Prevalence of Human Cytomegalovirus Infection among Human Immunodeficiency Virus Positive Women Receiving Antiretroviral Treatment at Federal Teaching Hospital Abakaliki, Ebonyi State, Nigeria

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Abstract

Background: Cytomegalovirus has been identified as the major cause of morbidity and mortality among neonates. It commonly occurs in immunocompromised and immunodepressed individuals.

Objectives: This study was conducted to determine the occurrence of CMV infection amongst HIV-positive women receiving antiretroviral treatment at Federal Teaching Hospital Abakaliki (FETHA). Socio-demographic factors associated with CMV and HIV were also examined. The population of volunteers present for the study was 124 Persons.

Study Design: Blood samples were collected from the study subjects and recent infection of CMV was investigated using the Enzyme Linked Immunosorbert Assay ELISA, which detects Immunoglobulin M (IgM).

Results: The result obtained indicated that 14 (11.3%) out of the 124 already confirmed HIV patients had recent/current CMV infection. Again, 18 of the patients were either pregnant or within two weeks of delivery, among which 3 (16.7%) were CMV positive. The study also showed that CMV and HIV co-infection predominates among the age group 26-30 years (15.6%), singles (13.9%), and unemployed (15.8%) of the tested population. The level of education had no effect on the rate of co-infection of CMV and HIV.

Conclusion: This study shows the rate of infection and reactivation of CMV among HIV positive patients receiving antiretroviral treatment at Federal Teaching Hospital Abakaliki, Ebonyi State, Nigeria. CMV infection predominates among the Single HIV-positive individuals, within the age group 26-30 years, followed by Civil Servants and the unemployed. We therefore recommend that serious and aggressive campaign be carried out in this region and elsewhere where CMV and HIV co-infection is endemic or prevalent in order to curtail the transmission of the disease and thus reduce the possible menace posed by this virus.

Keywords: Viral Co-infections; Cytomegalovirus; HIV; ELISA; Immunoglobulin M (IgM)

Background

Human Cytomegalovirus (CMV) has been established as the major cause of viral infection in pregnancy [1]. Infections of the foetus can result due to initial maternal infections, as well as, reinfecion and/or reactivation in HIV positive mothers [2]. CMV belongs to herpesviridae family of viruses. It possess double stranded DNA, that are linear in shape, and measures 235 kb in length [3]. Other components include viral envelope (containing glycoprotein of host membrane), and capsid. CMV, like other betaherpesviridae subfamily, are known to affect specified host cells, grows in fibroblast cells obtained from their host organisms, replicates slowly and can induce latent growth [4].

Cytomegalovirus (CMV) transmissions occur among people of all ages, races, and socioeconomic classes, throughout both the modernized and developing parts of the world [5,6]. A serological survey of over 20,000 women in London found 54.4% of these women were seropositive for CMV [7]. Africa has been reported to have the highest IgG prevalence rate of CMV. For instance Egypt and Western Sudan recorded prevalence rate of 96.0% and 72.2% respectively [8]. In Asia, Malaysia recorded 84.0% [9]. Similar studies in Lagos, Sokoto and Bida cities (all in Nigeria) showed CMV IgG rate of 98.7%, 97.2% and 84.2% respectively [10-12]. However, no such studies have been conducted in Ebonyi State, South-Eastern Nigeria.

CMV hardly cause infection in healthy individuals, but can manifest numerous disease syndromes in people who are immunocompromised (HIV and transplant patients). The extent of disease manifestation depends on the level of immunodepression. CMV causes reduced birth rate, increased death rate, and induces rejection of allograft in patients [13].
Human Immunodeficiency Virus (HIV)

Human Immunodeficiency Virus (HIV) is the aetologic agent of Acquired Immunodeficiency Syndrome (AIDS). The disease was discovered in 1981, and in 1983, HIV-1 was isolated. Since then, HIV has been transmitted worldwide, affecting different races and populations. HIV/AIDS, if left untreated leads to death of most carriers within a decade. The reason for this is because; the virus compromises the host’s immune system, enabling opportunistic infections to manifest [3,22,23]. Prevention of mother to child transmission of HIV is possible if the status of the mother is known. Studies have shown HIV sero-conversion during pregnancy [24].

The human body relies on the immune system to fight infections and to suppress malignant cells. White blood cells provide us with necessary soldiers called lymphocytes. The CD4-helper T-lymphocytes supervise the attack of immune system on foreign objects. HIV induces and causes the CD4-helper cells to produce copies of it (HIV); thereby leading to the depleting of CD4-cells. CD-4 cells have a normal range of 600-1500 per cubic milliliter of blood. If the concentration drops below 200, the host’s immunity weakens and the situation is termed Acquired Immunodeficiency Syndrome. Once the immune system is low, the body will be exposed to opportunistic and non-opportunistic infections.

Various diseases develop faster in HIV/AIDS patients than those without the virus. Among patients with Impaired Kidney Function (IKF) studies, 53.3% are HIV positive [25]. Risk factors and risk assessment for cardiovascular diseases are increased in HIV-positive patients with and without antiretroviral therapy. These factors include hypertension, measures of obesity, diabetes mellitus, dyslipidaemia, and homocysteine [26]. Electrocardiography is effectively used to detect cardiac Diseases. Electrocardiographic (ECG) and echocardiography abnormalities have been demonstrated in HIV-positive patients.

The manifestations of HIV infection in organs other than the heart mask the clinical evidence of cardiac disease in these subjects. An abnormal ECG was present in 175 (70%) of the 250 treatment-naive HIV-positive subjects, and 70 (35%) of the 200 HIV-negative subjects [27].

Nigeria has been labeled as the second country with highest population of HIV patients in Africa, only after South Africa [28]. It is also stated that within the Twenty-six years of HIV in Nigeria (1986-2011), about 3,459,363 patients have been being infected with HIV/AIDS. In 2011 alone, about 388,864 new cases occurred with 217,148 associated fatalities [28].

NACA identified the major factors driving the HIV/AIDS to include continuous multiple sexual partnership, commercialized and intergenerational sex, insufficient handling of sexual related diseases, substandard health care delivery, chronic and perpetual poverty, AIDS-related stigma and segregation [29].

Annual report of NACA [30] estimated that HIV transmission was highest in age group 30-33 years (4.4%) and lowest among the group 15-19 years. In males alone, age group 35-39 was highest (5.3%) and women of 30-34 years had 4.2%.

Pathogenesis and Pathology of CMV

The portal of entry include oral and respiratory routes [3], transplacenta [31], sexual contacts, contaminated fluids discharged from oral and pharyngeal secretions, tears, urine, cervix, seminal and vaginal fluids, feces [32], blood transfusion [33,34], and organ transplant. In immunocompetent hosts, HCMV either remains unnoticed or causes mild symptoms. Upon primary infection it establishes latent infection in a few cells. However, in certain situations where immunity is either immature or compromised, HCMV may reactivate and cause mortality and morbidity [35]. The mechanism of infection and reactivation of HCMV have been reported [35].

The magnitude of the CMV disease depends on the number/ frequency of exposure of an individual to the virus, and the level of immune-suppression. It is estimated that about one-third of CMV seronegative pregnant women transfer the virus to their child. CMV replicates in different organs and cells of the body, including liver, lungs, oesophagus, colon, kidneys, monocytes and T and B lymphocytes [36]. Manifestations in the GIT are fatigue, high fever, high level of monocytes, pneumonia, inflammation of the liver [37], severe pain in mouth or oesophagus when swallowing, blood in vomits (hematemesis), frequent stoolen (diarrhea), rupture of intestines, renal bleeding, and Ovarian diseases [38]. Among infants, about 10-15% of CMV carriers will experience neurologic disorders like hearing loss, bleeding [40] and Oophoritis [41] have also been documented. Unfortunately, it was discovered that intra-uterine transmissions still take place even with CMV positive antibodies [42].

Factors that determine severity and duration of CMV include development of cellular and humoral response, viral load and
infecting CMV strain [43], and genetic difference among CMV strains [44].

**Treatment of CMV**

Both prophylaxis and therapy have been widely administered against CMV diseases. Currently, the common drugs of choice are cidofovir (CDV), ganciclovir (GCV) and foscanet. GCV is a synthetic analog of deoxyguanosine and acts by competitive inhibition of deoxyguanosine. When inserted into a DNA strand by viral DNA polymerase, GCV slows chain elongation [45]. Forscanet inhibits viral DNA polymerase by binding to the pyrophosphate binding site and preventing cleavage of pyrophosphate from the deoxynucleotide triphosphate [46]. CDV is a monophosphate analog of cytosine that uses mammalian cellular kinase [47]; it acts by competitive inhibition of cytosine incorporation into the viral DNA strand inhibiting elongation of the strand. The incorporation of two (2) consecutive CDV molecules into a growing strand causes chain termination [48]. GCV is mostly effective for treatment and prevention/prophylaxis of CMV. PFA inhibits DNA polymerase and is recommended for treating CMV strains that are resistance to GCV. However, PFA is nephrotoxic, hence is not recommended during kidney transplant. CDV directly inhibits DNA polymerase and can be used in place of PFA [49].

**Prevention of CMV**

CMV infection has been associated with numerous neurologic debilitating effects, especially in infants and immunocompromised individuals. It is therefore necessary to prevent rather than to treat the disease. This could be achieved by avoiding transplantation of CMV seropositive blood, fluid or organ to seronegative patients [50]. Developing vaccines against CMV have met many setbacks because of inherent genetic variability among CMV strains. Recently, DNA capable of generating antibody response in healthy individuals has been produced [51].

To circumvent the obstacle associated with DNA vaccines, recombinant glycoprotein B vaccine against the viral envelop has been produced. This vaccine is more reliable because viral glycoprotein envelop is conserved in all the CMV strains [52]. The best method would be to vaccinate all women prior to their reproductive ages [53].

**Objectives**

To identify possible co-infection of HIV and Cytomegalovirus among pregnant and sexually active women, who are receiving treatment in FETHA.

To evaluate the socio-demographic factors (extent of age, educational, and occupational distribution) associated with co-infection of Cytomegalovirus and HIV among women.

**Study Design**

**Study area/population**

This study was carried out at the Federal Teaching Hospital Abakaliki (FETHA), located at Abakaliki Local Government Area, of Ebonyi State, Nigeria. The Hospital admits a large number of HIV patients from various parts of the state and its environs. The hospital also have HIV clinic known as AIDS Relief Clinic which made it a good study area. A total of 124 HIV sero-positive and sexually-active female patients between the ages of 15 and 45 were recruited for this study. Ethical approval was sought for, and obtained from the Research and Ethical Committee (REC) of FETHA. Pro-forma was distributed to the participants of the study in order to get their demographic data; and data was analyzed using descriptive statistics of percentages. All the volunteers were provided with consent forms to be recruited for the study. They all gave their consent orally and in written format.

**Sample collection**

About 5 ml of venous blood samples were aseptically collected from the HIV-sero-positive individuals. The samples were transported to FETHA laboratory in insulated carrier box maintained at room temperature. After sufficient coagulation, the sera were separated from the whole blood and the former stored in the freezer until usage.

**Detection of CMV using Enzyme Linked Immunosorbent Assay (ELISA)**

CMV was detected using the ELISA technique as was previously described [21,54]. Antibodies to CMV antigens were detected in the patient’s serum using Diagnostic Automation ELISA Cytomegalovirus IgM Kit [55]. The result was read using spectrophotometer at a wavelength of 450 nm.

**Results**

The incidence of CMV infection among HIV positive pregnant women is shown in Table 1. In the first trimester of pregnancy, 2 out of 7 HIV positive pregnant women tested positive for the presence of CMV infection while none of the pregnant women tested positive in the second and third trimesters. However, two weeks after delivery, 1 out of the 2 participants tested positive for CMV infection.

Table 2 shows the prevalence of CMV infection among HIV positive non pregnant women in relation to their marital status. CMV infection was most common among the married women and singles. Only one participant out of the 9 divorced women in this study tested positive for CMV infection. The distribution of CMV infection among the participants based on their educational and occupational status is shown in Table 3. CMV infection was common among the self-employed participants who had attained secondary education than in any other category.
Table 4 shows the distribution of CMV infection among different age groups. Highest occurrence was observed among the age group 26-30 (15.6%); and this was followed by age group 30-35 (10.0%) and age group 36-40 (10.0%).

Table 5 shows the experience of still-birth and previous blood transfusion prior to investigation. Twenty nine (29) patients attested to have experienced previous still birth. Among them, 2 (6.9%) were positive. With respect to blood transfusion, 3 (16.7%) out of 18 persons tested, displayed positive results.

Discussion

In this research work, blood samples were collected from a total of 124 volunteers. ELISA tests were carried out using the subjects’ sera. It was discovered that 14 (11.3%) persons among the tested population were positive for CMV IgM. This is in consonance with 14.8% positive tests obtained by [56] and 10.5% [1] while carrying out similar study.

The results also showed that all the individuals who participated in the research had some level of IgM antibody against CMV in their respectively sera. However, majority of the subjects (88.7%) had very low antibody titre. This means that the CMV viral dose circulating in their blood were not adequate to elicit disease. This discovery of ubiquitous nature of CMV is in consonance with the works of previous researchers that CMV has a worldwide distribution, with prevalence rate of 70%-100% [6,40,57,58]. The reason for this could be attributed to recent infection or reactivation due to diminishing immunity in the immune-compromised individuals.

Table 1 enumerated the incidence of CMV among HIV positive pregnant women. Within the study population of 124 persons screened, 18 people were pregnant, out of whom, 3 (16.7%) tested positive for CMV IgM antibody. This could be because of anaemia associated with most pregnancies. This deviates from other publications [59] and [60]. Which portrayed CMV prevalence in pregnancy to be 4% and 6% respectively? And those synergistic effects may worsen the immunological profile and could potentially translate into more rapid disease progressions in HIV infected persons [49].

Table 1: Incidence of CMV infection among HIV Positive Pregnant Women.

<table>
<thead>
<tr>
<th>Stage of pregnancy</th>
<th>No of Subject tested</th>
<th>CMV positive patient n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Trimester</td>
<td>7</td>
<td>2 (28.6)</td>
</tr>
<tr>
<td>Second Trimester</td>
<td>2</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Third Trimester</td>
<td>7</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Two (2) weeks after delivery</td>
<td>2</td>
<td>1 (50.0)</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>3 (16.7)</td>
</tr>
</tbody>
</table>

Out of 7 people who were in their first trimester tested 2(28.6%) tested positive to CMV. Early pregnancy has been associated with numerous physiological (hormonal and biochemical) changes which often favors opportunistic pathogens. However, literature supporting the relationship between early pregnancy (first trimester) and CMV manifestation/disease are scanty. The fact that CMV was not detected among those in their second and third trimesters does not rule out the possibility of CMV manifestations within these groups. A closer research among non-HIV positive pregnant women in Israel indicated that research, 75% of the pregnant women in their third trimester tested CMV IgM positive [61]. They also reported that vertical CMV transmission, in utero, increases with advancing pregnancy stages. Their result entailed that pregnancy alone can lower mothers’ immunity to opportunistic infections, including CMV. Two weeks after delivery, another patient showed positive result to CMV. The reason for this is not crystal clear. It could be as a result of serious bleeding or haemorrhage associated with delivery, or that the patient has had this infection before delivery.

In Table 2, prevalence of CMV among HIV-positive non pregnant women was tested. A total of 106 people were tested for CMV infection, and 11 (10.4%) were positive. Considering marital status, 33 spinster were examined and 4 (12.6%) reacted positively with CMV IgM kit. The reason for this could be that the spinster travel a lot in search of job opportunities, carrier growth, and even social activities. Changing of environments can predispose an individual to many opportunistic infections like CMV. Some of the singles also tend to have numerous intimate sexual partners. This result is in tandem with similar study which reported 13.6% prevalence of CMV in the single population as highest [62]. CMV have different strains, each capable of causing disease even among the carriers of the other strain(s) [43]. This might be the cause of re-infection or reactivation among the group.

Table 2: Prevalence of CMV infection Among HIV-positive non-pregnant women.

<table>
<thead>
<tr>
<th>Marital Status</th>
<th>No of tested subject</th>
<th>CMV positive subjects n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>33</td>
<td>4 (12.1)</td>
</tr>
<tr>
<td>Married</td>
<td>64</td>
<td>6 (9.4)</td>
</tr>
<tr>
<td>Divorced/ widower</td>
<td>9</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Total</td>
<td>106</td>
<td>11 (10.4)</td>
</tr>
</tbody>
</table>

The next group is the married population. Out of 64 persons tested, 6 (9.4%) were positive. Transmission of CMV to this group majorly had to do with sexual intercourse. Considering the fact that Ebonyi state is economically backward with low level of education, high level of poverty, and people still practice polygamy; a lot of the infection could have been transmitted to the women from their husband/ spouse. Hence, “Most of the new infection cases occurred among people who were not participating in high risk sex, a small group that include cohabiting or married partners. Condom use among this group is very low; hence infections contacted by one partner (due to recent or past activities) are easily transferred to the unsuspected partner [29].” Contrarily, other finding showed that sero-prevalence of CMV among women of child-bearing age
varies from 60% to over 80%, with inverse correlation to socio-economic levels [42].

Among the 9 widows/divorcees who participated, 1 (11.1%) tested positive. The cause of infection may largely be attributed to re-activation as a result of immuno-suppression. However other key factors could have played roles in the transmission, reactivation or re-infection.

In Table 3, distribution of CMV among various occupational groups and literacy level showed that among 47 primary school leavers who participated 4 (4.5%) were CMV positive. Next were 44 secondary school leavers with 7 (15.9%) of them tested positive. And 30 tertiary institution graduates were tested in which 3 (10.0%) persons were positive. The result therefore showed that level of education had no effect on the transmission of CMV infection. This is similar to other studies [6]. Again, the table also depicted the relationship between CMV infection and occupational status. Among the 18 civil servants tested, 4 (22.2%) were positive, 78 self-employed persons were screened and 7 (9.0%) were positive, and among 19 unemployed persons who participate 3 (15.8%) reacted positive. Following this trend, it appears the civil servants had the highest rate of CMV infection, followed by the unemployed and the self-employed groups respectively. CMV is prevalent among all the occupational groups in the area under study. Level of education has no effect on the transmission of CMV infection. Information regarding its presence and transmission are very scanty; and barely known to the general public.

Table 3: Distribution of CMV infection based on educational and occupational level.

<table>
<thead>
<tr>
<th>Level of Education Attended (population tested)</th>
<th>Occupation (population tested)</th>
<th>Primary (47)</th>
<th>Secondary (44)</th>
<th>Tertiary (30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Civil Servant (18)</td>
<td>-</td>
<td>2</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Self Employed (78)</td>
<td>3</td>
<td>4</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Un-Employed (19)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Student (9)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Table 4 clarifies the relatedness of age group to CMV infection. The age group 26-30 years comprising of 32 persons had the highest occurrence of CMV recent infection-5 (15.6%); whereas, those within 31 – 35 years old had 10% prevalence rate. In a close-related work, [62], age group 36 - 40 years with 22.2% as highest; although the research was conducted among non-HIV positive patients. The difference could have resulted due to increase in age which is relatively inversely proportional to individual’s immunity. Others reported that age range 18 - 24 years had the highest occurrence (45.5%) [34].

In Table 5, 18 persons who had previous blood transfusion were tested and the result showed that 3 (16.7%) were CMV positive. This is closer to 10.3% obtained by another study group [63]. It is however dissimilar from previous works [32-34]; which had 0.0%, 28.0% and 0.2% respectively.

Table 4: Age Distribution of CMV Infected Persons.

<table>
<thead>
<tr>
<th>Age group (Years)</th>
<th>No of Subject Tested</th>
<th>CMV positive subject n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-20</td>
<td>2</td>
<td>1 (50.0)</td>
</tr>
<tr>
<td>21-25</td>
<td>14</td>
<td>1 (7.1)</td>
</tr>
<tr>
<td>26-30</td>
<td>32</td>
<td>5 (15.6)</td>
</tr>
<tr>
<td>31-35</td>
<td>40</td>
<td>4 (10.0)</td>
</tr>
<tr>
<td>36-40</td>
<td>20</td>
<td>2 (10.0)</td>
</tr>
<tr>
<td>&gt;41</td>
<td>16</td>
<td>1 (6.3)</td>
</tr>
<tr>
<td>Total study population</td>
<td>124</td>
<td>14 (11.3)</td>
</tr>
</tbody>
</table>

Also in Table 5, when CMV and previous still-birth was evaluated among 29 subjects, 2 (6.9%) were positive. Preliminary studies investigating the relationship between CMV and still-birth are very scanty as at the time of compiling this report.

Table 5: Experience of still birth and blood transfusion prior to investigation.

<table>
<thead>
<tr>
<th>Response</th>
<th>Still Birth</th>
<th>Blood Transfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No tested</td>
<td>CMV + (%)</td>
</tr>
<tr>
<td>Yes</td>
<td>29</td>
<td>2 (6.9)</td>
</tr>
<tr>
<td>No</td>
<td>95</td>
<td>12 (12.6)</td>
</tr>
</tbody>
</table>

Although, this study did not critically examine the pathogenesis and complications associated with HCMV in HIV patients, such works has been documented. HCMV upon reactivation in HIV infected individuals can cause retinitis, neurological damage, gastrointestinal problems, hepatitis, pneumonitis and adrenalitis [64,65].

Some researchers have established the possible role of HCMV in carcinogenesis during the last decade. HCMV activated the IL-6-JAK-STAT3 pathway in PHH and HepG2 cells, favored cellular proliferation, induced primary human hepatocytes (PHH) transformation and enhanced HepG2 tumoursphere formation [66]. IL-6 levels are elevated in the serum of patients with chronic liver diseases and increase even more in patients who develop hepatocellular carcinoma [67,68]. Interestingly, high serum levels of IL-6 helped to predict the development of HCC in both hepatitis (HBV and HCV) infected patients [69].

Conclusions

In conclusion, our study has shown that individuals with HIV and AIDS are susceptible to CMV and other opportunistic infections. Rate of infection and reactivation of CMV among HIV positive patients is high among patients receiving antiretroviral treatment at Federal Teaching Hospital Abakaliki, Ebonyi State. CMV infection predominates among the Single HIV-positive
individuals, within the age group 26-30 years, followed by Civil Servants and Unemployed. Serious and aggressive campaign and attention need to be given to CMV infection and disease in order to curtail the transmission and reduce the possible menace posed by this virus.

Limitations of the Study

The limitations of this study include the small sample size. Our findings may not be widely generalizable as the data emanated from a relatively few patients who were attending the HIV relief Clinic of FETHA, the only tertiary hospital in Ebonyi State, at the time of research.

The researcher conducted only CMV IgM analysis, which was meant to discover recent infections and or reactivation. The exact times of infection were not determined.

There was no PCR analysis in order to determine the genetic makeup (strain) and biochemical analysis of the CMV present. This calls for further studies.

The study did not include the level of Hygiene maintained by the volunteers. However, the general level of hygiene practiced in Ebonyi State, is low.

Acknowledgement

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Ethical Approval

Ethical approval was sought for, and obtained from the Research and Ethical Committee (REC) of Federal Teaching Hospital Abakaliki (FETHA). All the patients endorsed their signatories and gave their consent for the research to be carried out.

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