SENSITIVITY PATTERNS, SEROTYPES OF CRYPTOCOCCUS NEOFORMANS AND DIAGNOSTIC VALUE OF INDIA INK IN PATIENTS WITH CRYPTOCOCCAL MENINGITIS AT KENYATTA NATIONAL HOSPITAL.

KAP conference 19\textsuperscript{th} March 2008: Dr Mohamed Hussein Jin.
SUPERVISORS:

PROF. E. AMAYO
Associate Professor & Consultant Neurologist

DR. G. REVATHI
Consultant Microbiologist

PROF. A. M. ADAM
Associate Professor & Consultant Neurologist

DR. J. O. JOWI
Consultant Neurologist
Cryptococcal meningitis caused by *Cryptococcus neoformans* is an important and fatal infection if not appropriately diagnosed and treated.

**The HIV pandemic:**
- Most important fungal cause of morbidity and death.
- Most common opportunistic infection of CNS.
- Most common cause of death after TB.
Incidence in HIV

- 6-10% Western world
- 15-30% Sub Saharan Africa
- Gender: Male > Female
- Fall in incidence of CM in HIV infected patients due to availability of ARV therapy.
Prevalence

- 30.8% Kenya. Most common cause of adult meningitis in HIV (32/104).
- 21% Zimbabwe, most common cause of adult meningitis.
- 13% South Africa
- 19% Rwanda
- 16.6% India

4 serotypes (A, B, C and D) based on antigenic determinants on the polysaccharide capsule.

- In HIV: sero-type A and D *(var-grubii, var-neoformans)*

- In immunocompetent— sero-type B,C *(var-gatii)*
Australia serotype A: 85% - 98% HIV + (chen et al)

Rwanda serotype A: 98.4%, B: 1.6% (Bogaerts et al)
CLINICAL CHARACTERISTICS

- Usually asymptomatic.
- Dissemination can occur following an immunosuppressive event.
- All organs are affected.
- Most common being CNS-meningoencephalitis
- Presentation mainly sub-acute.
- Mean CD4 count <70/ µl.
Symptomatology:

- Headache 76%
- Fever 65%
- Neck stiffness 25%
- Focal signs 15%
- Changes in level of consciousness
- Nausea, vomiting, irritability
- Papilloedema, hydrocephalus and multiple enhancing lesions more common with CN var-gatii.
DIAGNOSIS

INDIA INK

- Most important diagnostic test to rule out cryptococcal meningitis.
- Positive when $>10^3$ CFU/ml.
- In HIV sensitivity is above 70%.
- HIV negative sensitivity is 50%.
- Centrifuging the CSF improves the sensitivity of the test.
- Not a good guide of response to therapy.
CRAG

- Highly sensitive
- Positive in 98%
- False positives can occur
  - Infection with Trichosporon beigelii
  - Presence of rheumatoid factor eliminated by treatment with pronase.
CRAG (CONT’D)

- False negative – when acapsular strains of CN isolated.

- Serum CRAG also sensitive – mainly used to diagnose disseminated Cryptococcosis.

- Not recommended for follow up and treatment decisions- kinetics of the antigen clearance in CSF and serum are unpredictable.
CULTURE

- Strongest proof of CM.
- Rate of positivity in HIV is >70%
- Strongly correlates with the burden of the organism in CSF.
MOLECULAR IDENTIFICATION TECHNIQUES

- Will become a standard practice for diagnosis.
- Still remains beyond the scope of a clinical lab in a developing country.
SUSCEPTIBILITY TO ANTIFUNGALS

**Australia** (2000):
- 1/77 (1.2%) resistant to flucytosine,
- 1/77 (1.2%) to amph B,
- 3/77 (3.9%) to fluconazole

**Malawi:**
- Amph B-nil
- Fluconazole-6%
- 5 Flucytosine-nil

**Kenya:**
- Ampho B-nil
- Fluconazole-11.2%
- 5 Flucytosine-21%

*Chen et al*
BROAD OBJECTIVE

To determine the sensitivity patterns, serotypes of cryptococcus neoformans and diagnostic value of India ink stain in patients with cryptococcal meningitis at Kenyatta National Hospital.
SPECIFIC OBJECTIVES

1. To determine the relative sensitivity of India ink vs CRAG as a diagnostic tool.

2. To determine the culture yield of cryptococcus neoformans in patients with cryptococcal meningitis

3. To determine the sensitivity of Cryptococcus neoformans to Amphotericin B, fluconazole, miconazole and flucytosine
4. To determine the prevalent sero-type of Cryptococcus neoformans in the study group.

5. To determine the HIV status of patients with Cryptococcal meningitis.
MATERIALS AND METHODS

- **Study Design:** Cross-sectional analytic study.

- **Study Area:** Kenyatta National Hospital.

- **Study Population:** Patients admitted to the medical wards, Kenyatta National Hospital with clinical features of meningo-encephalitis and a CRAG positive on CSF studies.
Sample Size:

- Confidence Interval-CI-95%
- d-absolute precision-5%
- P-95% (Culture outcome in patient with Cryptococcal meningitis in Rwanda)
- $Z_{1-\alpha}$ standard error of mean = 1.64
- $n=$ sample size
- $n=Z^2xP(1-P)$
- $d^2$
- $n=51$
Sampling Technique

Consecutive patients with clinical diagnoses of meningo-encephalitis getting admitted to medical wards were screened using CSF CRAG and positive cases were recruited till the required sample size was reached.
Inclusion Criteria.

1. All patients admitted to the medical wards of KNH with clinical features of meningoencephalitis including acute, sub-acute and chronic presentations.
All patients with CSF CRAG positivity.
1 Patients in whom lumbar puncture was contraindicated:
   - Patients with papilloedema on fundoscopy.
   - Patients with focal neurological deficits.
   - Patients with space occupying lesions confirmed by CT-Scan.

2 Any patient or guardian who declined to give consent
RESULTS

4,475 Patients admitted to medical ward

459 Patients with meningocerebralitis

307 CSF obtained

61 CRAG positive

58 Cryptococcus neoformans

152 Excluded due to
- Focal neurological deficits
- Papilloedema on fundoscopy
- Prior Amphotericin B use
- Lack of consent
- Dry tap on LP

3 excluded
- 2 grew: T. mucoides
- 1 grew: T. beigeli
## Gender distribution

<table>
<thead>
<tr>
<th>Gender</th>
<th>Frequency(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>25 (43.1)</td>
</tr>
<tr>
<td>Female</td>
<td>33 (56.9)</td>
</tr>
</tbody>
</table>

Mean age: 37.22yrs (range: 20-63 yrs)
Marital status

- Single: 10.3%
- Married: 70.7%
- Divorced: 3.4%
- Widow: 15.5%
# HIV status

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Known</td>
<td>19</td>
<td>32.8</td>
</tr>
<tr>
<td>Newly diagnosed</td>
<td>38</td>
<td>65.5</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>HAART</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>16</td>
<td>27.6</td>
</tr>
<tr>
<td>No</td>
<td>3</td>
<td>5.2</td>
</tr>
</tbody>
</table>
Mode of presentation

- Acute: 17.20%
- Sub-acute: 67.20%
- Chronic: 15.50%
## Presenting complaints

<table>
<thead>
<tr>
<th>Complaint</th>
<th>Count</th>
<th>Percentage (multiple outcomes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>48</td>
<td>82.8</td>
</tr>
<tr>
<td>Vomiting</td>
<td>41</td>
<td>70.7</td>
</tr>
<tr>
<td>Visual disturbances</td>
<td>38</td>
<td>65.5</td>
</tr>
<tr>
<td>Nausea</td>
<td>34</td>
<td>58.6</td>
</tr>
<tr>
<td>Fever</td>
<td>28</td>
<td>48.3</td>
</tr>
<tr>
<td>Confusion</td>
<td>25</td>
<td>43.1</td>
</tr>
<tr>
<td>Unsteady gait</td>
<td>22</td>
<td>37.9</td>
</tr>
<tr>
<td>Dizziness</td>
<td>19</td>
<td>32.8</td>
</tr>
<tr>
<td>Somnolence</td>
<td>4</td>
<td>6.9</td>
</tr>
<tr>
<td>Stupor</td>
<td>4</td>
<td>6.9</td>
</tr>
</tbody>
</table>
Neck stiffness present in 55%
Kernig's positivity elicited in 34.5%
27(46.6%) had both headache and neck stiffness (menengism)
## Level of consciousness

<table>
<thead>
<tr>
<th>GCS</th>
<th>Frequency-n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-8 (comatose)</td>
<td>4 (6.9)</td>
</tr>
<tr>
<td>9-13 (semincomatose)</td>
<td>20 (34.5)</td>
</tr>
<tr>
<td>14-15 (alert)</td>
<td>34 (58.6)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>58 (100)</strong></td>
</tr>
</tbody>
</table>
## India ink sensitivity

<table>
<thead>
<tr>
<th>India Ink</th>
<th>Frequency(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>33 (56.9)</td>
</tr>
<tr>
<td>Negative</td>
<td>25 (43.1)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>58 (100)</strong></td>
</tr>
<tr>
<td>Culture outcome</td>
<td>Frequency (%)</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------------</td>
</tr>
<tr>
<td>POSITIVE</td>
<td>39 (67.2%)</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>19 (32.8%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>58</td>
</tr>
<tr>
<td>Culture</td>
<td>India ink</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>positive</td>
<td>29</td>
</tr>
<tr>
<td>negative</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>33</td>
</tr>
</tbody>
</table>
# Sensitivity patterns

<table>
<thead>
<tr>
<th>DRUG</th>
<th>SENSITIVITY STATUS n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive</td>
</tr>
<tr>
<td>Amphotericin B (n=39)</td>
<td>36 (92.3)</td>
</tr>
<tr>
<td>Fluconazole (n=39)</td>
<td>28 (71.8)</td>
</tr>
<tr>
<td>Miconazole (n=39)</td>
<td>37 (94.9)</td>
</tr>
<tr>
<td>Flucytosine (n=39)</td>
<td>19 (48.7)</td>
</tr>
</tbody>
</table>
Serotypes:

100% were serotype A
Discussion

Prevalence of CM: 19% (19% in Rwanda, 21.9% in Zimbabwe, 13% in S. Africa)
AIDS defining illness in 65.5% of patients.
Growth of Other fungi (T. beigeli, T. mucoides).
India ink

India ink sensitivity-56% (Zimbabwe-85%, Rwanda-83%, 72%-New Zealand)
Operator dependent
Depends on burden of organism in CSF
Only CRAG taken as gold standard
Severe cases excluded (papilledema, FND)
Culture:
(Australia 85%, Rwanda-53%-95%, Zimbabwe-85%)
Also depends on the burden of organism in the CSF.
Correlates well with the titers of CRAG.
Prior antifungal use in some patients.
Discussion cont......

Resistance to Ampho-B a concern.
Resistance to fluconazole not as high as earlier presumed but dose increment necessary.
Primary resistance to 5Flucytosine documented and a significant no with SDD.
Only serotype A identified.
Limitations:
CRAG used as the gold standard.
Patients with papilledema, focal deficits excluded.
CD4 counts, viral loads not done.
Crag titers not determined.
Recommendations

CRAG test be aggressively developed countrywide for diagnosis of Cryptococcal meningitis.
Formulation of policies for the usage of antifungals both in management as well as prophylaxis.
Further studies to correlate resistance patterns with clinical outcomes.
THANK YOU