The Effect of Co-infection with Hepatitis C Virus on Incidence of Anaemia, Liver Transaminases and Immunological Markers among HIV Patients on HAART in South West (Osun State) Nigeria

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Authors’ contributions

This work was carried out in collaboration between all authors. Author VOM designed the study and contributed to literature research. Author MAM carried out laboratory investigations. AuthorCAA assisted with analysis and literature search. Author RAA assisted in literature search. All authors read and approved the final manuscript.

ABSTRACT

Introduction: It is becoming clear that a major complication of HIV patients on HAART is co-infection with hepatitis C and its attendant sequelae such as liver cirrhosis and carcinoma. The aim is to determine the prevalence of anaemia, transaminitis in these co-infected patients.

Materials and Methods: Three groups of patients were studied. There were a total of 44 male and 106 females included in the study. No children were among. Those co-infected with both HIV and HCV (group I), HIV only (group II) and negative for both viruses (Group III). Each group consists of 50 patients each. HIV status was determined utilizing determine and Unigold to detect HIV antibodies. HCV was determined by detecting the anti-HCV antibody (IgG) using third generation ELISA kit from DIA.PRO, Italy. The haematological indices were determined using the Sysmex.
1. INTRODUCTION

The first clinically observed HIV/AIDS cases were in 1981[1] in the United States where young homosexual men with impaired immunity were presenting with Pneumocystis carinii pneumonia (PCP) and Kaposi’s sarcoma (KS). In 1983 the virus was isolated in a patient with lymphadenopathy and in 1984 the virus was identified in the laboratory [2]. Hepatitis C virus (HCV) and HIV are both blood borne diseases that are transmitted in similar ways as a result it is not unusual to find them occurring together. These include unprotected sexual intercourse, unscreened blood transfusions and sharing needles [3]. Persistent HCV infection can lead to cirrhosis of the liver and even liver cancer. When these viruses occur together in the same individual there is an increase in occurrence of more aggressive hepatic diseases (fibrosis, cirrhosis and carcinoma) including end stage liver disease (ESLD) [4]. This makes it particularly important to detect these co-infected individuals and administer the appropriate treatment for each component of the infection.

Accepted treatment for HCV is Rabavarin and pegylated interferon though this is thought to need improvement [5], while the effective treatment for HIV is highly active antiretroviral therapy (HAART). The problem with treating co-infection is drug drug interactions some of which could be life threatening [6].

In 1985 the ELISA kit was developed to detect the antibodies to HIV and thus established the ability to diagnose the disease condition. However following this, a diagnosis was initially not of much use to the medical status of the patient, death being only a matter of time. Since the introduction of highly active antiretroviral drugs (HAART) for the management of HIV/AIDS there has been a considerable improvement in the quality of life of these patients and a prolongation of the life span [7]. Also over time there has been a reduction in transmission of the HIV among high risk groups contributed to by, the use of HAART to prevent mother to child transmission (PMTCT) [8], the use of HAART itself both as treatment and prevention [9]and a general increase in the knowledge and awareness of methods of transmission through health education [10].

However this prolongation of life has led to the inevitable expression of complications of both the disease itself and those associated with HAART. Patients with HIV/AIDS have an increased risk of developing malignancies such as Kaposi’s sarcoma, non-Hodgkin’s lymphoma and also cervical carcinoma in women are not infrequent [11]. It should be mentioned at this point that hepatitis C itself has oncogenic properties with particular emphasis on hepatocellular carcinoma. High levels of alanine aminotransferase in hepatitis C viral infection has been associated with an increased risk in the development of hepatocellular carcinoma [12]. Some other important and sometimes potentially life threatening complications include hypertension and other related cardiovascular diseases, obesity and metabolic syndrome especially with those on HAART [13].

Anaemia is not uncommon among HIV/AIDS patients which could be due to the disease itself but also as a complication of treatment [14,15]. The haemoglobin level is an important parameter that needs to be determined routinely while patients are on therapy as there may be a need to switch from first line therapy as a result of persistent unexplained anaemia in patients already placed on HAART [15]. In well-established health care delivery services, there is usually a switch committee that investigates these patients and makes the decision on whether to switch therapy or not. It is thought

Results: No case of anaemia was detected. CD4 counts in group I patients (HIV /HCV positive) and group II patients were clearly reduced. The CD 4 counts were markedly reduced when compared to the controls (group III) P<0.005. The liver enzymes were markedly raised in co-infected patients.

Conclusion: The major observations in our group of co-infected patients was marked transaminitis and reduced CD 4 counts in co-infected patients. It is necessary to determine HCV genotypes to explain why our patients have not presented with increased cirrhosis and hepatic carcinoma.

Keywords: HIV; HCV; Co-infection CD4 counts anaemia.
that some of the HAART drugs interfere with haemopoiesis, the synthesis of the red cell component of cellular elements of blood. An example of this is Zidovudine a component of HAART which is a notorious cause of treatment induced anaemia [16] nevertheless HAART has improved the management of HIV in quantum leaps. Furthermore it is important to note the existence of different types of anaemia which could occur. This includes microcytic hypochromic anaemia, normochromic normocytic anaemia and megaloblastic anaemia. A reliable parameter of distinguishing these is the haematological indices, mean cell volume (MCV), mean haemoglobin concentration (MHC) and mean corpuscular haemoglobin concentration (MCHC). These would give an indication of the possible causes of anaemia and may influence treatment protocols. It has been found in previous studies that the probability of developing microcytic hypochromic anaemia in HAART - naïve patients is five times more than those on HAART [17].

It is well known that the gateway of the HIV virus into the individuals immune system is through the CD4 cells. Immune activation is now considered a main driving force for the progressive immune failure in HIV infection. During the early phases of infection, a rapid depletion of gastrointestinal CD4+ T cells occurs that is followed by a deterioration of the gut epithelium [18] and by the subsequent translocation of microbial products into the blood. Activation of innate immunity results in massive production of pro-inflammatory cytokines, which can trigger activation induced cell death among T lymphocytes. Moreover, persistent antigenic stimulation and inflammatory status causes immune exhaustion. The chronic immune activation also damages lymphoid tissue architecture, so contributing to the impairment of immune reconstitution. Other important complications are co-infection with ‘related’ viruses. Related in that these viruses share routes of infection with the HIV. These include hepatitis B, hepatitis C [19], E and delta [20]. They individually and collectively may contribute to and worsen the disease and treatment outcomes of co-infected patients [21].

It is of important to note that HBV co-infection with HIV is also common [22]. However there is more emphasis on co-infection with Hepatitis C virus because of the particularly damaging effects on the liver of HIV/HCV co-infection [21]. It has been mentioned in earlier studies carried out in this environment especially with regards to interpreting results that assumptions are inadvertently being made in such studies that the individuals are not confirmed to be co-infected with other viruses with similar routes of infection eghepatitis A, hepatitis B, hepatitis E, Delta which could individually and collectively contribute to the pathology [21]. It has been suggested that further studies be carried out in our environment on virology such that HIV positive patients are screened for a number of viruses at point of entry into the healthcare facility since the viruses tend to ‘travel’ together [21]. This would make interpretation of results of such investigations more meaningful.

Co-infection of HIV with hepatitis C as stated earlier is not uncommon [23]. The liver is usually the targeted organ and related symptoms are the cause of admission to hospital [24]. Pathological findings include liver fibrosis, particularly severe and aggressive cirrhosis and finally hepatocellular carcinoma. Prevalence rates of co-infection have been found to range from 16% in Ethiopia to 23.2% in Nigeria [25].

In summary this project is therefore designed to determine the prevalence of anaemia among our HIV/AIDS clients co-infected with hepatitis C. The project would also determine effects of co-infection on parameters such as the CD4 counts of these patients with the aim of determining if co-infection affects the CD4 counts and if so further compromising the immunity of these patients. Liver enzymes would also be determined and compared with controls. Since co-infection is associated with more severe disease, there could be a relation with patients who are co-infected presenting with more severe anaemia and higher alanine aminotransferase levels.

2. METHODOLOGY

Blood samples were collected from the 50 patients co-infected with hepatitis C and HIV, based on prevalence of 3.3% [26]. Fifty subjects living with HIV/AIDS who tested negative for hepatitis C virus were included concurrently. Finally 50 subjects seronegative for hepatitis C and HIV were included as negative control subjects. Informed consent was sought from all patients. Patients who consumed alcohol, indulged in smoking were not included in the study. A the time the only drugs they were on were HAART. There were a total of 44 male and 106 females included in the study. No children
were among the population studied. Their ages ranged from 20 years to 64 years.

Setting: The study is being carried out in Osogbo the capital of Osun state located in south west Nigeria. It is an urban setting with a population of 3,416,959 (en.wikipedia.org/wiki/List_of_Nigerian_states_by_population). The residents are majorly Yoruba however there are other tribes including Hausas, Igbos and those of Edo state origin. The weather is typically tropical with periods of heavy rain fall alternating with the dry season.

All participants were diagnosed as HIV positive by screening for the antibody using kits from two different sources Determine and Unigold.

For HCV, about 5 mls of blood sample was collected from every participant by venepuncture into EDTA vacutainer bottles (maker:BD, PL6,7BK,UK Ref. 367830). This was centrifuged at 1200G for 10 minutes and 1ml of plasma was harvested into 2 plain bottles for anti-HCV antibody detection with third generation enzyme immunoassay technique. The plasma from every participant was diluted with DILSPE (sample diluent prepared commercially by DIA.PRO, Sesto San Giovanni, Italy). Each sample was further diluted with DILAS (by DIA.PRO) alongside the negative controls in triplicate, the calibrator in duplicate and a positive control as provided by the kit manufacturer. After the micro plate is incubated and wells washed, all the wells were treated with enzyme conjugate except the first blanking well. The micro plate was incubated again and the chromogen/substrate mixture was added after the second washing.

The reactions were stopped with sulphuric acid and the optical density (OD) read at 450 nm immediately. The cut off value for each batch was determined and individual results were interpreted as negative (<0.9) and positive (>1.1) and equivocal (0.9-1.1).

where 20 µ/l of EDTA anticoagulated blood is mixed with 20 µ/l of CD4 antibody. The mixture was incubated in the dark and further diluted with 800 µ/l of diluent. This was presented to the probe of the cyflow which analyses the sample and a print out of results was generated for documentation.

For red cell indices: haematocrit, haemoglobin concentration, red cell count, red cell distribution width, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) shall be determined. The reagents involved include the diluents, the lyse and the clean. The anaemia would be classified using absolute values obtained from the indices mentioned above.

Statistical analysis will be carried out using SPSS software, frequency, mean, standard deviation and t-independent test.

Inclusion criteria 1) HIV positive patients 2) Patients who give consent 3) Only patients managed in our facility 4) Patients on HAART

Exclusion criteria 1) HIV negative patients 2) Patients who decline 3) Patients not on HAART

Limiting Factors: Lack of screening for other viruses with similar routes of infection such as HAV, HBV, hepatitis E and Delta viruses.

3. RESULTS

The total number of participants was 150. There were three groups of patients, those who were HIV positive and and HCV positive (Group I), the second group in which they were HIV positive and HCV negative (Group II) and the third group which served as the control group where the clients were both HIV and HCV negative (Group III). For group I the median age was 40.64±9.39, while for group II 39.22±8.62 and for group III 39.84±10.00 years. In all groups the majority were female the highest percentage of them being in group I at 80%, group II 68% and 64% in group III. For group I the mean aspartate transaminase level (AST or SGOT) was 21.38±32.32, while this was 13.32±11.76 in group II and and 7.70±4.82 for group III. For alanine transaminase (ALT) the mean for group I was 14.14±18.33 and 9.72±12.95 for group II and, 5.46±3.29 for group III.

For group I the mean haemoglobin was 11.10±1.67, for group II this was 11.43±1.92 and for group III was 11.59±1.67. Considering the haematological indices in group I, MCV was 83.88±44.54, MCH 29.16±9.35 and MCHC 30.78±9.06. In group II, MCV was 79.32±7.13, MCH was 25.31±2.69 and MCHC 31.70±1.47 while in group III MCV was 81.81±6.35, MCH 25.81±2.50 and MCHC 31.31.49±1.38. In group I (Table 2) where the clients were HIV positive and HCV positive the majority of patients fell in the age group 20-49 years and was found to be statistically significant (P<0.05). Sixty four percent
of the patients fell within this group. Determining the relationship between group I (HIV positive and HCV positive) and group III (both negative for HIV and HCV) all the parameters except for, haemoglobin (PCV), MCV and MCHC had a statistically significant relationship with the viral status of the patients (Table 3).

For group II and III statistical analysis using regression analysis the liver enzymes AST and ALT were found to be statistically significant (Table 4).

4. DISCUSSION

The importance of proper investigation and management of HIV/HCV co-infection cannot be overemphasized. Since the advent of highly active antiretroviral drugs (HAART) and subsequent prolongation of life HIV positive individuals, co-infection with hepatitis C virus and its sequelae have increasingly become well recognised complications of HIV/AIDS [27]. However it appears that our HIV patients in this environment co-infected with HCV do not appear to manifest with the same problems. Increased levels of the hepatic enzymes occur, however increased incidence of hepatic fibrosis, cirrhosis and liver carcinoma have not been documented. Either way it is important we observe our patients for these trends. It may be worthwhile noting that presently chronic hepatitis C virus infection is the leading indication of liver transplantation in the USA, and it has been estimated that the number of deaths from HCV infection in USA and Europe will within the next ten years triple and surpass deaths due to HIV [28]. Though these figures may not be automatically applicable to our resource limited environment in which the HIV positive individual is besieged with so many challenges, it could serve as an important and vital indicator as to where to direct our focus in the management of our HIV positive clients as they live longer. This is assuming that introducing HAART was the “first stage”.

Among all the groups studied it was found that the female sex were in majority. It has been reported severally over the years that the female sex is more vulnerable to HIV infection than the male sex [29] and it appears that the situation has not changed over the years. In one group the majority of participants were of the female sex as high as 80% (Table 1). Hence it only stands to reason that once there is co-infection with HCV, the higher prevalence of the female sex would also be reflected and therefore should not be unexpected (Fig. 2).

Table 1. Table showing the age range and sex distribution of the study group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>n=50</td>
<td>n=50</td>
<td>n=50</td>
</tr>
<tr>
<td>≤20 – 29yrs</td>
<td>7</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>30 -39yrs</td>
<td>16</td>
<td>26</td>
<td>21</td>
</tr>
<tr>
<td>40-49yrs</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>50-59yrs</td>
<td>11</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>≥60yrs</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>10</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>Female</td>
<td>40</td>
<td>34</td>
<td>32</td>
</tr>
</tbody>
</table>

The study revealed that the majority of patients were in the age range between 30-49 years (Table 1). This would be expected as sexual activity is well sustained within this age group (Fig. 1).

Patients co-infected with HCV tend to have lower CD4 counts than mono-infected HIV patients [30]. Some have even suggested lower immune restoration among these group of patients [30,31]. In our study the mean CD4 counts in groups I and II were only slightly different. In group I (patients co-infected) was 528.22±441.97/µl and those with just HIV infection (group II) had a mean of 519.32±378.75 a small but statistically significant difference (P=0.000). There are now multiple retrospective studies that suggest a possible benefit of HIV control and protection of CD4 cell counts to the liver of HIV/HCV co-infected patients. However, data are conflicting at times [32]. This factor along with the methodological limitations of these studies contribute to this. Even assuming a positive effect, HAART does not appear to fully correct the adverse effect of HIV infection on HCV-related outcomes. In the era of HCV direct antiviral agents, the timing of HIV and HCV therapies may have to be to be individualized in HIV/HCV co-infected patients given the variety of situations that could present.

The mean value in the control group (III) was 990.98±332.08 this being statistically significant (P=0.000). Overall there is a clear reduction in CD4 counts in co-infected patients. In a study carried out in India [33] majority of co-infected patients had mean CD4 counts of < 200/µl. However it was not stated in this study if the patients were under therapy as our patients are which would explain our higher CD4 counts. In
Drugs induced liver injury (DILI) is a well-known phenomenon associated with HIV infection and management. However, it has been found that coexisting infections e.g. hepatitis C virus may also have a role to play in this. This makes it imperative to measure liver enzymes once the diagnosis has been established both as a baseline and for monitoring. Intervention maybe necessary when grade 3 or 4 liver enzyme elevations occur. In our study, group I revealed mean aspartate transaminase levels of 21.38±32.32 and 13.32±11.76 in group II. This suggests that the hepatitis C component of the infection is responsible for the higher mean AST value observed in group I when compared to group II. It has been found that high levels of aspartate transaminase (AST) and alanine transaminase (ALT) are signs of liver injury which could result in fibrosis, cirrhosis and liver carcinoma [37] and were more likely to develop advanced liver disease. The mean values for ALT were 14.14±18.33 and 9.72±12.12.95 in groups I and II respectively. The higher levels of ALT in group I (Table 2) may also be attributed to the HCV component of the dual infection. These values should be observed with caution as it has been recognised that high values have been associated with higher tendency to develop hepatic complications including hepatic flares [38] more so in co-infected patients compared to mono-infected cases. Those with consistently lower levels are reported to less likely be associated with liver fibrosis [39]. In studies carried out in Sardinia, increase liver enzymes were an expected finding in co-infected patients especially those co-infected with HCV genotype 3 [38]. There may be need to investigate our patients further to determine the prevalent HCV genotype in our environment since it has been suggested that a particular genotype (genotype 3) is more likely to result in hepatic enzymes and thus more likely to develop liver disease [35].

**Fig. 1. Showing the age range**

*Key Group I=HIV Positive and HCV positive, Group II=HIV Positive and HCV Negative, Group III=HIV Negative and HCV Negative*
Table 2. Table showing the variables analysed according to groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>21.38±32.32</td>
<td>13.32±11.76</td>
<td>7.70±4.82</td>
<td>0.003</td>
</tr>
<tr>
<td>ALT</td>
<td>14.14±18.33</td>
<td>9.72±12.95</td>
<td>5.46±3.29</td>
<td>0.005</td>
</tr>
<tr>
<td>CD4</td>
<td>528.22±441.97</td>
<td>519.32±378.75</td>
<td>990.98±332.08</td>
<td>0.000</td>
</tr>
<tr>
<td>PCV</td>
<td>34.62±4.48</td>
<td>36.25±6.02</td>
<td>36.82±4.69</td>
<td>0.086</td>
</tr>
<tr>
<td>HB</td>
<td>11.10±1.67</td>
<td>11.43±1.92</td>
<td>11.59±1.67</td>
<td>0.379</td>
</tr>
<tr>
<td>MCV</td>
<td>83.88±44.54</td>
<td>79.32±7.13</td>
<td>81.81±6.35</td>
<td>0.687</td>
</tr>
<tr>
<td>MCH</td>
<td>29.16±9.35</td>
<td>25.31±2.69</td>
<td>25.81±2.50</td>
<td>0.002</td>
</tr>
<tr>
<td>MCHC</td>
<td>30.78±9.06</td>
<td>31.70±1.47</td>
<td>31.49±1.38</td>
<td>0.665</td>
</tr>
</tbody>
</table>

Fig. 2. Showing the gender distribution by groups

Key Group I=HIV Positive and HCV positive

Table 3. Table showing the comparison of variables between groups I and III

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th>Group III</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>21.38±32.32</td>
<td>7.70±4.82</td>
<td>0.004</td>
</tr>
<tr>
<td>ALT</td>
<td>14.14±18.33</td>
<td>5.46±3.29</td>
<td>0.001</td>
</tr>
<tr>
<td>CD4</td>
<td>528.22±441.97</td>
<td>990.98±332.08</td>
<td>0.000</td>
</tr>
<tr>
<td>PCV</td>
<td>34.62±4.48</td>
<td>36.82±4.69</td>
<td>0.018</td>
</tr>
<tr>
<td>HB</td>
<td>11.10±1.67</td>
<td>11.59±1.67</td>
<td>0.152</td>
</tr>
<tr>
<td>MCV</td>
<td>83.88±44.54</td>
<td>81.81±6.35</td>
<td>0.747</td>
</tr>
<tr>
<td>MCH</td>
<td>29.16±9.35</td>
<td>25.81±2.50</td>
<td>0.016</td>
</tr>
<tr>
<td>MCHC</td>
<td>30.78±9.06</td>
<td>31.49±1.38</td>
<td>0.585</td>
</tr>
</tbody>
</table>

Table 4. Table showing comparison of variables between groups II and III

<table>
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<tr>
<th>Variable</th>
<th>Group II</th>
<th>Group III</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>13.32±11.76</td>
<td>7.70±4.82</td>
<td>0.002</td>
</tr>
<tr>
<td>ALT</td>
<td>9.72±12.95</td>
<td>5.46±3.29</td>
<td>0.026</td>
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<tr>
<td>CD4</td>
<td>519.32±378.75</td>
<td>990.98±332.08</td>
<td>0.000</td>
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<tr>
<td>PCV</td>
<td>36.25±6.02</td>
<td>36.82±4.69</td>
<td>0.602</td>
</tr>
<tr>
<td>HB</td>
<td>11.43±1.92</td>
<td>11.59±1.67</td>
<td>0.662</td>
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<tr>
<td>MCV</td>
<td>79.32±7.13</td>
<td>81.81±6.35</td>
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<tr>
<td>MCH</td>
<td>25.31±2.69</td>
<td>25.81±2.50</td>
<td>0.332</td>
</tr>
<tr>
<td>MCHC</td>
<td>31.70±1.47</td>
<td>31.49±1.38</td>
<td>0.451</td>
</tr>
</tbody>
</table>

Note: GRP 1- HIV+VE/HCV+VE, GRP2- HIV+VE/HCV–VE, GRP 3- HIV–VE/HCV–VE
Anaemia could be a challenge in co-infected patients [17]. However, patients showed no features of anaemia probably because they were on HAART. It is important to note that despite the anaemia associated with HIV patients on HAART mentioned earlier in the introduction, other studies have shown that HAART naive patients are up to five times more likely to reveal features of anaemia than those patients on HAART [17]. From this view, the absence of anaemia in our study may not be so unexpected because all our patients are on HAART. Group I patients showed a mean value for haemoglobin concentration of 11.10±1.67 and group II patients 11.43±1.92. For the purposes of this study, the normal haemoglobin levels are taken as above 10.5 g/dl. (Table 2). The haematological indices were essentially within normal (Tables 3 and 4).

5. CONCLUSION

This study revealed marked reduction in CD 4 counts among co-infected patients. It also demonstrated elevation of the liver enzymes in these studies. Further studies will be needed especially to determine the HCV genotypes existing in our environment to assist in explaining why our patients have not shown an increased incidence in liver fibrosis, cirrhosis or hepatic carcinoma as in other parts of the world despite being HCV co-infected and on HAART for several years. Subsequent studies would include HCV mono-infected patients.

ETHICAL APPROVAL

This research work was given approval by the Lautech research ethics committee (Ref: LTH/REC/2014/08/16/178).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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