th>Males (%)</th>
<th>Females (%)</th>
<th>Total (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Self Stigmatization</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>154 (92.2)</td>
<td>148 (89.2)</td>
<td>302 (90.7)</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>10 (6.0)</td>
<td>14 (8.4)</td>
<td>24 (7.2)</td>
<td></td>
</tr>
<tr>
<td>Uncertain</td>
<td>3 (1.8)</td>
<td>4 (2.4)</td>
<td>7 (2.1)</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>$X^2=0.93; df=2; p=0.629$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Perceived Stigmatization</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>by others</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>116 (69.9)</td>
<td>114 (68.3)</td>
<td>230 (69.1)</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>36 (21.7)</td>
<td>33 (19.8)</td>
<td>69 (20.7)</td>
<td></td>
</tr>
<tr>
<td>Uncertain</td>
<td>14 (8.4)</td>
<td>20 (12.0)</td>
<td>34 (10.2)</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>$X^2=1.20; df=2; p=0.5478$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Perceived Discrimination</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>from others</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>119 (71.7)</td>
<td>130 (77.8)</td>
<td>249 (74.8)</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>44 (26.5)</td>
<td>34 (20.4)</td>
<td>78 (23.4)</td>
<td></td>
</tr>
<tr>
<td>Uncertain</td>
<td>3 (1.8)</td>
<td>3 (1.8)</td>
<td>6 (1.8)</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>$X^2=77; df=2; p=0.4137$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Possibility of respondents</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discriminating others</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>128 (77.1)</td>
<td>135 (80.8)</td>
<td>263 (79.0)</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>31 (18.7)</td>
<td>25 (15.0)</td>
<td>56 (16.8)</td>
<td></td>
</tr>
<tr>
<td>Uncertain</td>
<td>7 (4.2)</td>
<td>7 (4.2)</td>
<td>14 (4.2)</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>$X^2=0.83; df=2; p=0.4137$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[Figures in parenthesis are percentages]
Table 5:
Distribution of respondents according to responses to questions on rejection, dismissal and self withdrawal

<table>
<thead>
<tr>
<th>Questions</th>
<th>Males (%)</th>
<th>Females (%)</th>
<th>Total (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rejection by family or Immediate relationship</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>68 (41.0)</td>
<td>72 (43.1)</td>
<td>140 (42.0)</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>74 (44.6)</td>
<td>73 (43.7)</td>
<td>147 (44.2)</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Uncertain</td>
<td>24 (14.5)</td>
<td>22 (13.2)</td>
<td>46 (13.8)</td>
<td></td>
</tr>
<tr>
<td>X²=0.21; df=2; p=0.9025</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dismissal from work place</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>120 (72.3)</td>
<td>108 (64.7)</td>
<td>228 (68.5)</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>19 (11.5)</td>
<td>24 (14.4)</td>
<td>43 (12.9)</td>
<td></td>
</tr>
<tr>
<td>Uncertain</td>
<td>27 (16.3)</td>
<td>35 (21.0)</td>
<td>62 (18.6)</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>X²=2.24; df=2; p=0.0.3259</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self- withdrawal from duties</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>78 (47.0)</td>
<td>65 (38.9)</td>
<td>143 (42.9)</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>85 (51.2)</td>
<td>97 (58.1)</td>
<td>182 (54.7)</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Uncertain</td>
<td>3 (1.8)</td>
<td>5 (3.0)</td>
<td>8 (2.4)</td>
<td></td>
</tr>
<tr>
<td>X²=2.47; df=2; p=0.2908</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[Figures in parenthesis are percentages]

The fundamental issues that surround people with HIV/AIDS are stigma, discrimination and fear. The results of the respondents’ responses to questions on self stigmatization, perceived stigma, perceived discrimination from others and possibility of the respondents discriminating others is presented in Table 4. Of the 333 respondents interviewed, 302 (90.7%) gave responses that indicated self-stigmatization as a major barrier to VCT. Prevalence of perceived stigmatization by others was also high (69.1%) as a limiting factor to VCT and related services. Perceived discrimination of others, and the possibility of the respondents discriminating others or people infected with HIV/AIDS were high with positive responses of 74.8% and 79.0% respectively. The data indicated that the levels of stigma and discrimination were very high and well above 2/3 (66.7%) of the respondents in this study.
The high number of youths with only primary education (39%) coupled with those without basic education (4.5%) could have contributed to the relatively high levels of stigma and discrimination obtained in this study.

Table 5 shows the results of the respondents’ responses to questions on rejection by close family members, dismissal from work place and self withdrawal from duties following disclosure of HIV status. Of all the respondents 147 (44.2%) indicated that they will not be rejected by their love ones, but 140 (42.0%) believed they would be rejected by relatives and close family members, while 46 (13.8%) of them were either uncertain or did not respond to the question. There were no statistically significant (p > 0.05) differences in the responses of the male and female respondents to this question, suggesting that their views are similar. This study recommends a successful intervention program that should target and capture about 55.8 % of the 15-24 years age group constituting those who would be rejected by their love ones (42%) and the ones uncertain (13.8%) and subject them to a more rigorous awareness that should reverse the perceived rejection by close relatives and consequently promote VCT.

A total of 228 (68.5%) of all the respondents indicated that they would be dismissed from work place subsequent to positive HIV test results. This suggests that the fear of dismissal from work place for those employed or schooling in institutions constitutes a great obstacle that would discourage individuals from going to the VCT. The data indicates that HIV infected people are most likely to be discriminated at the work place than in the families. This finding should be of concern to the NACS authorities, because the PNG-HIV/AIDS Management and Prevention Act (PNG- HAMP Act”) prohibits requiring or coercing a person to be tested for HIV in relation to employment or contract work [18].

It is important that all HIV/AIDS awareness campaign should also stress the government’s policy of non discrimination of individuals with HIV/AIDS in the workplace, which is an important human right issue [18]. For the question on self withdrawal from duties, 143 (42.9%) of all the respondents, constituting of 78 (47.0%) males and 65 (38.9%) females indicated that they would withdraw from duties because of HIV +ve test. However, a total of 182 (54.7%) respondents made up of 85 (51.2%) males and 97 (58.1%) females indicated that they will not withdraw from duties; they intend to show self encouragement to continue with their normal duties. People should be educated and be encouraged to use the appropriate laws and legislations in PNG to challenge the HIV/AIDS related discrimination, stigma and denial that still exist in the society.
Table 6: Distribution of respondents according to responses to Lack of support from Health providers after HIV +ve results, fear of HIV +ve test and suicide elements

<table>
<thead>
<tr>
<th>Questions</th>
<th>Male (%)</th>
<th>Female (%)</th>
<th>Total (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lack of support after HIV + results</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>110 (66.3)</td>
<td>113 (67.7)</td>
<td>223 (67.0)</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>50 (30.1)</td>
<td>51 (30.5)</td>
<td>101 (30.3)</td>
<td></td>
</tr>
<tr>
<td>Uncertain</td>
<td>6 (3.6)</td>
<td>3 (1.8)</td>
<td>8 (2.4)</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>(X^2=1.05; df=2; p=0.5924)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fear of HIV + ve test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>100 (60.2)</td>
<td>105 (62.9)</td>
<td>205 (61.6)</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>54 (32.5)</td>
<td>58 (34.7)</td>
<td>112 (33.6)</td>
<td></td>
</tr>
<tr>
<td>Uncertain</td>
<td>12 (7.2)</td>
<td>4 (2.4)</td>
<td>16 (4.8)</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>(X^2=4.26; df=2; p=0.1187)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Suicide elements after HIV + test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>3 (1.8)</td>
<td>6 (3.6)</td>
<td>9 (2.7)</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>158 (95.2)</td>
<td>157 (94.0)</td>
<td>315 (94.6)</td>
<td></td>
</tr>
<tr>
<td>Uncertain</td>
<td>5 (3.0)</td>
<td>4 (2.4)</td>
<td>9 (2.7)</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>(X^2=1.11; df=2; p=0.5737)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Figures in parenthesis are percentages*

In Table 6, the results of respondents’ responses on the lack of support from health providers after HIV +ve results are presented. The majority of respondents, 223 (67.0%) with an almost an equal distribution by gender, 110 (66.3%) males and 113 (67.7%) females indicated that they will not get support from health providers following an HIV +ve test results. Support from health workers is crucial in ensuring acceptance and improvement in HIV testing. Special training should be made available for health workers to prepare them to be more receptive to VCT clients and to equip them with the required counselling and professional skills. Chi square tests of our data indicated that there were no statistically significant (p>0.05) differences between the responses of the male and female respondents, thus eliminating gender bias in the fear of HIV/AIDS stigma.
Table: 7 Distribution of respondents according to response on Knowledge of VCT centers & willingness to utilize their services

<table>
<thead>
<tr>
<th></th>
<th>Male (%)</th>
<th>Female (%)</th>
<th>Total (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Knowledge of Places</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With VCT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>127 (76.5)</td>
<td>130 (77.8)</td>
<td>257 (77.2)</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>39 (23.5)</td>
<td>36 (21.6)</td>
<td>75 (22.5)</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Uncertain</td>
<td>0 (0.0)</td>
<td>1 (0.6)</td>
<td>1 (0.3)</td>
<td></td>
</tr>
<tr>
<td>[X^2=1.15; df=2; p=0.5621]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Willingness to utilize VCT Services</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>148 (89.2)</td>
<td>153 (91.6)</td>
<td>301 (90.4)</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>18 (10.8)</td>
<td>11 (6.6)</td>
<td>29 (8.7)</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Uncertain</td>
<td>0 (0.0)</td>
<td>3 (1.8)</td>
<td>3 (0.9)</td>
<td></td>
</tr>
<tr>
<td>[X^2=5.07; p=0.0794]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[Figures in parenthesis are percentages]

Elements of suicide after HIV +ve test were indicated by few respondents (2.7%), with the breakdown of 5 (3.0%) males and 4 (2.4%) females.

Table 7 shows the results of respondents’ responses to knowledge about VCT centers, and their willingness to utilize services offered in the centers. A total of 257 (77.2%) respondents knew the locations of the VCTs. Only 8.7% of all the respondents were unwilling to utilize VCT services.

Summary of the views from the focus group:
People are careless and don’t care about this sickness; we can reduce large numbers of infections just like malaria cases if we raise awareness among the communities in the remote areas.

VCT has to be somewhere so that not everyone can see it.
We must arrange time such as midnight when nobody can see those infected visiting VCT.
It is our responsibility to get the VCT test because we don’t know whether we have the
virus or not; VCT test is a good thing. We must not disgrace infected people or gossip about them. We must encourage them. Our pastor has not done much on HIV awareness and needs to talk to the youths about it. Everybody must be involved in working together so as to raise awareness in the community. When a family member wants to go to a VCT site the others should all go with him or her as to give confidence and support. Visiting each house is the best form of awareness in the village. Active involvement of community leaders, the religious leaders in the campaigns against HIV/AIDS stigma & discrimination is recommended.

CONCLUSION:
Data obtained in the present study show that HIV/AIDS – Stigma & discrimination prevails in the areas surveyed and the influencing factors and barriers were identified. Awareness should be carried out carefully targeting the specific barriers identified in this study, with the aim of eliminating them. Active involvement of community leaders and religious leaders in the campaigns against HIV/AIDS stigma & discrimination is recommended. People who may suffer discrimination or dismissal from institutions or work place due to HIV +ve status need to be reminded of their rights to legally challenge such discriminatory actions.

ACKNOWLEDGEMENT:
Thanks to Mr Isu Aluvula, NCD HIV/AIDS Response Coordinator and to Mary Vagi, Secretary to Chairman, Motu-Koita Assembly for facilitating linkage with elders, religious leaders and counsellors of the study areas; to Dr Ripa for assisting with statistical analysis.

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PREVALENCE OF HYPOGLYCAEMIA AMONG PATIENTS PRESENTING WITH CHOLESTASIS OF INFANCY IN A NIGERIAN TEACHING HOSPITAL

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ABSTRACT:
In the paediatric age group, particularly in infancy, hypoglycaemia is a common metabolic problem complicating a variety of clinical conditions, and its coexistence may influence the outcome of the primary disease. This study assesses the prevalence of hypoglycaemia among patients presenting at the University of Benin Teaching Hospital, Benin City, Nigeria with cholestasis of infancy. Forty patients aged between 15 days and 12 months who presented with cholestasis of infancy were admitted and screened for hypoglycaemia, using Acutrend glucometer. For patients with low blood glucose values, blood samples were further analyzed, using the standard glucose-oxidase method. Of 2,835 patients admitted over a five-year period, 40 (1.4%) had cholestasis of infancy, giving an incidence of 14 cases per 1000 admissions, with a sex ratio of 2.1: 1 in favour of males. Nine (22.5%) of the 40 infants with cholestasis had at least one blood glucose concentration less than 2.6 mmol/L (hypoglycaemia). Of the nine hypoglycaemic infants, three (33.3%) had one blood glucose concentration less than 1.6 mmol/L (severe hypoglycaemia). Seven (77.8%) of the nine hypoglycaemic infants were diagnosed in the first 36 hours of admission. Lethargy and poor feeding were observed in three infants with severe hypoglycaemia. Six (66.7%) of the hypoglycaemic infants were below 3 months of age. Hypoglycaemia was observed among patients with cholestasis of infancy; the prevalence was higher among infants below 3 months of age.

Key words: Hypoglycaemia, Cholestasis, Infancy, Neonatal Cholestasis Syndrome.

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INTRODUCTION:
In the paediatric age group, particularly in infancy, hypoglycaemia is a common metabolic problem encountered in association with a variety of disorders, such as malaria, kwashiorkor and hepatic diseases (e.g., hepatitis, cirrhosis, metabolic liver disorders)[1-4]. The coexistence of hypoglycaemia and other diseases may influence the outcome or clinical course of the primary disease, if not appropriately addressed.
The term cholestasis refers to a group of disorders associated with bilirubin excretion and accompanied by a rise in serum conjugated bilirubin levels and often, bile salts and phospholipids [3]. Some of the key clinical features of cholestasis of infancy are: jaundice persisting beyond the age of 14 days, jaundice with elevated serum conjugated bilirubin fraction (> 2.0 mg/dl or > 20% of total bilirubin), variably acholic stool, dark urine that stain the diaper yellow, bilirubinuria, and hepatomegaly [4,5]. Clinically, the hallmark of cholestasis is itching but this may not be recognized in early infancy. Itching becomes apparent after the age of six months [5]. Before the age of six months, irritability is a common feature of itching. The estimated incidence of cholestasis of infancy is 1 in 2,500 infants [1,6]. Among the components of liver function tests, elevated Alkaline Phosphatase is one of the sensitive tests of cholestasis [7]. The liver plays an important role in the maintenance of blood glucose levels, which is highly regulated by insulin and insulin counter-regulating hormones. Thus, a patient with liver dysfunction manifesting with cholestasis of infancy may be at increased risk of developing hypoglycaemia [8,9]. It has been recommended that the initial investigation of an infant with a liver disease should focus on identification of associated abnormalities, such as hypoglycaemia that require urgent specific treatment [4,10]. In infants, hypoglycaemia is defined as blood glucose concentration of 2.5 mmol/L and below [11]. The association of cholestasis of infancy with hypoglycaemia has been documented [5,12]. Some studies have demonstrated links between hepatic dysfunction, hypoglycaemia and congenital hypopituitarism [13,14]. Although the mechanisms are not clearly understood, involvement of growth hormone and cortisol have been hypothesized [12,14,15]. Lablanc et al, [12] indicated that cortisol deficiency might be the main endocrine abnormality responsible for both hypoglycaemia and liver dysfunction. Reports of other studies support this view [16-18]. Thus, adrenal function tests should be requested for infants with hypoglycaemia and liver dysfunction [12,16,18]. On the other hand, link between mitochondrial respiratory chain enzyme deficiency, neonatal cholestasis and hypoglycaemia have been variously reported [13,19]. Although the biochemical mechanisms of occurrence of hypoglycaemia in association with cholestasis of infancy has
been studied, [12-19] the prevalence of hypoglycaemia among patients with cholestasis of infancy has not been well documented, especially in Nigeria. This study assesses the prevalence of hypoglycaemia among patients with cholestasis of infancy in the University of Benin Teaching Hospital (UBTH), Nigeria.

PATIENTS AND METHODS:
All infants (aged 15 days to 12 months) diagnosed with cholestasis of infancy and admitted between January, 2004 and December, 2008 into the under-five-paediatric ward of the Department of Child Health, University of Benin Teaching Hospital (UBTH) were recruited into the study after obtaining informed consent from the parents/caregivers. In this study, standard clinical features were used for the diagnosis of patients [4,5]. The glucometer (Acutrend meter product 128485) was used for measurement of blood glucose [18,19]. Laboratory confirmation of blood glucose level was requested for all infants with blood glucose level below 3.0mmol/L [20]. Appropriate monitoring of blood glucose level was observed for all the infants. For infants with at least one blood glucose level below 2.0 mmol/L, the glucose level was monitored for at least one day after the level had returned to normal or after the therapy had been discontinued. When the blood glucose level was below 1.6 mmol/L or the infant was symptomatic (irrespective of blood glucose value), intravenous administration of 10.0% glucose was started immediately [8,11]. Haematocrit values of all the infants were determined and recorded. Blood films were prepared and examined for malaria parasite, and liver function tests (LFT) were performed using standard procedures. All the infants were examined clinically for features of kwashiorkor and congenital conditions. In the present study, infants with blood glucose level of 2.5mmol/L and below were characterized as hypoglycaemic [11]. Appropriate statistical methods were used for analysis of data; odd ratios were computed and Z-test was used in ascertaining the significance of differences in proportions with p-value set at < 0.05.

RESULTS:
During the 5-year period covered by this study, a total of 2,835 children were admitted into the under-five ward in UBTH; 40 (1.4%) of them had cholestasis of infancy, giving an incidence of 14 cases per 1000 admissions. Of the 40 infants with cholestasis of infancy, 27 (67.5%) were males and 13 (32.5%) were females, giving a male-to-female ratio of 2.1:1. Nine (22.5%) of the 40 infants with cholestasis had at least one blood glucose concentration below 2.6mmol/L (hypoglycaemia). Of the nine hypoglycaemic infants, three (33.3%) had one blood glucose concentration below 1.6mmol/L (severe hypoglycaemia). The remaining 6 (66.7%) had one or more blood glucose concentration between 1.6 and 2.5 mmol/L. Seven (77.8%) of the hypoglycaemic infants
were diagnosed within the first 36 hours of admission while two (22.2%) were diagnosed between 36 and 72 hours of admission. Lethargy and poor feeding were present in the three infants with severe hypoglycaemia. Hypoglycaemia was observed only among infants who were between 15 days and 6 months old (Table 1).

Table 1: Age at presentation and prevalence of hypoglycaemia among patients with cholestasis of infancy

<table>
<thead>
<tr>
<th>Blood glucose concentration (mmol/L)</th>
<th>Age at presentation</th>
<th>&lt;1.6mmol/L (No (%))</th>
<th>1.6 – 2.5mmol/L (No (%))</th>
<th>&gt;2.5mmol/L (No (%))</th>
<th>Z-stat</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 days to &lt;3 months (n = 24)</td>
<td>3 (12.5%)</td>
<td>3 (12.5%)</td>
<td>18 (75.0%)</td>
<td>p= 0.072; a vs b</td>
<td></td>
</tr>
<tr>
<td>3 – 6 months (n = 10)</td>
<td>0</td>
<td>3 (30.0%)</td>
<td>7 (70.0%)</td>
<td>p&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>≥7 months (n = 6)</td>
<td>0</td>
<td>0</td>
<td>6 (100.0%)</td>
<td>p&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>Total (n = 40)</td>
<td>3 (7.5%)</td>
<td>6 (15.0%)</td>
<td>31 (77.5%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Gender distribution among the 40 infants with cholestasis of infancy

<table>
<thead>
<tr>
<th>Blood glucose concentration (mmol/L)</th>
<th>Gender</th>
<th>&lt;1.6mmol/L (No (%))</th>
<th>1.6 – 2.5mmol/L (No (%))</th>
<th>&gt;2.5mmol/L (No (%))</th>
<th>Odd ratio (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (n = 27)</td>
<td>2(7.4)</td>
<td>4 (14.8)</td>
<td>21 (77.8)</td>
<td>0.95 (1.31, 2.61)</td>
</tr>
<tr>
<td></td>
<td>Female (n = 13)</td>
<td>1 (7.7)</td>
<td>2 (15.4)</td>
<td>10 (76.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total (n = 40)</td>
<td>3 (7.5)</td>
<td>6 (15.0)</td>
<td>31 (77.5)</td>
<td></td>
</tr>
</tbody>
</table>

*CI = Confidence interval

None of the infants older than 6 months at the time of presentation had hypoglycaemia (Table 1). The prevalence of hypoglycaemia was 22.2% in males and 23.1% in females (Table 2). There was no association between the Serum Alkaline Phosphatase level and the occurrence of hypoglycaemia (Table 3). Similarly, there was no association between the Serum Aminotransferase levels and occurrence of hypoglycaemia (Table 4). Clinical examination of the males revealed normal external male genitalia and bilateral scrotal testis. The female infants also had normal female external genitalia. There was no pedal oedema. Total serum bilirubin concentrations ranged from 6.5 – 17.6 mg/dl with the conjugated fraction ranging from 1.5- 5.4 mg/dl and generally was more than...
20% of the total serum bilirubin concentrations. Serum albumin concentration ranged from 36 - 48 g/L. The blood glucose concentration obtained using the Acutrend glucometer correlated well with the values obtained from the hospital central laboratory.

Table 3: Distribution of serum Alkaline Phosphatase (ALP) levels among the 40 patients with cholestasis of infancy

<table>
<thead>
<tr>
<th>Blood glucose concentration in mmol/L</th>
<th>Serum ALP levels (U/L)</th>
<th>&lt;1.6mmol/L</th>
<th>1.6 – 2.5mmol/L</th>
<th>&gt;2.5mmol/L</th>
<th>z- statistics (p-value)</th>
<th>Odd ratio (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>160-250 (n = 28)*</td>
<td></td>
<td>1 (3.6)</td>
<td>4 (14.3)</td>
<td>23 (82.1)</td>
<td>a vs b = 1.001 (&gt; 0.05)</td>
<td>0.43</td>
</tr>
<tr>
<td>&gt;250 (n = 12)*</td>
<td></td>
<td>2 (16.7)</td>
<td>2 (16.7)</td>
<td>8 (66.6)</td>
<td>(1.34, 2.58)</td>
<td></td>
</tr>
<tr>
<td>Total (n = 40)</td>
<td></td>
<td>3 (7.5)</td>
<td>6 (15.0)</td>
<td>31 (77.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*CI = Confidence Interval

Table 4: Distribution of serum Alanine Transaminase (ALT) and Aspartate Transaminase (AST) levels among the 40 patients with cholestasis of infancy

<table>
<thead>
<tr>
<th>Blood glucose concentration in mmol/L</th>
<th>Serum ALP levels (U/L)</th>
<th>&lt;1.6mmol/L</th>
<th>1.6 – 2.5mmol/L</th>
<th>&gt;2.5mmol/L</th>
<th>z- statistics (p-value)</th>
<th>Odd ratio (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 - 99 (n = 31)*</td>
<td></td>
<td>2 (6.5)</td>
<td>3 (9.7)</td>
<td>26 (83.9)</td>
<td>c vs d = 0.698 (&gt;0.05)</td>
<td>0.24 (1.27, 2.65)</td>
</tr>
<tr>
<td>&gt;99 (n = 9)</td>
<td></td>
<td>1 (11.1)</td>
<td>3 (33.3)</td>
<td>5 (55.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (n = 40)</td>
<td></td>
<td>3 (7.5)</td>
<td>6 (15.0)</td>
<td>31 (77.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65 – 100 (n = 28)</td>
<td></td>
<td>1 (3.6)</td>
<td>2 (7.1)</td>
<td>25 (89.3)</td>
<td>e vs f = 0.000 (&gt;0.05)</td>
<td>0.12 (1.25, 2.67)</td>
</tr>
<tr>
<td>&gt;100 (n = 12)*</td>
<td></td>
<td>2 (16.7)</td>
<td>4 (33.3)</td>
<td>6 (50.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (n = 40)*</td>
<td></td>
<td>3 (7.5)</td>
<td>6 (15.0)</td>
<td>31 (77.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*CI = Confidence Interval
DISCUSSION:
The overall prevalence (22.5%) of hypoglycaemia among patients with cholestasis of infancy was 3.7 times lower than that reported by Leblanc et al in France [12]. The lower prevalence observed in the present study may be accounted for by differences in study population. Their study population was patients with either primary or secondary cortisol deficiency [12]. Although infants with cholestasis whose age was between 15 days and 3 months had a higher frequency of occurrence of hypoglycaemia than the other age groups, the difference was not statistically significant. This is in keeping with the report of Lablan et al [12] in which they observed that of six patients with hypoglycaemia associated with cholestasis of infancy, four were below three months of age. In the present study, although there were more males than females with cholestasis of infancy, the prevalence of hypoglycaemia was similar, suggesting that there was no gender difference in the frequency of occurrence of hypoglycaemia. Lablan et al [12] indicated that of the five infants with cholestasis, four (80.0%) were males. In contrast, the Gonc et al [18] reported two cases, one male (3 months of age) and one female (6 months of age), with both infants manifesting episodes of hypoglycaemia. These authors stated that cholestasis among their patients was due to primary or secondary cortisol deficiency [12,18]. It has been suggested that the age of appearance of the cortisol deficiency is an important predictor of occurrence of cholestatic hepatitis, and consequently, occurrence of hypoglycaemia [17,18,21]. It is assumed that cortisol deficiency manifesting in the neonatal or early infancy period causes cholestatic hepatitis. Five of six patients with isolated cortisol deficiency who presented beyond early infancy did not have cholestatic hepatitis [21]. Such an assumption cannot be made from the present study because it was not designed to identify the specific aetiology of cholestasis of infancy.

In the present study, serum levels of ALP, ALT and AST did not appear to influence the prevalence of hypoglycaemia. No specific explanation can be given for these findings. Whether this is related to the fact that hepatocyte mass in some metabolic disorders is lost by apoptosis rather than cell necrosis, resulting in serum Aminotransferases being only moderately elevated [1] and consequently, unrelated to occurrence of hypoglycaemia is not clear. Although the present study had some limitations, most authorities agree that the presence of conjugated hyperbilirubinaemia (after the age of 14 days) and bilirubinuria are indicative of cholestasis [4,5]. In conclusion, hypoglycaemia was observed among patients with cholestasis of infancy, especially in the first three months of life.
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MORTALITY PREDICTION USING POSSUM SCORING SYSTEM FOR LAPAROTOMY PATIENTS IN MULAGO NATIONAL REFERRAL HOSPITAL, UGANDA

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ABSTRACT:
Prediction of serious complications is an essential part of risk management in surgery. Knowing which patient to operate and those at high risk of dying contributes significantly to the quality of surgical care and cost reduction. The postoperative mortality of patients who underwent laparotomy in Mulago Hospital was studied using Physiological and Operative Severity Score for the enUmeration of Mortality and Morbidity (POSSUM). Consecutive patients who underwent laparotomy in Mulago Hospital were recruited and consent obtained. Patients operated were followed up to the 30th postoperative day. Postoperative deaths were promptly investigated and findings recorded. Follow-up of patients was conducted by phone and surgical review once a week in outpatient. Ethical approval was obtained from the Institutional Review Board (IRB) of Makerere University Medical School. Seventy-six patients participated and the observed mortality was 14.5% and the predictive value of POSSUM using Receiver Operative Characteristics (ROC) curve was 0.817 (95% Confidence Interval 0.711, 0.924) and the Hosmer and Lemeshow test predicted 18.2% of mortality and survival 100%. Postoperative mortality can be predicted in the modern management of surgery using POSSUM. It is markedly influenced by the preoperative, operative and postoperative conditions of the patients.

Key words: Postoperative mortality, POSSUM, Mulago Hospital, laparotomy, prediction

Received: January 2012; Accepted: February 2012
INTRODUCTION:
Prediction of serious complications and mortality are essential part of risk management in surgery [1]. Knowing which patient is at risk of developing complications or dying contributes to the quality of surgical care and cost reduction in surgery [1]. Doctors are legally bound to inform their patients the potential risks involved with a particular treatment [1, 2, 3]. It is therefore essential to identify and make appropriate decision on those patients who are at high-risk of developing serious complications or die [1, 2, 4, 5]. Postoperative complications and death are sometimes determined by the surgical procedures conducted [1,2,4,6-9]. The Department of surgery in Mulago Hospital had no defined guideline for predicting the outcome of surgery. The department used crude rates of morbidity and mortality as measures of performance of surgeons and firms. Because of the above scenario, surgeons did not have a reproducible and predictive score, which could be used to assess and make appropriate decisions on certain categories of patients especially while teaching and preparing for laparotomy. This gap in knowledge among the surgeons was thought could be over-come by using a scientifically accepted risk-predictor. Review of several studies that were conducted in Uganda to determine postoperative mortality found non that used POSSUM scoring equation.

The purpose of this study was to use POSSUM scoring model to predict postoperative mortality in the patients who underwent laparotomy in Mulago National Referral Hospital (MNRH).

SUBJECTS AND METHODS:
This was a prospective observational study design conducted over a period of 6-months at MNRH.

The study population consisted of 76 patients aged 13 years and above admitted and consented/assented for elective and emergency surgeries. Day-care surgery and those who died before laparotomy were excluded. The patients were assessed preoperatively, operatively and postoperatively to determine their clinical status and suitability. Each patient was scored with the physiological component of POSSUM just before the induction of general anesthesia [4, 9]. All the individual scores were computed and summed up to produce the POSSUM physiological score for each patient. The operative severity score was used on the patients who underwent laparotomy [4, 9].

The quality control was ensured by the principal investigator carrying-out all the preoperative and postoperative assessment, clinical examinations and measurements of variables. Laboratory investigations particularly blood investigations were done by the same standard operating methods and reported in standard Units (SI units).
Using the POSSUM equation for predicting mortality \( R^2 \) the post operative mortality of each individual patient was calculated by [1]:

\[
\text{Log} \left( \frac{R^2}{1-R^2} \right) = -7.04 + (0.13 \times \text{Physiological Score}) + (0.16 \times \text{Operative severity score}).
\]

The values obtained from these patients were subjected to a ROC curve and logistic regression analysis for prediction of mortality using the Hosmer and Lemeshow test [22].

Patients were followed up to the 30th postoperative day with a weekly review in surgical outpatient. Telephone contacts were used to follow-up some postoperative patients. In the event of postoperative death, postmortem examinations were conducted by a pathologist from Makerere University and findings summarized and recorded. Mortality was investigated and possible cause determined by autopsy examination and histology.

Statistical data analysis was conducted using the SPSS version 10.0 software. A student t-test was used to compare significant differences and chi-square/Fisher's exact test was used in testing association of categorical variables. ROC curve and logistic regression analysis using Hosmer Lemeshow test was used to predict the risk of mortality using POSSUM equation.

Ethical approval was obtained from the Institutional Review Board (IRB) of Makerere University Medical School and each patient consented/or assented to the study.

**RESULTS:**

Diagnoses for postoperative mortality: Eleven mortality with intestinal obstruction constituting 55%, abdominal trauma with generalized peritonitis (9%), perforated Peptic ulcer disease with generalized peritonitis (9%) and abdominal malignancy (18%) and surgical jaundice (9%) were the diagnosis of the patients. No statistical significant relationship was observed between diagnoses and mortality. However, Diabetes Mellitus \( (t=3.333, \ p=0.001) \), occupation (civil servants) was positively correlated and significant \( (t=2.720, \ p=0.008) \). Nature of surgery had a statistically significant effect on the operative scores \( (t=4.375, \ p=0.000) \). On average, the emergency operations had higher operative scores (23.39) than the rest of the elective patients (16.68). Similarly emergency surgery had a higher average physiological score (25.63) than the rest of the electives (24.4) though the difference was not statistically significant \( (p=0.595) \). Overall, the risk of mortality was increased by emergency surgery \( (t=0.129, \ p=0.134) \) though not significantly.

Consultants and senior Residents were the surgeons. Consultants operated 2 of the patients and all had a fairly good physiological status \( (\text{PS of 25 & 25}) \) but had very high operative scores \( (\text{OS of 26 & 30 respectively}) \). There was a negative correlation and a statistically insignificant relationship between consultant surgeon and mortality \( (t=-1.643, \ p=0.105) \).
The majority of the patients who died were operated by the senior Residents (9/11). In general, the Senior Residents operated patients with higher physiological scores (poorer physical condition). There was a positive correlation (t=2.765) and a statistically significant relationship between physiological score and senior Residents (p=0.007).

A statistically insignificant correlation between senior Residents and mortality (t=0.087 and p=0.228) was observed. The average physiological score (PS) observed overall was 25.22 while for mortality cases it was 38.5. This score was positively correlated and statistically significant with mortality (t=2.228, p=0.029) and ($\chi^2=15.862$, p=0.003).

The average score (OS) for the study was 21.2 while for mortalities was 27.9. This figure was far higher compared to that of the overall population. There was a positive correlation and a statistically significant relationship between mortality and OS (t=3.280, p=0.000) and ($\chi^2=14.605$, p=0.012).

The mean postoperative hospital stay for study population was 8.46 but for mortalities was 12.4 days. The postoperative hospital stay had a negative correlation (t= -2.894) to mortality but a statistically significant correlation (p=0.005).

The Predicted mortality using POSSUM Equation: The ROC curve showed that mortality formula had an area under the curve of 0.817 and the 95% confidence interval (CI) was ranging from 0.711 to 0.924. This means that prediction of mortality by this formula is good.

ROC curve for risk of Mortality ($R_2$) is presented in Figure 1. The area under the curve is 0.817 (95% CI is 0.711, 0.924). The range was considered good enough for the present study. Hosmer and Lemeshow test was used to assess the relationship between the observed and predicted mortalities. This statistical package showed that the mortality formula could with accuracy predict survival 100% (p=0.003) but only predicted death correctly in 18.2% (95% c.i. 1.014, 1.070). This meant that this test could adequately predict survival but not death. It therefore predicted death in only 2.6% as opposed to the observed 14.5%. This indicated that a number of patients could have had unexpected death due to perhaps other confounding factors such as poor postoperative management.

DISCUSSION:
Data obtained from the present study indicates that POSSUM can be used to predict the risk of mortality following laparotomy in MNRH. Assessing the preoperative, operative and postoperative indices following laparotomy was very useful while using POSSUM scoring system [4,6,10,11].

The mean postoperative hospital stay for mortalities were comparable to most studies conducted in Uganda [12,13,14]and diagnoses
were also similar to previous studies conducted here [12-19]. All patients who died had a mortality risk which was greater than 50%. The highest mortality rate was among patients of bowel surgery. This was perhaps due to inadequate preparation and poor choice of patients for operation by surgeons. The physiological and operative scores, diabetes mellitus, hospital stay and civil servants significantly increased the risk of mortality. Our result showed a positive correlation and a statistically significant relationship between these variables and mortality.

Most mortality was due to surgery for intestinal obstruction. This was consistent with other previously conducted studies in Mulago Hospital [12, 14-16] This in most cases occurred with emergency patients who were mainly operated by the senior Residents. Inadequate resuscitation and lack of experience by senior Residents may be the main reason for this unexpectedly high mortality. These patients had very high physiological and operative scores and these two factors were found to statistically (p=0.029, p=0.000 respectively) and significantly increased the risk of mortality.

The observed mortality rate in this study was comparable with other previous studies conducted here in Mulago [12,13,17-19]. All the emergency patients who died had very high physiological and operative scores. All the elective patients who died had very high operative scores and one of the patients had both carcinoma of the pancreas and diabetes mellitus. Diabetic status statistically (p=0.001) and significantly increased the risk of mortality. The nature of surgery had a significant effect on the operative scores. Emergency operations observed far more deaths and complications compared to electives (9:2) although this observation was found to be statistically insignificant (t=0.129, p=0.134). This meant that although the emergency operations significantly increased the physiological and operative scores, the differences between the two was not significant, therefore we suspect there were other confounding factors, which may be responsible for the mortality.

Inadequate resuscitation, poor surgical techniques by senior Residents and delayed surgical intervention in casualty theatres could perhaps be the reasons for these unexpected deaths. A study carried out in 168 hospitals in the state of Pennsylvania, USA involving 232,440 surgical patients indicated that a higher patient to nursing staff ratios was associated with higher risk-adjusted postoperative mortality rate [21]. This meant that nursing care alone could be an independent predictor of postoperative deaths [20,21]. These results indicated that factors such as hospital resources, the availability and training of medical staffs had a significant impact on the postoperative outcome (mortality and morbidity) of patients [20,21,22].

Consultant surgeons and senior Residents conducted surgeries that resulted in mortalities.
In general, the senior Residents operated patients with higher physiological scores (poorer physical condition). There was a positive correlation but a statistically insignificant relationship ($p=0.228$) between mortality and senior Residents. This is not new since studies in other countries have shown that higher-risk surgery performed independently by physician in training was shown to be related to poor postoperative outcome [22]. The UK government report, have drawn attention to the dangers of leaving high-risk procedures to trainee surgeons without supervision [22] The average physiological (PS) and operative scores (OS) for the mortalities were far higher than those for the other patients. This finding was consistent with the findings observed in the USA and UK [22] and Malaysia [11].

The mean postoperative hospital stay for the mortality was comparable with the findings in UK and USA [22], Malaysia [11] and previous studies conducted in Mulago Hospital [12, 17-19]. The postoperative hospital stay had a negative correlation but a statistically significant relationship to mortality. This meant that the longer patients lived postoperatively, the less
likely they were to die. With regards to the occupation (Civil servants) of the patients increasing the risks of mortality, there was no clear explanation to advance. The ROC curve showed that mortality formula had an area under the curve of 0.817 and the 95% confidence interval of 0.711 - 0.924. This meant that prediction of mortality by this formula was good and could be used to predict mortality for patients who underwent laparotomy.

CONCLUSION:
The POSSUM scoring system equation was used to predict postoperative mortality among the patients of MNRH. Poor choice of patients for laparotomy markedly increased the risk of mortality. Apart from the physiological and operative scores, factors such as duration of postoperative hospital stay, diabetic status of the patient and occupation especially civil servants significantly increased the risk of mortality.

RECOMMENDATIONS:
The POSSUM scoring system equation can be used in the department of surgery to predict the outcome of surgery following laparotomy. Physiological and operative scores together with duration of hospital stay, and diabetic status of the patient should be used to assess the suitability of the patient and also to predict the outcome of the laparotomy. POSSUM scoring system can be used for research and clinical management of laparotomy.

ACKNOWLEDGEMENT:
We wish to acknowledge the participation of the patients in the study and the financial support from the Uganda Ministry of Health towards this study.

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SOCIO-DEMOGRAPHIC FACTORS ASSOCIATED WITH SPUTUM POSITIVITY RATES FOR TUBERCULOSIS IN PATIENTS WITH COUGH IN SRINAGAR HOSPITAL, INDIA

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ABSTRACT:
More women die of tuberculosis than any other infectious disease. The social stigma of the disease adds to the burden of both men and women, particularly if the disease occurs in their youth. The objective of this study was to assess the various socio-demographic factors influencing sputum positivity rates in cough symptomatic. This was a cross sectional hospital based study in which a specially designed proforma was used to collect information regarding socio-demographic characteristics of sputum positive tuberculosis patients. Higher sputum positive rate was seen in chest symptomatic above 55 years of age. Sputum positivity rates were also high among illiterates, patients belonging to socio-economic class IV, patients who had no history of contact and had no family history of tuberculosis. Our data indicates the need for increased vigilance among the vulnerable groups in Srinagar.

Key words: cough symptomatic, sputum positivity, socio-demographic characteristics, tuberculosis

Received: November 2011; Accepted: March 2012

INTRODUCTION:
Tuberculosis (TB) continues to be one of the most important public health problems worldwide [1]. It kills more women in reproductive age group than all other causes of maternal mortality combined, and it may create more orphans than any other infectious disease. The indirect impact of TB on children is considerable, as nearly one-fifth of school-age children of TB patients either leave school or take up employment to help support their families [2]. According to Pandit [3], “TB is a
social disease”. The social stigma of the disease adds to the burden for both men and women. Men have to deal with the stigma at their work-places and in the community while women are ostracized in the house-hold and neighborhood [4]. The disruption caused to society and the economy is incalculable. A patient with TB takes an average of 3 or 4 months to recuperate, losing that much of income. The loss is disastrous for those struggling against poverty and under-development [5]. According to Biggs [6] the homeless, friendless dependent, dissipated vicious consumptive individuals are likely to be most dangerous to the community. This study was designed to assess the various socio-demographic factors of sputum positive patients. The objective was to assess the various socio-demographic factors influencing sputum positivity rates among patients screened for TB.

**PATIENTS AND METHODS:**

This was a hospital based cross sectional study carried out using a standardized protocol in the district TB centre chest disease hospital, Srinagar, India. A pretested structured proforma was used for data collection. The study was conducted from December 2008 to June 2010. During this period a total of 2810 patients were screened for TB and all of them were recruited for the study. Data was collected from all the patients with sputum TB positive reports. Pre-trained and properly briefed laboratory staff members collected appropriate information and filling of proforma for patients with sputum TB positive results. Information collected included age, sex, residential address, history of (H/O) contact, family H/O TB status, number of family members, and type of family, smoking habits, level of education, income and occupation. The educational, occupational and income status of the patients were assessed by the modified Kupuswamy’s method [7, 8]. This method is a socio-economic scale that uses three variables: education, occupation and income. The data so collected was subjected to statistical analysis.

Some modified terms have been used in the present study. “Cough symptomatic” refers to patient presenting with cough to the out-patient department (OPD) as defined by the Revised National Tuberculosis Programme in India (RNTCP).

“Suspected case” is a patient seen in the OPD with a history of cough of ≥ 2 weeks. “Smear positive case”, refers to a patient in the OPD with at least two sputum specimen positive for acid-fast bacilli (AFB) by Ziehl-neeelsen sputum microscopy. “Sputum positivity”, is when at least three AFB’s are seen in 100 Oil immersion fields of smear.
RESULTS:
A total of 70,000 new adult patients attended the OPD during the study period. The total number of patients referred to the laboratory for sputum microscopy was 2810, which is equivalent to 4.0% of the total OPD attendees. Among the 2810 cough symptomatic, 367 were sputum positive, giving sputum positivity rate of 13.1%. The highest number (31.1%) of patients screened in the OPD was in the ≥55 years age group (Table 1). Of the 367 patients with sputum positivity, the highest prevalence rate (26.4%) was among the cough symptomatic in the ≥55 years age group, followed by the 15 to 24 years age group (24.5%) and 25 – 34 years age group (24.0%). Gender distribution shows that more males (53.2%) were screened compared to females (46.8%). There was no difference in the prevalence of Sputum positivity rate among the male and female patients (Table 1).

Table 1: Distribution of patients according to age and gender

<table>
<thead>
<tr>
<th>Age groups (yrs)</th>
<th>Distribution of patients screened at OPD (n = 2810)</th>
<th>Distribution of patients with sputum positivity (n = 367)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;14</td>
<td>112 (4.0%)</td>
<td>19 (5.2%)</td>
</tr>
<tr>
<td>15 – 24</td>
<td>475 (16.9%)</td>
<td>90 (24.5%)</td>
</tr>
<tr>
<td>25 – 34</td>
<td>509 (18.1%)</td>
<td>88 (24.0%)</td>
</tr>
<tr>
<td>35 – 44</td>
<td>410 (14.6%)</td>
<td>39 (10.6%)</td>
</tr>
<tr>
<td>45 – 54</td>
<td>429 (15.3%)</td>
<td>34 (9.3%)</td>
</tr>
<tr>
<td>≥55</td>
<td>875 (31.1%)</td>
<td>97 (26.4%)</td>
</tr>
<tr>
<td><strong>Gender distribution</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>1495 (53.2%)</td>
<td>184 (50.1%)</td>
</tr>
<tr>
<td>Females</td>
<td>1315 (46.8%)</td>
<td>183 (49.9%)</td>
</tr>
</tbody>
</table>

Sputum positivity rate was higher in illiterates (61%) compared to the literates (39%). Regarding socio-economic status, the highest (86.1%) sputum positivity rate was among the class IV group. As can be seen in Table 2, sputum positivity rate was higher among the cough symptomatic in the nuclear family (56.4%) compared to the joint family (43.6%). Prevalence of sputum positivity rate was highest (66.2%) among the smokers and lowest (3.3%) among the past smokers. Sputum positivity rate among patients with H/O contact was only 9.8%, while Sputum positivity rate was 26.2% among patients who had family H/O TB.
Table 2: Distribution of patients according to social status and risk factors

<table>
<thead>
<tr>
<th></th>
<th>Distribution of patients screened at OPD (n = 2810)</th>
<th>Distribution of patients with sputum positivity (n = 367)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Literacy status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterates</td>
<td>1866 (66.4%)</td>
<td>224 (61.0%)</td>
</tr>
<tr>
<td>Literates</td>
<td>944 (33.6%)</td>
<td>143 (39.0%)</td>
</tr>
<tr>
<td><strong>Educational status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterates</td>
<td>1866 (66.4%)</td>
<td>224 (61.0%)</td>
</tr>
<tr>
<td>Primary</td>
<td>124 (4.4%)</td>
<td>16 (4.4%)</td>
</tr>
<tr>
<td>Secondary</td>
<td>603 (21.5%)</td>
<td>102 (27.8%)</td>
</tr>
<tr>
<td>Higher</td>
<td>217 (7.7%)</td>
<td>25 (6.8%)</td>
</tr>
<tr>
<td><strong>Socioeconomic status (SE)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class II</td>
<td>125 (4.4%)</td>
<td>15 (4.1%)</td>
</tr>
<tr>
<td>Class III</td>
<td>393 (14.0%)</td>
<td>35 (9.5%)</td>
</tr>
<tr>
<td>Class IV</td>
<td>2275 (81.0%)</td>
<td>316 (86.1%)</td>
</tr>
<tr>
<td>Class V</td>
<td>(0.6%)</td>
<td>1 (0.3%)</td>
</tr>
<tr>
<td><strong>Family type</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuclear</td>
<td>1549 (55.1%)</td>
<td>207 (56.4%)</td>
</tr>
<tr>
<td>Joint</td>
<td>1261 (44.9%)</td>
<td>160 (43.6%)</td>
</tr>
<tr>
<td><strong>Life style</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
<td>843 (30.0%)</td>
<td>112 (30.5%)</td>
</tr>
<tr>
<td>Past smokers</td>
<td>140 (5.0%)</td>
<td>12 (3.3%)</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>1827 (65.0%)</td>
<td>243 (66.2%)</td>
</tr>
<tr>
<td><strong>Risk factors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H/O contact</td>
<td>174 (6.2%)</td>
<td>36 (9.8%)</td>
</tr>
<tr>
<td>Family H/O TB</td>
<td>620 (22.1%)</td>
<td>96 (26.2%)</td>
</tr>
<tr>
<td>Patients with no history of contact and family history</td>
<td>2016 (71.7%)</td>
<td>235 (64.0%)</td>
</tr>
</tbody>
</table>

**DISCUSSION:**

In our study observing high sputum positivity rates in productive age groups is in conformity to earlier findings reported by Godoy et al [9]. The highest sputum positivity found in age groups 55 and above in our study is noteworthy because sometimes TB as a cause of cough in older groups may be ignored and chest symptomatic in this age group may be treated for other chronic respiratory tract infections (like asthma, chronic bronchitis, emphysema) which are of major importance in the upper decades of life [10]. A high suspicion /vigilance for the older age-groups attending the health facility with history of cough are required.

In our study we observed no difference in sputum positivity rates among the male and
female patients, which is consistent with earlier study by Chandrasekhar et al.[11] Sputum positivity rate in our study was high among illiterates as compared to literates. This is in contrast to studies by Krishnada et al [12] and Olumuyiwa et al [13].

The higher rates in illiterates may be attributed to lack of community awareness. Interestingly among the literates, higher sputum positivity rate was observed in those having secondary level of education which is consistent with the findings from the study by Olumuyinwa et al [13]. Highest sputum positivity rate was prevalent among patients in the socio-economic class IV; these findings are consistent with observations report by Krishneda et al [12]. Sputum positivity rates were higher among the nonsmokers compared to smokers; this indicates that attention should also be given to non-smokers as well. Patients with no H/O contact and negative Family H/O of TB are another high risk groups that need immediate attention for screening in OPD settings [1, 5]. This is reinforced with the findings of the present study that recorded high sputum positivity rate among chest symptomatic having no H/O contact and with no family H/O TB. Thus, this emphasizes the need for vigilance in those who have no H/O contact and or no family H/O TB.

**CONCLUSION & RECOMMENDATIONS:**

Higher sputum positivity rates were seen in age group above 55 years. High sputum positivity rates were found in illiterates and those belonging to SE Class IV. Patients with no H/O contact and/or no family H/O TB are also susceptible to risk of acquiring TB.

Our data indicates that there should be high suspicion and increase vigilance for cough symptomatic among all groups, which include patients above 55 years, illiterate, belonging to the lower SE class; and also to those with no H/O contact and/or with no family H/O TB.

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CASE REPORTS

EROSIVE LICHEN PLANUS – A CASE REPORT
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ABSTRACT:
Lichen planus (LP) is a common disorder in which auto-cytotoxic T lymphocytes trigger apoptosis of epithelial cells leading to chronic inflammation. Oral Lichen Planus (OLP) can be a source of severe morbidity and has a small potential to be malignant. The diagnosis of OLP can be made from the clinical features if they are sufficiently characterized, but biopsy is recommended to confirm the diagnosis and to exclude dysplasia and malignancy. This is a case report of erosive lichen planus in a female patient, age 60 years.

Keywords: Lichen planus, Oral, Skin
Submitted August 2011, Accepted January 2012

INTRODUCTION:
Oral lichen planus (OLP) is a common disorder that affects stratified squamous epithelium virtually exclusively. It is seen worldwide, mostly in the fifth to sixth decades of life, and is twice as common in women as in men [1]. This article is a case report of erosive lichen planus located in the buccal mucosa of a 60 year old female patient. The diagnostic approach, clinical feature and various treatment modalities are discussed.

CASE REPORT:
A 60 year old female patient from Kannur, Kerala presented with the chief complaint of burning sensation in the left side of the cheek for the past 12 years. The burning sensation is aggravated when eating spicy foods. There was no burning sensation in the eyes and other parts of the body. She consulted several physicians and in each case was prescribed...
The clinical history indicated that the patient is hypertensive (for the past 12 years) and diabetic (2 years). She was on appropriate medications for these conditions.

On intra oral examination, the Left buccal mucosa revealed a reddish lesion interspersed with greyish white lines (Figure 1).

Anterio-posteriorly, the lesion extended from left commissure to the retromolar area. Superio-inferiorly it extended up to the buccal vestibule. The lesion on palpation was tender (Visual Analog Scale 8), and non scrapable. No such lesion was seen elsewhere in the mucosa.

So based on the history given by the patient and the clinical examination carried out a provisional diagnosis of erosive lichen planus on left buccal mucosa was made. Punch biopsy was taken from the lesion under local anaesthesia, and the specimen was sent for histo-pathological examination.

Microscopically the section showed stratified squamous atrophic epithelium which was separated from connective tissue.
The connective tissue showed infiltration of lymphocytes & few eosinophils (Figure 2). Final diagnosis of lichen planus was made. The patient was asked to apply triamclobacinetone 0.1 % ointment over the lesion three times a day for 3 weeks. Patient reported back after 3 weeks with complete healing of the lesion.

**DISCUSSION:**

Lichen planus is a benign condition that affects either the skin or the lining of the mouth. Erosive lichen planus is one type of lichen planus, although not as common as the reticular form, more significant for the patient because the lesions are usually symptomatic. Clinically, there are atrophic, erythematous aees with central ulceration of varying degrees. The periphery of the atrophic regions is usually bordered by fine, white radiating striae [2]. OLP can present as small, raised, white, lacy lesions, papules, or plaques, and can resemble keratotic diseases such as leukoplakia [2]. Atrophic lesions and erosions are the forms most likely to cause pain. The most common sites affected are the buccal mucosae, tongue (mainly the dorsum), gingiva, labial mucosa, and vermilion of the lower lip [3]. About 10% of patients with OLP have the disease confined to the gingiva Erythematous lesions that affect the gingiva cause desquamative Gingivitis [4].

**Malignant potential of OLP:** At least three studies using strict diagnostic criteria have shown a significant risk of malignant transformation of OLP to squamous cell carcinoma [5]. Malignant potential of erosive lichen planus is more when compared to other types of lichen planus.

**Diagnosis:** OLP that presents with classic white lesions may be diagnosed correctly if there is classic skin or other extraoral lesions. However, an oral biopsy with histopathological examination is recommended both to confirm the clinical diagnosis and particularly to exclude dysplasia and malignancy.

**Management of OLP:** Treatment of OLP depends on symptoms, the extent of oral and extra-oral clinical involvement, medical history, and other factors. In the case of patients with lichenoid lesions, the suspected precipitant should be eliminated. Patients with reticular and other asymptomatic OLP lesions usually require no active treatment. Symptomatic lesions require treatment depending on their severity which can be divided into 3 steps namely primary, secondary and tertiary line of treatment [4]. Primary line of treatment is indicated for mild to moderate symptomatic cases, topical applications of Triamcinalone acetonide 0.1% cream or Fluocinonode 0.05% gel or Dexamithazine 0.5mg/5ml in orabase
Secondary line of treatment is indicated for lesions which is not respond to topical treatment. In such cases Local Injection of 0.2 to 0.4ml Triamcinalone acitonide should be given or Systemic Prednisolone 40 to 80 mg daily is usually sufficient to achieve a response; drug toxicity requires that it should be used only when necessary, at the lowest dose, and for the shortest time, possible [4]. Systemic Prednisolone should be taken either for brief periods of time, (5–7 days) and the dose should be reduced by 5–10 mg/day gradually over 2–4 weeks [4]. Tertiary line of treatment should be used for severe cases that do not respond to short term prednisolone. More protracted course of Prednisolone should be given [5]. In order to reduce the side effects of corticosteroids, Immunosuppressant such as Azathioprine 50 to 100mg / day, cyclosporine 50mg should be prescribed [6,7].

**Other Modalities:** Resection has been recommended for isolated plaques or non-healing erosions, because it provides excellent tissue specimens for histopathological confirmation of diagnosis, and is said to cure localised lesions [8]. Free soft-tissue grafts have also been used for localised areas of erosive OLP [8].

**CONCLUSION:** OLP is an immunological disease which appears clinically in different types. The erosive lichen planus variant is one with highest malignant potential. It is important to identify the lesion clinically and histopathologically at the earliest and treat the condition in the early stage.

**REFERENCES:**

ACTINIC LICHEN PLANUS OF LIP – A CASE REPORT

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Running title: Actinic lichen planus

ABSTRACT:
Actinic lichen planus is a rare variant of lichen planus seen commonly in tropical and subtropical countries in the dark complexioned individuals. It manifests in the sun exposed areas of the face, neck and limbs. Though many cases have been reported in the skin, few lesions associated with the lip have been reported. The Lip is highly susceptible to actinic changes increasing the chances of malignancies. This is an unusual case report of 32 year old female patient where the lip lesion was seen mimicking discoid lupus erythematoses

Key Words: actinic lichen planus, erosive lip lesion, photosensitivity, lichen planus of lip,

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INTRODUCTION:
The lips occupy prominent position on the face of a person. The abnormalities can be obvious and be a source of embarrassment for the patient. Since lips form the gateway to the oral cavity, lesions affecting the oral cavity and dermatologic diseases are seen manifesting in the lips. Due to the position, lack of keratin covering, thinner epithelium, smaller amounts of melanin, and decreased secretions from sebaceous and sweat glands, all of which normally protect skin from radiation, are not present in lips making it more susceptible to actinic damage [1].

CASE REPORT:
A-32- year old female patient who reported to Department of Oral Medicine and Radiology, Yenepoya Dental College, with the chief complaint of red erosive lesions on the lower lip.
with periods of exacerbations on exposure to sunlight and remissions for the past 25 years. These lesions initiated as papules which ulcerated and later became infected and gave out purulent discharge. The present lesion was seen since past two weeks. The patient did not give any history of consuming any medications associated with the occurrence of the lesions. She had consulted doctors before who had prescribed her steroid topical preparations- mometasone cream (Momate-F cream) that gave relief during exacerbative episodes. The patient had burning sensation associated with the lip lesions. On extraoral examination there were no cutaneous lesions, only lesions present on the lips. The lower lips showed diffuse involvement from the vermilion upto the lower border of the lip and the lesion appeared atrophied in the centre, and hyperpigmented at the periphery. There was presence of epithelial tags throughout the erosive lip lesions. There were no whitish striae around the lesions. The upper lip showed presence of violaceous papules at the midline region associated with depigmentation [Figure 1].

Intraorally, there were radiating whitish striae in the buccal mucosa bilaterally at the level of occlusal plane associated with increased melanin pigmentation [Figure 2]. There were carious lesions present though no amalgam restorations were present. There was mild to moderated plaque accumulation with no ulcerations of any mucosal surfaces present. Based on the history and clinical presentation a provisional diagnosis of actinic lichen planus of the lip was made. However discoid lupus erythematoses (DLE), actinic chelitis of lip and erythema multiforme minor also shows similar clinical features. The reasons for these are presented in the discussion. Incisional biopsy of the buccal mucosa as well as lip region was done. The histological picture showed the presence of an atrophied stratified squamous epithelium and underlying connective tissue showing dense chronic inflammatory infiltrate with extensive melanin pigmentation and melanophages suggestive of a healing lichen planus lesion [Figure 3]. The lip lesion histologically atrophied epithelium and connective tissue presented with chronic inflammatory cells consistent with the findings present with the intraoral section.

The patient was given a systemic methyl prednisolone (Tab Zempred 8.0mg), a total dose of 12.0mg given in divided doses for a period of 2 weeks and topical clobetasole ointment (Clonate ointment 20 mg) to be applied on the lip lesions only for a period of 3 weeks. After three weeks there was complete remission of erosive lesions, however depigmentation was present [Figure 4].
Figure 1: Erosive lesions seen on entire lower lip along with epithelial tags. Upper lip shows violaceous papules.

Figure 2:
A. Right buccal mucosa shows healing lichen planus with post inflammatory pigmentation.
B. Left buccal mucosa shows healing lichen planus with post inflammatory pigmentation.

Figure 3:
A. Atrophied epithelium with dense chronic inflammatory infiltrate, increased melanin pigmentation with melanophages-healing lichen planus.
B. Atrophied epithelium with connective tissue with dense chronic inflammatory infiltrate.

Figure 4: Complete remission of erosive lesion.
DISCUSSION:
Lichen planus (LP), first described by Erasmus Wilson in 1869 and is related to a T-cell mediated immune response whose etiology is not correctly understood [2]. The mucosal lesions are more chronic in nature and persist for many years. The reticular form occurs more frequently and is characterized by white lacy streaks known as Wickham's striae, due to histologic folding of stratum granulosum [2, 3]. The reticular form usually causes no symptoms, it involves the posterior mucosa bilaterally, upper and lateral surfaces of the tongue, the gums and the palate [2, 4]. The erosive form is not as common as the reticular form and is painful, interfering with chewing. Clinically, erosive lichen planus manifests as atrophic and erythematous areas frequently surrounded by radiating thin striae [2, 4]. In certain cases, the epithelium may separate if erosion is severe, resulting in a relatively rare form of the disease known as bullous lichen planus [2]. Concomitant lesions usually are found, and lesions may change forms during the course of the disease [5]. Lesions regress with post inflammatory hyperpigmentation as seen in the present case in both right and left buccal mucosa.

Actinic lichen planus (ALP), also known as lichen planus tropicus, is a rare variant of LP that typically affects children or young adults with dark skin that live in tropical or subtropical regions [6, 7]. Lichen planus actinicus seen in sun exposed areas of the face, dorsum of the hands and arms, lips, V-shaped area of the chest and nape of the neck [8].

Four morphologic patterns have been clinically described in the literature [9]. The atrophic type is the most common form and is accompanied by hyperpigmentation. The second type, which is the dyschromic type, shows small, white angular papules that coalesce into plaques on the neck and dorsa of the hands. The classic plaque-like form presents as violaceous papules, and the pigmented form can be seen as melasma-like patches on the face and neck [10]. Our case may represent the classic form of ALP. Unlike classic lichen planus, pruritus, the Koebner phenomenon, and mucous membrane involvement are not commonly seen in all types of ALP [11]. Here, ultraviolet radiation appears to be an important inciting factor under the influence of genetic or other factors (hormonal, toxic, or infectious factors,). Hepatitis viral infection (B and C) is also reported to be a trigger factor in the occurrence of ALP [9, 12 – 15]. On histopathologic examination, epidermal atrophy may be more prominent in lichen planus actinicus which was also seen in our case. DLE was considered as one of the differential diagnosis where in lip involvement is frequent [16].

Clinical manifestations in DLE include well-demarcated discoid lesions or a diffuse cheilitis wherein lesions typically tend to spread from
the vermilion to the surrounding lip skin, obscuring the limits of the vermilion. This feature is useful in differentiating lupus erythematoses from lichen planus of the lip and from other types of cheilitis, as lichen planus lesions are characteristically limited to the vermilion area. The term “lupus cheilitis” is a different or special manifestation instead of a typical lupus erythematoses lesion on that location [16, 17]. Most common mucosal presentation of chronic lupus erythematoses is the oral discoid lesion which presents as a well-demarcated, round or irregular red area that can be atrophic or ulcerated, with white radiating keratotic striae and telangiectases [18]. Lesions are more often asymmetrically distributed in the oral cavity in palate, buccal mucosa, tongue which helps to differentiate it from lichen planus where lesions are bilaterally present [16]. Actinic cheilitis is most commonly seen on the lower lip of middle-aged and older (40 to 80 years), fair-skinned men, who have had excessive exposure to sun during their lives [19]. Actinic cheilitis presents as rough, scaly lips with fissures and ulcerations [20]. Commonly seen as a single lesion, but multiple lesions also occur. The initial sun-induced lesion is whitish-gray or brown, annular and the lip vermilion border becomes indistinguishable and shows generalized atrophy. Plasticity is lost. Marked folds appear along the vermilion perpendicular to the long axis of the lip and often dryness is associated with it. The lower lip especially the labial aspect is more commonly affected due to the angle of the UV rays striking the vermilion surface. Palpation is important in diagnosis because actinic cheilitis has a fine; “sandpapery” feel to it [21]. Erythema multiforme minor also causes erosive lesions on lips along with mucous membrane involvement is limited to only one site and usually it is the oral mucosa alone that is affected [22]. Intraoral lesions occur predominantly on the non keratinized mucosa and more pronounced on anterior parts of mouth. The lips are also commonly affected and are swollen and cracked, bleeding and crusted. Oral lesions progress through diffuse widespread macules to blisters and ulceration although only ulceration may be seen at presentation. The patient manifests with fever and malaise at the time of examination [23]. In the present case, there were whitish striae present bilaterally in the buccal mucosa and erosive lesions in the lip similar to the clinical presentation of DLE, but there were no other cutaneous lesions and a negative lab investigation for antinuclear antibody was seen. Further, as the patient was already taking steroidal medication both topically and systemically immunoflorescence testing would not yield any kind of results, so it was not done. DLE of the lip has been associated with malignancy and there are reports of actinicus lichen planus and actinic cheilitis [8,20, 24] also turning into squamous cell carcinoma. Squamous cell carcinoma of the lips has higher
metastatic potential than cutaneous squamous cell carcinoma [25]. Several therapies have been tried with variable results for ALP, including bismuth, arsenic compounds, and topical corticosteroid preparations. Treatment with antimalarial agents or intralesional corticosteroids combined with sunscreens has shown good results with prolonged remission [14, 15].

CONCLUSION:
As in the present case, diagnosis of lesions of lip would pose a diagnostic challenge to the practitioners when they do not occur in their characteristic form, however differential diagnosis of these lesions are important. Since lesions present in prominent areas of the face are highly susceptible to actinic changes and undergoing malignant transformation, their treatment should be done aggressively not only to prevent morbidity and mortality, but also to maintain patients’ social acceptance and self-esteem.

REFERENCES:


ORAL SUBMUCOUS FIBROSIS AS A PRECURSOR OF MALIGNANCY - A CASE REPORT

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Running title: Oral Submucous Fibrosis- Malignant transformation

ABSTRACT
Oral submucous fibrosis (OSF) is a high risk precancerous condition predominantly occurs in Indians and other population of the Indian subcontinent with certain oral habits. Betel quid (BQ) chewing is a popular oral habit with potential links to the occurrence of oral cancer. In patients with submucous fibrosis, the oral epithelium becomes atrophic and thereby becomes more vulnerable to carcinogens. Since the ingredients of BQ, tobacco are crucial for tumour initiation, promotion and progression, exposure to these toxicants simultaneously has been shown to markedly potentiate the oral cancer incidence in OSF patients. The rate of malignant transformation of OSF has been estimated to be 4.5%. Most cases with malignant transformation in OSF had occurred gradually over a long period of time.

Key words: Oral sub-mucous fibrosis, Betel quid, Areca nut, Chemical carcinogenesis.

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INTRODUCTION:
Oral submucous fibrosis (OSF) is a high risk precancerous condition of the oral mucosa that predominantly affects people of South-East Asian origin [1]. In patients with submucous fibrosis, the oral epithelium becomes atrophic and thereby becomes more vulnerable to carcinogens. The atrophic epithelium shows first an intercellular oedema and later epithelial atypia associated with moderate epithelial hyperplasia [2]. From then on, carcinoma may develop any time. OSF should be regarded as a condition that causes predisposition to the development of oral cancer [2]. The possible
The precancerous nature of OSF was first mentioned by Paymaster in the year 1956 [2]. A malignant transformation rate of 11.7% was reported with OSF which was seen predominantly in males (87%) [3]. The malignant transformation rate of OSF has been reported to be around 7.6% over a 17-year period [4]. Here we report a rare case of OSF which had turned into malignancy within a short period of only six months, in a thirty year old patient. The ethical clearance for the publication of the case report was obtained from the patient & from the concerned authority.

CASE REPORT:
A 30 year old male patient reported to our department with a chief complaint of burning sensation in the oral mucosa associated with difficulty in mouth opening since one year. There was difficulty on swallowing food and increased burning sensation on intake of spicy food. He had the habit of chewing gutkha three packets per day, since last six years.

Intraoral examination revealed diffuse blanching of left (Figures 1) as well as the right buccal mucosa and was slightly opaque with presence of thick fibrotic bands which were palpable bilaterally and running in vertical direction. Mouth opening was 20mm along with reduced flexibility of cheek. The area was found to be leathery & non tender on palpation. Incisional biopsy of the left buccal mucosa showed stratified squamous orthokeratinized epithelium with atrophy of the rete ridges, the connective tissue showed dense collagenous tissue with hyalinization suggestive of OSF grade II (Figure 2). Antioxidant was prescribed for the patient. Habit counselling was done for quitting gutka chewing habit. The frequency of gutka chewing habit was reduced following the habit counselling but was not completely stopped. Intralesional steroid injection (Betnesol 4mg/1ml vial) was given biweekly. Also patient was advised to do mouth exercises. The mouth opening was improved following the intralesional steroid injections and mouth exercises. The patient was under follow up once in a month for 6 months with no further change. Furthermore after about 6 months, there was an ulcerative growth (measuring about 4 × 3 cm) noticed on the left buccal mucosa extending 3cm from the corner of mouth to the retromolar area showing irregular and indurated margins. It was firm in consistency and fixed to underlying tissue. Mouth opening was reduced to one finger width. Left submandibular lymph node was enlarged, fixed, firm and non-tender on palpation. Orthopantomograph showed the presence of alveolar bone loss in relation to lower left second and third molar (Figure 3). Incisional biopsy was performed and was sent for histopathology. It was then diagnosed as well differentiated squamous cell carcinoma (Figure 4). The patient underwent segmental
mandibulectomy along with excision of the lesion.

Figure 1: Blanching on left buccal mucosa.
Figure 2: Histopathology showing stratified squamous orthokeratinized epithelium with atrophy of the rete ridges suggestive of grade II OSF.
Figure 3: Orthopantomograph showing alveolar bone loss in relation to lower left second and third molar teeth.
Figure 4: Histopathology shows keratin pearls suggestive of well-differentiated squamous cell carcinoma.

DISCUSSION:
OSF is a chronic insidious disease that affects the oral mucosa as well as the pharynx and the upper two-thirds of the oesophagus. It is a well-recognized potentially malignant condition of the oral cavity. Besides being regarded as a precancerous condition, it is a seriously debilitating and progressive disease [5]. Once initiated, the disease is not amenable to reversal at any stage of the disease process even after cessation of the habit. It causes significant morbidity (in terms of loss of mouth function as tissues become rigid and mouth opening becomes difficult) and mortality (when transformation into squamous cell carcinoma occurs) [6].
The strongest risk factor for OSF is the chewing of betel quid (BQ) containing areca nut [5]. The amount of areca nut in betel quid and the frequency and duration of chewing betel quid are clearly related to the development of OSF [7]. The direct contact of the quid mixture with oral tissues results in their continuous irritation by various components, including biologically active alkaloids (arecoline, arecaidine, arecolidine, guvacoline, guvacine), flavonoids (tannins and catechins) and copper [6]. These chemical components present in areca nut modulate lysyl oxidase enzyme [6]. This increases collagen production and decreases collagen degradation thus leading to an increased fibrosis [6]. The copper content of areca nut is high and the possible role of copper as a mediator of fibrosis is supported by the demonstration of up regulation of lysyl oxidase in OSF biopsies [8]. The fibrosis in the soft tissues leads to trismus, difficulty in eating and even dysphagia as also reported in the present case.

It has been suggested that the areca nut ingredients have tumour-promoting activity [9]. Chemical carcinogenesis is a complex multi-step process including initiation, promotion and progression of tumour [10].

The most important and decisive event of chemical carcinogenesis is the interaction between presumed carcinogens and cellular macromolecules such as DNA, proteins and lipids [11]. Normal oral mucosal epithelial cells are continuously subjected to the attack of genotoxic agents present in betel quid [12]. Antioxidants such as cellular Glutathione (GSH), N-acetyl-L-cysteine (NAC) and enzymes such as glutathione peroxidase, catalase and superoxide dismutase can form conjugates with Reactive Oxygen Species (ROS) and reactive intermediates, thereby degrading reactive toxic species and protecting the critical cellular macromolecules [9]. Repeated and continuous exposure of oral mucosal cells to BQ ingredients, however will lead to the impairment of cellular-defence systems [9].

An excessive amount of ROS, reactive metabolic intermediates from BQ and tobacco can attack cellular DNA and induce various kinds of DNA damage. If the DNA-damaged cells are subsequently induced by proliferative agents to replicate, the DNA damage will remain permanently in the cells, and thereby leading to the formation of mutated “initiated” cells [9]. The further promotion and progression of such initiated cells can lead to the occurrence of oral pre-cancerous lesions and clinical tumours. Since the ingredients of BQ, tobacco have been shown to exert genotoxicity and are crucial for tumour initiation, promotion and progression, exposure to these toxicants simultaneously has been shown to markedly potentiate the oral cancer incidence in OSF patients [13]. Epithelial atrophy in OSF patients increases the penetration of carcinogenic
ingredients of BQ and thereby subsequently increasing the incidence rate of oral cancer [2]. It was also observed that gutkha chewing was preferred by people in the age group 11-30 years [14]. It has been reported that the onset of OSF changes occurred earlier with gutkha chewing compared to only areca nut chewing [14]. These findings clearly document the hazard of gutkha chewing.

Since people take to gutkha chewing at a comparatively younger age and as it requires a shorter duration of chewing to precipitate OSMF, there may be an increased risk developing malignant changes in such OSF cases [14].

CONCLUSION:
The case reported here had a history of chewing gutkha which contains both arecanut & tobacco. This has led to the development of OSF and further progression to oral cancer. Documentation of such cases is highly important to make the people aware of the possible hazardous effects of chewing habits and to prevent further progression of OSF to malignancy.

REFERENCES:
BURULI ULCER IN THE PROXIMAL RIGHT THIGH AND GROIN: A CASE REPORT

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ABSTRACT:
We report a case of histopathologically proven Buruli ulcer (BU) in a 25-year-old man which was found at the proximal right thigh and groin – an unusual site of occurrence. Laboratory results including Gram and ZN stains were negative while a culture on Lowenstein Jensen media at 33°C from the tissues produced a positive growth of Mycobacterium ulcerans. Histology of the edges of the ulcer showed a granulomatous lesion consistent with BU. This highlights the differentiation of Buruli ulcer from tropical ulcer and, to a lesser extent other forms of skin malignancies and benign skin lesions. The ulcer presented by the young man was Buruli ulcer.

Key words: Buruli Ulcer, Gulu Hospital, Diagnosis, tropical ulcer

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INTRODUCTION
Buruli ulcer (BU) is caused by Mycobacterium ulcerans [1]. It is one of the most neglected but treatable tropical diseases [1,2]. The causative organism is from the family of Mycobacteriaeae which causes tuberculosis and leprosy but Buruli ulcer has received the least attention compared to other two diseases [1,2]. Infection leads to extensive destruction of skin and soft tissues with the formation of large ulcers usually on the legs or arms [2]. Patients who are not treated early often suffer long-term functional disability such as restriction of joint movement as well as the obvious cosmetic problems [1,2,3,4,5]. Early diagnosis and treatment are vital in preventing such disabilities [6,7,8,9]. Limited knowledge of the disease, its focal distribution and its occurrence mainly amongst poor rural communities contribute to low reporting of cases [9,10]. In 1897, Sir Albert Cook, a British physician working at Mengo Hospital in Kampala, Uganda described skin ulcers that were consistent with Buruli ulcer (BU) [1]. In the 1960s, many cases occurred in Buruli County (now called Nakasongola District) in Uganda,
giving rise to the most widely used name for the disease – Buruli ulcers [8,9]. Since 1980, the disease has emerged rapidly in several parts of the world, particularly in West Africa [9]. We discussed in this paper the characteristic clinical features, investigations and compared Buruli ulcer with tropical ulcer.

CASE REPORT
A 25 year old male peasant farmer from Koch Goma Sub County in Nwoya district presented to Gulu Hospital in February 2006 with a one month’s history of an ulcer in the right thigh. He had moved to several health facilities for treatment but without any improvement. He gave a history that the ulcer started with a small nodule at the upper right thigh which he kept on scratching because it was itchy and three weeks later it developed into an ulcer. He also reported to have been cultivating crops in the nearby swamps for many months and that very often he made sleeping mats from papyrus reeds which he normally collected from the swamps for which proceeds from their sale were his additional family income. On examination, he was in good general condition with a large ulcer in the proximal right thigh, measuring over 25cm in the longest diameter and was covering the anterior and medial portions of the thigh. The wound had an undermining edge, non tender, no palpable inguinal lymph nodes and the base was not fixed to the underlying structures, the patient was afebrile and the floor of the ulcer was clean. Laboratory examination was conducted on swabs which were taken from the floor and edges of the ulcer. One was used for microscopy: Gram stain was performed for general bacterial infections while the Zhiel Neelsen (ZN) was performed specifically for Acid Alcohol Fast (AAFB) Bacilli. The other swabs were used for culture both for general bacteria on blood agar and MacConkey agar while the third swab was inoculated on Lowenstein Jensen medium for mycobacterium. Both Gram and ZN stains revealed no mycobacterium. However the culture result revealed the presence of mycobacterium ulcerans. Similarly, biopsy of the ulcer edges was done and the histology result was comparable with the culture results. The patient was treated with Rifampicin (10mg/kg once a day) and Streptomycin (15mg/kg once a day) because previous studies showed that the 2 drugs were bactericidal to tubercle bacilli [9, 21]. This treatment was for eight weeks and thereafter, the ulcer was skin grafted and the rehabilitation process completed.
Table 1: Clinical characteristic comparison between Buruli and Tropical ulcers [10,13,16,17]

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Buruli ulcer</th>
<th>Tropical ulcer</th>
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<tbody>
<tr>
<td>Pain</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Undermining edges</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Lower limb (site)</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Inguinal nodes</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Generalized symptoms e.g. Fever</td>
<td>–</td>
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+ present; – absent

Fig. 1: Buruli ulcers in Gulu Regional Referral Hospital (Uganda)
This patient was a 25 year old boy who presented with Buruli ulcer to Gulu Regional Referral hospital and was managed in surgical department. He was treated with anti TB drugs (Rifampicin (10mg/kg once a day and Streptomycin 15mg/kg once a day) and wound dressing until when it was clean enough for skin grafting. Skin grafting was undertaken and patient's wound healed completely. The patient's condition could not be diagnosed by most health workers he visited before coming to Gulu Hospital. Secondly the ulcer occurred at an unusually uncommon site in the body of this patient (Normally, buruli ulcer occurs at exposed parts of the limbs.

Fig 2: Tropical Ulcers in a 17 year old in Gulu Regional Referral Hospital
This patient was a 17 year old boy who presented with tropical ulcer to Gulu Regional Referral hospital and was being managed in the outpatient surgical department. He was treated with broad spectrum antibiotics and daily dressing of the wound until when it was clean enough for skin grafting. His wound was skin grafted and it healed completely.
DISCUSSION:
The true incidence of Buruli ulcers is not well known and although it was first described in Uganda in the sixties, it had literally been eradicated from the country [1]. This case report presents a particularly unique fact that it is a rare site (groin) of occurrence of the disease in this particular area of Uganda [1, 2, 3]. M. ulcerans produces a destructive toxin, mycolactone, which causes tissue damage and inhibits the immune response [2, 9, 10]. The toxic effects of the toxin explain most of the virulence of this organism [9, 10]. Buruli ulcer frequently occurs near water bodies – slow flowing rivers, ponds, swamps and lakes; cases have also occurred following flooding [9, 10]. Exposure risk factors of economic and social activities that take place near water bodies are the major source of infections [9, 10]. The disease can affect any part of the body, but in about 90% of cases the lesions are on the limbs, with nearly 60% of all lesions on the lower limbs [9]. In Uganda, socio-cultural beliefs and practices strongly influence the health-seeking behaviours of people affected by BU [8, 9]. The first recourse is often traditional treatment. In addition to the high cost of surgical treatment, fear of surgery and concerns about the resulting scars and possible amputations may also prevail [1 - 5, 9, 10]. Disfiguration stigma is a problem that also prevents people from seeking early treatment and the long hospital stay, huge losses in productivity for adult patients which affects children’s educational opportunities [1, 9, 10]. The possible differentials in this case report could be tropical ulcer and other rare skin lesions. Tropical ulcer though has distinct clinical features, such as presentation with painful ulcers below the knee, usually the ankle [11, 12, 13]. They are often initiated by minor trauma, and subjects with poor nutrition and poor hygiene are at higher risks [12, 14, 16, 17]. Once developed, the ulcer may become chronic and stable, but also it can run a destructive course with deep tissue invasion and osteitis [12, 13, 16]. Unlike Buruli ulcer in acute stage, tropical ulcers are very painful and aggravated by standing up [14, 16, 17]. Walking often causes venous congestion, leading to bleeding which can be quite severe. Pain and bleeding are both relieved by firm bandaging [16, 17]. The pain and rapid rate of spread are characteristic of tropical ulcer; the thick rolled edge and the continuation into a chronic condition complete the clinical picture [15, 16, 17]. Lesions begin with inflammatory papules that progress into vesicles and rupture with the formation of an ulcer [16, 17]. One of the major problems with tropical ulcers is the spread of infection leading to generalized septicemia or infection with tetanus and gas gangrene which are further complications [16, 17]. The majority of tropical ulcers develop in the lower leg, overlying the tibia or the fibula and in the first 5 days, until a pustule discharges, there are usually no significant
radiological findings [16,18]. When the ulcer has developed there is usually marked soft tissue thickening, visible in profile radiography or with ultrasonography [15,16,17]. As the ulcer spreads, the earliest radiological finding in bone is a periosteal reaction which is localized immediately beneath the ulcer [16,17,18]. In most cases this begins as a minimal layered, fusiform periosteal reaction, but occasionally "sunray" spicules develop [16,17,18]. The periosteal reaction then blends with the original cortex to produce a thickened sclerotic layer which may be as much as 2.5 cm in thickness [18].

This ivory periosteal reaction can eventually involve the whole circumference of the bone and extend above and below the site of the ulcer and as it does so; it often becomes wavy and irregular, varying in width and in outline [18]. In some patients the next development is "ulceration" or, more strictly, sequestration of a localized superficial area of the periosteal new bone [18,19]. The sequestrum is sharply defined and may be separated from the underlying cortex and may eventually be discharged, resulting in a clear-cut, saucer-shaped defect [18,19]. The sharpness of this deficit is of considerable importance, because it is the main clue from which the radiologist can try to differentiate an infective process from malignant degeneration: when there is malignancy, the edge of the bone defect will be less regular and less well defined [18,19]. The same bone or adjacent bones may be affected some distance from the ulcer and may show periosteal reaction, with irregular widening and bony spicules quite remote from an ulcer in the anterior soft tissues and this presumably results from the soft tissue reaction in the leg [19,20]. A characteristic finding in some cases is calcification of the interosseous membrane between the tibia and the fibula [19]. Septic arthritis may occur depending on the locality of the ulcer [18,19,20]. When healing occurs there is considerable residual deformity, not only due to contraction of the skin and underlying soft tissues, but also because there may have been tendon and joint damage [13,14,19,20].

A flexion deformity of the knee or "talipes equinovarus" can develop and if scarring continues, the tibia and fibula may be drawn together by contraction and the bones may become thin and atrophied [19,20]. When ulceration is extremely chronic, deformity of the lower leg occurs and the bones may elongate and bow anteriorly or laterally [20]. The greatest convexity lies beneath the ulcer, the site of the osteoma and lymphoedema may result from the scarring [19,20].

Radiologically, the differential diagnosis of tropical ulcer in its early stages will include almost any cause of a localized periosteal response, but tropical ulcers may be distinguished because of the local soft tissue ulceration overlying them [18,19,20].
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Competing Interest: None

REFERENCES


BILATERAL CHOLESTEROL GRANULOMA OF MAXILLARY SINUS: A CASE REPORT

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ABSTRACT

Cholesterol Granuloma [CG] is a rare pathology found in the paranasal sinuses and is usually associated with middle ear infections. The etiology of sinonasal CG is not yet known. The clinical manifestations are nonspecific. Most patient presents with nasal discharge, facial pain and nasal obstruction similar to current case. The additional feature observed in the present case was bilateral CG of the maxillary sinus which was extending into ostiomeatal complex and into the oral cavity, whose clinical, imaging and histological characteristics were unique.

Key words: Cholesterol granuloma, maxillary sinus, ostiomeatal complex, Caldwell luc.

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INTRODUCTION:

Cholesterol granuloma (CG) is a foreign body reaction. It is mainly due to the presence of Cholesterol crystals and the components of the granulation tissue formed during the inflammatory process. CG is a fibrous granulation tissue which consists of cholesterol clefts, foreign body giant cells, foam cells and macrophages filled with hemosiderin pigments [1]. It usually develops in association with chronic middle ear diseases and affects the mastoid antrum and air cells within the temporal bone. It can rarely develop in the Paranasal sinuses [2]. There are only few case reports where there was involvement of both maxillary antrum. The present case is unique
since it had bilateral involvement of CG which was extending into the oral cavity. Most of these cases have been treated with radical operative techniques, including Caldwell luc operations similar to our case [2].

CASE REPORT:
A 60-year-old male patient reported to the Department of Oral Medicine and Radiology (DOMR), Yenepoya Dental College and Hospital, with a complaint of a growth arising from the socket of recently extracted tooth. The tooth was extracted four weeks back and after one week the patient noticed a slow progressive painless growth in the same region. The patient complained of mild pain in the left maxillary sinus with intermittent nasal discharge since four months. Extra oral examination revealed a mild facial asymmetry in left middle 1/3rd of face with tenderness and firmness in the left maxillary sinus region. Intraoral examination revealed a well defined tooth like, yellow colored, pedunculated growth, measuring about 1.5 X 1.0 CM in the left alveolar process of third molar region. The surface of the growth appeared rough with indentation of opposite tooth cusps. On palpation, growth was non tender, mobile, soft in consistency and was attached to alveolar socket [Fig 1].

Intraoral periapical radiograph showed unclear socket with loss of continuity in the floor of the maxillary sinus [Fig 2]. Computed Tomography revealed complete hazziness in the left maxillary sinus extending into superior and middle concha and a well defined polyp in right maxillary sinus region [Fig 3]. The growth was excised followed by curettage of maxillary sinus through Caldwell Luc approach. The excised specimen was subjected to histopathological examination by using hematoxylin-eosin stain. It revealed cluster of cholesterol clefts surrounded by multinucleated giant cells and there was granulation tissue formation with infiltrate of lymphocytes and plasma cells in connective tissue [Fig 4].

DISCUSSION:
Cholesterol granuloma is rare sinus benign tumor. It was first described by Graham and Michael in 1978 [1]. It occurs mainly in middle age males with a history of rhinitis, facial pain, headache and nasal obstruction [2].

There are several schools of thoughts for pathogenesis of CG which includes impairment of drainage, disturbed ventilation, and hemorrhage into bony cavities with haemolysis, which leads to cholesterol precipitations [3]. This substance, coming from the destroyed cellular membranes of the erythrocytes, crystallizes because of the slow drainage of the sinus causing the inflammatory process of the adjacent tissues and the formation of the granulomas [4].
The symptoms of the CG are notably non-specific and are similar to many inflammatory sinopathies. Some authors recognize the clear golden yellow rhinorhea as the only specific sign of the disease [5].

The present case had initial rhinorhea later nasal obstruction, facial pain and growth protruding to oral cavity after traumatic extraction of tooth. The differential diagnosis of sinonasal CG includes mucocele, cysts, neoplasm and chronic inflammatory processes of the sinus mucosa [5]. Water’s and CT images shows cyst-like or a massive opacification of the sinus. In some cases growth even involves the osteo-meatal complex similar to our case. The radiological features could be similar to chronic inflammatory polyp.
of the sinus, antro-chonal polyp and benign neoplasm. Histological examination is usually used to reveal the rare diagnosis of cholesterol granuloma [6].

The CG of the maxillary sinus is a difficult disease to diagnose without the auxiliary help of histological examination because of the non-specific symptoms and the endoscopic and radiological signs similar to other diseases (neoplastic or inflammatory). Surgical excision of the lesion, through Caldwell Luc approach is recommended [6]. The present case is the rare case with an unusual feature of CG herniating into oral cavity through the extracted socket. The characteristic clinical features and bilateral involvement of both the antrum prompted us to report the current case.

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REFERENCES
FOREIGN BODY PENETRATION: A MISSED DIAGNOSIS

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Running title: Foreign body reaction.

ABSTRACT:
Healing of the injured site is a complex biological process of carefully orchestrated cellular events. Presence of any foreign body at the site of injury delays the healing along with inducing biological response such as inflammation, infections, allergic reactions, toxic events and tissue alterations. Such body reactions against an exogenous materials depends upon the mode of entry, chemical composition of material, quantity of material its physical form and also depends upon the body site. A careful history, clinical examination and imaging techniques should be considered for patients with any suspected penetrating injuries.

Key words: foreign body reaction, traffic accident, wood fragments, tissue response.

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INTRODUCTION:
The term foreign body reaction is used for the tissue response to extraneous materials that becomes implanted in or beneath dermis [1]. Such reactions occur as a result of direct accidental penetration of the exogenous materials or due to iatrogenic cause, both inducing an inflammatory reaction. Epidemiological profile reveals Road Traffic Accident (RTA) is one among the top five causes of morbidity and mortality in South-East Asian countries [2]. A careful history
surrounding any suspected penetrating injury is essential, taking into account the timing and the type of material involved. Diagnosis depends on combination of through history taking supported by external and oral cavity examination followed by radiographic aids. Foreign bodies like wood fragments, if present, act as infectious nidus, which is not revealed by imaging study [3]. A wound that fails to heal continues to cause pain with movement or exhibit persistent purulent discharge, may suggest the presence of a foreign body.

This case report describes the clinical feature of foreign body reaction that develops after traumatic implantation of wood following RTA and the limited role of radiographs in diagnosis of such reaction.

**CASE REPORT:**
A 37-year old male patient reported to department of Oral Medicine and Radiology with chief complains of swelling on the right middle third of face since one month. Medical history reveals that the patient was involved in a road traffic accident three months back and was hospitalized immediately because of loss of consciousness. He reported injuries to the upper extremities and bleeding from nasal cavity for which primary management was provided. No injury to teeth and jaws was reported, however treatment was provided for the soft tissue laceration of mid-facial region. The patient was discharged after an observational period of 24hrs. He was asymptomatic for 2 months except complaining of stuffiness below the right malar region. Extraoral examination after two months revealed Scar mark on forehead and below middle canthus of right eye. A diffuse oval swelling on the right middle third of face and obliteration of right nasolabial fold was also noticed (Figure 1). Extension of swelling was 2.0 cm below the middle canthus of right eye to base of upper lip superior-inferiorly & 3.0 cm laterally form the right ala of nose. Surface and color of skin over the swelling was similar as adjacent side with no sign of trauma. On palpation swelling was woody hard centrally and soft at the periphery with diffuse margins. The temperature of the swelling was raised with tenderness positive. Intraoral examination revealed presence of root stumps in relation to maxillary right premolars, maxillary left second molar and mandibular left first molar teeth. A solitary irregular ulcerative lesion was noticed on the right upper buccal vestibule near the periapical region of maxillary right central, lateral incisors and canine (Figure 2).

The surface of the lesion appeared to be hypervascular with irregular tissue projections and purulent discharge at the periphery. The central area of lesion showed presence of yellowish mass of size 1.0 × 1.0 cm which on palpation was woody hard in consistency and non mobile. Surrounding area elicited
fluctuation with purulent discharge suggestive of cystic lesion. Tenderness was positive over the lesion and also with maxillary right central, lateral incisors and canine, on percussion. Intraoral periapical radiographic examination and maxillary occlusal radiograph revealed presence of only root stumps in relation to maxillary right both premolars with no other contributory findings (Figure 3 and 4). Hence the hard yellowish mass was retrieved. The implanted material was wooden pieces 4 to 5 in number of varying size (Figure 5). Thus, suggestive of penetrating injury during the time of accident and missed during preliminary examination, causing a foreign body reaction over a period of three months.

DISCUSSION:
When exogenous materials penetrate body tissue, there is usually a phase of acute inflammation in response to the injury. Persistent presence of such inert substance within tissue results in accumulation of monocytes, tissue macrophages, epithelioid histocytes and giant cells with fibroblastic reaction to lay down new connective tissue around the area of foreign body deposition [1]. Thus, penetration injuries may result in formation of implanted cysts mixed with granulomatous response. Such response is different from immune specific granulomatous inflammatory reactions based on duration, dynamics, severity and evolution. The clinical presentation of body reaction may vary based on physical composition of material, size, their non digestible characteristics and site of injury. Tissue reactions to foreign materials are commonly encountered in oral cavity against large number of dental materials. The more common iatrogenic instances such as accidental penetration of metallic restoration or endodontic sealers and fillers are already reported in literature which induces toxic reaction [4,5]. Occurrence of pyogenic infections with vegetative matters is also common in oral cavity [6]. Unusual foreign bodies like wooden stick in oral cavity are rarely reported [7]. Review of the literature indicates foreign body reaction with aluminum silicate and black plastic tapes in oral cavity [8].

As seen in present case, such foreign body reactions are complicated by infections, especially with traumatic inoculation of wooden splinters [9,10]. This may be due to introduction of sporotrichosis or mycotic organisms manifested as cellulitis, abscess or draining sinus. Diagnosis of such reaction depends on careful history taking as the tissue reaction may be associated with delayed symptoms which may apparently been forgotten by the patient or left unrevealed, as in the present case. Hence, if the history of trauma is significant, the possibility of a mass associated with a long standing foreign body should be considered.
Figure 1: Diffuse oval swelling on the right middle third of face and obliteration of right nasolabial fold

Figure 2: Ulcerative lesion was noticed on the right upper buccal vestibule near the periapical region of 11, 12 & 13

Figure 3 - Intraoral periapical radiographic in relation to 14 and 15 shows root stumps in relation to 14 and 15 missing with no other contributory findings.

Figure 4 - Maxillary occlusal radiograph was non contributory.

Figure 5 - Wooden pieces 4 to 5 in number of varying sizes
A history of trauma in the remote past must be treated with skepticism with essential clinical and radiological examination of suspected penetrated injury. Imaging technique may be helpful but may not yield consistent results in certain radiolucent materials like glass, wooden splinters or vegetative matters [12]. In such case sonography is an accurate imaging modality; however this was not performed, as the presence of foreign body was not suspected due to unrevealed history of penetrated injury [13]. It was difficult to judge as to whether or not to explore the lesion, but based on location and accessibility, the suspicious embedded material was retrieved revealing presence of numerous wooden splinters. This indicates that a thorough patient history and careful imaging in suspected cases remains the keys for diagnosis of retained foreign bodies. Dentist should be familiar with their features and include them in the differential diagnosis of tissue masses, mainly in the presence of trauma history.

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