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Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

A list of non-standard Abbreviations should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml).

The Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

The presentation of the case study should include the important information regarding the case. This must include the medical history, demographics, symptoms, tests etc. Kindly note that all information that will lead to the identification of the particular patient(s) must be excluded.

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The Acknowledgments of people, grants, funds, etc should be brief.

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Interest of spiramycin in the treatment of toxoplasmosis in Dakar

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Toxoplasmosis is an anthropozoonosis of medical and veterinary importance, due to the protozoan *Toxoplasma gondii*. Oocysts shed by felids play a key role in parasite transmission as they contaminate meat-producing animals, vegetables and water consumed later by humans. This study tried to study the effectiveness of spiramycin in the treatment of toxoplasmosis in the laboratory of the Abass NDAO Hospital’s (CHAN) in human immunodeficiency virus (HIV)-positive women (82 cases) using the enzyme immunoassay method solid phase (EIA). It reveals a negativity of IgM antibodies and progressive regression of IgG antibodies more visible in three women as high (between 50 and 1000 International Units (IU)/ml) and in three other unreliable in the first immunoglobin M (IgM) serology by a seroconversion at the second serology with a stabilization of immunoglobin (IgG) 10 IU/ml. The effectiveness of current treatment has been demonstrated by low return rate IgG antibodies to 10 IU/ml in those who received 3 million IU of Rovamycin at a dose of 2 tablets for day to one month. These first results need to be followed by more extensive investigations.

Key words: Prevalence, treatment, toxoplasmosis, *Toxoplasma gondii*.

INTRODUCTION

*Toxoplasma gondii* is the agent of a cosmopolitan anthropozoonosis: toxoplasmosis. This intracellular parasite maintains an optional heteroxenous cycle between cats (definitive hosts) and other warm-blooded animals (intermediate hosts).

Toxoplasmosis is almost always asymptomatic but can be severe in immunocompromised individual or after congenital transmission. The medical and veterinary importance of toxoplasmosis drives for 50 years numerous epidemiological studies to identify the reservoirs and modes of transmission of the parasite (Try et al., 2000).

The consumption of raw or undercooked meat containing cysts of the parasite and the ingestion of oocysts with fruits contaminated with faeces of cats are the two main modes of contamination. More recently, the consumption of water contaminated with oocysts was identified as a risk factor for toxoplasmosis in Brazil (Bahia - Oliveira et al., 2003). Waterborne outbreaks have been causing symptomatic toxoplasmosis sometimes fatal in Panama (Benenson et al., 1982), Canada (Bowie et al., 1997) and Brazil (Taverne, 2002). The seroprevalence of human toxoplasmosis varies according to geographical areas.

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Europe, it is 30 to 50% in majority of the countries in central and west and becomes less than 30% in the north. Low prevalences are recorded in North America, Southeast Asia and some African countries (Niger, South Africa) (Try et al., 2000). The highest prevalence (> 60%) occurs primarily among the countries bordering the Gulf of Guinea and Latin America. These differences are mainly due to the larger survival of oocysts in humid climates. There are few infections in areas where cats are absent (Dubey et al., 1997). Oocysts have a central role in transmission of the parasite, because they infect humans directly, or indirectly through animals for slaughter. From the perspective of assessing the risk of toxoplasmosis associated with oocysts, it is necessary to determine the prevalence of oocysts in the environment. This is only possible with methods specific and sensitive detection, because the probability of isolating oocysts in naturally contaminated random sample is very low. Study the prevalence of toxoplasmosis in herbivores is also an interesting way to indirectly assess the prevalence of oocysts in the environment. The objective of the work presented here is part of the concern quite recent to evaluate the effectiveness of spiramycin in the treatment of toxoplasmosis in infected women in Dakar. To do it, they will all receive the same treatment with spiramycin (Rovamycine 3 million International Units (IU)) 1 tablet morning and evening and serology control every month to assess their serological profile and ultrasound control every 2 months.

METHODOLOGY

Immunooassay solid phase Orgenics SA (Immunocombs) was used based on ELISA principle insoluble support shown by the combs and a developing tank with a pre-serum dilution for the determination of immunoglobulin M (IgM) antibodies accordance with the manufacturer's instructions. The positivity threshold of 10 IU/ml for immunoglobulin G (IgG) and CombScals (card color matching concentrations) to determine the title. The IgM follows the same principle, but here the test is qualitative. The serological profiles of sera from patients already infected after treatment with spiramycin a month apart was later determined.

RESULTS

The study population consisted of 82 toxoplasmosis serology-positive women at the laboratory of the Abass NDAO Hospital. They all received treatment and met free serological tests. 82 patients were infected in the first serology (S1): 38 (46.34%) with either a recent active infection is an old chronic infection; 29 (35.36%) is a recent active infection; 10 (12.2%) had a recent infection or early non-specific IgM antibodies fixation; 4 (4.87%) had a former active or chronic infection and one (1.22%) had a recent infection or early attachment non-specific IgM antibodies (Table 1).

For IgM antibodies (44/179 or 24.58%) with 39 (21.79%) positive and frankly dubious 5 (2.79%) in the first serology (S1) carriers (Table 2), they were all negative in the second serology (S2). It shows that 39.65% (71 positive/179 tested) were positive for IgG antibodies to S1 with a title between 10 and 50 IU/ml (68, including 64 for assessment of pregnancy) and between 50 and 100 IU/ml (3 total for assessment of pregnancy) (Table 3). To S2, it shows that 90 (24%) were carriers (74 positives/82 tested) with 89 (02%) title between 10 and 50 IU/ml (73/82, including 68 for assessment of pregnancy.

Table 1. Positive results of the first serology according to the type of antibody.

<table>
<thead>
<tr>
<th>Reasons for consultation (S1)</th>
<th>Positive serology</th>
<th>IgM+/IgG-</th>
<th>IgM+/IgG+</th>
<th>IgM+IgG+</th>
<th>IgM+IgG+</th>
<th>IgM+/IgG+</th>
<th>IgM+/IgG+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nber</td>
<td>%</td>
<td>Nber</td>
<td>%</td>
<td>Nber</td>
<td>%</td>
<td>Nber</td>
</tr>
<tr>
<td>Review of pregnancy</td>
<td>73</td>
<td>89.02</td>
<td>5</td>
<td>6.1</td>
<td>38</td>
<td>46.34</td>
<td>25</td>
</tr>
<tr>
<td>Repeat abortion</td>
<td>9</td>
<td>10.28</td>
<td>5</td>
<td>6.1</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Divers (bilan)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>82</td>
<td>100</td>
<td>10</td>
<td>12.2</td>
<td>38</td>
<td>46.34</td>
<td>29</td>
</tr>
</tbody>
</table>

T: rate; Nber: Number.

Table 2. Distribution of serological tests based on reasons for consultation and IgM antibodies.

<table>
<thead>
<tr>
<th>Reason for consultation</th>
<th>Serological examination</th>
<th>IgM antibodies rate (S1)</th>
<th>Positive</th>
<th>Traces</th>
<th>Négative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nber</td>
<td>%</td>
<td>Nber</td>
</tr>
<tr>
<td>Review of pregnancy</td>
<td>165</td>
<td></td>
<td>30</td>
<td>16.76</td>
<td>5</td>
</tr>
<tr>
<td>Repeat abortion</td>
<td>13</td>
<td></td>
<td>9</td>
<td>5.03</td>
<td>0</td>
</tr>
<tr>
<td>Autres (bilan)</td>
<td>1</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>179</td>
<td></td>
<td>39</td>
<td>21.79</td>
<td>5</td>
</tr>
</tbody>
</table>

T: rate; Nber: Number.
Table 3. Distribution of serological tests based on reasons for consultation and IgG antibodies.

<table>
<thead>
<tr>
<th>Reason</th>
<th>IgG antibodies rate (S1)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T&lt;10 UI/ml</td>
<td>10≤T&lt;50 UI/ml</td>
<td>50≤T&lt;100 UI/ml</td>
<td>T≥100 UI/ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nber %</td>
<td>Nber %</td>
<td>Nber %</td>
<td>Nber %</td>
<td>Nber %</td>
</tr>
<tr>
<td>Review of pregnancy</td>
<td>98 54.75</td>
<td>64 35.75</td>
<td>3 1.67</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Repeat abortion</td>
<td>9 5.03</td>
<td>4 2.23</td>
<td>0 0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Divers (bilan)</td>
<td>1 0.56</td>
<td>0 0</td>
<td>0 0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>108 60.34</td>
<td>68 37.98</td>
<td>3 1.67</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

T: rate; Nbre: Number.

Table 4. Positive serology results of the second depending on the type of antibody

<table>
<thead>
<tr>
<th>Reason</th>
<th>Positives serology</th>
<th>IgM+/IgG-</th>
<th>IgM-/IgG+</th>
<th>IgM+/IgG+</th>
<th>IgM+-/IgG-</th>
<th>IgM+/IgG+</th>
</tr>
</thead>
<tbody>
<tr>
<td>(S2)</td>
<td>Nber %</td>
<td>Nber %</td>
<td>Nber %</td>
<td>Nber %</td>
<td>Nber %</td>
<td>Nber %</td>
</tr>
<tr>
<td>Review of pregnancy</td>
<td>69 93.24</td>
<td>0 0</td>
<td>69 93.24</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Repeat abortion</td>
<td>5 6.76</td>
<td>0 0</td>
<td>5 6.76</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Total</td>
<td>74 100</td>
<td>0 0</td>
<td>74 100</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
</tbody>
</table>

T: rate; Nbre: Number.

Table 5. Distribution of positive serology based on IgG titer in the first control.

<table>
<thead>
<tr>
<th>Reason</th>
<th>IgG antibodies rate (S2)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T&lt;10 UI/ml</td>
<td>10≤T&lt;50 UI/ml</td>
<td>50≤T&lt;100 UI/ml</td>
<td>T≥100 UI/ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nber %</td>
<td>Nber %</td>
<td>Nber %</td>
<td>Nber %</td>
<td>Nber %</td>
</tr>
<tr>
<td>Review of pregnancy</td>
<td>4 4.88</td>
<td>68 82.92</td>
<td>1 1.22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Repeat abortion</td>
<td>4 4.88</td>
<td>5 6.1</td>
<td>0 0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>8 9.76</td>
<td>73 89.02</td>
<td>1 1.22</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

T: rate; Nbre: Number.

Table 6. Distribution of positive serology based on IgG titer in the second control.

<table>
<thead>
<tr>
<th>Reason</th>
<th>Positives serology</th>
<th>IgM+/IgG-</th>
<th>IgM-/IgG+</th>
<th>IgM+/IgG+</th>
<th>IgM+-/IgG-</th>
<th>IgM+/IgG+</th>
</tr>
</thead>
<tbody>
<tr>
<td>(S3)</td>
<td>Nber %</td>
<td>Nber %</td>
<td>Nber %</td>
<td>Nber %</td>
<td>Nber %</td>
<td>Nber %</td>
</tr>
<tr>
<td>Review of pregnancy</td>
<td>69 93.24</td>
<td>0 0</td>
<td>69 93.24</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Repeat abortion</td>
<td>5 6.76</td>
<td>0 0</td>
<td>5 6.76</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Total</td>
<td>74 100</td>
<td>0 0</td>
<td>74 100</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
</tbody>
</table>

T: rate; Nbre: Number.

Table 3. Distribution of serological tests based on reasons for consultation and IgG antibodies.

Table 4. Positive serology results of the second depending on the type of antibody

Table 5. Distribution of positive serology based on IgG titer in the first control.

Table 6. Distribution of positive serology based on IgG titer in the second control.

DISCUSSION

The disappearance of IgM antibodies between the first and second serology would not rule on the effectiveness of treatment of toxoplasmosis Spiramycin because it obeys the normal kinetics of these antibodies. However, the disappearance of IgM antibodies for 5 patients then traces the development of IgG antibodies at a rate equal to 10 IU/ml in three of them, including two women for assessment of pregnancy and 1 abortion record repeatedly could attest to the effectiveness of treatment for preventing infection to develop. This efficiency is more significant in three women to balance pregnancy with a high level of IgG antibodies (between 50 and 100 IU/ml), two of them which have seen their rates drop to the
second serology (between 10 and 50 IU/ml) and the last third serology. This study is the first of its kind in Dakar. These preliminary results may attest to the effectiveness of treatment undertaken. They are supported by Ajzenberg et al., (2010), in France who assessed the impact of treatment in patients with AIDS, who were cerebral toxoplasmosis and received specific treatment. He also arrived at the same conclusion by the marked improvement in CD4+ lymphocytes compared with those whose cause of immunosuppression was another. Gilbert et al., (2001) had shown that the treatment of the mother with spiramycin was effective because of the concentration of the product in the placental tissue and lack of teratogenic effects as fetal infection is not proven.

Couvreur and Leport (1998) had shown that spiramycin is not effective on fetal damage. Brézin et al., (2003), McAuley et al., (1994) and Roizen et al., (1995) proved the treatment of congenital toxoplasmosis to be ineffective. Few studies have been reported so far in the world. Although it has not been registered with congenital toxoplasmosis studies, Ndiaye et al., (2004) placed the risk of fetal infection to 4.88%, that is, a thousand pregnancies among toxoplasmosis serology-negative women. However, Baden et al., (2003) demonstrated the efficacy of treatment with cotrimoxazole on toxoplasmosis and other opportunistic infections in 417 heart transplant. However, it would be interesting to do a large-scale study, selecting patients, normalizing molecules and doses taken and assessing according to the different clinical forms recorded treatment.

**REFERENCES**


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**Table 7. Distribution of positive serology based on IgG titer in the second control.**

<table>
<thead>
<tr>
<th>Reason</th>
<th>10≤T&lt;50 UI/ml</th>
<th>50 ≤T&lt;100 UI/ml</th>
<th>T≥100 UI/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td><strong>Percent</strong></td>
<td><strong>Number</strong></td>
<td><strong>Percent</strong></td>
</tr>
<tr>
<td>Review of pregnancy</td>
<td>69</td>
<td>93.24</td>
<td>0</td>
</tr>
<tr>
<td>Repeat abortion</td>
<td>5</td>
<td>6.76</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
<td>89.02</td>
<td>0</td>
</tr>
</tbody>
</table>

**T:** rate; **Nbre:** Number.

---

**Conclusion**

Treatment of toxoplasmosis based on the administration of spiramycin (Rovamycine 3 million IU) in one tablet twice a day appears to be effective in the light of the results obtained in this study. All toxoplasmosis serology-positive who received treatment are cured with a threshold rate IgG antibodies (10 IU/ml) indicating an immunological memory. But, as a precaution, it would be interesting to extend this to a much larger population corroborating treatment with different clinical cases of toxoplasmosis in order to make a final decision.
Characterization of *de novo* colonic stricture(s) due to Crohn’s disease

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The development of colonic stenosis is a rare complication of Crohn’s disease (CD) without a postsurgical anastomose history. The management and long term follow-up results of colonic stricture due to CD have not been clearly defined. In this study, we aimed to characterize *de novo* colonic stricture due to CD. We evaluated 702 patients with CD to investigate for colonic stricture. Colonic stricture was considered to exist when passage of a standard colonoscope was not possible and diagnosed radiologically and endoscopically in this study. Of the 702, 14 had colonic stricture according to the above definition criteria. Of the 14, 8 were male. Stricture diagnosed date varied from 0 to 13 years after the CD was diagnosed. Localization of the strictures differed from rectum to the ceacum. Of the 14, 3 patients had more than 1 stricture. Pathologic examination of the stricture showed no dysplasia or malignancy at the beginning and during the follow-up. *De novo* colonic stricture due to CD is a rare condition according to the presented study’s results. Distribution of the stricture varied from the rectum to ceacum without an increased colonic cancer risk. We observed antifibrotic role of thiopurines and biologics in this study, limitedly.

**Key words:** Chron’s disease, stricture, colon, thiopurines, biologics, surgery.

INTRODUCTION

Intestinal stricture is one of the main causes of hospitalization and cost in patients with Crohn's disease (CD) (Lichtenstein et al., 2009). It occurs more frequently due to the surgical anastomoses (Cosnes et al., 2005; Louis et al., 2007; Oostenbrug et al., 2006). Although *de novo* colonic stenosis is a rare complication of CD (less than 1%), it must be a typical complication by time because of transmural involvement of the bowel wall (Lichtenstein et al., 2009; Cosnes et al., 2005; Louis et al., 2007; Oostenbrug et al., 2006). It was reported that shortened tubular colon and colonic stricture as strong risk factors for the development of colorectal cancer (CRC) (Farraye et al., 2010), thus directed biopsies of strictures is always recommended, with people now aware that development of any stricture is an ongoing dynamic pathologic process which includes both fibrotic and antifibrotic components (Strong et al., 2007; Lichtenstein et al., 2006; Vermeire et al., 2007; Van Assche et al., 2004; Hanauer et al., 2002). Thus, thiopurines and particularly biologics might be good options before giving a surgical decision. However, there is a concern regarding biologic and their rapid healing effect which may lead to perforation on the effected bowel segment (Vermeire et al., 2007; Van Assche et al., 2004; Hanauer et al., 2002). In this study, we aimed to characterize *de novo* colonic stricture due to CD by giving long...
Table 1. Overall results of the patients with colonic stricture due to Crohn’s disease (CD). Laboratory results shown at the stricture diagnosed date. *: age at stricture diagnosed date.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (year)*</th>
<th>Type of the disease</th>
<th>Localization of the stricture (s)</th>
<th>Stricture diagnose date – IBD diagnose date</th>
<th>WBC</th>
<th>PLT</th>
<th>CRP</th>
<th>ESR</th>
<th>Main symptom</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>38</td>
<td>Colitis</td>
<td>Rectum–length: 4 m</td>
<td>3 years</td>
<td>5420</td>
<td>343000</td>
<td>0.3</td>
<td>14</td>
<td>none</td>
</tr>
<tr>
<td>F</td>
<td>33</td>
<td>Colitis</td>
<td>Ascendan</td>
<td>5 years</td>
<td>5300</td>
<td>263000</td>
<td>1.0</td>
<td>10</td>
<td>none</td>
</tr>
<tr>
<td>M</td>
<td>28</td>
<td>ileocolitis</td>
<td>Transvers/ left colon / sigmoid</td>
<td>8 years</td>
<td>7500</td>
<td>468000</td>
<td>128</td>
<td>26</td>
<td>Abdominal pain, weight loss, fever</td>
</tr>
<tr>
<td>M</td>
<td>30</td>
<td>ileocolitis</td>
<td>Rectum / descendan</td>
<td>6 months</td>
<td>14000</td>
<td>789000</td>
<td>12</td>
<td>19</td>
<td>Weight loss</td>
</tr>
<tr>
<td>M</td>
<td>28</td>
<td>ileocolitis</td>
<td>Rectum</td>
<td>13 years</td>
<td>9000</td>
<td>645000</td>
<td>2.5</td>
<td>20</td>
<td>none</td>
</tr>
<tr>
<td>F</td>
<td>45</td>
<td>ileocolitis</td>
<td>Ascendan</td>
<td>1 year</td>
<td>7000</td>
<td>136000</td>
<td>22</td>
<td>29</td>
<td>Abdominal pain</td>
</tr>
<tr>
<td>M</td>
<td>38</td>
<td>ileocolitis</td>
<td>Ascendan</td>
<td>1 year</td>
<td>10800</td>
<td>312000</td>
<td>-</td>
<td>9</td>
<td>diarrhea</td>
</tr>
<tr>
<td>M</td>
<td>21</td>
<td>ileocolitis</td>
<td>Splenic flexura</td>
<td>4 years</td>
<td>5000</td>
<td>316000</td>
<td>0.3</td>
<td>10</td>
<td>diarrhea</td>
</tr>
<tr>
<td>M</td>
<td>35</td>
<td>ileocolitis</td>
<td>Ascendan</td>
<td>Presented with stricture and active luminal disease</td>
<td>7700</td>
<td>309000</td>
<td>4.1</td>
<td>20</td>
<td>Abdominal pain, weight loss</td>
</tr>
<tr>
<td>F</td>
<td>19</td>
<td>ileocolitis</td>
<td>Rectum / ascendan / descendan</td>
<td>5 months</td>
<td>5300</td>
<td>571000</td>
<td>0.2</td>
<td>12</td>
<td>Weight loss, rectal bleeding</td>
</tr>
<tr>
<td>F</td>
<td>35</td>
<td>Colitis</td>
<td>Rectum</td>
<td>5 years</td>
<td>5900</td>
<td>242000</td>
<td>1</td>
<td>25</td>
<td>Abdominal pain</td>
</tr>
<tr>
<td>M</td>
<td>66</td>
<td>Colitis</td>
<td>Transvers – length: 5 cm</td>
<td>Presented with stricture and active luminal disease</td>
<td>8900</td>
<td>221000</td>
<td>1.2</td>
<td>7</td>
<td>Abdominal pain</td>
</tr>
<tr>
<td>F</td>
<td>42</td>
<td>Colitis</td>
<td>Sigmoid</td>
<td>6 months</td>
<td>8200</td>
<td>518000</td>
<td>0.3</td>
<td>33</td>
<td>diarrhea</td>
</tr>
<tr>
<td>M</td>
<td>25</td>
<td>ileocolitis</td>
<td>Ascendan</td>
<td>2 years</td>
<td>8700</td>
<td>402000</td>
<td>2.01</td>
<td>5</td>
<td>diarrhea</td>
</tr>
</tbody>
</table>


long term follow-up results.

METHODOLOGY

We performed a retrospective study among patients with CD at our inflammatory bowel disease (IBD) Center in Ankara, Turkey. In this center, we have used chart review system since 1995. The study was approved by Ankara Yüksek İhtisas Hospital Ethics Committee and confidentiality of records was maintained according to the guidelines issued by the health authorities. The diagnosis of CD was established when clinically, endoscopically and radiologically findings were supported by histologic evidences and exclusion of other disorders known to cause intestinal inflammation. Mycobacteria were excluded by tissue staining and cultures because of high incidence of tuberculosis in Turkey. The diagnosis of colonic stricture was performed endoscopically and radiologically. A colonic stricture was considered to exist when passage of a standard colonoscope was not possible. Biopsy and histologic examination of stricture was performed in each patient at the initial diagnosis and during the follow-up term. Date of the CD diagnosed, stricture diagnosed, localization of the stricture (s), number, length, and type of the stricture (s), biopsy results, type of the therapy before and after the stricture diagnosed, and response to therapy were all evaluated in each patient. These patients are still being followed at IBD center.

RESULTS

Of the 702 patients with CD, 14 had colonic stricture and 6 were female (Table 1). All patients had one or more than one complaints in his or her history such as abdominal pain, diarrhea, weight loss or fever at the presentation. Mean age was 34.5 years. Stricture diagnose date varied from 0 to 13 years after the CD was diagnosed. Two cases presented by colonic stricture at the initial diagnosis. Localization of the strictures was differed from rectum to the caecum. Of the 14 patients, 3 had more than 1 colonic stricture. Two patients had 3 colonic strictures and 1 had 2 strictures. Pathologic examination of the colonic stricture showed no dysplasia or malignancy during the follow-up. Of the 14 patients, 4 had only colitis without ileum involvement; left colon in 2 cases, one with sigmoid and one with transvers colon involvement.

All patients with or without dilation had put on mainly azotiopurine plus oral steroid with mesalazine after the diagnosing of colonic stricture (Table 2). Therapy results are as follows: 7 patients showed partial response; 4 had complete resolution; and 3 had no response after at least 6 months therapy. One of the non-responders had 3 colonic strictures as shown in Figures 1 and 2. He had 9 years history and used azotiopurine plus steroids irregularly. Abdominal
Table 2. Therapy response in IBD patients with colonic stricture.

<table>
<thead>
<tr>
<th>No.</th>
<th>Therapy before the stricture diagnosed</th>
<th>Therapy after the stricture diagnosed</th>
<th>Therapy response for the obstruction resolve</th>
<th>Maintenance therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AZA</td>
<td>CS+AZA</td>
<td>relief</td>
<td>AZA</td>
</tr>
<tr>
<td>2</td>
<td>AZA</td>
<td>BUD+AZA</td>
<td>Partial response</td>
<td>AZA</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>AZA</td>
<td>Non-response</td>
<td>Surgery and resection</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>CS+AZA</td>
<td>Non-response</td>
<td>Surgery and resection</td>
</tr>
<tr>
<td>5</td>
<td>BUD+AZA</td>
<td>CS+AZA</td>
<td>relief</td>
<td>Surgery and resection</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>CS+AZA</td>
<td>Partial response</td>
<td>AZA</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>CS+AZA</td>
<td>Non-response</td>
<td>Surgery and resection</td>
</tr>
<tr>
<td>8</td>
<td>AZA</td>
<td>CS+AZA</td>
<td>relief</td>
<td>AZA</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>CS+AZA</td>
<td>Partial response</td>
<td>Biologics (ADA) : Response at 6 month by clinical, endoscopic and MRI enteroclysis</td>
</tr>
<tr>
<td>10</td>
<td>AZA</td>
<td>CS+AZA</td>
<td>relief</td>
<td>AZA</td>
</tr>
<tr>
<td>11</td>
<td>AZA</td>
<td>CS enema plus AZA</td>
<td>Partial response</td>
<td>AZA</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>AZA</td>
<td>Partial response</td>
<td>AZA</td>
</tr>
<tr>
<td>13</td>
<td>-</td>
<td>AZA</td>
<td>Partial response</td>
<td>AZA</td>
</tr>
<tr>
<td>14</td>
<td>-</td>
<td>CS + AZA</td>
<td>Partial response</td>
<td>AZA</td>
</tr>
</tbody>
</table>

MSZ: mesalazine, CS: corticosteroids, BUD: budesonide, AZA: azathioprine, SLZ: salazopyrine, ADA: adalimumab. All patients were on mesalazine therapy.

tomography showed that colonic wall was thickened, particularly in the right colon with 22 and 12 mm in transvers and 9 mm in sigmoid colon. Small bowel contrast examination showed thickened wall in the distal part of ileum. Double contrast examination of the colon showed 3 long strictures involved in transvers, left and sigmoid colon (Figures 1 and 2). The lengths of the strictures were 3, 6 and 2 cm, respectively. Colonoscopy showed active colon mucosal inflammation with obstructive stenosis. Biopsy and histologic examination showed no dysplasia. After the full discussion with the patient and his hesitation to the surgery, biologic therapy (adalimumab: 160 mg sc, then 14 days later: 80 mg sc and 14 days later: 40 mg every 2 weeks sc) was started. An oral steroid therapy was added (40 mg/day prednisolone for 1 month, then gradually decreased for the last 2 months and stopped). His condition was dramatically improved. Four months later, magnetic resonance imaging enterography was performed and showed significant improvement on the narrowing segments, particularly in the sigmoid and right colon (Figures 3 and 4). He had no complaints for the last 24 months during the adalimumab therapy. However, colonoscopy performed at the last examination and showed that a narrowing segment in the middle of the left colon and endoscopy failed to pass. Thus, the patient was sent to the surgery for the resection of the involved colon segments.

DISCUSSION

Intestinal stricture which is an ongoing dynamic pathologic process includes fibrotic and antifibrotic components. There is a belief that intestinal fibrosis is preventive from the perforation of the effected bowel wall during the natural course of the disease in CD. So, the management of colonic stricture due to CD is a controversial issue and has long been debated between gastroenterologists and surgeons (Strong et al., 2007; Lichtenstein et al., 2006; Vermeire et al., 2007; Van Assche et al., 2004; Hanauer et al., 2002). Efficacy of biologics on stenosing forms of CD has not been established, so far. The main concern is that biologic agents might increase stricture rate and severity because of rapid mucosal healing induced fibrosis (Vermeire et al., 2007; Van Assche et al., 2004; Hanauer et al., 2002). Others considered that biologics might decrease intestinal wall thickness rapidly and leads to colonic perforation.

Our patients’data in this study was obtained from the records of a tertiary referral center and contains every stage of CD. Patients had most likely moderate or severe stage of the disease. To
the best of our knowledge, for the first time, efficacy and safety of biologics was questioned in this study with 2 cases (numbers 3 and 9). We observed that biologic agents might be a safe therapy option in patients with colonic stenosing disease due to CD, provided patients should be carefully followed during the biologic therapies.

The incidence of colon cancer in patients with Crohn’s colitis was reported to be 2 to 7%. Thus, colonoscopy with biopsies and brushing to evaluate for malignancy was highly recommended particularly when the disease was diagnosed at an older age, after longer disease duration, and with more extensive colon involvement (Lakatos et al., 2011; Yamazaki et al., 1991; Maykel et al., 2006). In our study, we showed that pathologic examination of the stricture revealed no dysplasia or
malignancy.
Fifty percent of the patients had elevated CRP (C reactive protein) or ESR (erythrocyte sedimentation rate) which reflected to active luminal disease. The number of 702 patients with CD is not a limitation in this study. So, 14 patients with colonic stricture due to CD in this study cannot be explained by the limited number of study population.

Conclusion

De novo colonic stricture due to CD is a rare condition and the distribution of the stricture varied from the rectum to ceacum. Biologics and tiopurines are reasonable alternatives to the surgery before giving a surgical decision.

REFERENCES


Figure 4. After the 4 months adalimumab injection and short term oral steroid therapy, magnetic resonance imaging enterography showed significantly improvement on the narrowing segment in left colon.
Review

The “ENT – TEN”: Ten rules of thumb in otorhinolaryngology that every doctor must know – A review

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This review, titled ‘THE ENT TEN’, is a review of ten important rules of thumb in otorhinolaryngology that every doctor must know. It is a collection of vital tips and pearls that if every doctor, especially family physicians, knew, would significantly increase the rate of early diagnosis of potentially life-threatening conditions and improve the quality of life of patients with several non life-threatening but disabling conditions. It is suggested that all doctors memorize these points.

Key words: Otorhinolaryngology, rules of thumb, early diagnosis, preventing disability, improving quality of life, deafness.

INTRODUCTION

“If you do not remember anything in otorhinolaryngology, remember the “ENT – TEN”. They will stand you in good stead throughout your practice”. These were among the first words some of us heard as we began postgraduate training in Otorhinolaryngology. The origin of the expression “ENT – TEN” is obscure but it refers to a collection of ten important “rules of thumb” in otorhinolaryngology. Though just learning them at that time, it soon became obvious that they were vital pieces of information that if every doctor, especially family physicians, knew, would significantly increase the rate of early diagnosis of potentially life-threatening conditions and improve the quality of life of patients with several non life-threatening but disabling conditions. For most physicians, exposure to Otorhinolaryngology in Medical School is not adequate, therefore knowledge of these tips and pearls are invaluable. This is a review of these points.

RULE NO 1: IF A MOTHER SAYS THAT HER CHILD IS DEAF, SHE IS USUALLY RIGHT

Mothers are very important screening agents and could facilitate early diagnosis of hearing loss in Children. Hearing is important for speech development, and it is vital that hearing loss is diagnosed early. This will allow the provision of suitable support and aids to facilitate the development of speech and communication. In an effort to achieve this, children in developed countries are subjected to regular screening tests. For example, in the UK it was done at 7 months, 2 to 4 years and after school entry. However, recent evidence has suggested that a first screening test at age 7 months is too late as 0.1% of live-born babies will have a severe hearing impairment. Thus screening of all neonates has been proposed (Culpepper, 1998). This involves otoacoustic emission testing shortly after birth and may also involve brainstem
RULE NO 2: A UNILATERAL FOUL SMELLING NASAL DISCHARGE IN A CHILD IS DUE TO A FOREIGN BODY UNTIL PROVEN OTHERWISE. IN AN ADULT IT IS IMPORTANT TO RULE OUT A TUMOUR

The scenario is very common: distressed parents with a child with a unilateral foul smelling nasal discharge. They have visited a lot of hospitals and clinics and the child has been treated for a wide variety of conditions ranging from very simple to complex, but all to no avail. When they appear in an Otorhinolaryngology clinic, however, it is a spot diagnosis. A unilateral nasal discharge in a child is nearly always due to a foreign body. Nasal foreign bodies are very common in children. They may be inorganic or organic. Inorganic foreign bodies include buttons, beads, metal, plastic and stones. Often asymptomatic, they may be discovered only accidentally during an examination for an unrelated complaint. Examples of organic foreign bodies are sponge, rubber, paper, foam, wood, peas, seeds and nuts. These are irritants and do lead to an inflammatory reaction causing a nasal discharge. The discharge is initially mucoid, but eventually becomes mucopurulent and foetid, and may also be blood stained. The diagnosis is confirmed on examination and it is usually possible to remove the foreign body in the clinic without the need for general anaesthesia. If the child is uncooperative, if the clinic attempt fails or if the foreign body is situated posteriorly in the nasal cavity, general anaesthesia will be required for removal. Post operative nasal decongestants and systemic antibiotics are often necessary to prevent the possible complication of sinusitis. It is essential to make this diagnosis early as the inflammation will persist for as long as the foreign body remains and Sinusitis may complicate the problem. Also, deposits of calcium and magnesium carbonates and phosphates may take place around the foreign body to form a rhinolith, which will require removal under general anaesthesia.

However, in an adult, especially if older than forty, the possibility of a sino-nasal tumour must be kept in view. Sinonasal tumours may masquerade as sinusitis because if the tumour blocks the ostiomeatal complex, as it often does, there is a disturbance of normal sinus physiology and the setting up of an inflammatory process in the sinuses. In addition, chronic nasal sepsis has been implicated as increasing the risk of squamous cell carcinoma. Thus any adult who presents with persistent or recurrent mucopurulent discharge must be thoroughly assessed to rule out underlying malignancy (Donald, 1997). This point is important as there is reported a typical delay in diagnosis of 8 months or more due to failure of identification of the early signs (Osguthorpe, 1994; Alvarez et al., 1995).

RULE NO 3: DEAFNESS IN THE ELDERLY CAN USUALLY BE HELPED AND RECENT ONSET HEARING LOSS IN AN ADULT SHOULD NOT BE IGNORED AS IT MAY BE AN EARLY SIGN OF A SERIOUS CONDITION

Deafness in the elderly is usually due to presbyacusis. This is the lessening of the acuity of hearing that characterizes old age. It is due to a progressive degeneration in the auditory system with ageing. It is said that moderate hearing impairment (45 dB hearing level averaged over 0.5, 1, 2 and 4 kHz) occurs in 4% of the age group 51–60 but in 18% of those aged 71–80 (Roland et al, 2001). 20 million Americans have impaired hearing, and approximately 75% of these people are over age 55. In the 25-year period between 1976 and 2000, the number of persons below age 75 increased by 23%, the number between age 75 and 84 increased by 57%, and the number over age 84 increased by 91%. Indeed, by the year 2030, the elderly will comprise 32% of the population, an increase of 250%. As many as 60 to 75% of these older persons will have clinically significant hearing loss (Mills and Lambert, 2003). The typical presentation is difficulty in hearing which is worse in the presence of background noise. Unfortunately, when an attempt is made to increase the pitch of the noise, the patient perceives it as being too loud, a phenomenon known as recruitment. This change could be so disturbing that many patients eventually become socially isolated and even depressed. While there is no curative treatment, it is possible, after assessing the degree of disability, to provide a hearing aid and rehabilitate the patient (Gates et al., 1990). The previously common drawbacks of a poorly-performing digital aids are being overcome by the superior performance of the recent digital aids. Indeed, as a result of excellent advances in hearing aid technology in combination with improved fitting techniques, nearly every older hearing impaired person can benefit (Mills and Lambert, 2003). Rehabilitation in the form of speech reading or auditory training as well as the use of...
accessory aids (for example, an induction coil fitted to the telephone or television which transmit sound to the patient’s aid when activated.

Recent onset hearing loss in an adult could occur as an isolated symptom or in conjunction with other head and neck symptoms. It could be due to causes as benign as cerumen impaction as well as to sinister causes like malignancies. As a matter of fact many head and neck malignancies present with a bewildering array of symptoms in which hearing symptoms feature prominently. Thus at the back of the physician’s mind, when assessing a patient with hearing loss must linger the fact that it could be due to a malignancy and he should seek to rule it out, especially when in conjunction with other head and neck symptoms, particularly in patients older than 40. A notable example is the hearing loss found in patients with nasopharyngeal carcinoma, the early diagnosis of which is made difficult by the poor accessibility of the nasopharynx to routine physical examination.

The early symptoms are often confusing and treated as non–life-threatening for some time before the diagnosis is made. Generally, symptoms fall into four categories: aural, nasal, neck and miscellaneous accounted for by cranial nerve involvement (facial pain, diplopia). The classic presentation at the time of diagnosis is a neck mass and conductive hearing loss, often with bloody nasal discharge. One study has that ear symptoms occur first in 29% of patients (Neel et al., 1983). At the time of admission 44 to 53% of patients present with hearing loss (Dickson, 1981). The hearing loss is accounted for by the fact that nasopharyngeal carcinoma often arises from the lateral wall of the nasopharynx, near the fossa of Rosenmüller. As the mass enlarges, it obstructs the Eustachian tube orifice and induces a serious otitis media.

RULE NO 4: ANYBODY WANTING TO PUT ANYTHING INTO HIS/HER EAR SHOULD FEEL FREE TO PUT HIS/HER ELBOW

Obviously ironic, this means that no one should put anything into his ears; a basic rule of ear hygiene that every physician should teach. This prohibition includes the insertion of cotton tipped swabs, bobby pins, fingernails, any other object or even water into the ears. It is not necessary to clean the ears because the ears are self cleansing. The habitual cleaning of the ears with cotton tipped swabs and the frequent exposure of the external auditory meatus to water predisposes to infection and cerumen impaction. Normally, the external auditory meatus is a well-protected and self-cleansing structure. Cerumen (“earwax”) is produced by the cerumen and sebaceous glands in the skin of the meatus and forms a protective film in which fatty acids, lysozymes, and the creation of an acid milieu effectively protect the skin of the ear canal. Self-cleansing of the ear canal, with natural removal of accumulated cerumen is normally accomplished by epithelial migration from the tympanic membrane toward the external meatus. Accumulation of cerumen represents the most common and routine otologic problem (Jung and Jinn, 2003). It may interfere with the clinician’s view of the tympanic membrane, cause hearing loss and discomfort, or become a source of infection. Although cerumen impaction may result from other causes such as excessive production, it most commonly results from a disturbance of the normal self-cleansing mechanism during routine attempts to remove cerumen with cotton swabs. This displaces the cerumen towards the tympanic membrane predisposing it to obstruction and impaction. Swelling of the plug, after contact with water worsens the obstruction.

Otitis externa is one of the most common diseases in clinical practice (Jung and Jinn, 2003). Acute diffuse otitis externa, a bacterial infection, is the most common form and is caused by the removal of the protective lipid film from the canal thus allowing bacteria to enter especially in the presence of a wet canal. It usually begins with itching in the canal and skin maceration and local trauma from scratching the canal with a cotton swab, bobby pin, fingernail, or other object sets up a vicious cycle. Preventive measures against otitis externa therefore include counseling patients not to touch or place any objects such as cotton swabs, paper clips, or any other objects into the canal. Swimmers are instructed to use earplugs and are advised to use alcohol-vinegar (1:1) drops after swimming.

RULE NO 5: A PAINFUL SWOLLEN EYE COULD BE SECONDARY TO AN UNDERLYING SINUS INFECTION. WHEN THAT HAPPENS, VISION IS AT RISK AND THERE IS A DANGER OF INTRACRANIAL SPREAD OF INFECTION. URGENT INTERVENTION IS NEEDED

The spread of infection involving the orbital structures is the most common complication of sinusitis. Due to the fact that the orbital contents are separated from the ethmoidal labyrinth only by the thin lamina papyracea, direct extension of infection into the orbit is common. In addition, the ethmoidal veins may become thrombophlebitic resulting in the spread of infection into the orbit. Purulent frontal sinusitis also may result in orbital complications. The floor of the frontal sinus frequently, is the path of least resistance for the infection because it usually is the thinnest wall. The first indication of orbital involvement usually is inflammatory edema of the eyelids with progression of the cellulitis, erythema, progressive proptosis, and fever occurring. Early in the process, extraocular muscle function and results of fundoscopic examination usually are normal but as the cellulitis...
progresses chemosis increases, ophthalmoplegia may develop and funduscopic examination may show mild vascular congestion. Although the fever may increase, the patient usually is not particularly toxic. As the disease progresses, an abscess may form along the lamina papyracea or within the periorbita. This is followed by abscess formation within the orbit thus worsening the proptosis and chemosis. Ophthalmoplegia occurs, vision reduces and eventually there is a cavernous sinus thrombosis. This is heralded by the onset of swelling of the contralateral eye (Chandler et al., 1970).

In the same manner, infection may gain access to the intracranial space by direct extension through a defect in the posterior wall of the frontal sinus caused by infection or through diploic frontal vessels. The subdural space may be involved even when no infection of the intervening tissues exists. Evidence of nuchal rigidity in a patient with sinusitis should alert the physician to the possibility of an intracranial complication.

**RULE NO 6: A UNILATERAL SORE THROAT THAT RADIATES TO THE EAR IS A SINISTER SYMPTOM. RULE OUT A MALIGNANCY**

Otalgia or earache is a common symptom and it is important to keep in mind that while it may be caused by primary disorders of the ear, it may also be secondary to disease from other sites in the head and neck which share the same sensory innervation (referred pain). Otalgia may arise from a primary neuralgia of any of the sensory nerves, although it is most common in the glossopharyngeal nerve. Shingles of the ear (herpes zoster oticus—VII, IX and X cranial nerves) will also usually cause otalgia. Pain can be referred to the ear through:

1. Second and third cervical nerves (C2 and C3), for example in cervical spondylosis
2. Trigeminal nerve (cranial nerve V): In dental disease such as tooth impaction, caries and abscess, temporomandibular joint dysfunction, nasopharyngeal and salivary gland disease.
3. Glossopharyngeal nerve (cranial nerve IX): Oropharyngeal infections, such as pharyngitis, tonsillitis and quinsy, oropharyngeal tumours and post-tonsilllectomy.
4. Vagus (cranial nerve X): In carcinoma of the larynx and hypopharynx.

Thus, full history and thorough examination of the head and neck is mandatory in all cases of otalgia. The examination should include the ears, the temporomandibular joints, the neck and the oral cavity. Particular attention should be paid to the tongue base, pharynx and larynx especially if the patient has concomitant sore throat, as pathology here can be catastrophic if overlooked. In cases where doubt exists, scans (CT and MRI), and a panendoscopy (endoscopy of the entire upper aero-digestive tract) may be appropriate. It is also noteworthy that thorough examination of these areas is also indicated in cases of unexplained intractable otalgia (Roland et al., 2001).

**RULE NO 7: A PAINFUL SWOLLEN CHEEK IS DUE TO DENTAL DISEASE UNTIL PROVEN OTHERWISE**

The cheek is the side of the face forming the lateral wall of the mouth and there is much pathology that could manifest with a swollen cheek. The differential diagnosis of cheek swelling is a very long list and so diagnosis is often difficult. The list includes conditions like infection, cysts of all kinds including sebaceous, salivary gland, bone, malignancy of the many structures related to the cheeks and the oral cavity, soft tissue injuries, fractures, haematomas and even scar tissue from maxillofacial trauma, allergic reactions, lymphadenopathy, pathology in other head and neck structures such as the teeth, nose and paranasal sinuses, the salivary glands, the ears, the eyes and even systemic conditions like heart failure, renal failure and ovarian hyperstimulation syndrome that you may not be thinking of. The puzzle is more difficult to solve for physicians than for dental surgeons since most physicians have not had a good enough exposure to oral anatomy, physiology and pathology. If the swelling is painful, much of the puzzle is solved as we all know that the pain may be due to an inflammation. In any case, otolaryngologists are taught to always examine the teeth especially in cases of cheek swelling as most will be found to be of dental origin. A good working principle for the physician, who most time is usually not thinking of the teeth, therefore, is to consider all painful swollen cheeks as of dental origin and refer such cases to a dental surgeon.

**RULE NO 8: IN ALL CASES OF FACIAL NERVE PALSY AND MENINGITIS, RULE OUT EAR DISEASE; WHEN EAR DISEASE CAUSES FACIAL NERVE PALSY RULE OUT TUBERCULOSIS OF THE EAR AND MALIGNANT OTITIS EXTERNA**

Facial nerve dysfunction dramatically affects a patient's quality of life from the functional consequences of impaired facial motion as well as the psychological impact of a skewed facial appearance, while meningitis threatens life. It is therefore extremely important to be able to pinpoint the underlying pathology. Failure to do so promptly may lead to disastrous consequences. The causes of facial nerve palsy and meningitis are many and as the clinician works to seek for the source of the pathology he must not forget to rule out ear pathology. Both the facial nerve and the middle cranial fossa are intimately related to the ears and infection of the ear can be complicated by facial nerve palsy and intracranial sepsis.
The following brief review of anatomy reminds us of how it is so.

After passing through the internal auditory meatus on the posterior face of the petrous temporal bone, the nerve enters its canalicular segment, within the internal auditory canal. It pierces the meatal foramen to enter the labyrinthine segment. The labyrinthine segment is notable in that it is the narrowest portion of the fallopian canal, where it averages <0.7 mm in diameter, occupies the canal to the greatest proportional extent, and is lined by a fibrous annular ligament. As a result, it is believed that infections or inflammations causing edema of the facial nerve within this region can lead to temporary or permanent paralysis of the nerve, such as in Bell palsy.

The labyrinthine segment traverses between the cochlea and the vestibular labyrinth. After making its first genu (bend) at the geniculate ganglion, it becomes the tympanic segment, so called because it travels within the middle ear space. In addition to bony dehiscence from pathology, natural falloppian canal dehiscences have also been described in cadaver specimens, a majority of which occurred in the tympanic segment leaving the nerve in this area especially prone to injury. In more than 80% of cases, the dehiscences involved the portions of the canal adjacent to the oval window.

This portion of the nerve is approximately 10 mm long. The facial nerve then travels posteriorly, just superior to the oval window and stapes and curves inferiorly at its second genu, just posterior to the oval window, pyramidal process and stapedial tendon and anterior to the horizontal semicircular canal. It is this portion of the nerve that is most susceptible to injury during surgery because processes such as cholesteatoma frequently erode the bone covering the facial nerve in this region, leaving it precariously exposed. After the second genu, the nerve traverses the synonymously named vertical, descending, or mastoid segment en route to the stylomastoid foramen. After passing through the stylomastoid foramen, it becomes extracranial (Probst et al., 2006).

When facial nerve palsy accompanies ear disease, thorough examination should be done and microbiology specimens be taken to rule out tuberculosis. Multiple tympanic membrane perforations are highly suggestive of tuberculosis. Malignant otitis externa, a potentially life-threatening necrotizing external ear infection that proceeds to involve the skull base should be suspected in elderly poorly controlled diabetics or other immunocompromised patients.

**RULE NO 9: A PATIENT WITH HOARSENESS OF MORE THAN 3 WEEKS DURATION SHOULD BE SENT FOR A THOROUGH ENT EVALUATION**

Voice disorders always have causes. Something must be abnormal or atypical in the way in which the vocal folds function to produce disordered voice. Hoarseness is defined as a perceived change in the voice and indicates an abnormality of the vocal cord. Because only the slightest change in contour, thickness, or vibratory characteristics of the vocal cord results in hoarseness, glottic larynx cancers often come to medical attention while still at an early stage. This is the most important reason why persistent hoarseness must never be ignored since at this early stage the tumour is eminently curable. Patients with supraglottic cancers, however, typically present at a more advanced stage because tumors are bulkier before voice changes, dysphagia, airway compromise, or aspiration become apparent. Thus even hoarseness that presents after these symptoms must be urgently attended to. In addition, a multitude of laryngological conditions can cause voice problems. Some of these conditions demonstrate a visible organic pathology on an initial routine ear, nose, and throat (ENT) exam, either with a mirror or fiberoptics. Other conditions do not. Therefore, it is extremely important not to dismiss a patient’s claim of “hoarseness,” specifically in the absence of a visible pathology. Any voice condition, but specifically when hoarseness is present calls for thorough laryngeal examination as soon as possible. Delays in arriving at a diagnosis can result in medical complications and potential legal consequences, as well as delays in treatment (Korovin and Gould, 1995).

**RULE NO 10: ALL PATIENTS WITH NECK MASSES MUST BE SUBJECTED TO A THOROUGH HEAD AND NECK EVALUATION PRIOR TO ANY INVASIVE PROCEDURE. AVOID DOING AN OPEN BIOPSY ON A NECK MASS**

The most important element in the evaluation of a neck mass is the age of the patient. The differential diagnosis of a neck mass in adults and children is significantly different. In adults, the incidence of malignancy is high, and the protocol for the evaluation of a neck mass is well established (Lee and Helmus, 1970). Although malignancy is always a concern in a child, the incidence is low, and there are no established guidelines for the diagnosis of a pediatric neck mass (Wetmore et al., 1991). Most pediatric neck masses are inflammatory or congenital and resolve spontaneously or after appropriate medical therapy. In contrast, a neck mass in an adult over the age of 40 should be considered neoplastic in origin unless proven otherwise. The duration, growth pattern, and absence or presence of pain are important aspects of the history. A review of symptoms of head and neck disease is important and symptoms such as change in voice, hoarseness, difficulty with swallowing, and ear pain are important symptoms to note in addition to systemic symptoms such as fever, night sweats, and weight loss. The social history, such as history of alcohol and drug
use, smoking and recent travel should also be taken into consideration.

The physical examination should include a systematic investigation of all mucosal and submucosal areas of the head and neck. The mass itself should be assessed carefully. The location of the neck mass is particularly important in congenital and developmental masses because these masses typically appear in consistent locations. For example, a lateral neck mass in a child is suggestive of a branchial cleft cyst or laryngoecele whereas a midline neck mass is more suggestive of a thyroglossal duct cyst. The location also may be helpful in assessing adult patients. A neck mass located in the supraclavicular region of an older adult should focus the physician’s attention to metastasis from a primary lesion located in a site other than the upper aerodigestive tract (for example, a gastrointestinal or pulmonary source). An isolated posterior triangle lymph node should raise suspicion of a nasopharyngeal carcinoma.

A needle biopsy is preferred in adults if a biopsy is required for diagnosis. Open biopsy is not recommended because this interrupts both the tumor and lymphatic channels. A primary malignancy and metastatic neck lesion should be treated in a coordinated fashion. Coordinated treatment decreases the risk of wound necrosis, local recurrence and distant metastasis. If the diagnosis is a metastatic malignancy, endoscopy of the entire upper aerodigestive tract (panendoscopy) and biopsies of any suspicious mucosal areas and any likely primary sites should be performed before any definitive treatment is planned.

CONCLUSION

In conclusion, it is reasonable to say that though the above ten points are of course not the only ‘rules of thumb’ or the only important points to note in otorhinolaryngology (indeed there are loads of them), these ten paint the common scenarios that physicians often encounter. Thus it is not too much to suggest that all physicians memorize and pass on these points whether or not they are otorhinolaryngologists. Not just for the sake of those whose health we have pledged to protect but also to protect ourselves.

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REFERENCES


Indigenous knowledge of communities around Lake Victoria Basin regarding treatment and management of tuberculosis using medicinal plants

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This survey was aimed to determine the indigenous knowledge of communities around Lake Victoria Region regarding the treatment and management of Tuberculosis. Opinion leaders suggested the names and locations of known Traditional Medical Practitioners (TMPs) in the study locale. A sample of 102 TMPs from Kenya, Uganda and Tanzania residing around Lake Victoria Basin in East Africa participated in the study. Snow ball sampling technique was used to draw 22 TB patients claimed to have been treated by TMPs. It was established that local people have remarkable detailed knowledge of species identity, characteristics and their uses in the treatment and management of Tuberculosis. The main parts of the plants used include the root, bark, leaves and seeds in various combinations. It is concluded that local people have vast knowledge regarding the treatment of tuberculosis which is largely confined to the elderly, exploit the medicinal plants non-sustainably and use crude plant extracts as concoctions for treating and/or managing TB. It is recommended that traditional knowledge should be documented and top priority be given to the conservation of the habitat by launching special programs for raising people’s awareness about sustainable utilization of medicinal plant species and conservation.

Key words: Indigenous knowledge, medicinal plants, rural community, treatment of tuberculosis, sustainable use, conservation.

INTRODUCTION

There is abundant literature which indicates that rural communities across the world and especially Lake Victoria Region depend heavily on plant diversity and have traditionally made judicious selection of these plants for various purposes including control of various ailments affecting human and their domestic animals (Heinrich,2000; Mahmood et al., 2011a; Joshi et al., 2010). Traditional medicines have been defined as a sum of knowledge, skills and practices based on theories, beliefs and experiences indigenous to different cultures in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical or mental illness (Mahmood et al. 2011b). In many developing countries, a large part of the population, especially in rural areas depends mainly in traditional medicines for their primary health care (Mahmood et al., 2011d). In fact, a global review of phytomedicine in relation to ethnology reveals that the science of plants in the early days was based on the utilitarian approach (Wallis, 2005).

This is evident because there are several records of

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highly priced plant species which have been mentioned several times in literature (Joshi et al., 2010). These communities collect useful plant resources from various habitats and utilize them using indigenous knowledge and practices.

The global development of the art of making judicial selection of plants that can be used for curative purposes are found in Indus civilization dating back to 900 BC and the second Millennium BC (Ali, 2008). There is also a lot of evidence contained in hymns found in the Rigveda as well as the Atharvaveda which contains the records of useful plants (Rajan et al., 2005). In other studies, a total of 341 different plants species are documented in the Charaka Samhita (900 BC), as useful in the management of human health (Ali, 2005). In the Susrita Samhita, there are a total of 395 plant species listed for the same purpose (Majumdar, 1971). It is evident that other scholars, from the East Asian Region in this field, have over 70 species of plants with the list currently being approximated to 600 plants that are used in Ayurvedic (Namjoshi, 1979). Such a culture depending on Mother Nature has been practiced for over 2000 years (Namjoshi, 1979).

In the African context, the literature pertaining to the use of plants as food and curative purposes dates back to about 1600 BC among ancient Egyptian culture (Diop, 1989). An Egyptian medical treatise (papyrus), drawn up in the Thebes, during the aforementioned period, contains an inventory of 700 plants used in medicine (Pelt, 1979; Diop, 1989). In West Africa in comparatively more recent times, amongst most communities, for example the Yoruba prior to the European civilization, it was mandatory that a young boy before initiation into adulthood had to learn the names of all the useful plants in relation to future uses by the pupil in life (Rodley, 1971).

Most indigenous and local communities are situated in areas where the vast majority of plant species are readily available either for free or at minimal cost which majority of rural poor communities in the developing and the developed world can afford (Samie et al., 2005). Of the entire world flora, 250,000 species have been identified and used for curative purposes (Patwadham et al., 2005). This number represents only 15% of those species that have been effectively investigated and found to be useful (Okeke, 2005). Consequently, there are a whopping 85% of potentially useful plants that could be used for curative purposes which are yet to be investigated.

In addition, there is evidence that the search for plants with therapeutic activities has been a continuous process in the world over (Dimayuga and Garcia, 1991). For example, in Mexico, several field surveys have been carried out to isolate and elucidate active compounds in plants. Laboratory tests of the mentioned plants against Gram-positive bacteria have revealed high anti-bacterial activities. There is also evidence that most of the plant preparations are known to treat chronic diseases that are caused by non-bacterial pathogens (Patwadham et al., 2005).

Tuberculosis (TB), which is a chronic condition requiring prolonged treatment, is an old human disease whose infection rate has often been dreaded in the human population, is increasingly becoming a world-wide problem because of the emergence of multi-drug-resistance (MDR) TB, for which treatment is beyond the reach of most African countries (Anyangwe et al., 2006; Kamuoliratanakul et al., 1999). Estimates indicate that about one third of the world's population is exposed to TB and is responsible for approximately three million deaths each year (Anyangwe et al., 2006). It is also estimated that eighty one million new cases of TB occur each year and Africa has the highest incidence rate (WHO, 2002).

Sub-Saharan Africa has a much higher rate than other African states (WHO, 2012). At the regional podium, Uganda has a prevalence rate of 65%, with Kenya and Tanzania around 57% (Bercion and Kuaban, 1999). There is a little contest that these figures portray the Lake Victoria region a tuberculosis endemic zone.

Furthermore, recent statistics indicate that women are more affected by TB than men. The disease kills more than 2,700 women each day (Anyangwe et al., 2006). This translates to over one million women killed each year. Moreover, the women are killed in their most productive years because of hormonal changes, nutritional deficiency and stress during pregnancy. The situation has been aggravated by the recently reported extensively drug resistant TB (XDR TB), which is resistant to both the first and second-line drugs, and is hence threatening to make TB impossible to treat especially in cases of co-infection with HIV/AIDS (Bloom, 2006; Thorn, 2006; Wright et al., 2006; WHO, 2012; CDC report, 2005).

Against this background, the research questions that constitute the problem addressed in this paper were: What is the range of indigenous knowledge regarding the treatment and management of Tuberculosis among the rural communities and how did they acquired such knowledge? Specifically, how did indigenous peoples know what plants to use and combine in their traditional treatment, especially when so many are poisonous or have no effect when ingested?

The purpose of this paper was to investigate the indigenous knowledge of communities around Lake Victoria Region regarding the treatment and management of Tuberculosis using medicinal plants. The study specifically sought to: (1) find out the extent to which local people know the symptoms and causes of TB; (2) determine the type of plant species used by the sampled TMPs to treat and manage TB; (3) determine the parts of plant species commonly used. The study also traced the TB patients who had ever visited TMPs to find out the extent they considered the treatment effective and the cost effective.

MATERIALS AND METHODS

The methodology used was a cross-sectional survey that employed mixed methods that incorporated qualitative and quantitative
approaches. The study which commenced in March 2007 covered three purposively selected districts from each of the three East African countries of Kenya, Uganda and Tanzania. In Kenya, the study sites were: Teso, Siaya and Kisii Counties. In Uganda the districts covered were Mukono, Mayuge and Mbarara. In Tanzania, the districts covered were Musoma, Magu, and Sangerema. The study locales were purposively sampled using the criteria of high prevalence rate of TB infections, ethnic diversity of residents and known Traditional Medical Practitioners (TMPs) in the area. The study used a combination of snowball/network sampling technique to reach 32 TMPs in Kenya, 31 TMPs in Uganda and 39 TMPs in Tanzania, making a sample of 99 TMPs. In addition, the study reached 3 TB patients in Kenya, 3 in Tanzania and 16 in Uganda, making an overall sample size of 122.

The qualitative approach involved the use of interview guides and ethnographic and case studies for specialists in Traditional Medicine and questionnaires for consumers of the traditional medicines. In this study, observations were made of the behavior of TMPs during their treatment exercises as well as appropriateness of their working environment and TB diagnostic techniques. Samples of mentioned and identified plants by the Traditional Medical Practitioners (TMPs) were collected from the study area and taken to the Department of Plant and Microbial Sciences, Kenyatta University in Kenya were identified by the university taxonomist.

The main dependent variables for the study were: Level of knowledge of plant species used to treat tuberculosis (measured in terms of ability identify the medicinal plant species by local names and the type of concoctions made); Knowledge of the major symptoms or signs of TB. These were compared with clinically known clinical signs; Parts of plants used and Medicinal preparation (juice, ash). The independent variables were country of residence age, sex and educational level.

The data obtained was edited and analyzed using Statistical Package for Social Sciences (SPSS) version 20. The relationship between age, level of education, sex, country of origin and knowledge of signs of tuberculosis and medicinal plants used to treat tuberculosis were assessed using Pearson correlation coefficient and the Chi-Square statistical technique.

RESULTS AND DISCUSSIONS

The 99 TMPs who participated in the study demonstrated a good understanding of the symptoms of tuberculosis which they claimed to treat and manage using medicinal plant species. Figure 1 indicates the most frequently mentioned signs of TB by the TMPs. The signs that were used to diagnose TB were; labored breathing, loss of weight and tiredness, dry persistent cough, dry lips and coughing blood, amongst others.

The following are the most frequently mentioned signs of TB across the region:

1) Dry lips, 24.2% (Kenya 3.0%, Tanzania 20.2% and Uganda 1.0%).
2) Coughing sputum, 17.2% (Kenya 1.0%, Tanzania 13.1% and Uganda 3.0%).
3) Dry persistent cough, 11.1% (Kenya 3.0%, Tanzania 1.0% and Uganda 7.1%).
4) Loss of body weight and tiredness, 70.1% (Kenya 7.1%, Uganda 3.0%).
5) Medical diagnosis, 9.1% (Kenya 2.0%, Uganda 7.1%).
6) Labored breathing/shortness of breath, 7.1% (Kenya 5.1%, Uganda 2.0%).
7) Coughing blood, 5.1% (Kenya 3.0%, Tanzania 2.0%).
Table 1. Medicinal plants used to treat Tuberculosis.

<table>
<thead>
<tr>
<th>Medicinal plant</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entada abyssinica</td>
<td>Kenya and Tanzania</td>
</tr>
<tr>
<td>Albizia coriaria</td>
<td>Uganda</td>
</tr>
<tr>
<td>Warbugia ugandensis</td>
<td>Uganda, Kenya and Tanzania</td>
</tr>
<tr>
<td>Rubia cordifolia</td>
<td>Uganda and parts of Kenya</td>
</tr>
<tr>
<td>Mangifera indica</td>
<td>Uganda</td>
</tr>
<tr>
<td>Zanthoxylum chatybeum</td>
<td>Uganda and Kenya</td>
</tr>
<tr>
<td>Eucalyptus spp.</td>
<td>Kenya, Kenya and Tanzania</td>
</tr>
<tr>
<td>Entada abbyssinica</td>
<td>Kenya and Tanzania</td>
</tr>
<tr>
<td>Acasia hoki</td>
<td>Uganda</td>
</tr>
<tr>
<td>Gurcina spp.</td>
<td>Uganda and parts of Kenya</td>
</tr>
</tbody>
</table>

(8) Night fevers and loss of appetite
(9) Chest pains
(10) Night sweats, about 4.0%.

The information carried in Figure 1 indicates that the level of knowledge of TMP regarding the signs of tuberculosis is fairly well distributed across the Lake Victoria Basin. The TMPs in the region concurred that the common signs of tuberculosis are coughing sputum, dry persistent cough, loss of appetite and dry lips. The signs which were predominantly reported by TMPs in Kenya and Tanzania were coughing blood and fever at night. The signs mentioned by TMPs in Uganda and Kenya alone were chest pains, labored breath/shortness of breath, loss of weight, night sweats and medical diagnosis.

The sex breakdown of TMPs and their level of knowledge regarding the common signs of tuberculosis were also computed. The symptoms of tuberculosis frequently mentioned by the TMPs by sex in decreasing order of mention were: dry lips; coughing sputum, persistent dry cough, chest pain, loss of weight, fever at night and coughing blood. It was established that there was a slight positive and non-significant difference between the level of knowledge of TMPs by sex. The only notable difference was that night sweat was mentioned exclusively by females while the medical history and loss of appetite was only mentioned by males. Thus, there was no significant difference between the frequency of mention of the signs of tuberculosis and sex ($\chi^2 = 20.455$, df = 10, $P = 0.321$).

The overall impression is that the symptoms of TB according to the traditional health care practitioners who participated in the study were nearly similar to the general clinical allopathic symptoms though disparities existed across the study locales. In some cases there was mixing up as most respiratory diseases initially express themselves alike. Some TMPs mentioned loss of body weight and coughing blood as the common symptoms of TB, while the common symptoms of respiratory tuberculosis according to published literature includes malaise, weight loss, fever and night sweats, over three weeks cough, breathless chest pain (Schreider, 2006).

It was established that the people residing along the Lake Victoria Region have a good knowledge of useful plant species especially the knowledge on medicinal plant species. Table 1 summarizes the wide spectra of plant species (initially given in local names but later given scientific names) reported as being used by TMPs across the Lake Victoria Basin.

The information in Table 1 indicates that TMPs across the entire Lake Victoria Region have a wide knowledge of medicinal plant species used for the treatment and management of Tuberculosis. In terms of ethnic distribution of these plants in Kenya, Warbugia ugdensis was widely used among the Kisi, in Kisii District; Luo in Siaya/Bondo District and Ateso in Teso Districts. In Uganda, the ethnic distribution spread among the communities living in Mayuge and Mbarara. In Tanzania, the TMPs were located in Geita Districts.

Entada abbyssinica was also used in Kenya among the Kisi, Siaya and Teso communities, Tanzania within Musoma and Bunda Districts and rarely used among the communities in Uganda. Instead, the most commonly used plant species to treat TB in Uganda are Rubia cordifolia and Psidium guajava in Mayuge District and Albizia coriara; Acacia hoki; Garcinia species in Mbarara Districts. This finding is consistent with those of Kunjani et al., (2011), Mahmood et al., (2011c) and Martin, (1995) who concur that local communities have rich indigenous knowledge which needs to be saved in black and white.

It was further established that a large proportion of TMPs did not cultivate any of these medicinal plant species due to cultural considerations, misconceptions regarding the role of herbal treatment. Some of these misconceptions included the perception that people who planted these medicinal plants in their homesteads were practicing witchcraft. It was therefore evident that traditional beliefs and practices are also deeply rooted in their culture in such a way that they attribute most of the complicated ailments and other misfortunes to supernatural origin due to soul loss, spells or curses casts by evil spirits by their displeasure. The local people use the medicinal plant species and their parts for the treatment of ailments following the traditional practices.

There was no statistically significant correlation between the level of knowledge of the plant species used to treat tuberculosis and the age of the TMPs. The implication is that most of this useful knowledge has been passed over to the younger generation. However, it was found that there was a significant difference between the level of knowledge of plant species used to treat TB and the sex of the TMPs ($\chi^2 = 46.6$, df = 35, $P<0.001$). It was evident that female TMPs had better knowledge than the male counterparts. Further, the TMPs whose main occupation was practicing treatment using medicinal plants and/or farming and nursing had better knowledge regarding the medicinal plants used to treat TB, than those with other occupations or businesses.
There was a moderately weak correlation between the TMPs level of knowledge about the signs of TB and the media through which they learned how to treat TB. A majority of TMPs acquired their knowledge on how to treat TB from their parents, relatives and other healers. A large proportion of TMPs reported that they acquired their skills through a combination of parental and other healers’ knowledge. A minority reported that they acquired the skills through books belonging to healers associations. In fact, the only TMPs who reported that they had acquired their skills through books and healers association were the more educated lot. These were largely concentrated in Kenya among the Kisii community and Uganda among the communities around Mukono and Mayuge Districts.

The different parts of the plant species used for making the herbal medicines are summarized in Table 2. It is noted that the most frequent part of plant species used to treat TB were: whole plant parts mentioned by 32 TMPs followed by roots/tubers and then leaves as well as seeds. The least frequently part of the plant species was the stem.

In terms of inter-country comparisons, TMPs in Kenya use more of whole plant followed by roots/tubers to treat TB. In Uganda, the most frequently used plant parts are roots/tubers followed by whole plant. In Tanzania, the most frequently plant parts are whole plant, followed by leaves and seeds. This finding confirms the observation made by Storr (1995) that roots are the most potent parts of some plants and uncontrolled root harvesting for medicine has severe effect to herbal plants especially when they are in low stock.

The most popular medicinal preparations across all the three countries are: decoction, paste, juice and ash from burnt plant parts. This finding is consistent with the observation made by Kunjani-Joshi et al., (2011) that popular preparations are infusion, decoction, paste or juices. The medicinal uses of the species vary from one district or village to the next district.

Figure 2 carries information on how the recovery rate of TB was known to the TMPs in various study locales. From the figure, it is evident that there are various ways of assessing whether or not their TB patient had recovered after undergoing treatment. About two-thirds of TMPs knew about the recovery of their TB patients either through self-reporting or through other TB patients referred to the TMPs by those who had recovered. It was only in Siaya/Bondo and Teso in Kenya where the patients reported to TMPs that they had recovered as a result of the herbal treatment. Some patients who had recovered from TB were still undergoing treatment for other diseases unrelated to TB as at the time of the study. Most of the patients who knew their recovery status through laboratory testing were from Uganda. None of the Previous TB patients from Tanzania reported that they had undergone laboratory testing.

The TMPs were also unanimous that the recovery rate of patients seeking tuberculosis treatment depended on whether or not they had taken the prescribed dosage regularly and for the recommended duration (an average of about six months). A tracer of some of the previously treated patients who had recovered testified that they had recovered as a result of the herbal treatment. This confirms that most of the local herbalist had a good mastery of indigenous knowledge of the medicinal plants used to treat and/or manage tuberculosis in the Lake Victoria Region in East Africa.

There is little doubt that many traditional practitioners have good knowledge and extensively use medicinal plant species and use the plants and their parts to treat various diseases including TB. The commonly used plant parts to prepare decoctions, paste, juice, and powder for treatment were the bark, roots and whole plant which exposes these plant species to extinction. Because of the therapeutic and economic implications of herbal medicines, the plant species used are subjected to destructive harvesting by greedy traders. A negligible percentage of the TMPs made any effort to cultivate the medicinal plant species in their homesteads. Domestication of medicinal wild varieties is constrained by a number factor including misconceptions, attitudes and unawareness on the specific propagation conditions. For example, some assume that domestication either makes other villagers relate the practice to witchcraft or merely lessen medical potency of wild plants (Katende, 1995).

It is apparent that the plant species which were collected from the wild, especially from common areas might become locally extinct when their habitats are destroyed or modified. Of grave concern is the fact that most of the knowledge is concentrated among the older generation of the TMPs and that most of the plant species were not being harvested sustainably. This finding is consistent with Adhikari et al., (2010) who reports that uprooting of Aloe spp and Asparagus racemosus for medicine caused large scale soil erosion in Maradavally forests. They pointed out that unfortunately, localized threats to such simple species is hardly addressed on the grounds that the effect does not conform to UICN red list criteria for declaring an organism threatened species (IUCN, 2007). The critical point of concern here is that even if a species is not categorized a threatened species to IUCN scales, its scarcity to a particular community must have local impact.

### Table 2. Most frequently used parts of plant species to treat TB in Lake Victoria Region.

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Kenya</th>
<th>Uganda</th>
<th>Tanzania</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves and seeds</td>
<td>7</td>
<td>5</td>
<td>10</td>
<td>22</td>
</tr>
<tr>
<td>Stem</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>Roots/tubers</td>
<td>9</td>
<td>11</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>Whole plant</td>
<td>12</td>
<td>9</td>
<td>11</td>
<td>32</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>31</td>
<td>39</td>
<td>102</td>
</tr>
</tbody>
</table>
that deserves to be addressed locally. Whenever a medicinal plant becomes unavailable, its use is overtaken by less important species, or else, complex concoctions of unpopular medicinal plants are formulated (Arjurn et al., 2009).

Furthermore, because a majority of the rural and urban poor have strong attachment to herbal medicine and also combine spiritual beliefs with therapeutic efficacy, there is need for value addition to ensure the toxicity and sustainability concerns are addressed through systematic research not only in the Lake Victoria Region but also in the Eastern African Region. This concern is also echoed by Kunjani et al., (2011) who observes though some initiatives have already been taken for the conservation and sustainable utilization of the useful species, less priority is given to conserve these resources in an integrated manner.

The causes of tuberculosis were also well known by the TMPs and were consistent with those medically established ones. These included known causes such as: smoking; overcrowding/contacts; bacterial diseases and inheritance (Anyangwe et al., 2006). The study also found that more than three quarters of the TMPs learned about the symptoms, causes and treatment of tuberculosis through parents/relatives of the older generation and dreams. A negligible percentage learned about the treatment of tuberculosis through modern print and electronic media and other sources such as journals/publications, internet or healers associations. The few TMPs who updated their knowledge regarding tuberculosis treatment were the most educated with at least tertiary level of formal schooling.

Among the sampled TMPS, 74% of the older people and 58% of the new or younger generation used medicinal plants and their products to cure various ailments including Tuberculosis. There was a moderately positive and significant relationship between the TMPs’ levels of knowledge about the signs of TB and the age of TMPs (r=0.48, P≤0.05). The TMPs with more appropriate knowledge were in the advanced age groups of 50 years and above. This finding is consistent with the finding by (Schulters, 1986) who expresses fear that those who hold this useful information may be buried with the information.

**CONCLUSION AND RECOMMENDATIONS**

This study reveals that the study area is rich with medicinal plants and it is a common trend to use these plant species in local healthcare system especially in the treatment and management of Tuberculosis. A large proportion of TMPs have a rich wealth of diverse indigenous knowledge regarding the symptoms and causes of tuberculosis. Of graver concern is that the TMPs use plant parts such as stem and roots which not only expose these precious medicinal plant species to extinction but also lead to environmental degradation.

It is strongly recommended that major thrust should not only be directed towards documenting and conserving...
traditional knowledge but also undertaking an intensive inventory and documentation of useful plant species, their chemical constituents, habitats and potential utilization as raw materials. The study indicates that there seem to be a good potential for their sustainable utilization.

Assessment of herbal or simple medicinal plant species with locally important medicinal value could be better achieved by considering local uses linked to these. This can be achieved by involving communities whose survival is affected by either a loss or abundance of individual plant species in their environments.

As most plants are extracted for their roots and or tubers, total uprooting of non-timber plants for medicine can be reduced significantly through chemical profiling of leaves for possible presence of same active chemotypes of the root. Harvesting of leaves for medicine can have less deteriorating effect due to fast proliferation cycles.

There is a need to establish a link between communities who are dependent on plan species for their primary healthcare and researchers on ex-situ conservation of locally important medicinal plants.

Medicinal plants with market value should be treated as important resources for sustainable development through commercial cultivation. Entada abbyssinica, Eucalyptus spp and Warburgia ugandensis which were the most popular plant species among the sampled TMPs in the Lake Victoria Region of East Africa are proposed for commercial cultivation.

Finally, top priority should be given to the conservation of the habitat by launching special programs for raising people’s awareness about sustainable utilization of medicinal plant species and environmental conservation. Therefore, emphasis should be given to implement some pilot programmes for plantation, domestication and cultivation of useful plant species not only found to treat TB, but also other diseases.

ACKNOWLEDGEMENT

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REFERENCES


A study on resistance loss of multidrug resistant (MDR) *Pseudomonas aeruginosa* strains after treatment with dilutions of acridine orange

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This study investigated the loss of resistance of multidrug resistant (MDR) *Pseudomonas aeruginosa* strains after exposure to dilutions of acridine orange. Five pure axenic strains of *P. aeruginosa* coded PA\(_1\) to PA\(_5\) obtained from five infected human sources which included middle ear, urethra, trachea, wound and urine were obtained from the Medical Microbiology Department of the University of Benin Teaching Hospital, Nigeria and stocked on sterile Nutrient agar slants. Slant cultures were sub-cultured aseptically on sterile MacConkey and Blood agar plates and incubated aerobically at 37°C for 24 h to confirm for *P. aeruginosa*. Gram staining and oxidase test were carried out on resulting colonies. Antibiotic sensitivity test was done by agar disc diffusion method on all confirmed strains on sterile Mueller-Hinton agar plates before and after treatment with acridine orange (AO). *P. aeruginosa* strains that showed ≤50.0% reduction in resistance markers (RM) after treatment with 0.35, 0.55, 0.75 and 0.95 µg/ml dilutions of AO were noted. Minimum inhibitory concentration (MIC) assay was done using gentamicin on PA\(_5\) strain with all four dilutions. All five strains showed 100% resistance against augmentin, nalidixic acid, nitrofurantoin, cotrimoxazole, amoxicillin and tetracycline. Sensitivity was recorded for ofloxacin and gentamicin with 14.6±9.5 and 8.4±4.4 mm zones of inhibition, respectively for all the strains except strain PA\(_3\) which was resistant to 8 (100.0%) of antibiotics used. Strains PA\(_1\), PA\(_2\), PA\(_4\) and PA\(_5\) were each resistant to 6 (75.0%) of the antibiotics tested. There was loss of RM of 52.1±18.6 and 54.7±37.6% to ofloxacin after treatment with 0.35 and 0.55 µg/ml dilutions, respectively by all MDR *P. aeruginosa* strains. Loss of RM to gentamicin by strains PA\(_1\), PA\(_2\), PA\(_4\) and PA\(_5\) after 0.35 µg/ml acridine orange treatment was recorded as 0.0, 61.5, 58.3 and 60.0%, respectively with a mean±standard error (SE) of 45.0±15.0%. With 0.55 µg/ml dilution, 97.6±28.3% loss of RM was recorded while less than 45.0 and 35.0% loss of RM were recorded for 0.75 and 0.95 µg/ml dilutions, respectively. Acridine orange dilutions of 0.35 and 0.55 µg/ml recorded two-fold (5 µg) and four-fold (2.5 µg) reduction in MIC of gentamicin, respectively. The implications of these findings are discussed.

**Key words:** Resistance loss, *Pseudomonas aeruginosa*, treatment, dilutions, acridine orange

**INTRODUCTION**

*Pseudomonas aeruginosa* is a highly invasive and toxigenic aerobic Gram negative bacterium. It is non-sporeng, non-capsulated and usually motile with the help of one or two flagella. The organism readily grows over a wide range of temperature and media. The reasons for the preeminence of this microorganism as a human pathogen range from its adaptability, its innate resistance to many antibiotics, disinfectants and its virulence factors (Aendekerk et al., 2005). The organism is a danger and threat to patients with cystic fibrosis or AIDS and other...
immune disorders as well as those requiring long-term hospitalization. *P. aeruginosa* is an opportunistic pathogen with innate resistance to many antibiotics and disinfectants (Shahid et al., 2003). The microorganism is physiologically versatile and flourishes as a saprophyte in multiple environments including sinks, drains, respirators, humidifiers, and disinfectant solutions (Govan and Deretic 1996). *P. aeruginosa* is notorious for its resistance to antibiotics as it maintains antibiotic resistance plasmid (R) factor (Radi and Rahman, 2010). These plasmids are transmissible to sensitive bacteria which make them acquire resistance to antibiotics and have the ability to undergo recombination through conjugation, transformation and transduction. Multidrug active efflux systems have recently been recognized in a number of bacteria as efficient mechanisms of resistances in *P. aeruginosa* by which antibiotics are expelled from the cells by membrane transporter proteins, the so called drug efflux pumps (Lomovskaya et al., 2001).

Infections due to *P. aeruginosa* are seldom encountered in healthy adults. The organism has become increasingly recognized as the etiological agent in a variety of serious infections in hospitalized patients especially those with impaired immune defenses (Neu, 1993). The indiscriminate use of antimicrobial drugs partly in hospital in patients leads to the suppression of drug susceptible organisms in the gut flora and favors the persistence and growth of resistant bacteria including *P. aeruginosa*. The closed environment of hospitals favors the transmission of such resistant strains through personnel, fomites and by direct contact (Melnick and Adelberg, 1998).

*P. aeruginosa* is the most common and lethal pathogen responsible for urinary tract infections (UTI), ventilator associated pneumonia in intubated patients with directly attributable deaths reaching 38% (Mansouri et al., 2011). Multidrug resistant (MDR) *P. aeruginosa* is found to be resistant to a very large number of antibiotics (Bonomo and Szabo, 2006; Manikandan et al., 2011). Resistance may be due to interplay of various interactions including beta-lactamases, mutations, decreased permeability and the activities of efflux pumps (Defez et al., 2004; Abdi-Ali et al., 2007) and presence of drug resistant plasmids (Ranbar et al., 2007).

*P. aeruginosa* is a serious threat in clinical medicine since most isolates are resistant simultaneously to many antibiotics at very high levels (Mukherjee et al., 2011). Elimination of these resistance markers (RM) by known pharmacological compounds would be advantageous in successful therapeutic control of various infections caused by this pathogen (Mukherjee et al., 2011).

There are a number of reports demonstrating the ability of various chemical and physical agents to increase the rate of loss of plasmid DNA from bacteria (Sonstein and Baldwin, 1972; Stanier, 1984; Otajevwo, 2012). Antibiotics such as mitomycin, rifampicin, novobiocin and flavophospholipol as well as DNA intercalating dyes (such as acridine orange, ethidium bromide, acriflavine and ascorbic acid) have been shown to cure many plasmids (Ramesh et al., 2000). Acridine orange has been shown to cure F-plasmids from *Escherichia coli* and it is suggested that this dye interferes with plasmid replication, stimulating the entire plasmid loss (Salisbury et al., 1972).

It has also been reported that acridine orange and ethidium bromide are better curing agents for *Pseudomonas cepacia* than sodium dodecyl sulphate (SDS) and elevated temperature (Kumar and Surendran, 2006). Stanier (1984) reported that the elimination of plasmids by dyes and other agents reflects the ability of such agents to inhibit plasmid replication at a concentration that does not affect the chromosome. Acridine orange, ethidium bromide, mitomycin and SDS failed to cure the plasmid of *Pseudomonas putida*, though the phenotypic characteristics changed and the plasmid was cured at a frequency of 2.63% when acridine orange and elevated temperature (40°C) were used together (Stanier, 1984).

Ingram et al. (1972) found that drug resistance of *P. aeruginosa* could be eliminated by treatment with SDS and Pattmakik et al. (1995) reported that acridine orange could not affect *P. aeruginosa* due to impermeability of its cell wall while ethidium bromide and SDS cured antibiotic resistance plasmid at a concentration of 1 to 2% and 700 to 3000 µg/ml for SDS and ethidium bromide, respectively. Al-Amr et al. (1999) treated an isolate of *P. aeruginosa* with 1000 µg/ml dilution of acridine orange and reported that there were no cured cells as the plasmid profile of cured cells was the same as that of untreated samples with a conclusion that acridine orange had no effect on *P. aeruginosa* as a curing agent.

Treatments that increase frequency of elimination of plasmids will certainly enhance sensitivity (effectiveness) of antibiotics in situ. There is dearth of literature on antibiotic sensitivity improvement (through loss of RM) using dilutions of chemical agents such as acridine orange. Hence, the aim of this work was to study the RM loss of MDR *P. aeruginosa* strains after treatment with clinical dilutions of acridine orange with the following objectives:

1. Determine the sensitivity profile of *P. aeruginosa* strains (from selected diseased sites of the human body) before treatment with dilutions of acridine orange after incubation for 24 h at 37°C.
2. Determine the distribution of multidrug RM among the selected pathogenic strains of *P. aeruginosa*.
3. Determine *P. aeruginosa* strains that showed ≤50.0% loss of their RM after 0.35 µg/ml acridine orange dilution treatment and incubation at 37°C for 24 h.
4. Determine *P. aeruginosa* strains that showed ≤50.0% loss of their RM after 0.55, 0.75 and 0.95 µg/ml acridine orange dilution treatments and incubation at 37°C for 24 h.
5. Determine the distribution of ≤50.0% loss of RM among MDR *P. aeruginosa* strains after treatment with...
0.35, 0.55, 0.75 and 0.95 µg/ml dilutions of acridine orange.

(6) Determine acridine orange dilutions’ effect(s) on the minimum inhibitory concentration (MIC) of gentamicin (an aminoglycoside) on an MDR P. aeruginosa strain from midstream urine.

MATERIALS AND METHODS

Sampling

Five pure axenic strains of P. aeruginosa coded PA₁ to PA₅ were obtained from the Medical Microbiology Department of University of Benin Teaching Hospital, Edo State, Nigeria. Bacterial pathogens which were isolated from ear swab, urethral swab, tracheal (throat) swab, wound swab, and mid stream urine samples, respectively, were inoculated aseptically (in pure form), on sterile nutrient agar slants and incubated at 37°C for 24 h. All strains were appropriately labeled.

Processing of samples

Colonies from resulting stocked cultures were re-confirmed by sub-culturing them on sterile nutrient agar (LabM, UK), MacConkey agar (LabM, UK) and Blood agar plates and inoculated plates were incubated at 37°C for 24 h. Purity test was done on all prepared agar plates before use to guarantee their sterility. Resulting colonies were inoculated on the selective medium centrimbre agar and incubated appropriately.

Finally, oxidase test and gram staining were done on resulting colonies according to scheme provided by Cowan and Steel (1993). Colonies were stocked on sterile nutrient agar slants and kept at 4°C in the refrigerator for further use. The set up was appropriately labeled. All isolated bacterial pathogens were subjected to antibiotics sensitivity testing before treatment with dilutions of acridine orange.

Antibiotic sensitivity testing

Antibiotic sensitivity testing on each of the five pure strains of P. aeruginosa was carried out using the disc diffusion method on sterile Mueller-Hinton agar (MHA) plates (Bauer et al., 1966). A loop full of each colony was picked aseptically using a flame wire loop and placed in the centre of sterile MHA plates. This was then spread all over the plates applying the caution of not touching the edges of the plates. The seeded plates were allowed to stand for about 2 min to allow the agar surface to dry. A pair of forceps was flamed and cooled and used to pick antibiotic multidisc (Abitek, Liverpool) containing augmentin (30 µg), ofloxacin (5 µg), gentamicin (10 µg), nalidixic acid (30 µg), nitrofurantoin (200 µg), cotrimoxazole (25 µg), amoxicillin (25 µg) and tetracycline (25 µg). Discs were placed at least 22.0 mm from each other and 14.0 mm from the edge of the plate (Ochei and Kolhatkar, 2008). Antibiotics discs were selected on the basis of their clinical importance and efficacy on P. aeruginosa. Plates were allowed to stand for 10 min tunes before incubation (Mbata, 2007).

The plates were incubated at 37°C for 24 h. A reference control strain of P. aeruginosa NCTC was inoculated in the same way on another plate with the same antibiotic discs and incubated at the same temperature and time. At the end of incubation, the diameters of the zones of inhibition from one edge to the opposite edge were measured to the nearest millimeter using a transparent ruler (Byron et al., 2003). Strains that showed resistance against three antibiotics and above were termed multiple drug resistant strains (Jan et al., 2002) and were noted and used further.

Preparation of dilutions of acridine orange

For treatment of MDR P. aeruginosa strains, sub-clinical acridine orange concentrations of 0.35, 0.55, 0.75 and 0.95 µg/ml were used. These concentrations (dilutions) were chosen in line with non-toxic laboratory concentrations of 0.25 to 1.0 µg/ml prescribed by Wurmb-Schwark et al. (2006) for ethidium bromide which have been reported to possess curing potentials. Hence, acridine orange dilutions of 0.35, 0.55, 0.75 and 0.95 µg/ml were prepared using RV/O where stock or original concentration of acridine orange used was 1.0 mg/ml or 1000 µg/ml. To obtain 0.35 µg/ml dilution therefore, 0.14 ml of stock reagent was added to sterile 9.86 ml of Mueller-Hinton broth. To obtain 0.55 µg/ml acridine orange dilution, 0.11 ml stock acridine orange solution was added to sterile 19.78 ml Mueller-Hinton broth. Also to obtain 0.75 µg/ml dilution, 0.30 ml stock solution was added to sterile 19.70 ml Mueller-Hinton broth and 0.38 ml stock solution of acridine orange was added to sterile 19.61 ml of Mueller-Hinton broth to obtain 0.95 µg/ml dilution. All dilutions were effected in sterile universal bottles containing sterile 20.0 ml of Mueller-Hinton broth each.

Growing broth culture of MDR P. aeruginosa strains

A colony of each MDR strain was aseptically picked from its slant stock culture using flamed and cooled wire loop and inoculated into sterile 10 ml Mueller-Hinton broth. Inoculated broths were incubated at 37°C for 18 h. The resulting turbid broth culture was then diluted according to a modified method of Shirtliff et al. (2006). Using a sterile pipette, 0.1 ml of broth culture was mixed with 99.9 ml (1:200 dilution) of sterile Mueller-Hinton broth. This was properly mixed and was used as working inoculum and should contain 10⁸ to 10⁹ organisms, which was used within 30 min (Ochei and Kolhatkar, 2008).

Treatment of MDR pathogenic strains with prepared acridine orange dilutions

The treatment of MDR P. aeruginosa pathogenic strains with the prepared dilutions of acridine orange was done according to a modified method of Byron et al. (2003). Using a sterile pasteur pipette, 0.5 ml aliquot of each diluted overnight broth culture of MDR pathogen was added to 4.5 ml sterile molten Nutrient agar. The various prepared dilutions (one at a time) of acridine orange were then added in 0.5 ml volume. The set up was properly mixed and labeled. The set up for each dilution was then poured on top of sterile hardened or set 2% Nutrient agar plates and left to set.

The same antibiotic multidisc used before treatment were then picked (using flamed and cooled pair of forceps) and impregnated on the set agar overlay plates. Plates were incubated at 37°C for 24 h. Measurement of diameters of zones of inhibition was taken and recorded (NCCLS, 2000).

Determination of MIC of gentamicin after acridine orange treatment

Serial doubling dilutions of gentamicin, the antibiotic to which a P. aeruginosa strain (isolated from mid stream urine) recorded more than 50.0% RM loss was carried out. The antibiotic being used for this assay was sterilized by filtration before use. Sterile test tubes, numbering 13 were set up on a test tube rack and labeled 1 to 13.

Using a sterile pipette, 1 ml of diluted broth was dispensed into tubes 2 to 10, 11 and 13. Into tube 12, 2.0 ml of diluted broth culture was pipetted. Tube 11 was the inoculum control, tube 12 was the broth control and 13 was the drug control. Into tubes 1, 2 and 13, 1 ml of the working antibiotic solution was pipetted. Serial doubling dilutions of antibiotic solution were separately prepared in
nutrient broth to get reducing concentrations of the anti-
biotic using 100 to 0.4 µg/ml as a standard for MIC assay
(Ochei and Kolhatkar, 2008). All tubes were incubated at
37°C for 18 h. Turbidity (cloudiness) in the growth medium
indicated growth. Tube 11 showed turbidity and tubes 12
and 13 showed no growth. The lowest concentration
showing no growth was the MIC of the antimicrobial agent
as effective against P. aeruginosa strains. All MIC results
before and after treatment with dilutions of acridine orange
were recorded accordingly.

RESULTS

Table 1 shows P. aeruginosa strains PA1, PA2, PA3, PA4, and PA5 isolated from ear swab, urethral swab, tracheal swab, wound swab and midstream urine, respectively and their sensitivity reactions to augmentin, ofloxacin, gentamicin, nalidixic acid, nitrofurantoin, cotrimoxazole, amoxicillin and tetracycline. All five strains showed 100.0% resistance against augmentin, nalidixic acid, nitrofurantoin, cotrimoxazole, amoxicillin and tetracycline. Sensitivity was recorded for oflaxacin and gentamicin with mean±standard error (SE) zones of inhibition of 14.6±9.54 and 8.4±4.40 mm, respectively for all P. aeruginosa strains with exception of PA3 which did not respond to the tested drugs at all.

The occurrence of multidrug resistance P. aeruginosa strains after antibiotic susceptibility testing is shown in Table 2.

Strains PA1, PA2, PA4 and PA5 isolated from ear swab, urethra swab, wound swab and mid stream urine, respectively were each resistant to 6 (75.0%) of the total antibiotics tested. Interestingly, all four P. aeruginosa strains were each sensitive to ofloxacin and gentamicin. Only strain PA3 (isolated from tracheal swab) resisted 8 (100.0%) or the entire antibiotics used.

In Table 3, the percentage loss of RM due to 0.33 µg/ml acridine orange curing effect is shown. Loss of 50% and above of RM was recorded for P. aeruginosa strains PA1 (with 78.5% loss of resistance to ofloxacin), PA2 (with 80.0 and 61.5% loss of resistance to ofloxacin and gentamicin, respectively), PA4 (with 50.0 and 58.3% loss of resistance to ofloxacin and gentamicin, respectively), PA5 (with 53.0% loss of resistance to ofloxacin and gentamicin, respectively), and PA3 (with 56.0% loss of resistance to ofloxacin).
Table 3. *P. aeruginosa* strains that showed ≤50.0% loss of their resistance biomarkers after treatment with 0.35 µg/ml dilution of acridine orange and incubation at 37°C for 24 h.

<table>
<thead>
<tr>
<th>MDR strain</th>
<th>AUG (%)</th>
<th>OFL (%)</th>
<th>GEN (%)</th>
<th>NAL (%)</th>
<th>NIT (%)</th>
<th>COT (%)</th>
<th>AMX (%)</th>
<th>TET (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA1</td>
<td>Before</td>
<td>0.0</td>
<td>14.0</td>
<td>14.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>0.0 (0.0)</td>
<td>25.0 (78.5)</td>
<td>19.0 (35.7)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>PA2</td>
<td>Before</td>
<td>0.0</td>
<td>15.0</td>
<td>13.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>0.0 (0.0)</td>
<td>27.0 (80.0)</td>
<td>21.0 (61.5)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>PA3</td>
<td>Before</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>PA4</td>
<td>Before</td>
<td>0.0</td>
<td>16.0</td>
<td>12.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>0.0 (0.0)</td>
<td>26.0 (50.0)</td>
<td>19.0 (58.3)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>PA5</td>
<td>Before</td>
<td>0.0</td>
<td>19.0</td>
<td>5.0</td>
<td>17.0</td>
<td>0.0</td>
<td>0.0</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>0.0 (0.0)</td>
<td>25.0 (31.5)</td>
<td>8.0 (60.0)</td>
<td>21.0 (23.5)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>14.0 (16.6)</td>
</tr>
</tbody>
</table>

OFL: Ofloxacin; GEN: gentamicin; AUG: augmentin; NAL: nalidixic acid; NIT: nitrofurantoin; COT: cotrimoxazole; TET: tetracycline; AMX: amoxicillin.

Table 4 shows percentage loss of RM due to 0.55 µg/ml curing effect of acridine orange. Loss of 50% and above of RM was recorded for *P. aeruginosa* strains PA1 (with 50.0% loss to gentamicin), PA2 (with 160.0 and 76.9% loss to ofloxacin and gentamicin respectively), PA3 (with 83.3% loss to gentamicin) and PA5 with 58.8 and 180.0% loss of RM of ofloxacin and gentamicin, respectively. Strain PA3 maintained 100.0% resistance to 8 (100.0%) of the antibiotics used while the remaining four strains maintained 100.0% resistance to 6 (75.0%) of the antibiotics used after treatment with 0.55 µg/ml acridine orange.

Data on 50% and above reduction in RM in *P. aeruginosa* strains after treatment with 0.75 µg/ml acridine orange are shown in Table 5. *P. aeruginosa* strains PA1 and PA3 recorded 50 and 120.0% loss, respectively in RM to gentamicin. Strain PA3 recorded less than 35.0% loss in RM to ofloxacin and gentamicin. Strains PA4 and PA5 each recorded less than 20% loss in resistance to ofloxacin. *P. aeruginosa* strain PA3 showed 100.0% resistance to 8 (100.0) of drugs used, while other strains recorded 100.0% resistance to augmentin and tetracycline. There were slight losses in RM for nalidixic acid, nitrofurantoin, cotrimoxazole and amoxicillin as recorded for PA1, PA4 and PA5.

The effect of 0.95 µg/ml acridine orange treatment of the *P. aeruginosa* strains on the selected antibiotics is shown in Table 6. Only *P. aeruginosa* strain PA5 recorded 120.0% loss in RM gentamicin. Strain PA1 showed less than 25.0% loss of ofloxacin and gentamicin. Strain PA3 recorded less than 10.0% and less 40.0% loss of ofloxacin and gentamicin respectively. Strain PA4 showed less than 10.0% loss to gentamicin while PA5 recorded 120.0% loss in RM to gentamicin. Strains PA1, PA2, PA4 and PA5 recorded 100.0% resistance loss to 6 (75.0%) of the antibiotics used after acridine orange treatment, while strain PA3 showed 100.0% loss to 8 (100.0%) of the drugs used for the susceptibility testing.

Presented in Table 7, is the effect of subclinical dilutions of acridine orange on the MIC of gentamicin as it inhibited the growth of the growth of...
Table 4. *P. aeruginosa* strains that showed ≤50.0% loss of their resistance markers after treatment with 0.55 µg/ml dilution of acridine orange and incubation at 37°C for 24 h.

<table>
<thead>
<tr>
<th>MDR strain</th>
<th>AUG (%)</th>
<th>OFL (%)</th>
<th>GEN (%)</th>
<th>NAL (%)</th>
<th>NIT (%)</th>
<th>COT (%)</th>
<th>AMX (%)</th>
<th>TET (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PA1</strong></td>
<td>Before</td>
<td>0.0</td>
<td>14.0</td>
<td>14.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>0.0 (0.0)</td>
<td>18.0 (28.5)</td>
<td>21.0 (50.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td><strong>PA2</strong></td>
<td>Before</td>
<td>0.0</td>
<td>10.0</td>
<td>13.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>0.0 (0.0)</td>
<td>26.0 (160.0)</td>
<td>23.0 (76.9)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td><strong>PA3</strong></td>
<td>Before</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td><strong>PA4</strong></td>
<td>Before</td>
<td>0.0</td>
<td>20.0</td>
<td>12.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>0.0 (0.0)</td>
<td>24.0 (20.0)</td>
<td>22.0 (83.3)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td><strong>PA5</strong></td>
<td>Before</td>
<td>0.0</td>
<td>17.0</td>
<td>5.0</td>
<td>17.0</td>
<td>0.0</td>
<td>0.0</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>0.0 (0.0)</td>
<td>27.0 (58.8)</td>
<td>14.0 (180.0)</td>
<td>21.0 (23.5)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>14.0 (16.6)</td>
</tr>
</tbody>
</table>

OFL: Ofloxacin; GEN: gentamicin; AUG: augmentin; NAL: nalidixic acid; NIT: nitrofurantoin; COT: cotrimoxazole; TET: tetracycline; AMX: amoxicillin.

Table 5. *P. aeruginosa* strains that showed ≤50.0% loss of their resistance markers after treatment with 0.75 µg/ml dilution of acridine orange and incubation at 37°C for 24 h.

<table>
<thead>
<tr>
<th>MDR strain</th>
<th>AUG (%)</th>
<th>OFL (%)</th>
<th>GEN (%)</th>
<th>NAL (%)</th>
<th>NIT (%)</th>
<th>COT (%)</th>
<th>AMX (%)</th>
<th>TET (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PA1</strong></td>
<td>Before</td>
<td>0.0</td>
<td>14.0</td>
<td>14.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>0.0 (0.0)</td>
<td>18.0 (28.5)</td>
<td>21.0 (50.0)</td>
<td>8.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>11.0 (0.0)</td>
<td>6.0 (0.0)</td>
</tr>
<tr>
<td><strong>PA2</strong></td>
<td>Before</td>
<td>0.0</td>
<td>20.0</td>
<td>13.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>0.0 (0.0)</td>
<td>26.0 (30.0)</td>
<td>17.0 (30.7)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td><strong>PA3</strong></td>
<td>Before</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td><strong>PA4</strong></td>
<td>Before</td>
<td>0.0</td>
<td>20.0</td>
<td>12.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>0.0 (0.0)</td>
<td>23.0 (15.0)</td>
<td>12.0 (0.0)</td>
<td>19.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td><strong>PA5</strong></td>
<td>Before</td>
<td>0.0</td>
<td>19.0</td>
<td>5.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>0.0 (0.0)</td>
<td>21.0 (10.5)</td>
<td>11.0 (120.0)</td>
<td>0.0 (0.0)</td>
<td>13.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
</tbody>
</table>

OFL: Ofloxacin; GEN: gentamicin; AUG: augmentin; NAL: nalidixic acid; NIT: nitrofurantoin; COT: cotrimoxazole; TET: tetracycline; AMX: amoxicillin.

MDR *P. aeruginosa* strain **PA5** (isolated from midstream urine) or the effect of the dilutions in RM reduction or otherwise, of **PA5** strain. Whereas 0.35 µg/ml acridine orange dilution caused a two-fold (5.0 µg) reduction in gentamicin MIC (which is 10 µg), 0.55 µg/ml dilution caused a four-fold (2.5 µg) reduction. Both 0.75 and 0.95 µg/ml acridine orange dilutions recorded no effect (no change) in the MIC of gentamicin.

In Table 8, the summary and distribution of 50.0% and above loss in RM is presented. *P. aeruginosa* strains **PA2** and **PA5** recorded 160.0 and 58.8% loss in RM, respectively to ofloxacin with mean loss of 54.7±37.6% after treatment with 0.55 µg/ml acridine orange. This was followed by mean loss of 52.1±18.6% to ofloxacin after treatment with 0.35 µg/ml acridine orange. Strains **PA1**, **PA3**, **PA4** and **PA5** recorded 50.0, 76.9, 83.3 and 180.0% loss in RM, respectively to gentamicin with mean loss of 97.6±28.3% after treatment with 0.55 µg/ml acridine orange.
Table 6. *P. aeruginosa* strains that showed ≤50.0% loss of their resistance markers after treatment with 0.95 μg/ml dilution of acridine orange and incubation at 37°C for 24 h.

<table>
<thead>
<tr>
<th>MDR strain</th>
<th>AUG (%)</th>
<th>OFL (%)</th>
<th>GEN (%)</th>
<th>NAL (%)</th>
<th>NIT (%)</th>
<th>COT (%)</th>
<th>AMX (%)</th>
<th>TET (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA&lt;sub&gt;1&lt;/sub&gt; Before</td>
<td>0.0</td>
<td>14.0</td>
<td>14.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>PA&lt;sub&gt;1&lt;/sub&gt; After</td>
<td>0.0 (0.0)</td>
<td>17.0 (21.4)</td>
<td>16.0 (14.2)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>PA&lt;sub&gt;2&lt;/sub&gt; Before</td>
<td>0.0</td>
<td>20.0</td>
<td>13.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>PA&lt;sub&gt;2&lt;/sub&gt; After</td>
<td>0.0 (0.0)</td>
<td>21.0 (5.0)</td>
<td>18.0 (38.4)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>PA&lt;sub&gt;3&lt;/sub&gt; Before</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>PA&lt;sub&gt;3&lt;/sub&gt; After</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>PA&lt;sub&gt;4&lt;/sub&gt; Before</td>
<td>0.0</td>
<td>20.0</td>
<td>12.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>PA&lt;sub&gt;4&lt;/sub&gt; After</td>
<td>0.0 (0.0)</td>
<td>20.0 (0.0)</td>
<td>13.0 (8.3)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>PA&lt;sub&gt;5&lt;/sub&gt; Before</td>
<td>0.0</td>
<td>19.0</td>
<td>5.0</td>
<td>17.0</td>
<td>0.0</td>
<td>0.0</td>
<td>12.0</td>
<td>0.0</td>
</tr>
<tr>
<td>PA&lt;sub&gt;5&lt;/sub&gt; After</td>
<td>0.0 (0.0)</td>
<td>26.0 (36.8)</td>
<td>11.0 (120.0)</td>
<td>21.0 (23.5)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>14.0 (16.6)</td>
<td>0.0 (0.0)</td>
</tr>
</tbody>
</table>

OFL: Ofloxacin; GEN: gentamicin; AUG: augmentin; NAL: nalidixic acid; NIT: nitrofurantoin; COT: cotrimoxazole; TET: tetracycline; AMX: amoxicillin.

Table 7. The effect of acridine orange dilutions on the minimum inhibitory concentration (MIC) of gentamicin on a MDR *P. aeruginosa* strain isolated from midstream urine after 24 h incubation at 37°C.

<table>
<thead>
<tr>
<th>Acridine orange dilution (µg/ml)</th>
<th>New MIC after AO treatment (µg)</th>
<th>80.0</th>
<th>40.0</th>
<th>20.0</th>
<th>10.0</th>
<th>5.0</th>
<th>2.5</th>
<th>1.25</th>
<th>0.63</th>
<th>0.33</th>
<th>Inoculum control</th>
<th>Sterile broth control</th>
<th>Drug control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.35</td>
<td>5.0 (2-fold)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.55</td>
<td>2.5 (4-fold)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.75</td>
<td>No change</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.95</td>
<td>No change</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Gentamicin was chosen for MIC Assay because it was one of the two antibiotics that recorded ≤50.0% reduction in resistance markers. PA<sub>5</sub> (*P. aeruginosa* isolated from midstream urine) was selected and used for MIC assay on the basis of organism that showed up to ≤50.0% reduction in resistance markers for all four dilutions.

Table 8. Distribution of ≤50.0% loss of resistance markers among multidrug resistant *P. aeruginosa* strains after treatment with 0.35, 0.55, 0.75 and 0.95 μg/ml dilutions of acridine orange.

<table>
<thead>
<tr>
<th>MDR strain</th>
<th>0.35%</th>
<th>0.55%</th>
<th>0.75%</th>
<th>0.95%</th>
<th>0.35%</th>
<th>0.55%</th>
<th>0.75%</th>
<th>0.95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA&lt;sub&gt;1&lt;/sub&gt;</td>
<td>78.5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>50.0</td>
<td>50.0</td>
<td>0.0</td>
</tr>
<tr>
<td>PA&lt;sub&gt;2&lt;/sub&gt;</td>
<td>80.0</td>
<td>160.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>61.5</td>
<td>76.9</td>
<td>0.0</td>
</tr>
<tr>
<td>PA&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>PA&lt;sub&gt;4&lt;/sub&gt;</td>
<td>50.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>58.3</td>
<td>83.3</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>PA&lt;sub&gt;5&lt;/sub&gt;</td>
<td>0.0</td>
<td>58.8</td>
<td>0.0</td>
<td>0.0</td>
<td>60.0</td>
<td>180.0</td>
<td>120.0</td>
<td>120.0</td>
</tr>
<tr>
<td>Mean±SE</td>
<td>52.1±18.6</td>
<td>54.7±37.6</td>
<td>0.0</td>
<td>0.0</td>
<td>45.0±15.0</td>
<td>97.6±28.3</td>
<td>42.5±12.1</td>
<td>30.0±16.1</td>
</tr>
</tbody>
</table>

orange. This was followed by mean loss of less than 50.0% to gentamicin after treatment with 0.75 μg/ml acridine orange. This was followed by mean losses of 45.0±15.0, 42.5±12.1 and 30.0±16.1% all to gentamicin after treatment with 0.35, 0.75 and 0.95 μg/ml, respectively.

The highest and lowest mean loss of RM therefore, were 97.6±28.3 and 30.0±16.1% after treatments with 0.55 and 0.95 μg/ml, respectively and both to gentamicin. There was no reduction in RM to ofloxacin after 0.75 and 0.95 μg/ml acridine orange treatments. In all, mean loss
of 50.0% or more of RM was recorded after treatment with 0.35 µg/ml (52.1±18.6%) and 0.55 µg/ml (97.6±28.3%) to gentamicin.

**DISCUSSION**

The antibiotics susceptibility profile of all five strains of *P. aeruginosa* before acridine orange treatment in this study showed that the five strains were sensitive to ofloxacin and gentamicin with mean±SE zones of inhibition of 14.6±9.5 and 8.4±4.4 mm, respectively. This means only 2 (25.0%) of the antibiotics recorded positive reactions when tested on the pathogenic organisms. By extension, this also implies that 6 (75.0%) of the antibiotics were completely resisted by the five *P. aeruginosa* strains. This is rather alarming as findings confirm the MDR nature of *P. aeruginosa* as reported by earlier authors (Radi and Rahman, 2010; Mukherjee et al., 2011). Findings in this study (in terms of antibiotic sensitivity profile) is disturbing because it ostensibly suggests that infections or diseases caused by MDR *P. aeruginosa* in the study environment can be treated successfully with only ofloxacin or gentamicin or a combination of both. This place the low income patients at a serious disadvantage as they may not be able to afford ofloxacin which is expensive and which showed almost twice sensitivity reaction compared with gentamicin.

Particularly worrisome, is the total resistance against augmentin, amoxicillin and cotrimoxazole because these drugs are used routinely to treat a myriad of human diseases. This same worry with particular reference to augmentin has been expressed by some authors (Oluuremi et al., 2011). It was not clear as to whether the site from where the pathogens were isolated had any direct or indirect effect on the antibiotograms of the strains as recorded in this study. However, it may be possible that pH changes or variations from site to site and presence/absence of oxygen could affect the response of *P. aeruginosa* (a strict aerobe) to relevant antibiotics it is exposed to *in vitro*.

A pathogen is MDR when it is resistant to three or more antibiotics at any given time (Jan et al., 2002). Based on results obtained in this study, *P. aeruginosa* strains isolated from ear swab, urethral swab, wound swab and midstream urine were each resistant to augmentin, nalidixic acid, nitrofurantoin, cotrimoxazole, amoxicillin and tetracycline (6 drugs) representing 75.0% of the antibiotics tested. Only strain PA3 (isolated from tracheal swab) resisted eight drugs representing 100.0% of antibiotics tested. This finding re-establishes the MDR nature of *P. aeruginosa* strains irrespective of the source or site from where they are isolated. The high prevalence of multiple antibiotic resistant *P. aeruginosa* strains in this study is a possible suggestion that very large population of *P. aeruginosa* organisms has been exposed to several antibiotics which is consistent with report of earlier studies(Oluuremi et al., 2011).

Acridine orange dilutions of 0.35, 0.55, 0.75, and 0.95 µg/ml were used to treat and cure the five pathogenic strains of *P. aeruginosa* with the intent of reducing their RM significantly or eliminating them completely. The loss of 50 to 100% of RM after treatment with acridine orange subclinical dilutions of 0.35, 0.55, 0.75 and 0.95 µg/ml was used as the basis of establishing the curing effects of these dilutions. The use of 50% and above loss in RM as a criterion to determine the extent of plasmid curing was according to the scheme provided by Akortha et al. (2011). Stanier et al. (1984) reported that the elimination of plasmids by dyes and other agents reflects the ability of such agents to inhibit plasmid replication at a concentration that does not affect the chromosome.

It has been reported that acridine orange and ethidium bromide are better curing agents for *P. aeruginosa* than sodium dodecyl sulphate (SDS) and elevated temperature (Kumar and Surendran, 2006). Earlier studies reported no effect of acridine orange on plasmids of *P. aeruginosa* (Pattnakik et al., 1995; Al-Amir et al., 1999). Reports of some other earlier studies stated a reversion in the resistance of some multiple drug resistant strains following exposure to acridine orange (Naomi, 1978; Darini, 1996; Adeleke and Odetola, 1997).

*P. aeruginosa* strain PA1 (isolated from ear swab) recorded 78.5% loss of RM to ofloxacin after 0.35 µg/ml treatment with acridine orange. For the same strain, there was zero loss of RM to ofloxacin after 0.55, 0.75 and 0.95 µg/ml treatments with the agent. This somewhat implies that 0.35 µg/ml is a choice dilution of the agent which may enhance antibiotic sensitivity of this MDR *P. aeruginosa* strain of ofloxacin.

In case of strain PA2 (isolated from urethral swab), RM losses of 80.0 and 160.0% to ofloxacin were recorded after 0.35 and 0.55 acridine orange treatments, respectively. Strain PA4 (isolated from wound swab) recorded 50.0% loss of RM to ofloxacin after 0.35 µg/ml treatment with the curing agent. There was zero loss of RM to ofloxacin by strain PA5 with 0.35 µg/ml treatment and 58.8% loss of RM to the same drug after 0.55 µg/ml acridine orange treatment. The mean±SE loss of RM of 52.1±18.6 and 54.7±37.6% to ofloxacin by all the MDR *P. aeruginosa* strains after 0.35 and 0.55 µg/ml acridine orange treatments, respectively, tends to suggest potency of either or both dilutions in the enhancement of sensitivity to ofloxacin in the course of treatment. In a related study, Otajewuo (2012) reported 0.35, 0.85 and 0.95 µg/ml dilutions of homodium (ethidium) bromide as significant enhancers of a multidrug uropathogenic *Escherichia coli* strain to some selected antibiotics.

Some authors have used dilutions of a non-antibiotic compound-thioridazine to reduce RM in some strains of *P. aeruginosa* (Mukherjee et al., 2011). Loss of RM to gentamicin by strains PA1, PA2, PA4 and PA5 after 0.35 µg/ml acridine orange treatment was recorded as 0.0, 61.5, 58.3 and 60.0%, respectively with a less than 50.0% mean±SE dilution (that is 45.0±15.0%). After 0.55 µg/ml treatment with acridine orange, PA1, PA2, PA4 and PA5
strains showed loss of RM of 50.0, 76.9, 83.3 and 180.0%, respectively, with a mean±SE percentage loss of 97.6±28.3. Acridine orange dilutions of 0.75 and 0.95 µg/ml recorded less than 45.0 and 35.0% loss of RM, respectively. These findings also suggest 0.55 µg/ml dilution and to a lesser extent, 0.35 µg/ml as potential enhancers of antibiotic sensitivity in MDR P. aeruginosa pathogens.

Sensitivity enhancement effect of the subclinical acridine orange dilutions on the minimum inhibitory concentration (MIC) of gentamicin as it affected MDR P. aeruginosa strain (isolated from midstream urine) showed a two-fold (5.0 µg) and four-fold (2.5 µg) reductions in MIC of gentamicin as recorded for 0.35 and 0.55 µg/ml acridine orange dilutions, respectively. Some authors had reported similar findings on MDR Staphylococcus aureus strains (Otajevwo and Momoh, 2013). A fast and accurate determination of MIC can ensure optimal effective treatment of patients while at the same time avoiding overprescription. This will save money for health care providers as well as reduce development of resistance (NCCLS, 2000; McGowan and Wise, 2001).

In this study, the MIC of gentamicin (an aminoglycoside) which is 10 µg (based on long standing research) was reduced to 5 µg (two fold reduction) and 2.5 µg (four fold reduction) by acridine orange subclinical dilutions of 0.35 and 0.55 µg/ml, respectively as tested on a multiple resistant drug strain of P. aeruginosa isolated from the urinary tract of a patient. A similar report had been made by an author which stated that 0.35, 0.45, 0.75, 0.85 and 0.95 µg/ml dilutions of homodium bromide reduced the MIC of gentamicin to 2.5, 5, 5.25 and 2.5 µg, respectively when tested on an MDR strain of uropathogenic E. coli (Otajevwo, 2012). The implication of findings in this study is that when doses of either 0.35 or 0.55 µg/ml or both are incorporated into the manufacture of gentamicin or any other related aminoglycoside, and then administered to a patient diagnosed to be suffering from a disease caused by any MDR P. aeruginosa strain, a better result in terms of outcome (cure of the disease) may be achieved as it will require four times its concentration to function in vivo. In a related work, Kohler (2010) showed that the resistance of P. aeruginosa to tetracycline efflux was reduced from MIC of 0.032 to 0.004 µg/ml (eight-fold reduction) by treatment with phenothiazine. Crowle et al. (1992) demonstrated that non-toxic concentrations of phenothiazine in the lungs achieved complete elimination of Mycobacterium tuberculosis. In a related study, some workers had reported the capacity of an aqueous methanolic plant extract-epidiosbulbin - E- Acetate (EEA) to decrease the MIC of antibiotics against MDR bacteria thus making antibiotic treatment more effective (Shiram et al., 2008).

Conclusion

All five MDR P. aeruginosa strains used in this study showed 100.0% resistance against augmentin, nalidixic acid, nitrofurantoin, cotrimoxazole, amoxicillin and tetracycline. Fortunately, all five MDR (but one, PA3) strains showed sensitivity to ofloxacin and gentamicin. This finding implies that augmentin and amoxicillin for example, which are used routinely to treat a gamut of human diseases may not yield good result in terms of curing human diseases due to MDR P. aeruginosa and therefore, their routine prescription should be discouraged. Sensitivity to ofloxacin and gentamicin on the contrary, suggests that either of the two drugs or a combination of both may prove successful in tackling human diseases due to MDR P. aeruginosa. Four of the five strains were each resistant to 6 (six) of the antibiotics tested and this qualifies them as multiple drug resistant organisms. This finding re-establishes the MDR nature of P. aeruginosa strains irrespective of source or site they are isolated from. The high prevalence of multiple antibiotic resistant P. aeruginosa strains in this study therefore is a possible suggestion that very large population of P. aeruginosa organisms has been exposed to several antibiotics (that is, drug abuse). Symptomatic and asymptomatic patients are advised therefore to desist from self medication for any justifiable reason. They should ensure drugs are taken based on prescription by a qualified doctor after proper evaluation.

Acridine orange of 0.35 and 0.55 µg/ml recorded loss of RM of 52.1±18.6 and 54.7±37.6% to ofloxacin, respectively and then loss of RM of 45.0±15.0 and 97.6±28.3% to gentamicin, respectively. This is suggestive of the potency of either or both dilutions in the enhancement of sensitivity to ofloxacin and gentamicin in the course of therapy in vivo as they may be able to eliminate drug resistance plasmids.

Finally, acridine orange dilutions of 0.35 and 0.55 µg/ml recorded two- and four-fold reductions in MIC of gentamicin, respectively when tested on a MDR strain of P. aeruginosa. Again, the aforementioned dilution if incorporated into drug regimens may produce better effect and results in terms of cure of a disease which will require four times the concentration of the new MICs to function in vivo. A decrease in the MIC of an antibiotic will make the particular antibiotic more effective in treatment of a Pseudomonas disease.

SUGGESTIONS FOR FURTHER STUDIES

The determination of resistance loss by MDR P. aeruginosa strains after treatments with 0.35, 0.55, 0.75 and 0.95 µg/ml acridine orange dilutions may not be totally convincing. It is recommended therefore, that the effects of these dilutions in the pathogens at the molecular level be probed further by carrying out plasmid profiling before and after treatment to see at what dilution(s) there is partial or total elimination of drug resistance “R” plasmid bands.
REFERENCES


Risk factors for common cancers in Nigeria: Knowledge, attitudes and practice among secondary school students in Kaduna, Nigeria

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Cancer is an important cause of morbidity and mortality worldwide and is increasingly becoming a major public health issue in developing countries including Nigeria. The objective of this study was to assess the knowledge, attitudes and practice of cancer risk factors among secondary school students with the aim of promoting healthy lifestyles. A structured self questionnaire was administered to 405 senior secondary school students who consented to participate. Data obtained were analysed using MINITAB and Statistical Package for Social Sciences (SPSS) statistical packages. A significant proportion (27.9%) did not know that cancer can result from habits learned in youth and there was poor knowledge about sexually related risk factors (early sexual exposure, repeated sexually transmitted infections, promiscuity). There was inaccurate knowledge about causes of cancer with 18.8% believing that cancer is caused by evil spirits. The commonest cancer risk factors practiced by the students were early sexual exposure (11.6%), smoking (9.6%), alcohol ingestion (6.9%), and promiscuity (6.4%). There is room for improvement of knowledge and attitudes about cancer risk factors among adolescents in order to minimize adoption of risky lifestyles. There is a need to educate adolescents on cancer risk factors and integrate promotion of healthy lifestyles in health-related activities targeted at young people.

Key words: Cancer risks, factors, lifestyles, adolescents, Kaduna.

INTRODUCTION

Cancer is an important cause of morbidity and mortality worldwide and is increasingly becoming a major public health issue in developing countries including Nigeria. Cancer morbidity and mortality are increased by human immunodeficiency virus (HIV) which is associated with higher incidences of various types of cancer. The most common types in Nigeria include cancer of the cervix and breast among women and cancer of the urinary bladder and prostate among men, while malignant lymphoma is common in both sexes. (Afolayan, 2004).

The risk factors for various types of cancer are well documented and include many habits or lifestyles which are commonly adopted in youth. General risk factors for cancer include a diet high in saturated fat and low in fresh fruit and vegetables, physical inactivity, tobacco use and alcohol consumption especially in excessive quantities. (National Cancer Institute, 2007; Unwin and Alberti, 2006). Specific risk factors for various cancers include tobacco use including smoking and chewing tobacco for cancers of the lung, mouth, bladder, cervix, oesophagus, pancreas, kidney and larynx; skin bleaching for skin cancer; alcohol consumption for cancers of the liver, breast, oesophagus, mouth and throat; poor diet especially low roughage and high fat diets for cancers of

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the colon, uterus and prostate; exposure to radiation or harmful chemicals for leukemia, and cancers of the thyroid, breast, lung, skin and stomach; some viruses and bacteria are also risk factors for cancer including human papilloma virus for cervical cancer, hepatitis B or C for liver cancer, human T-cell leukemia/lymphoma virus for leukemia or lymphoma, and helicobacter pylori for stomach cancer or lymphoma in the stomach; oestrogens used for hormone replacement therapy may increase the of breast cancer; and genetic predispositions as occur with cancers of the breast, ovary, prostate and colon and melanomas (National Cancer Institute, 2007). Recreational/hard drugs have also been implicated in causation of cancer e.g. marijuana and lung cancer (Han et al., 2010), cocaine, amphetamines (and a few other drugs) and non-Hodgkin’s Lymphoma (Nelson et al., 1997). There has also been a report that cocaine and marijuana use by parents significantly increases the risk of rhabdomyosarcoma in their children (Grufferman et al., 1993).

Other risk factors include early sexual exposure, multiple sex partners and repeated or poorly treated sexually transmitted infections which increase the risk of developing cervical cancer (Emembolu and Ekwempu, 1988). Promiscuity is also a risk factor for HIV which increases the risk of developing various types of cancer in both men and women. Early menarche and late menopause, low parity, late age at first full term pregnancy and non-breastfeeding are important risk factors for breast cancer (Okobia and Bunker, 2005; Okobia et al., 2006). Although cigarette smoking is the main risk factor for bladder cancer, it is also associated with schistosoma haematobium infestation (Jankovic and Radosavljevic, 2007) which is endemic in some parts of Nigeria (Ochicha et al., 2003).

Most of the risk factors for common cancers in Nigeria are avoidable habits or lifestyles which are commonly adopted during adolescence. This study was carried out to assess the knowledge, attitudes and practice of adolescents in a secondary school in Nigeria with regard to cancer risk factor. There is anecdotal evidence that risky lifestyles are increasingly being adopted by youth in the country and the study aimed to document this in the study population, with a view to minimizing the problem thus reducing risk of cancers. This information will provide a guide for developing health promotion and education activities targeted at young people with a view to preventing cancers later in life and promoting healthy lifestyles.

### METHODOLOGY

A structured questionnaire was administered to senior secondary school (4 to 6th year) students in Kaduna, Northern Nigeria by one of their teachers between September 2006 and March 2007. The school authority had given permission for the study to be carried out. The sample was one of convenience and only students who had consented to take part in the study were included. Information was obtained about their age and other biosocial characteristics, their knowledge about, and attitudes towards cancer and cancer risk factors, and their practice of any cancer risk factors. Rates and comparative analyses including student’s t-tests and \( \chi^2 \) tests were carried out on the data obtained using MINITAB and Statistical Package for Social Sciences (SPSS) statistical software packages. Statistical tests were two-sided and an association was considered statistically significant with \( p \) values of \(< 0.05\).

### RESULTS

The total number of questionnaires that were distributed was 500, out of which 405 (81%) were completed and returned. The mean age of the students was 16.22 years with a range of 12 to 22 years, and a standard deviation of 1.43. They were all senior secondary school students; 153 (38.1%) were in their 4th year, 152 (37.8%) in their 5th year, 97 (24.1%) were in their 6th (final) year while 3 did not respond to the question. There were 126 girls (33.3%) and 253 boys (66.8%).

### Knowledge and attitude

Only 2.7% said they had never heard of cancer. Table 1 shows the general knowledge about cancer among the students.

There was no significant difference in knowledge between boys and girls in terms of having ever heard of cancer, knowledge that cancer is not restricted to any race, and knowledge that habits learned in youth can lead to cancer later in life. However, more girls (3.2% compared to 0.8% of boys) did not know that cancer is not just a disease of old people only \( (\chi^2 = 6.4, p = 0.04) \), and more girls (9.2% compared to 0.8% of boys) did not know that cancer can occur in any age group including babies \( (\chi^2 = 10.55, p = <0.01) \). Similarly, there was no
significant knowledge between students in the different classes in terms of having ever heard of cancer, knowledge that cancer is not just a disease of old people and knowledge that cancer is not restricted to any race. However, knowledge that cancer can affect any age group including babies was significantly higher ($\chi^2 = 6.6$, $p = 0.04$) in the higher classes (77.7% in 6th year, 65.1% in 5th year, 62.3% in 4th year). Knowledge that habits learned in youth can lead to cancer later in life was also significantly higher ($\chi^2 = 10.34$, $p = <0.01$) in the higher classes (82.8% in 6th year, 73.7% in 5th year, 64% in 4th year).

There was no significant association between mean age of the students and their general knowledge about cancer and their knowledge of cancer risk factors.

The occupation of the students’ parents was medical (doctor, pharmacist, nurse) among 15 (4%) of the fathers and 37 of the mothers (9.7%). Knowledge of about cancer and cancer risk factors was not significantly associated with the father’s occupation or the mother’s occupation (medical or non-medical). The knowledge of risk factors for common cancers in Nigeria among the students is as shown in Figure 1.

Majority of the students (46.7%) knew that cancer was not caused by evil spirits but many (34.5%) did not know whether or not evil spirits were involved in the aetiology of cancer while some (18.8%) felt that evil spirits cause cancer.

Many of the students (51.9%) said they knew someone who had cancer and the commonest types of cancer mentioned included those of the breast (59.5%), lungs (18.5%), skin (8.3%), and leukaemia (4.9%). Others included cancers affecting the liver, brain, kidney (1% each), bone, leg, scrotum, intestine, stomach, vagina, ear (0.5% each), heart (1.5%), and umbilicus (0.5%). One student (0.5%) also named fibroid as a type of cancer.

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**Figure 1.** Knowledge of cancer risk factors.
Knowing a cancer victim was not significantly associated with the sex or class of the student.

Many of those who said they knew someone who had cancer said they did not know what caused it (30.6%). Figure 2 shows the perceived causes of breast cancer which was the most commonest type mentioned by students who said they knew someone who had cancer.

The perceived causes of other types of cancer included menstruation and adultery for vaginal cancer, transfusion of infected blood for leukaemia, radiation from mobile phones and use of sharp objects in the ear for ear cancer, excess salt intake and ingestion of maggi (bullion cubes) for stomach and intestinal cancer, and using an affected person’s clothes for skin cancer. Skin bleaching, smoking and excessive alcohol ingestion were correctly perceived to cause cancer in the skin, lungs and liver, respectively.

Majority of the students (89.5%) felt that it was possible to minimize the risk of cancer by making the right lifestyle choices, while 3.6% felt that this was not possible and 6.9% did not know. The attitude towards lifestyle choices was not significantly associated with the sex or class of the student.

Some students (22.9%) believed that cancers have no treatment while others (22.1%) did not know, but the majority (55%) knew that cancers can be treated. Significantly, more boys (59.3%) compared to girls (46.6%) knew that cancers can be treated ($\chi^2 = 6.9, p = 0.03$). More students in the 4th year (29.6%) and the 5th year (23.3%) believed that cancers have no treatment compared to those in the 6th year (11.7%) with these differences being statistically significant ($\chi^2 = 10.3, p \leq 0.01$).

Majority (81.6%) agreed with the statement that cancer treatment is more likely to succeed if started early in the course of the disease while others (13.5%) unsure, and a few (4.9%) felt that the statement was not true. There was no significant difference between boys and girls in terms of knowledge that cancer treatment is more likely to succeed if started early. More students in the 6th year (95.7%) agreed that cancer treatment is more likely to succeed if started early compared to those in the 5th year (80.5%) and the 4th year (74.5%) with these differences being statistically significant ($\chi^2 = 18.5, p \leq 0.01$).

The mean age of students who felt that cancer treatment was more likely to succeed if started early in the course of the disease was significantly higher than the mean age of those who did not agree with this statement. There was no association between mean age of the students and other attitudes towards cancer as shown in Table 2.

Some students (24.5%) felt that they could indulge in...
cancer risk factors, because they knew people who indulged in some of the things that are said to cause cancer and yet did not have cancer; some (16.8%) were neutral; while majority (58.7%) disagreed. This attitude was not significantly associated with sex or class of the student. Many students (47.4%) felt that one only needs to be careful and avoid cancer risk factors, if someone in their family had had cancer while some were neutral (18.9%) and others (33.8%) disagreed with this opinion. This attitude was not significantly associated with the sex of the student but was significantly associated with the class ($\chi^2 = 15.3$, $p \leq 0.001$) with less 6th year students (33%) agreeing with this opinion than 5th year (44.6%) and 4th year students (58.4%).

Significantly more girls knew that skin bleaching (83.6% compared to 64.80% of boys), early sexual exposure (25.2% compared to 20% of boys), and promiscuity/multiple sex partners (30.5% compared to 26.6% of boys) are risk factors for cancer while more girls did not know that repeated or poorly treated sexually transmissible infections (49.2% compared to 37.5% of boys) are risk factors for cancer. Knowledge that smoking (97.4, 96.9 and 88.2% of 6, 5 and 4th year students, respectively), chewing tobacco (87.8, 75.8, and 68.2% of 6, 5 and 4th year students, respectively), and exposure to chemicals and radiation (79.4, 61, and 55.3% of 6, 5 and 4th year students, respectively) are risk factors for cancer, and was significantly higher with higher classes. Details are shown in Table 3.

### Practice

The most common cancer risk factors practiced by the students are early sexual exposure, smoking, alcohol ingestion and promiscuity/multiple sex partners as shown in Table 4.

The mean age at first sexual intercourse was 12.9 years with a range of 10 to 19 years, and a standard deviation of 2.5. There was a slight difference in the mean age at first sexual exposure for boys (12.9 years) compared

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### Table 3. Association between student's sex and class, and knowledge of cancer risk factors.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Student's sex</th>
<th>Student's class</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\chi^2$ value</td>
<td>p-value</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.3</td>
<td>0.52</td>
</tr>
<tr>
<td>Chewing tobacco</td>
<td>2.4</td>
<td>0.31</td>
</tr>
<tr>
<td>Alcohol</td>
<td>2.3</td>
<td>0.31</td>
</tr>
<tr>
<td>Hard drugs</td>
<td>2.1</td>
<td>0.35</td>
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<tr>
<td>Skin bleaching</td>
<td>16.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Early sexual exposure</td>
<td>10.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Promiscuity</td>
<td>15.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Repeated/Poorly treated STIs</td>
<td>7.8</td>
<td>0.02</td>
</tr>
<tr>
<td>Poor diet</td>
<td>0.9</td>
<td>0.63</td>
</tr>
<tr>
<td>Exposure to chemicals or radiation</td>
<td>3.6</td>
<td>0.16</td>
</tr>
<tr>
<td>Not breastfeeding</td>
<td>5.7</td>
<td>0.06</td>
</tr>
<tr>
<td>Inheritance</td>
<td>2.6</td>
<td>0.27</td>
</tr>
<tr>
<td>Evil spirits</td>
<td>2.7</td>
<td>0.27</td>
</tr>
<tr>
<td>No cause</td>
<td>5.6</td>
<td>0.06</td>
</tr>
</tbody>
</table>

### Table 4. Practice of cancer risk factors among students and duration of such practices.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Number (%)</th>
<th>Mean duration in years (standard deviation)</th>
<th>Range (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td>39 (9.6)</td>
<td>3.38 (2.1)</td>
<td>0.3 – 8.0</td>
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<tr>
<td>Chewing tobacco</td>
<td>5 (1.2)</td>
<td>0.5 (*)</td>
<td>0.50 – 0.5</td>
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<tr>
<td>Alcohol ingestion</td>
<td>28 (6.9)</td>
<td>2.64 (1.6)</td>
<td>0.3 – 6.0</td>
</tr>
<tr>
<td>Hard drugs</td>
<td>9 (2.2)</td>
<td>3.40 (1.5)</td>
<td>2.0 – 5.0</td>
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<tr>
<td>Skin bleaching</td>
<td>5 (1.2)</td>
<td>2.04 (2.8)</td>
<td>0.1 – 4.0</td>
</tr>
<tr>
<td>Early sexual exposure</td>
<td>47 (11.6)</td>
<td>4.58 (2.4)</td>
<td>5.0 – 11.0</td>
</tr>
<tr>
<td>Promiscuity/multiple sex partners</td>
<td>26 (6.4)</td>
<td>4.60 (2.5)</td>
<td>0.5 – 11.0</td>
</tr>
<tr>
<td>Repeated/poorly treated STIs</td>
<td>2 (0.5)</td>
<td>1.00 (0)</td>
<td>1.0 – 1.0</td>
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</tbody>
</table>

*Only one student gave the duration of this practice so standard deviation could not be calculated.*
CI knowledge of cancer risk factors or lightly smoking 5.

- The number of sexual partners was 4.1 with a 5.

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students in the higher classes who may have already adopted risky habits or lifestyles. Similarly, knowledge of cancer risk factors was significantly higher among students in higher classes. It is important that students be given information about health-promoting habits and lifestyles at early stages before they make their choices.

There was poor knowledge that early sexual exposure, multiple sex partners, and repeated/poorly treated sexually transmitted infections are cancer risk factors. This is an important gap as cervical cancer is the leading cancer among Nigerian women (Afolayan, 2004) and one of the ways this disease can be prevented is through safer sexual practices. Majority of the students also did not know that cancer can run in families and this is important as even those who are at higher risk due to genetic predisposition may not make the right choices, thus further increasing their risk of developing cancer. More girls knew that skin bleaching, early sexual exposure and promiscuity are cancer risk factors as compared to boys probably resulting from deliberate targeting of women and girls with such information. It is important to include men and boys as target audiences for such information as these risk factors affect both sexes as the men/boys are involved in transmission of sexually transmitted infections and in the use of skin bleaching agents.

Inaccurate knowledge about the aetiology of cancer was common, with many students even believing that cancer is caused by evil spirits (18.8%). Even among those who knew cancer victims, knowledge of its causation was largely inaccurate. However, majority of the students felt that the risk of cancer can be minimized by making the right lifestyle choices. This attitude may form a basis for promotion of healthy lifestyles among these students if they are given accurate information. Accurate information will also help to change the attitude that some students had about indulging in cancer risk factors, because they knew people who indulged in some of the things that are said to cause cancer and yet did not have cancer or that one only needs to be careful and avoid cancer risk factors if someone in their family had had cancer.

The commonest cancer risk factors practiced by the students were early sexual exposure (11.6%), smoking (9.6%), alcohol ingestion (6.9%), and promiscuity (6.4%). These are of concern as they are risk factors for development of cervical cancer, breast cancer, prostate cancer and liver cancer which are the common cancers in the country.

Older students, students in higher classes and boys were significantly more likely to be involved in habits or lifestyles that increase their risk of developing cancer later in life such as smoking, alcohol ingestion, early sexual exposure and sexual promiscuity. This highlights the need to provide information as early as possible to prevent adoption of these risky lifestyles. Previous studies have reported healthier lifestyles among people who had more knowledge of the risks associated with certain lifestyles (Brown et al., 2006; Fiala and Brazdova, 1996; Schinke et al., 1996).

About 18% of the students reported being sexually active with more boys reporting that they were sexually active. Early age at first sexual exposure and having multiple sexual partners, reported by some students, are important risk factors for development of cervical cancer later in life. Similarly, there is an increased risk of contracting HIV with both early sexual exposure and multiple sexual partners and this may result in development of various types of cancer (National Cancer Institute, 2007).

Secondary school students are increasingly being targeted for health education and promotion activities in the efforts to prevent HIV infection and such activities can be modified to include information on how to live healthy lives and avoid cancer risk factors as much as possible in order to minimize the risk of cancer later in later in life. Adolescents and young adults should be given the opportunity to make informed choices about their life-styles and their health. There is also a need to educate parents, teachers youth leaders and other community leaders on these issues so that they can provide adequate support and information to these young people. School health clubs (consisting of teachers and students), religious organizations, youth-friendly health services and the media, all have important roles to play in promoting healthy lifestyles.

REFERENCES


An insight review on immunopathogenesis of bovine and human mycobacteria infections

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Mycobacterium is one of the first infectious agents to spring to mind in connection with chronic or persistent infections. The causative organism of bovine tuberculosis is Mycobacterium bovis (M. bovis), a member of the Mycobacterium tuberculosis complex (MTBC), which includes Mycobacterium tuberculosis (M. tuberculosis), M. bovis, Mycobacterium africanum (M. africanum), Mycobacterium microti (M. microti), Mycobacterium canetti (M. canetti), Mycobacterium caprae (M. caprae) and Mycobacterium pinnipedii (M. pinnipedii), and many of the species and subspecies of MTBC show specific host association. Immunity against mycobacteria is multifactorial and it is believed that the host innate immunity provides initial resistance to mycobacteria before the adaptive cell-mediated immunity fully develops. There are still many unsolved problems associated with the pathogenesis and immune response to tuberculosis. Therefore multi-disciplinary approach to develop more complete understanding of the pathogenic strategies is mandatory. Special consideration to bovine tuberculosis might help scientists to devise proper mechanisms to prevent human tuberculosis as they are closely related.

Key words: Granuloma, immune evasion, immunity, mycobacteria, pathogenesis.

INTRODUCTION

Tuberculosis (TB) remains a major cause of mortality and morbidity worldwide. Currently, a third of the world’s population is infected with Mycobacterium tuberculosis, the causative agent of TB, and annually there are 10 million new cases of clinical TB and approximately 2 million deaths. TB kills more individuals each year than any other bacterial pathogen, and alarmingly, current control practices have not been able to significantly reduce the incidence over the past 15 years (World Health Organization (WHO), 2010). The global incidence rate of TB per capita fell at a rate of 2.2% between 2010 and 2011 (WHO, 2012), with Sub-Saharan Africa displaying the highest annual risk of infection, probably catalyzed by the human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) pandemic (Corbett et al., 2003).

The causative organism of bovine tuberculosis is Mycobacterium bovis, a member of the M. tuberculosis complex (MTBC), which includes M. tuberculosis, M. bovis, Mycobacterium africanum, Mycobacterium microti, Mycobacterium canetti, Mycobacterium caprae and Mycobacterium pinnipedii, and many of the species and subspecies of MTBC show specific host association (Smith et al., 2006). The most notable member of the

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complex is \textit{M. tuberculosis}, the most important bacterial pathogen of human. In contrast to \textit{M. tuberculosis} which is largely host restricted to humans, \textit{M. bovis} is primarily maintained in bovine, in particular, domesticated cattle, although the pathogen can frequently be recovered from other mammals, including humans (Smith et al., 2006).

In developing countries, the conditions for \textit{M. bovis} transmission to humans not only exist unchanged, but the human population has a greater vulnerability due to poverty, HIV and reduced access to health care (Ayele et al., 2004). Bovine TB has been reduced/eliminated from domestic cattle in many developed countries by the application of a test-and-cull policy that moves (Amanfu, 2006; Thoen et al., 2006). In Africa, although bovine TB is known to be common in both cattle and wildlife, control policies have not been enforced in many countries due to cost implications, lack of capacity, and infrastructure limitations (Amanfu, 2006; Renwick et al., 2007).

Pathogenesis of human and bovine tuberculosis occurs in a similar way, beginning with bacterial entry to host lungs by inhalation and bacteria phagocytosis by alveolar macrophages. Establishment of a chronic infection status is accomplished due to mycobacterial virulence factors that allow it to enter and survive within the host phagocytic cells. It is well known that macrophages play an important role in tuberculosis pathogenesis, being the first defense line, the niche for the bacteria and the main control mechanism (Uziel et al., 2011). When disease develops, the associated granulomatous pathological changes are seen mainly in the lower and upper respiratory tract and because of this pattern, it is considered that infection most often follows aerosol exposure to \textit{M. bovis} (Neill et al., 2001). Modeling of bovine tuberculosis believed to help a lot in the production of effective vaccine for human (Van Rhijn et al., 2008). Therefore this review highlights on the immunopathology of bovine and human mycobacteria infections.

**NATURE OF MYCOBACTERIA**

According to the latest list of bacterial names with standing in nomenclature, there are more than 100 recognized species in the genus \textit{Mycobacterium} (Euzéby, 2004). A number of species of mycobacteria are important pathogens of animals or humans. Human tuberculosis is chiefly associated with infection with the species \textit{M. tuberculosis}, although \textit{M. africanaum} is also important in some regions. Bovine tuberculosis is caused by intracellular infection with the acid-fast bacterium, \textit{M. bovis} (Pollock et al., 2005). In cattle, exposure to this organism can result in a chronic disease that jeopardizes animal welfare and productivity, and in some countries leads to significant economic losses (Pollock and Neill, 2002). \textit{M. tuberculosis}, \textit{M. bovis}, \textit{M. africanaum}, \textit{M. canetti} and \textit{M. pinnipedii} together with \textit{M. microti} (associated with infection of rodents) form a very closely related phylogenetic group and may be referred to collectively as the \textit{M. tuberculosis} complex (MTBC). Human infection with members of the MTBC produces an indistinguishable clinical picture and the individual species cannot be distinguished from each other based on microscopic examination of stained tissues or other clinical specimens (Annon, 2003).

**Virulence factors**

Early secretory antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10) are potent IFN-\(\gamma\) inducing antigens of tuberculous mycobacteria. These two proteins are co-secreted and form a tight 1:1 complex upon export (Renshaw et al., 2002). Genes for ESAT-6 and CFP-10 are absent in many environmental, non-tuberculous mycobacteria as well as in the TB vaccine strain, \textit{M. bovis} bacille calmette guerin (BCG). Use of ESAT-6 and/or CFP-10 as antigens in IFN-\(\gamma\)-based TB assays enhances specificity when compared to use of \textit{M. bovis} purified protein derivative (PPD) (Buddle et al., 2003). ESAT-6 has also been used to discriminate between cattle naturally infected with \textit{M. bovis} and cattle sensitized/injected with environmental, non-tuberculous strains of mycobacterial or vaccinated for paratuberculosis.

**Route of infection and lesion distribution**

With some exceptions, it is agreed that cattle become infected with \textit{M. bovis} by either oral or respiratory routes. The oral route is likely most important in calves nursing tuberculous cows. In the late 1990’s, surveys of tuberculous cattle in Great Britain (Phillips et al., 2003) revealed that 67% of tuberculous lesions were within the lungs and pulmonary lymph nodes (tracheobronchial and mediastinal). Although many studies demonstrate a tendency toward lesion development in pulmonary lymph nodes rather than in lungs, meticulous examination often reveals lesions in the lungs; most < 1 cm in diameter.

**IMMUNE RESPONSES TO MYCOBACTERIAL INFECTION**

Immunity against mycobacteria is multifactorial and dependent on the balance between an inflammatory response that allows the host to develop a granuloma, which contains the microorganism and an anti-inflammatory response that restricts the extent of the granuloma and allows contact of effectors T-cells with the infected cells resulting in the killing of the infecting pathogen.
(Villa assembling-Ramos et al., 2003).

Innate immunity

It is believed that the host innate immunity provides the initial resistance to infections with intracellular pathogens before the adaptive type 1 cell-mediated immunity fully develops. The major cellular components involved in innate immunity include phagocytes, macrophages, neutrophils, dendritic cells (DCs), natural killer (NK) cells, γδ T cells, and soluble mediators released by these cells serve as a linker to cell-mediated immunity (Wolf et al., 2008). Macrophages and DCs have been suggested as important in inducing the immune response, though they are likely to have different roles in immunity for killing or T cell stimulation, respectively (Hope et al., 2004).

Macrophages

Classical activation of macrophages is well known since 1964 and demonstrated that M. bovis BCG or Listeria monocytogenes infection in a mouse model increased macrophage microbicidal activity in a stimulus dependent manner, but not antigen specific (Uziel et al., 2011). Alveolar macrophages resident within the lung are considered to be the main cellular host for mycobacteria in vivo and the major role of these cells is the rapid killing of the invading organism. This is due to the release of toxic reactive oxygen and nitrogen intermediates or killing by lysosomal enzymes following fusion with the bacterial phagosome. The type of receptor that is engaged by the bacteria can influence the response generated within the macrophage (Liebana et al., 2000).

The receptor molecules that have been implicated in the uptake of mycobacteria include mannose receptors that bind mannosylated molecules on the bacterial surface, Fc receptors binding opsonised cells and complement receptors. The use of complement receptor three (CR3) by Mycobacterial species may be advantageous for the bacterium, as triggering this receptor does not induce the release of potentially cytotoxic reactive oxygen intermediates (Liebana et al., 2000). Binding to the mannose receptor has also been suggested as a possible safe route of entry for mycobacteria that facilitate their intracellular survival. Following uptake into the phagosome, phago-lysosome fusion occurs followed by the destruction of bacteria and the processing and presentation of bacterial antigens to T cells in the context of MHC molecules.

The stimulation of T cells in this way activates the adaptive arm of the immune response and induces IFN-γ release and CD8+ T cell cytolytic capacity that can further enhance the anti-microbial defence system (Lopez et al., 2003). Effects on cytokine synthesis and expression of molecules on the cell surface of macrophages have been reported in some studies in the interaction of mycobacteria and antigen presenting cells. Macrophages infected with M. tuberculosis preferentially secrete pro-inflammatory cytokines including TNF-α, IL-1 and IL-6 (Giacomini et al., 2001; Hickman et al., 2002). Infected macrophages are known also to secrete chemokines including IL-8, RANTES and MCP-1 which would aid the recruitment of lymphocytes to the lung and granuloma formation, thus leading to containment of the mycobacteria (Peters and Ernst, 2003).

In addition M. tuberculosis infected macrophages secrete IL-10, rather than IL-12, which could act to suppress Th1 responses (Giacomini et al., 2001; Hickman et al., 2002). IL-10 may also inhibit export of MHC class II molecules to the cell surface, which would, in turn, down-regulate T cell responses. Reduced MHC class II expression in M. tuberculosis infected macrophages has been reported and proposed to be linked to stimulation of TLR2 by mycobacterial lipopeptides (Noss et al., 2001). A reduced ability of these cells to signal T lymphocyte activation combined with recruitment of cells to form granulomas may help mycobacterial persistence within the host. However it is known that stimulation of macrophages by other components of the immune response, such as IFN-γ or TNF-α released by T cells, can enhance macrophage microbicidal activity (Giacomini et al., 2001; Hickman et al., 2002).

Dendritic cells

Dendritic cells (DCs) are a system of cells that are specialized for the presentation of antigen to T cells. They are the most potent of the antigen presenting cells and are central to the initiation of immune responses in naive animals. They originate in the bone marrow but recent investigations suggest that they may be derived from either myeloid or lymphoid precursors (Tizard, 2004).

DCs are a trace population in most tissues but notably form networks underlying major body surfaces such as skin, trachea and intestine, where their function is the uptake of antigens, and after migration to the draining lymphnodes, the presentation of processed antigen. A number of properties have been established that are critical to the function of DC as the ultimate antigen-presenting cell population. These include the ability to effectively take up antigen by a number of routes, which may include endocytosis by clathrin-coated pits, macropinocytosis or phagocytosis depending on the maturation stage of the cell. The interaction of DC and mycobacteria augments their expression of surface molecules that are involved in the interaction with T cells,
notably MHC II and the costimulatory molecules CD40 and CD80 (Hope et al., 2004; Tizard, 2004). Taken together this suggests that infected DCs have an augmented capacity to stimulate mycobacteria reactive T cells. Also of importance for the interaction with T cells and modulation of immune responses by DC is the altered cytokine profile that is observed following mycobacterial infection of these cells (Hope et al., 2004).

Infection of DC with either M. tuberculosis or BCG is associated with increased expression of IL-12, TNF-α, IL-1 and IL-6 (Giacomini et al., 2001). These cytokines play major roles in protective anti-mycobacterial immune responses. As noted, IL-12 secreted by DC can potenti ate IFN-γ and TNF-α secretion by T cells and this in turn may serve to enhance the anti-microbial activity of macrophages to destroy invading bacilli (Hickman et al., 2002). In addition to the production of proinflammatory cytokines, mycobacterial infection of DC is also associated with the secretion of IL-10, which may inhibit the cellular response to mycobacterial through the down-regulation of IL-12 secretion (Giacomini et al., 2001; Hickman et al., 2002). This may serve to limit the extent of DC and macrophage activation and thus regulate the potentially damaging immune response that occurs in tissues in vivo.

Like non-activated macrophages, DCs are reported to provide an environment within which mycobacteria can survive and replicate (Tailleux et al., 2003a). DCs may therefore be a reservoir for Mycobacteria in vivo, particularly within lymph nodes to which they have migrated following the initial response to mycobacterial infection (Tailleux et al., 2003b). Thus, survival and/or replication of mycobacteria within the DC is likely to induce T-cell activation but may also eventually lead to granuloma formation and persistence of infection. This would potentially contribute to disease pathogenesis. In contrast, uptake by macrophages may lead to lower T-cell base immune responses and a failure to control infection. Alternatively, the mycobacteria may be killed leading to disease resolution. Thus, it may be advantageous in terms of immunity to have an extended range of cells that are permissive for infection, each with differing functions that should allow more effective clearance of the invading pathogen (Kaufmann and Schaible, 2003).

**Natural killer (NK) cells**

NK cells are a type of cytotoxic lymphocyte that is a major component of the innate immune system. These cells have been implicated in early immune responses to a variety of intracellular pathogens, including mycobacteria, through their capacity to rapidly produce IFN-γ and other immunoregulatory cytokines (Brill et al., 2001). NK cells are hypothesized to be important in the initiation and regulation of various immune responses and it has been shown that NK cells induce a granulomatous response to a glycolipid fraction of M. tuberculosis cell wall (Taniguchi et al., 2003).

**Neutrophils**

Polymorphonuclear cells, principally neutrophils, are the first phagocytes to arrive from circulation and attempt to eliminate invading pathogens via oxygen-dependent and oxygen-independent mechanisms. The former mechanism results from the generation of reactive oxygen species, whereas the latter mechanism reflects the capacity of neutrophils to degranulate and release preformed oxidants and proteolytic enzymes from granules (Lacy and Eitzen, 2008). Neutrophils have been implicated in the control of mycobacterial infections (Martinaeu et al., 2007), but the mechanisms by which they exert direct protective functions are not completely resolved (Kisch et al., 2002). Mycobacteria-infected macrophages acquired the contents of neutrophil granules and their antimicrobial molecules by the uptake of apoptotic neutrophil debris, which was trafficked to endosomes and co-localized with intracellular bacteria (Tan et al., 2006). Neutrophils may play an important role in the transition from innate to adaptive immune responses by producing critical cytokines and chemokines (Sawant and McMurry, 2007).

**γδ T cells**

Human T cells expressing γδ TCR represent a unique lymphocyte population with an unusual tissue distribution and antigen recognition pathway. Conditions that lead to responses of γδ T cells are not fully understood and current concepts of γδ T cells as first line of defense or bridge between innate and adaptive responses are also still vague (Holtmeier and Kabelitz, 2005). Murine studies have indicated that the induction of γδ T cells in the immune response against TB precedes that of conventional CD4+ and CD8+ cells, hence plays an important role in modulating the effectors’ response against tuberculosis. Intranasal infection of mice with BCG resulted in an early accumulation of γδ T cells in the lungs, and the peak of γδ T cells expansion at 7 days post infection preceded the 30 day peak of qβ T cells (Deli et al., 2003), suggesting that γδ T cells in the lungs might help to control mycobacterial infection before the onset of adaptive immunity. Studies using γδ TCR knockout mice indicate that γδ T cells may be involved in the regulation of granuloma formation, which is critical for the control of mycobacteria (Ehlers et al., 2001).
Adaptive immunity

In cattle, both humoral and cell-mediated responses can be induced following *M. bovis* infection. Several studies have shown that protective immunity to TB is dependent on the adaptive TH1 immune responses (Ngai et al., 2007). It is mediated by macrophages, DCs, T cells and their interactions, which depends on the interplay of cytokines produced by these cells (Berrington and Hawn, 2007). The adaptive immune response is initiated when mycobacteria infected DCs mature and migrate to local lymph nodes (LN), where recognition by T cells takes place (Flynn, 2004). The hallmark of chronic infections such as TB is the significant delay between infection and the induction of the adaptive immune response, which allows early growth of the pathogen and the establishment of persistent infection. Recently, it was demonstrated that activation of *M. tuberculosis*-specific CD4⁺ T cells is dependent on trafficking of bacteria from the lung to local LN, and that delayed dissemination from the lung to sites of antigen presentation accounts for the lag in the initiation of adaptive immunity (Triccas and Davenport, 2008; Wolf et al., 2008) (Figure 1). While the precise mechanisms for this delay are unclear, it has been suggested that low levels of antigen in early infection may help evade immune recognition and that some threshold level of antigen is required to stimulate the T-cell response (Russell et al., 2007). On the other hand, late migration of activated T cells to the lung was suggested to contribute to the delay in the onset of adaptive immunity (Wolf et al., 2008).

Cell mediated immunity (CMI)

Numerous studies performed in humans and various species of animal have demonstrated the central role of cell mediated immunity in the resolution of mycobacterial infections. The key players in anti-mycobacterial immune responses are T lymphocytes and antigen presenting cells. Both CD4⁺ and CD8⁺ T cells are implicated in the response; these cells produce IFN-γ and display cytolytic activity against mycobacteria infected cells. Responses mediated by γδ T cells are also involved in the response to mycobacteria. Central to the induction of immune responses to invading pathogens are the antigen presenting cells (Ngai et al., 2007). Robust delayed type hypersensitivity (DTH) and IFN-γ responses are elicited upon experimental and natural infection with *M. bovis*. The bias of the immune response to *M. bovis* is a T helper type-1 response as evidenced by IFN-γ, IL-12, and TNF-α production to pathogen-associated antigen (Flynn, 2004).

Immunohistological examination of early granulomatous
lesions induced by experimental *M. bovis* infection of cattle has shown T-cells to be among the first cells involved in the reaction (Cassidy et al., 2001). This has pointed to the importance of cell mediated immune responses in bovine tuberculosis, a concept supported by both field and experimental studies which recognize a complex spectrum of immune activity with early domination by T-cell driven responses. These observations have led to a recent expansion of studies to dissect the early CMI response in bovine tuberculosis (Pollock et al., 2001).

All of the main T-cell subsets (γδ T-cells, CD4+ and CD8+ αβ T-cells) have been shown to be involved in the anti-mycobacterial immune response in cattle (Figure 2) (Buddle et al., 2002). Study of the dynamics of lymphocyte subsets in the circulation of cattle infected experimentally with *M. bovis* has revealed a sequential involvement of γδ then CD4+ and later in the infection a more prominent involvement of CD8+ T-cells. T-helper type-1 (TH1) type of immune response, was characterised by production of IFN-γ, which is deemed to be essential for the activation of macrophage microbicidal pathways. In *M. bovis* infected cattle, CD4+ T-cells appear to be the most dominant cell population producing IFN-γ leading to the activation of macrophage anti-mycobacterial capabilities, with CD8+ T-cells having a greater involvement in the lysis of infected cells (Liebana et al., 2000). Effective immune responses are believed to primarily rely on CMI or TH1 responses that involve macrophages, dendritic cells and an adaptive T cell response. These responses are controlled by cytokines released from antigen-specific T cells with the pivotal cytokine of this response being IFN-γ. Evidence indicating that IFN-γ plays a significant role primarily comes from studies using IFN-γ knockout mice. Mice deficient in IFN-γ production quickly succumb to infection. Other pro-inflammatory cytokines, such as IL-12 and TNF-α like play a role in the TH1 response and granuloma formation (Flynn and Chan, 2001). The culmination of the CMI response is granuloma formation in which the bacteria are walled off, presumably to prevent their spread (Ulrichs and Kaufmann, 2006) (Figure 2).

The precise contributions of the TH1 response to immunity and pathology have not been delineated. TH-helper type-2 (TH2) responses induced by *M. bovis* infection are thought to inhibit type-1 T cell responses and thus contribute to pathology (Tylers et al., 2007). These responses are characterized by the production of cytokines such as IL-4, IL-5 and IL-10. It has been suggested that during the course of infection, cattle convert...
convert from a predominant TH1 response early after infection to a TH2 like response and that this conversion correlates with increased pathology (Welsh et al., 2005).

**Humoral immunity**

Since the organism is an intracellular pathogen, the serum components are thought not to get access to the pathogen and hence, may not play any protective role. However, this view has been challenged by recent studies, showing that the humoral immune response has shown effectiveness against other intracellular pathogens (Glatman-Freedman, 2006), suggesting that it may contribute to protective immunity to tuberculosis. Studies has shown that monoclonal antibodies against surface antigens of *M. tuberculosis* give rise to protective immunity in mice and prolong their survival after infection with lethal doses of *M. tuberculosis* or *M. bovis* through a more organized and compact granuloma formation where the bacilli were contained (Chambers et al., 2004).

It is also known that mycobacteria specific antibodies can both influence mycobacterial dissemination and modulate potentially detrimental inflammatory tissue responses (Maglione et al., 2007). Generally, antibodies seem to have an opsonizing role and thereby improve phagocytosis by macrophages or the cytotoxic actions of killer lymphocytes. The ability of human antibodies induced by *M. bovis* BCG vaccination has been studied recently and internalization of BCG by phagocytic cells was significantly enhanced in post-vaccination serum samples. Furthermore, the inhibition effects of neutrophils and macrophages on mycobacterial growth were significantly enhanced by BCG-induced antibodies. BCG-induced antibodies were shown to significantly enhance the cell-mediated immune response with an increased proliferation and IFN-γ production in mycobacterium specific CD4+ and CD8+ T cells. Mycobacterium specific antibodies seem capable of enhancing both innate and cell mediated immune responses to mycobacteria (De Valliere et al., 2005). It is increasingly recognized that B cells can exert an influence on T cells (Lund et al., 2006) and are an important constituent of granuloma architecture (Tsai et al., 2006). It is important to note that intracellular pathogens can also be found in the extracellular space during their life cycle, either before entering the host cells or after cell death, and then can easily be reached by antibodies, preventing their dissemination (Hiwa et al., 2007).

**IMMUNE EVASIVE MECHANISMS**

*M. tuberculosis* invades and replicates in macrophages, cells of the host innate defense system designed to eliminate pathogenic microorganisms, through a variety of immune evasion strategies. The use of non-activating complement receptors (CR) to enter into macrophages may be advantageous for the bacterium, since engagement of these receptors does not induce the release of cytotoxic reactive oxygen intermediates (ROI) (Lie bana et al., 2000). The ability of pathogenic mycobacteria to adapt to the hostile environment of macrophages has been instrumental in its success as a pathogen. Mycobacteria interfere with host trafficking pathways by modulating events in the endosomal/phagosomal maturation pathway to create a protective niche (Houben et al., 2006). The mycobacteria containing phagosome, while connected to the endocytic pathway, does not fuse with lysosomes or mature into phagolysosomes (Nguyen and Pieters, 2005). By blocking its delivery to lysosomes, the mycobacterium is able to avoid the acidic proteases of lysosomes; avoid exposure to the bactericidal mechanisms within lysosomes; prevent degradation and hence processing and presentation of mycobacterial antigens to the immune system (Pieters, 2001).

Another mechanism by which mycobacteria could interfere with phagolysosomal fusion is by retention of an important host protein called tryptophan aspartate containing coat protein (TACO), also known as coronin 1 on the phagosome, thereby behaving as self antigens. TACO represents a component of the phagosome coat, and retention of TACO prevents phagosomes from fusing with lysosomes, thereby contributing to the long-term survival of bacilli within the phagosome (Nguyen and Pieters, 2005). The recognition of infected macrophages by CD4+ T cells depends on constitutively expressed major histocompatibility complex (MHC) class II on professional antigen presenting cells (APCs), level of which is upregulated upon activation with IFN-γ. One mechanism by which *M. tuberculosis* avoids elimination by the immune system after infection is through the inhibition of MHC II expression or antigen processing or presentation by macrophages (Fulton et al., 2004). Inhibition of MHC II expression or antigen processing does not require viable bacilli and can be achieved by exposure to bacterial lysate (Noss et al., 2000).

**PATHOGENESIS**

The pathogenesis of bovine tuberculosis is not as well understood as the pathogenesis of tuberculosis in humans. Advances in the field of human tuberculosis have been made using various small animal models of *M. tuberculosis* infection (Mitchell and Waters, 2006). The host response to the tubercle bacillus is complex and broad, involving all aspects of the immune system (Flynn and Chan, 2001). The organism has evolved to avoid
immune clearance and induce chronic lesions ensuring transmission by infectious aerosol droplets (North and Jung, 2004). Paradoxically, lesions (that is, granulomas) are elicited as a mechanism to limit spread of the bacillus, thereby preventing early demise of the host (Mitchell and Waters, 2006). Although granulomas limit the spread of the pathogen, they contribute to tissue damage. The balance between controlling bacterial spread and tissue damage may represent the most significant biological challenge to the host immune response (Tyler et al., 2007). Inflammatory response induced by persistent presence of the mycobacterium in the tissue is characterized by granuloma, a distinctive pattern of chronic inflammatory reaction. Granulomatous formations, that surround infected cells and caseous necrosis, are an evidence of cellular response against mycobacteria infection (Tonya et al., 2005) (Figure 3).

Granuloma is multi-cellular structure where there is predominate macrophages, multinucleated giant cells, lymphocytes and necrosis. These macrophages respond to bacterial infections by the process of phagocytosis; the engulfment of bacteria (Algood et al., 2005). Activated macrophages become efficient at phagocytosis and bacterial killing due to the presence of T cells and cytokines. The granuloma serves three major purposes; it is a barrier to dissemination of bacteria throughout the lungs and other organs, a local environment in which immune cells can interact to kill bacteria, and a focus of inflammatory cells that prevent inflammation from occurring throughout the lungs (Roach et al., 2001).

Phagocytes are attracted to sites of infection via the release of chemokines and cytokines (for example, IFN-γ) by a variety of cell types. Infected macrophages release various chemokines (for example, IL-8, MIP-2, IP-10, and MCP-1) that attract macrophages, neutrophils and T cells to sites of infection (Mitchell and waters, 2006). Additionally, macrophages produce cytokines (for example, IL-12, TNF-α) that both up- and down-regulate adaptive immunity (Flynn and Chan, 2001; Tonya et al., 2005). The immunopathology of granuloma formation is complex and appears to develop in distinct stages of advancement. Bovine models of M. bovis infection have shown that lesions, induced following experimental infection, are generally indistinguishable from natural cases of infection (Neill et al., 2001). In an experimental study, a deer was infected with virulent M. bovis via intratonsilar inoculation. Gross lesions were seen in the upper and lower respiratory tract and associated lymph nodes at 6 to 23 weeks post infection.

Histologically, there are different types of granulomas. Initially, epithelioid cells may be surrounded by an acellular necrotic region, with a ring of B and T cells. The granulomas can displace parenchymal tissue and may necrotize, caseate, and/or calcify. Caseous granulomas might turn calcified during chronic or latent infection. Other types of granulomas may not have a necrotic area and are composed primarily of macrophages and a few lymphocytes. Host-pathogen interactions in the granuloma over the course of infection lead to adaptive changes of the tubercle bacilli, phenotypes of the host immune cells, and levels of the immune mediators they produce. These features allow for the formation of a wide spectrum of granuloma structures even within a single human host, therefore implying the presence of several unique micro environments for M. tuberculosis as well as for the immune response (Algood et al., 2005).
Generally, granulomatous lesions were small pale yellow in colour with a caseous core. Microscopically, lesions were observed with macrophage, giant cells (containing acid-fast bacilli) and neutrophilic debris in evidence. As lesion development progressed, there was more extensive necrosis, consisting of intact and degenerate neutrophils, macrophages and lymphocytes. Some mineralization and fibrosis was also seen (Pollock et al., 2006). Previous studies classified granulomatous lesions in lymph nodes from calves experimentally infected with *M. bovis* into four developmental stages (Linda et al., 2006).

1. Stage I (initial): Irregular, unencapsulated cluster of epithelioid macrophages, with interspersed lymphocytes and few admixed neutrophils. Langhans’ giant cells may be present. No necrosis is present.
2. Stage II (solid): Partially or completely thinly encapsulated granuloma composed primarily of epithelioid macrophages. Haemorrhage is often noted along with infiltration of lymphocytes, neutrophils and often Langhans’ giant cells. Minimal necrosis may be present, and is generally composed of necrotic inflammatory cells.
3. Stage III (minimal necrosis): Fully encapsulated granuloma with central necrosis which is caseous and mineralized. Epithelioid macrophages surround necrosis admixed with Langhans’ giant cells. A peripheral zone of macrophages mixed with clusters of lymphocytes and scattered neutrophils extends to the fibrous capsule.
4. Stage IV (necrosis and mineralization): Thickly encapsulated, large and irregular, multicentric granuloma with prominent caseous necrosis and extensive islands of mineralization comprising the greatest area of the lesion. Epithelioid macrophages and multinucleated giant cells surround the necrosis, with clusters of lymphocytes distributed more densely near the peripheral fibrotic capsule.

The granuloma is an elaborated aggregate of immune cells found in non-infectious as well as infectious diseases. It is a hallmark of tuberculosis (TB). Predominantly thought as a host-driven strategy to constrain the bacilli and prevent dissemination, recent discoveries indicate that the granuloma can also be modulated into an efficient tool to promote microbial pathogenesis (Guirado and Schlesinger, 2013). It would be unreasonable to call the TB granuloma an unsuccessful host defense, as it successfully contains the infectious focus in more than 90% of cases. The 10% of individuals that progress toward TB disease suffer from a disbalanced inflammatory reaction, be it due to too little innate or adaptive immunity or due to unrestrained hypersensitivity reactions (Ehlers and Schaible, 2013).

Trehalose-6, 6 dimycolate (TDM), the mycobacterial cord factor is the most abundant cell wall lipid of virulent mycobacteria, is sufficient to cause granuloma formation, and has long been known to be a major virulence factor (Lang, 2013).

According to Shaler et al. (2013) the true nature of the granuloma still remains to be defined, it is now clearly evident that the granuloma is not just a host-mediated entity of segregation and rather, it is a dynamic battlefield bearing the scars left both by the pathogen and the host immune response. The same authors stated that it may have been originally destined to restrain bacterial dissemination; *M. tuberculosis* efficiently hijacks the granuloma to provoke the generation of an immunologically sheltered niche to reside within and persist until the situation is favorable to bacterial transmission.

**CONCLUSION**

The immune response to bovine tuberculosis is apparently multifaceted and there are still many unsolved problems associated with the pathogenesis and immune response to tuberculosis. There is considerable benefit in drawing from knowledge of the immunopathological mechanisms elucidated in studies of tuberculosis in humans and in laboratory animal models. These can provide new insights into many of the fundamental mechanisms shared with the bovine disease. The variation of immunopathological responses in a variety of susceptible hosts makes the understanding challenging. Although our knowledge is still limited it is already clear that mycobacterium employs novel pathogenic strategies for both replication and persistence *in vivo*.

It is already clear that a more complete understanding of the pathogenic strategies of this highly successful intracellular organism will elucidate novel feature of host immune response. A broad, multi-disciplinary approach in comparative pathology, immunology and molecular biology will be required for the understanding of comprehensive pathogenesis of the disease. Clear contribution of both the humoral and cell mediated immunity in protection against mycobacterium need to be elucidated. The future research on identification and detail understanding of how host cell regulate mycobacterial infection will be of exquisite importance to develop biomarkers and therapeutics. Rapid advancement and explosion of research nourishes our hope for a giant leap in better diagnosis and treatment of mycobacterial infections in the future.

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Review

Cardiac tumors in children- A review

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Primary cardiac tumors despite the potential for more frequent recognition with the advent of echocardiography are rare. The incidence of cardiac tumors ranges from 0.0017% to 0.28% in the general population. Primary cardiac tumors are much less common than metastatic tumors of the heart; and of the primary cardiac tumors, over 75% are benign, 3 of which are made up of myxomas, rhabdomyomas and fibromas. The most common primary sites are the lung, breast and cutaneous melanoma. The common malignant tumors are rhabdomyosarcoma and angiosarcoma.

Key words: Tumors, children, myxomas, rhabdomyomas, fibroma, lipoma.

INTRODUCTION

Cardiac tumors are benign or malignant neoplasms arising primarily in the inner lining, muscle layer, or the surrounding pericardium of the heart. Cardiac tumors can be primary or metastatic. Primary cardiac tumors are rare in paediatric practise with a prevalence of 0.0017% to 0.28% in autopsy series. In contrast, the incidence of cardiac tumors during fetal life has been reported to be approximately 0.14% (McAllister, 1979; Nadas and Ellison, 1968; Holley et al., 1995). The vast majority of primary cardiac tumors in children are benign, while approximately 10% are malignant. Secondary malignant tumors are 10-20 times more prevalent than primary malignant tumors (Lam et al., 1993).

Rhabdomyomas are the most common cardiac tumor during fetal life and childhood (Holley et al., 1995) (Table 1). This is usually followed by teratomas, fibroma and hemangioma (Holley et al., 1995). Cardiac tumors may present in fetal or post-natal life. The presenting features depend on the size and location of the mass. The manifestations of a cardiac tumor in fetal life include arrhythmia, congestive heart failure, hydrops and rarely stillbirth. In postnatal life, cardiac tumors may affect the integrity and function of the adjacent cardiac structures leading to severely compromised blood flow due to inflow or outflow tract obstruction, cyanosis, murmur, respiratory distress, myocardial dysfunction, valvular insufficiency, arrhythmias and sudden death (Nadas and Ellison, 1968; Groves et al., 1992).

The diagnosis of cardiac tumors can be established in symptomatic patients, but in rare cases sudden death is the presenting feature. Echocardiography or Magnetic Resonance Imaging is usually adequate to facilitate the diagnosis of cardiac tumors. Tumor biopsy, with histological assessment, remains as the gold standard for confirmation of the diagnosis.

Due to the progressive nature of pregnancy, fetal cardiac tumors are expected to grow antenatally and it is not unusual for cardiac lesions to be missed at an early obstetric scan. Some tumors can be detected from 20 weeks onwards but the majority will develop later in the course of the pregnancy. Most fetal cardiac tumors will be readily detectable in the late second or third trimester.

EPIDEMIOLOGY AND NOMENCLATURE

1. Primary cardiac tumors are rare
2. The most common primary cardiac tumor is the
Sudden Infant Death Syndrome (SIDS) is diagnosed in the first month of life. A quarter of primary cardiac tumors are malignant, the vast majority being sarcomas. Embolization, obstruction, and arrhythmogenesis are the chief modes of presentation. Sudden death is not common.

**General clinical features**

Cardiac tumors are diverse in clinical presentation and atrial myxomas in particular may cause systemic symptoms mimicking collagen vascular disease, malignancy or infective endocarditis. There are several clinical features that are seen commonly with cardiac tumors:

1. Embolization: This occurs frequently. Either the tumor itself, or adherent thrombus may dislodge and migrate; multiple emboli may mimic vasculitis or endocarditis, while larger fragments may lead to cerebrovascular events. Right sided tumors naturally embolise to the lungs producing pleuritic symptoms and possibly right heart failure.
2. Obstruction: Atrial tumors, once they are large enough, may result in obstruction of atrioventricular valvar flow, and in particular, may mimic valvar stenosis. Symptoms are markedly paradoxical and may relate to body positions; frequent chest pain, breathlessness and syncope.
3. Arrhythmias: Intramyocardial and intracavity tumors may both affect cardiac rhythm, either through direct infiltration of the conduction tissue, or through irritation of the myocardium itself. The presence of serious ventricular arrhythmias should always lead to a search for structural heart disease and very infrequently a tumor may be found.

<table>
<thead>
<tr>
<th>Incidence (%)</th>
<th>Children</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myxoma</td>
<td>15</td>
</tr>
<tr>
<td>Lipoma</td>
<td>-</td>
</tr>
<tr>
<td>Papillary fibroelastoma</td>
<td>-</td>
</tr>
<tr>
<td>Angioma</td>
<td>5</td>
</tr>
<tr>
<td>Fibroma</td>
<td>15</td>
</tr>
<tr>
<td>Haemangioma</td>
<td>5</td>
</tr>
<tr>
<td>Rhabdomyoma</td>
<td>45</td>
</tr>
<tr>
<td>teratoma</td>
<td>15</td>
</tr>
</tbody>
</table>


**Histiocytoid cardiomyopathy**

This is a rare but distinctive arrhythmogenic disorder caused by a neoplastic or hamartomatous proliferation of cardiac cells with some Purkinje cell characteristics. The synonyms of histiocytoid cardiomyopathy are Purkinje cell hamartoma, infantile cardiomyopathy, oncocytic cardiomyopathy, isolated cardiac lipoidosis, myocardial conduction system hamartoma, foamy myocardial transformation and congenital cardiomyopathy. Histiocytoid cardiomyopathy occurs predominantly in the first two years of life; 20% of cases are diagnosed in the first month, 60% in the first year and less than 3% after two years of life.

Histiocytoid cardiomyopathy is an arrhythmogenic disorder; over 70% of patients present with a spectrum of arrhythmias and electrical disturbances including; paroxysmal atrial tachycardia, atrial fibrillation, premature ventricular contractions, Wolff-Parkinson-White syndrome and right or left bundle branch block. Approximately 20% of patients present as sudden death and often such cases have been misclassified as Sudden Infant Death Syndrome (SIDS).

**BENIGN TUMORS OF PLURIPOTENT MESENCHYME**

**Cardiac myxoma**

Myxoma is a neoplasm composed of stellate to plump cytologically bland mesenchymal cells set in a myxoid stroma. Myxoma represents one of the most common benign cardiac tumors; are usually solitary and develop in the atria, 75% originating in the left atrium and 15-20% in the right atrium. They characteristically arise from or near the interatrial septum at the border of the fossa ovalis membrane (Bruce, 2007). Multiple acronyms for this condition are LAMB (lentigines, atrial myxoma, mucocutaneous myxoma and blue naevi) and NAME (naevi, atrial myxoma, myxoid neurofibromata and ephelides).
Recent nomenclature, however, suggests that they should be brought together under a broader category of Carney complex, named after the physician who first described the familial nature of this disorder (Carney et al., 1986).

Clinical manifestations are legion, both cardiac and systemic. Symptoms include breathlessness, fever, weight loss, syncope, haemoptysis and sudden death. Murmurs are frequently present, as is evidence of pulmonary hypertension, right sided cardiac failure and pulmonary embolization. Anemia, erythrocyte sedimentation rate and less frequently, the characteristic “tumor-plop” may be detected. This is heard as a loud but rather dull sound as the tumor prolapses into the left ventricle, and may be confused with a third heart sound. The method of choice for treatment is surgical resection on cardiopulmonary bypass.

Papillary fibroelastoma

This is an endocardial based papilloma lined by endothelial cells with proteoglycan rich avascular stroma, usually rich in elastin. This is a rare and benign tumor representing less than 10% of primary cardiac tumors and is the most common tumor of cardiac valves. Until recently, these tumors were considered to be benign and insignificant, but recent autopsy studies have demonstrated a high incidence of embolization; and the “sea anemone” appearance, with a short attaching pedicle, is typical. The clinical diagnosis of papillary fibroelastoma can be difficult because embolic complications can mimic a variety of underlying diseases.

HAEMANGIOMA

Haemangiomas are benign tumors composed predominantly of blood vessels. The histologic classification includes three types; the cavernous, capillary and arterio-venous haemangioma. Most cardiac haemangiomas are discovered incidentally but patients may present with dyspnoea on exertion, arrhythmias, right-sided heart failure, pericarditis, pericardial effusion and failure to thrive. This affects all age groups and accounts for 5% to 10% of all benign tumors. Haemangiomas undergo spontaneous regression with a good prognosis. However, their clinical course may be unfavourable in infants due to high-output cardiac failure, haemorrhage from ruptured vessels and thrombocytopenia.

BENIGN TUMORS WITH MYOFIBROBLASTIC DIFFERENTIATION

Cardiac fibroma

Cardiac fibroma is a rare primary heart tumor composed of fibroblasts or myofibroblasts with a matrix containing collagen. It almost exclusively occurs within the myocardium of the ventricles or ventricular septum. It is unclear whether it is a hamartoma or a true neoplasm. Because most cases occur in infants and children, it is likely to be congenital; with size varying from 1 to 10 cm. Cardiac fibroma may invade the ventricular muscle, replace the working myocardium and may result in intractable congestive heart failure or cyanosis; and rarely, a cardiac fibroma may extend into the ventricular conduction system causing ventricular arrhythmias (Isaacs, 2004; Becker, 2000; Marlin-Garcia et al., 1984).

Cardiac fibromas usually remain dormant and spontaneous regression rarely occurs, therefore total surgical resection is normally recommended. Large tumors can be resected subtotally and heart transplantation is done if there is progressive loss of working myocardial fibres (Burke et al., 1994; Geha et al., 1967).

Inflammatory myofibroblastic tumor

Inflammatory myofibroblastic tumor is composed of myofibroblasts accompanied by a variable number of inflammatory cells including lymphocytes, macrophages, plasma cells and eosinophils. The synonyms are plasma cell granuloma, inflammatory pseudotumor and possibly inflammatory fibrosarcoma. This tumor is characterized by fibroinflammatory and pseudomembranous appearance (Coffin et al., 1995; Demirkan et al., 2001); mostly occurring in children and young adults with a slight female gender predilection (male-to-female ratio being 3:4). Common site of occurrence is the lungs. Extrapulmonary sites have also been reported and they include the mesentery, genitourinary tract, gastrointestinal tract, retroperitoneum, pelvis, head and neck, trunk and extremities (Coffin et al., 1995; Ko et al., 2005).

CARDIAC LIPOMA

Cardiac lipomas are benign tumors composed of mature, white adipocytes. This occurs exclusively in adults; but when it occurs in children, accounts for less than 2% of heart tumors. Cardiac lipomas may occur anywhere in the heart but there is a predilection for the pericardium and epicardial surfaces. Other sites include the ventricular septum and cardiac valves. They are commonly silent but may rarely cause arrhythmias and atrioventricular block (Val-Bernal et al., 2000; Ashar and van Hoeven, 1992).

OTHER BENIGN TUMORS

Angioma

These tumors are extremely rare, occurring principally in the interventricular septum. They are visualised as
subendocardial nodules, having 2 to 4 cm diameter. Coronary angiography reveals a characteristic “tumor-blush”. Total surgical excision is not feasible due to the highly vascular nature of the tumor (Leonard, 2001).

**Teratoma**

Cardiac teratoma is a rare tumor of the heart and pericardium (Ali et al., 1994). Teratomas are the second most common tumor in the fetus and neonate after rhabdomyoma (Flyer, 1980; Isaacs, 1997; Isaacs, 1997). Most commonly, these tumors are detected in the pericardial activity attached to the pulmonary artery and aorta (Uzon et al., 1996). The tumor size within the heart varies from 2 to 9 cm in diameter and intrapericardial tumors as large as 15 cm have been reported (Carter et al., 1982; Roberts, 1997). Cardiac and pericardial teratomas are easily detected in the fetus and neonate by echocardiography as heterogeneous and encapsulated cystic masses. Treatment is by dissecting the teratoma from the great vessels.

**MALIGNANT TUMORS**

**Primary malignant cardiac tumors**

Up to a quarter of all cardiac tumors may exhibit some features of malignancy. 95% of these primary malignancies are sarcomas, 5% being lymphomas (Roberts, 1997). Sarcomas are more common in adults and most commonly located in the right atrium. The clinical course is usually aggressive with extensive local infiltration, intracavity obstruction and death.

**Angiosarcoma**

Angiosarcomas are the most common primary cardiac malignancy and more common in males. 80% of these tumors originate in the right atrium or pericardium (Roberts, 1997; Herrmann et al., 1992). Clinical picture includes right-sided heart failure, pericardial disease, pleuritic chest pain, dyspnoea and pericardial effusion. Some patients also present with fever, weight loss, and lassitude appear before signs of cardiac involvement (Leonard, 2001).

**Rhabdomyosarcoma**

Rhabdomyosarcomas are the second most common primary malignancy of the heart; originating from the striated muscle. These malignancies mostly occurs in adults, affecting the children rarely. Most common presenting symptoms are fever, anorexia, malaise and weight loss and the prognosis is usually poor (Leonard, 2001; Isaacs, 1997).

**SECONDARY MALIGNANT (METASTATIC) CARDIAC TUMORS**

Secondary cardiac tumors may be epicardial, myocardial or endocardial, but the vast majority are epicardial. Metastasis is rarely limited solely to the heart. The development of tachycardia, arrhythmias, cardiomegaly or heart failure in a patient with carcinoma should raise the suspicion of cardiac metastases. Rarely, cardiac involvement may be the first clinical feature of malignancy.

Macroscopically, carcinomatous metastatic tumors are multiple small, discrete and firm nodules. Carcinomas are more frequent than sarcomatous infiltrations. Melanoma shows a special affinity to spread to heart with equal distribution to all four chambers (Gibbs et al., 1999). Leukemias and lymphomas may cause intramyocardial infiltration, haemorrhagic pericardial effusion, but occasionally they remain asymptomatic (Isaacs, 1997; Carter et al., 1982). The majority of metastatic tumors remain silent but some may present with arrhythmias, cardiac failure or pericardial effusion.

**DIAGNOSTIC EVALUATION OF CARDIAC TUMORS**

Diagnosis and differential diagnosis of cardiac tumors often presents a challenge for the physician. Cardiac tumors, either benign or malignant, are difficult to diagnose due to their rarity, variety and nonspecificity of the symptoms that they may cause. Patient's history, clinical examination and blood tests rarely lead to an immediate diagnosis of the tumor; therefore, suspicion of this condition is critical for the correct and timely diagnosis of a cardiac tumor. Furthermore, beyond the
performance of imaging techniques, histological evaluation via biopsy is essential for the final diagnosis to be established.

MANAGEMENT

Therapy of benign malignant tumors is surgical resection and the urgency to intervene is determined by the symptoms of the patient and the type of the tumor.

1. Myxomas are indicated for immediate surgical resection regardless of symptoms, because of the high risk of embolic and cardiac complications.
2. Papillary fibroelastomas are surgically removed if the tumors are larger than 1 cm.
3. For small, immobile tumors in the left ventricles, conservative management and close follow-up is advocated.
4. Lipomas and lipomatous hypertrophy are surgically managed.
5. Rhabdomyomas usually do not require surgical management, since they tend to regress spontaneously.
6. Sarcomas are managed by surgical resection and chemotherapy is used as an adjuvant to help decrease tumor.

Finally, regarding cardiac manifestations due to metastatic extra cardiac cancer, priority is given to the management of the primary focus of the disease and the cardiovascular complications that are manifested (that is, percutaneous balloon pericardiectomy in cases of cardiac tamponade, radiotherapy and chemotherapy in cases of tumors that obstruct flow in the venae cavae).

CONCLUSION

Although cardiac tumors are rare, they are increasingly recognised ante mortem, permitting earlier diagnosis and treatment. The most likely etiology of a cardiac mass is a thrombus or vegetation. If a cardiac mass represents a tumor, its etiology can be determined by considering the histology based likelihood, the age of the patient at time of presentation, tumor location and tissue characterization by non-invasive imaging. Echocardiography is a vital non-invasive method of detecting and diagnosing cardiac tumors since it provides the precise anatomic location and hemodynamic impact of most cardiac tumors. In the current era, for benign cardiac tumors, an early diagnosis and appropriate treatment is not only possible, but often curative. Unfortunately the outcome for malignant primary tumors, even despite early diagnosis and aggressive treatment, remains dismal. Fortunately these tumors are exceedingly rare and seldom encountered in clinical practise.

REFERENCES


**UPCOMING CONFERENCES**

*Keystone Symposia — Sensing and Signaling of Hypoxia: Interfaces with Biology and Medicine, Breckenridge, USA*

![Keystone Symposia](image1)

*7th International Symposium on Molecular Insect Science, Amsterdam, The Netherlands*

![7th International Symposium on Molecular Insect Science](image2)
January 2014
Keystone Symposia — Sensing and Signaling of Hypoxia: Interfaces with Biology and Medicine, Breckenridge, USA

10th International Conference of Management and Behavioural Sciences, Bhopal, India

August 2014
International Conference on Biomedical Engineering and Systems, Prague, Czech Republic

September 2014
Life Sciences Baltics 2014 (LSB 2014), Vilnius, Lithuania
International Journal of Medicine and Medical Sciences

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