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Immunoglobulin G Specific Antibody Level against Ebola Viral Glycoprotein and Nucleoprotein in Ebola Virus Disease Survivors and Their Relatives

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Abstract

Ebola virus disease is a complex zoonosis that is highly virulent in humans. Despite its sorely pathogenic and lethal nature, survivors of this infection and even asymptomatic cases are able to develop both humoral and cellular immunity against several Ebola virus (EBOV) proteins. We aimed at determining immunoglobulin G (IgG) antibodies level against two Ebola viral antigens, the glycoprotein and the nucleoprotein in Ebola survivors and their relatives. Anti-EBOV glycoprotein (GP) and nucleoprotein (NP) IgG antibodies were quantified using ELISA. We enrolled 199 participants in two different sites as follow: 91 survivors at the Loreto clinic and 70 survivors with 38 relatives of Sierra Leone Association of Ebola Survivors Bombali Branch (SLAESB) tested for anti-EBOV NP and anti-EBOV GP IgG antibodies. Our findings revealed that the median anti-EBOV IgG level among survivors was 5.7128 U/ml [IQR: 2.793 - 7.783] for anti-EBOV GP IgG and 4.431 U/ml [IQR: 2.083 - 7.696] for anti-EBOV NP IgG. Survivors relatives had a median anti-EBOV GP IgG level of −0.7128 U/ml [IQR: −0.903 to −0.04327] and −2.711 U/ml

[IQR: −4.01 to −1.918] for anti-EBOV NP IgG. We observed that IgG levels in survivors were higher than in relatives with a significant difference of about 0.0001. The median value of anti-EBOV IgG level among seropositive relatives was 0.7043 U/ml [IQR: 0.5686 to 3.716] for anti-EBOV GP IgG and 4.05 U/ml [IQR: 0.2765 to 7.759] for anti-EBOV NP IgG respectively. Interestingly, we observed that 3.30% of Loreto clinic survivors did not developed anti-EBOV NP IgG antibodies; also about 10% survivors of the SLAESB were not reactive to anti-EBOV NP IgG and 1.43% of these survivors did not express antibodies against the Ebola viral glycoprotein. Our work is consistent with previous published studies showing heterogeneity in both survivors and asymptomatic cases of Ebola infection developing adaptive immunity against EBOV proteins.

Keywords

Author Summary

Ebola virus disease, formally known as Ebola Haemorrhagic Fever, is a severe, often fatal illness in humans. The West Africa outbreak presented unprecedented features and has been recorded as the largest and the most complex Ebola outbreak since its discovery with a case fatality rate of approximately 70%. More than 28,610 confirmed, probable, and suspected cases have been recorded with 11,308 deaths between December 2013 and March 2016. Although our understanding of how host immunity controls the infection is still limited, we do know that antibodies have the ability to ensure protection against Ebola virus disease infection. In this piece of work, we determine immunoglobulin G (IgG) antibodies level against two Ebola viral antigens (Glycoprotein and Nucleoprotein) in Ebola survivors and household contacts considered here as family members who live in the same compound with an EBOV infected person or took care of EBOV infected person at home. We observed a wide variability of antibodies level among close contacts of infected person and even survivors. However, with a different disease course, asymptomatic subjects could elicit a similar immune pattern as in survivors.

1. Introduction

Antibodies play a crucial role in host defense against viruses, both by preventing infection and by controlling viral replication [1]. It is reported that antibodies also exert their antiviral effects by crystallizable fragment (Fc)-mediated effector mechanisms alongside their capacity to neutralize viruses [2] [3]. This involves a bridge between innate and adaptive immune systems, wherein antibodies form immune complexes that drive numerous innate immune effector functions, including antibody-dependent cellular cytotoxicity, antibody-dependent comple-

ment-mediated lysis, and antibody-dependent phagocytosis [4]. Several mechanisms modulate antibody-mediated effector functions against virally infected cells and can either protect viral replication or enhance infected cell clearance [5]. This phenomenon has been described in Ebola virus disease infection where the ability of antibodies to provide protection from a lethal Ebola virus (EBOV) challenge has been demonstrated in the context of pre- and post-exposure administration of EBOV glycoprotein (GP) specific monoclonal antibodies (mAbs) [6]. It is reported that Ebola virus disease (EVD) survivors develop both humoral and cellular immunity against several EBOV proteins, including GP, secreted GP (sGP), nucleoprotein (NP) and matrix protein VP40 [7].

Ebola virus genome is mainly constituted of seven genes. The viral RNA contains information about eight proteins: VP24, VP30, VP35, VP40, L, NP, sGP and GP1/2. Each of the protein expressed is known for its multi-functionality, essentially VP35 and GP that present multi-functionality in the pathogenesis process and in the inhibition of immune responses in the host [8]. The only virally expressed protein on the virion surface of EBOV is the glycoprotein (GP) that is critical for attachment to host cells and catalysis of membrane fusion [9]. Ebola virus matrix protein or nucleoprotein (NP) is a key component of the viral ribonucleoprotein complex and the most abundant. It has a distinct function in the replication cycle where it plays critical roles in protecting viral RNA from degradation and in mediating genome encapsidation during virus assembly. At present, all research has focused on these primary activities of NP, and any secondary roles remain to be determined [10].

Studies show that immunological events very early in an Ebola virus infection determine the control of viral replication and recovery or catastrophic illness and death [11]. Recovery from infection is related to orderly and well regulated humoral and cellular immune responses, characterized by the early appearance of immunoglobulin M (IgM) and immunoglobulin G (IgG), followed by activation of cytotoxic cells at the time of antigen clearance from blood. By contrast, fatal outcome is associated with impaired humoral responses and an early activation of T cells unable to control virus replication, followed by considerable intravascular apoptosis [12].

Although many studies have been conducted to understand the role of immunity against Ebola virus disease, the extent of asymptomatic EBOV infection is still unclear. Several studies reported a wide variability of antibodies level among close contacts of infected person and even survivors [11] [13] [14]. This high variability may be ascribed to the different antigens targeted. Thus, we aimed at determining immunoglobulin G (IgG) antibodies level against two Ebola viral antigens (Glycoprotein and Nucleoprotein) in Ebola survivors and household contacts or relatives.

2. Methods

2.1. Study Area and Sample Population

During the last 2013-2016 Ebola outbreak, we conducted a study in Makeni

town situated in the northern region of Sierra Leone. This capital town of the Bombali District in Sierra Leone experienced 1050 confirmed. Ebola virus disease cases during this unprecedented outbreak [15]. The Ethical Review Board of the Ministry of Health and Sanitation of Sierra Leone approved this study. Participants and/or guardians of children enrolled in this study gave their approval by signing or fingerprinting a written consent form according to the Helsinki declaration. We collected demographic data and information about clinical history using questionnaires before obtaining 4 ml of peripheral blood by venepuncture.

Overall, we enrolled 199 participants in two different sites described as follow:
- The first set was made of 91 Ebola survivors follow up at the Loreto clinic. All the 91 survivors were tested for anti-EBOV NP IgG antibodies and 41 of them were tested for both anti-EBOV NP and anti-EBOV GP IgG antibodies.
- The second set was made of 70 Ebola survivors recruited with the help of Sierra Leone Association of Ebola Survivors Bombali Branch (SLAESB) and 38 relatives tested for anti-EBOV NP and anti-EBOV GP IgG antibodies.

The inclusion criteria for Ebola survivors was to be a person who was infected by Ebola virus, presented signs and symptoms of the disease, tested Ebola positive by real time polymerase chain reaction and later on recovered. Relatives or household contacts were family members who live in the same compound with an EBOV infected person and or took care of EBOV infected person at home.

It should be noted that these activities were carried out in an emergency context where reagent and consumable supplies were highly challenging.

2.2. Laboratory Testing

Ebola virus RNA detection using real time PCR in survivors of the Loreto clinic

Given that, this was the first West Africa Ebola outbreak and our first experience with Ebola Virus Disease (EVD), we thought one way of ensuring our safety was to ascertain if viremia is cleared after onset of symptoms in survivors. Ebola nucleic acid detection was performed using real time RT-PCR on the 91 enrolled survivors at Loreto clinic. Viral ARN extraction was done after sample inactivation with TRIzol under a biosafety cabinet level 3; we used reagents of a commercial Kit PureLink Viral RNA/DNA Kits, *Invitrogen life technologies.* Ebola virus genetic material was quantified using a commercial kit RealStar Ebolavirus RT-PCR Kit 1.0 of Altona Diagnostic, according to the manufacturer's instructions.

Anti-EBOV IgG quantification using ELISA

Anti-Zaire Ebola Glycoprotein (ZEBOV-GP) IgG antibodies and Anti-Zaire Ebola Nucleoprotein (ZEBOV-NP) IgG antibodies were quantified using Enzyme-Linked Immunosorbent assay (ELISA) of a commercial kit Alpha Diagnostic International [ADI], Texas, USA, according to the manufacturer's instructions. Positive control and calibrators provided by the kit were used in each

test run.

To perform ELISA in these two sets of samples, optimal sample dilution was previously determined at 1:500 as described by Mafopa *et al.*, 2017 [16].

2.3. Statistical Analysis

We collected data and entered them in a customized template in EpiData software version 3.1. Statistical analyses were done using GraphPad Prism 7.04. $p < 0.05$ was considered statistical significant.

3. Results

3.1. Ebola Survivors of Loreto Clinic

Of the overall participants enrolled, 58.24% were women (53/91) and 40.66% were men (37/91). The other 1.01% (1/91) enrolled did not inform us on their gender.

We performed EBOV nucleic acid detection in Loreto clinic survivors and the presence of EBOV nucleic acid was not detected (Table 1). To be sure that participants were indeed EBOV survivors, anti-EBOV NP IgG antibodies screening using quantitative ELISA technique was performed. Surprisingly, 3.30% (03/91) of these survivors did not developed anti-EBOV NP IgG antibodies.

Anti-EBOV IgG antibody activity variation related to gender

Our results shown that there was no statistical difference ($p = 0.3443$) between median anti-EBOV NP IgG level among female (7.827 U/ml, IQR: 5.098 - 8.599) and male survivors (6.698 U/ml, IQR: 1.598 - 7.803) as described in Figure 1.

Table 1. Plasmatic Ebola viral RNA detection by Real time RT-PCR and Anti-EBOV NP IgG detection months after onset of symptoms.

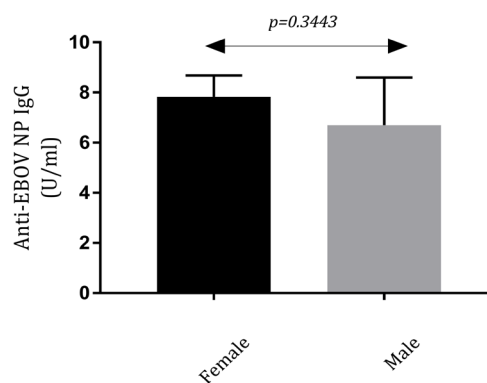|	Positive	Negative	Total (N)
First RT-PCR (Performed during symptoms onset)	91	0	91
Second RT-PCR (Performed months after recovery)	0	91	91
ELISA (Anti-EBOV NP IgG detection)	88	03	91

Figure 1. Anti-EBOV NP IgG activity variation among female and male survivors.

3.2. Anti-EBOV IgG Antibody Activity Variation Related to Gender

Survivors of this specific objective were aged 2 to 65 years old with an average of 30 years old. We enrolled 15.38% infants (2 to 12 years), 9.9% adolescents (above 12 to 19 years) and 69.23% adults (above 19 to 65 years) and 5.49% did not inform us. Adults were more representative. We used one-way ANOVA test with p statistical significant at 0.05. Figure two (Figure 2) shows that anti-EBOV NP IgG detection among survivors revealed that the median antibodies level among the age groups varied slightly (p = 0.0462) with teenagers having the highest median antibodies level of 8.417 U/ml [IQR: 8.205 - 8.883]; while infants and adults had a median antibodies level of 4.876 U/ml [IQR: 1.598 - 7.803] and 7.105 U/ml [IQR: 3.917 - 8.659] respectively. We unexpectedly observed that anti-EBOV NP IgG titers were lower in children compare to adolescents (p = 0.0171, Figure 3).

Of the 91 survivor samples collected at Loreto clinic, anti-EBOV NP and anti-EBOV GP IgG titration was done on 41 samples. These survivors expressed more immunoglobulins directed against the viral glycoprotein. The median IgG titter was 9.029 U/ml [IQR: 6.773 - 10.54] and 6.601 U/ml [IQR: 4.895 - 7.23] respectively for anti-EBOV GP IgG and anti-EBOV NP IgG (p = 0.0010, Figure 4).
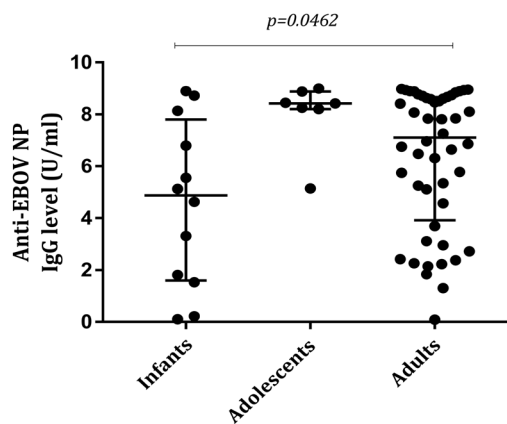

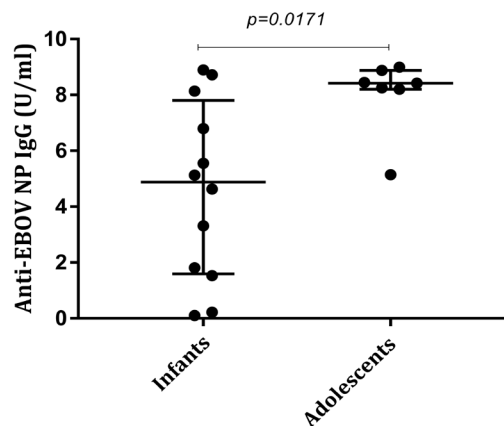**Figure 2.** Anti-EBOV NP IgG Antibody level related to age.

Figure 3. Anti-EBOV NP IgG activity variation among children and adolescent survivors.
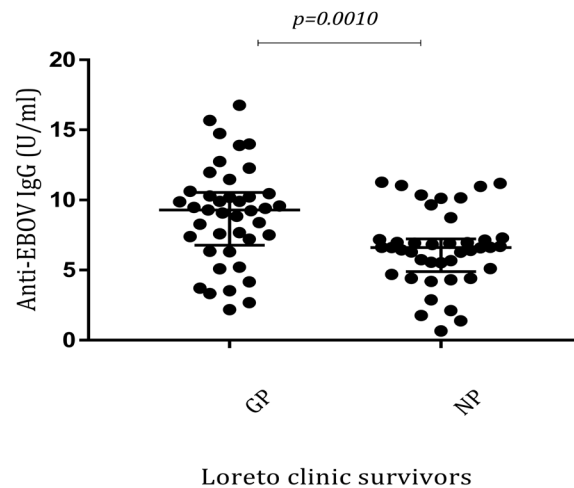
Figure 4. Anti-EBOV IgG titter in Loreto clinic survivors.

3.3. Ebola Survivors and Family Members of the Sierra Leone Association of Ebola Survivors Bombali Branch (SLAESB)

Samples collected with the help of Makeni Ebola survivors association were made of 70 survivors and 38 relatives. The average age of survivors was 28 years and the one of relatives was 22 years.

3.4. Anti-EBOV NP IgG Level in Survivors and Their Relatives

Interestingly, we observed a significantly higher anti-EBOV NP IgG antibody level in EBOV survivors compare to those of their family members (p < 0.0001) with median antibodies level of 4.431U/ml [IQR: 2.085 - 7.696] in survivors and −2.711 U/ml [IQR: −4.08 to −1.918] in their relatives. As expected, the prevalence of anti-EBOV NP IgG was higher among Ebola survivors (63/70, 90%) than among household contacts (8/38, 21.05%) with p < 0.0001 (**Figure 5**).

3.5. Anti-EBOV GP IgG Level in Survivors and Their Contacts

Anti-EBOV GP IgG detection revealed that the median antibody level was 5.176 U/ml [IQR: 2.793 - 7.783] in survivors and −0.7128 U/ml [IQR: −0.903 to −0.04327] in their relatives. These results varied significantly (p < 0.0001, **Figure 6**). The proportion of asymptomatic cases (in order words seropositive cases) with anti-EBOV GP IgG antibodies among household contacts was 13.16% (5/38). We noticed that anti-EBOV GP IgG prevalence in survivors was 98.57% (69/70) with only 1.43% (1/70) of survivors who did not express antibodies against the Ebola viral glycoprotein.

3.6. Anti-EBOV NP IgG and Anti-EBOV GP IgG Level in Survivors

There was no significant difference (P = 0.1702, **Figure 7**) in immunoglobulin G level directed toward the Ebola nucleoprotein (median = 4.431 U/ml, IQR: 2.085 - 7.696) or the Ebola glycoprotein (median = 5.711 U/ml, IQR: 2.793 - 7.783) in survivors enrolled with the help of the Makeni Ebola survivors association. We
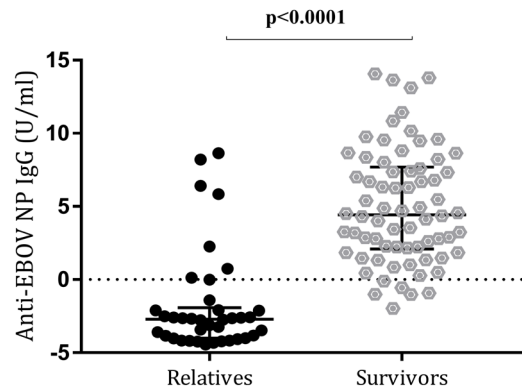
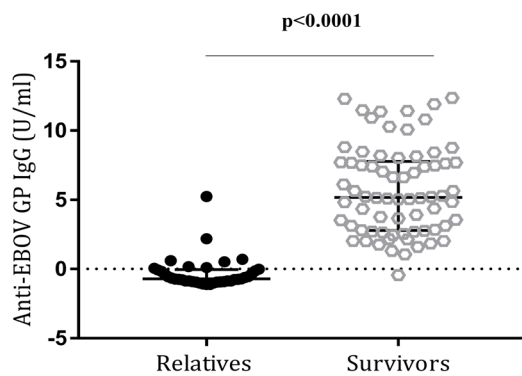**Figure 5.** Anti-EBOV NP IgG level in survivors and their contacts.

Figure 6. Anti-EBOV GP IgG level in survivors and their contacts.
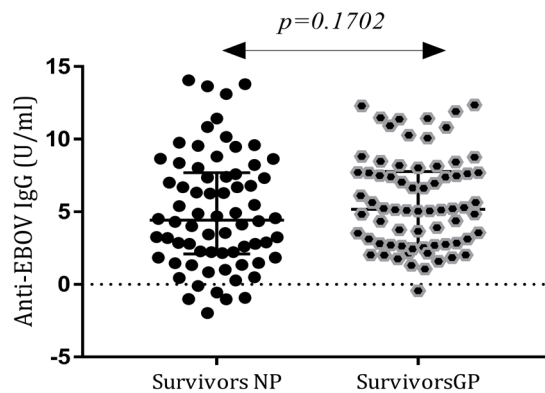

Figure 7. Anti-EBOV IgG level in survivors of SLAESB.

observe that about 10% (7/70) of these survivors did not express antibodies against the Ebola viral nucleoprotein and 1.43% (1/70) did no present both antibodies against the nucleoprotein and the glycoprotein.

3.7. Anti-EBOV NP IgG and Anti-EBOV GP IgG Level in Relatives of Survivors

Family members who took care of survivors from the Ebola association expressed more anti-EBOV NP IgG. We observed in Ebola seropositive asympto-

matic cases that level of immunoglobulin G directed towards the Ebola nucleo-protein (median = 4.04 U/ml, IQR: 0.2765 - 7.759) was higher than the level of IgG (median = 0.7043 U/ml, IQR: 0.5686 - 3.716) directed against the Ebola gly-coprotein (p < 0.0001, Figure 8). About 7.89% (3/38) of the asymptomatic cases expressed both anti-EBOV NP IgG and anti-EBOV GP IgG.

3.8. Comparison of Anti-EBOV NP IgG and Anti-EBOV GP IgG Level in the Two Sets of Survivors

When comparing the two sets of samples, we observed that anti-EBOV IgG level against the nucleoprotein were elevated in Loreto clinic survivors (median = 6.601, IQR range: 4.894 - 7.23) compare to those of SLAESB (median = 4.431, IQR range: 2.085 - 7.696, Figure 9) with a significant difference of p = 0.0177. On the contrary, there was no statistical different in level of IgG directed toward the glycoprotein between the two sets of samples (median = 5.176, IQR range: 2.793 - 7.783 and 5.139, IQR range: 4.022 - 5.695 respectively for LCS and SLAESB survivors) (Figure 10).
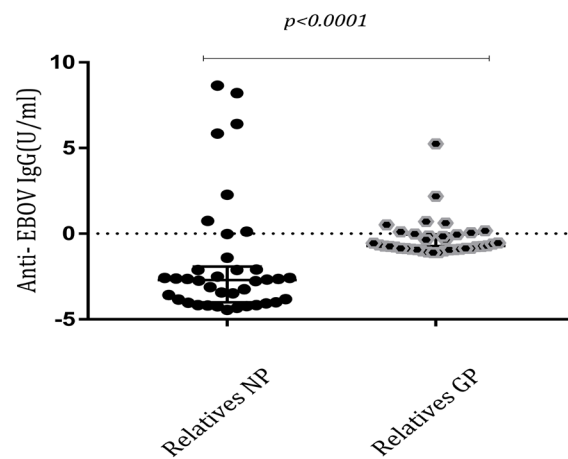

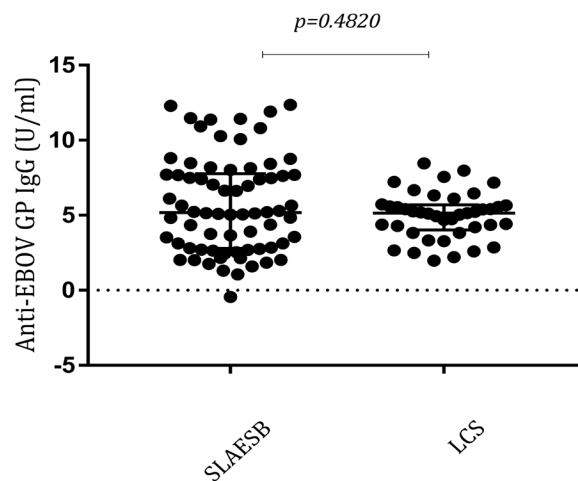**Figure 8.** Anti-EBOV NP IgG and Anti-EBOV GP IgG level in survivors' contacts.

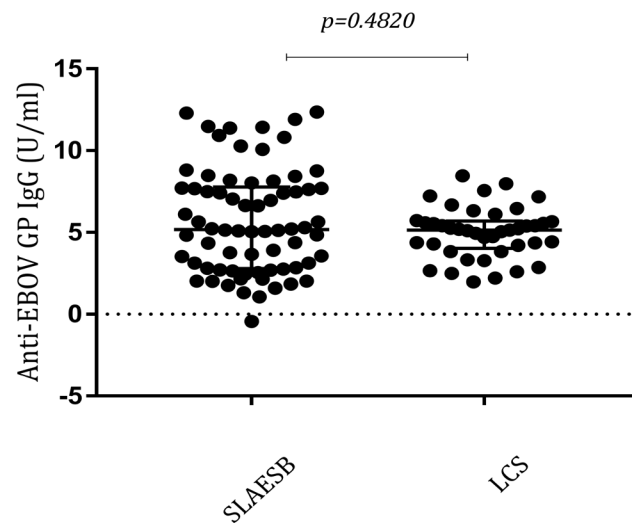**Figure 9.** Anti-EBOV NP IgG level in the two sets of survivors.

$p=0.4820$

Figure 10. Anti-EBOV GP IgG level in the two sets of survivors.

4. Discussion

4.1. Anti-EBOV-IgG Prevalence

Our findings show that anti-EBOV IgG was detected in both Ebola virus disease survivors and their family members, although at different prevalence rates, suggesting that the immunity, previously reported as persistent among survivors, may be important for protection that allowed subclinical disease among household contacts [17]. Anti-EBOV IgG positive proportion among survivors (98.57% for anti-EBOV GP IgG and 90% for anti-EBOV NP IgG) was higher than in asymptomatic household contacts (13.16% for anti-EBOV GP IgG and 21.05% for anti-EBOV NP IgG) with a significant difference of about 0.0001. It means in order words that immunological response, which occur early in survivors, compare to patients with fatal outcome lead to a more robust antibody response since they encountered high viral load than in asymptomatic patients [11]. Our findings are consistent with the study of Colavita *et al.* 2019 [18] where they demonstrated that in the late phase of infection, survivors showed high level of pro-inflammatory mediators, which plays a role in the immune system. Thus Anti-EBOV IgG production is strongly stimulated to control virus replication and disease progression. The presence of anti-EBOV IgG in plasma sample of some household contacts is indicative that they came across the virus but were able to clear the infection. This could be due to host factors or low viral load in their blood stream given enough time for a robust humoral immune response and subsequent viral clearance. This low viremia could be because in some cases virus infection resulted in the synthesis of viral proteins without the production of progeny virus [19]. In addition, as reported by Richardson *et al.* in 2016 [20], Ebola infection, like many other viral infections, may present a spectrum of clinical manifestations, including minimally symptomatic infection. Thus, a significant portion of Ebola transmission events may have gone undetected during

the outbreak.

4.2. How Long Ebola Virus Could Stay in the Blood Stream of Survivors?

Many studies reported that Ebola virus could persist in body fluids including semen and ocular fluid months after disease onset [21] [22]. We though one way of ensuring our safety was to ascertain the fact that it is stated that plasmatic Ebola virus is cleared two weeks to 21days after onset of symptoms in survivors [23] [24]. We performed Ebola nucleic acid detection using real time RT-PCR on 91 enrolled survivors of Loreto clinic although samples were collected 70 to 308 days after symptoms onset (average = 180 days). The presence of Ebola virus nucleic acid was not detected. In a study conducted by Y. Liu *et al.* (2018) [25] on serological investigation of laboratory-confirmed and suspected Ebola virus disease patients during the late phase of the Ebola outbreak in Sierra Leone, RNA could be detected as early as on day 1 after disease onset, and as late as day 36 post disease onset. Our results are in accordance with the findings of this study. Nevertheless, to be sure, that participants were indeed Ebola survivors, we decided to performed anti-EBOV NP IgG antibody screening using ELISA technique. Surprisingly, we observed that 3.30% (03/91) of these survivors did not develope anti-EBOV NP IgG antibodies. We also found that about 10% (7/70), survivors of the Sierra Leone Association of Ebola Survivors Bombali Branch were not reactive to anti-EBOV NP IgG and 1.43% (1/70) of these survivors did not express antibodies against the Ebola viral glycoprotein. These results are perhaps suggesting the issue of false positive or the fact that some people cheated using survivor cards of their fellow to have the provision made for them. Our results are consistent with the findings of Halfmann *et al.* (2019) [15] who observed in their study the lack of detectable antibodies level in some survivors (2.3%). They stated that it could also reflect immune defects resulting in low and/or short-lived antibody responses or could be due to technical errors or miscommunication during sample collection.

4.3. Anti-EBOV IgG Antibody Activity Variation Related to Gender

We performed anti-EBOV IgG detection among survivors aiming at observing if there could be any variation of antibody level related to gender. Our results shown that there was no statistical difference (p = 0.3443) between Anti-EBOV NP IgG level among female and male survivors. It has been reported that there is currently no evidence related to biological differences in female or male sex that increases Ebola virus transmission and vulnerability; rather, there are differences in the level of exposure between men and women. In fact, women's increased exposure can be due to time spent at home and their responsibility for caring for the sick. For men, increased vulnerability to the virus can be attributed to their responsibility for caring for livestock and time spent away from home, as most known sources of the index cases have been infected in the process of hunting [26].

4.4. Anti-EBOV IgG Antibody Activity Variation Related to Age

Although the first suspected case of the 2013-2016 outbreaks is believed to be a 2-year-old child in Guinea [27] [28], children comprise a small percentage of all cases globally. They typically represent only a minority (approximately 10%) of cases and are most often spared of exposure to Ebola virus disease infection during recognized human Ebola outbreaks [29]. It is reported that either the people who take care of ill individuals, in a healthcare setting or at home, or those who handle the remains of individuals who have died, are those with highest risk of contracting the infection. However, there is limited evidence on whether children have different disease severity or prognosis compared with adults [30].

In the western Africa outbreak, children less than 15 years of age were approximately 14% of all reported confirmed and probable Ebola virus disease cases. Case fatality for children less than 15 years of age was 73.4%, 66.1% among those of 15 to 44 years of age and 80.4% among those older than 45 years [31] [29]. This data are similar to those observed during an outbreak in Northern Uganda, Gulu district, in the year 2000 to 2001 caused by *Soudan Ebola virus* (SUDV), where 9% of laboratory-confirmed cases were children less than 18 years of age.

Ebola survivors' ages in our study ranged between 2 to 65 years old with an average age of about 30 years old. We grouped them into three major categories: 15.38% children (2 to 12 years), 9.9% adolescents (above 12 to 19 years) and 69.23% adults (above 19 to 65 years). The fact that adults' survivors were more representative confirms they were the one more exposed to the virus in this outbreak. These figures correlate with the above-mentioned findings.

Our findings shown that anti-EBOV IgG level in children (median = 4.876 U/ml, IQR: 1.298 - 7.803) was lower than in adolescents (median = 8.417 U/ml, IQR: 8.205 - 8.883) and in adults (median = 7.105 U/ml, IQR: 3.917 - 8.659) with a significant difference of $p = 0.0171$ and $p = 0.0462$ respectively. Children immune system is immature compare to teenagers and adults' immune system and thus most often less active when challenged by a pathogen. These results differed from those of Mc Elory *et al.*, 2014 [32] where IgG levels were higher in samples from pediatric patients than in samples from adult patients. Based on the study of Zeger and collaborators in 1975 [33] on Serum immunoglobulins level in healthy children and adults, Mc Elory and collaborators [32] stated that this difference is notable because children usually have slightly lower levels of total IgG than adults do. For them, the higher IgG levels might suggest a higher degree of immune activation, perhaps secondary to other infectious co-existing conditions, which are likely to be present in children living in a rural area of Africa. Consistent with this theory, high levels of malarial parasitemia have been associated with higher total levels of IgG in children in The Gambia [34]. It should be highlighted that the study of Mc Elory and collaborators [32] was carried out in Uganda during the 2000-2001 outbreak, which was not the first Ebola virus

disease epidemic in this country. It is possible that the immune system of these children had already encountered Ebola virus among many other infectious agents. Reason why IgG response against Ebola virus is more pronounced in Uganda children than in children leaving in Sierra Leone who experienced Ebola virus infection for the first time.

4.5. Immunoglobulin G Response to EBOV Viral Proteins

Generally, our results showed that about 34.21% (13/38) of survivors' relatives or household contacts enrolled with the help of Sierra Leone Association of Ebola Survivors Bombali Branch expressed IgG towards either EBOV NP or EBOV GP. The prevalence of anti-EBOV NP IgG and anti-EBOV GP IgG antibodies were respectively 21.05% (8/38) and 13.16% (5/38). About 7.89% (3/38) of the asymptomatic cases expressed both anti-EBOV NP IgG and anti-EBOV GP IgG. Survivors' relatives expressed more anti-EBOV NP IgG than IgG directed against the Ebola glycoprotein (p < 0.0001). Sakabae and co-authors (2018) [35] performed analysis of CD8+ T cell response during the 2013-2016 Ebola epidemic in West Africa. They demonstrated a relatively low abundance of GP-specific CD8+ T cells, yet much higher levels of NP- and VP40-specific CD8+ T cells in Ebola survivors. Although with a different disease course, asymptomatic subjects could elicit a similar immune pattern as in survivors. In addition, studies have stated that the split between development of GP-specific antibodies and development of NP and VP40 specific CD8+ T cells may indicate that the two branches of adaptive immunity are differentially shaped by distinct Ebola virus proteins and may complement each other to maximize immunity [7].

Our findings are in contrast with what Hoff and collaborators (2019) [36] obtained when conducting a serosurvey to determine seroprevalence against multiple Ebola virus antigens among health care workers of Boende Health Zone, Democratic Republic of the Congo, the site of the 2014 EBOV outbreak. Their results shown overall, 41.4% of enrolled participants were reactive to at least one Ebola virus protein. Seroreactive participants to anti-glycoprotein immunoglobulin G (IgG) were more representative (28.1%) than those seroreactive for anti-nucleoprotein IgG (15.8%) and for anti-VP40 IgG (9.5%). Hoff *et al.* [36] stated that the high proportion of anti-EBOV GP IgG might be due to unrelated nonspecific binding, with the high degree of glycosylation in EBOV GP lending itself to such nonspecific recognition. They also added that alternately, it might be that GP is more sensitive than other viral proteins to the presence of cross-reactive antibodies directed against related viruses. Thus, anti-EBOV GP IgG alone may not be sufficient as a marker for demonstration of previous exposure, especially in asymptomatically infected or otherwise unrecognized EVD survivors [37].

We noticed that anti-EBOV GP IgG and anti-EBOV NP IgG seroreactive rates in SLAESB survivors were 98.57% (69/70) and 90% (63/70) respectively. There was no significant difference (p = 0.1702) in immunoglobulin G levels directed toward the Ebola nucleoprotein (median = 4.431 U/ml) or the Ebola glyco-

protein (5.7128 U/ml). On the contrary, Loreto clinic survivors expressed more immunoglobulins directed against the viral glycoprotein. The median IgG titter was 9.029 U/ml (IQR: 6.773 - 10.54) and 6.601 U/ml (IQR: 4.895 - 7.23) respectively for anti-EBOV GP IgG and anti-EBOV NP IgG (p = 0.0010). Our data in Ebola survivors are in contrast with those of Sakabae and collaborators (2018) [35]. Their findings indicate instead the immunodominance of the EBOV NP-specific T cell response while suggesting that EBOV NP inclusion in vaccine design along with the EBOV GP would best mimic survivor responses and help boost cell-mediated immunity during vaccination. In addition, although targeting only two Ebola viral proteins, our results corroborate with previous studies that reported the development of both humoral and cellular immunity against the different EBOV proteins in Ebola survivors [12] [32] [35]. Nevertheless, when comparing the two sets of samples, we observed that anti-EBOV IgG level against the nucleoprotein were elevated in Loreto clinic survivors (median = 6.601, IQR range: 4.894 - 7.23) compare to those of SLAESB (median = 4.431, IQR range: 2.085 - 7.696) with a significant difference of p = 0.0177. There was no statistical difference in levels of IgG directed toward the glycoprotein between the two sets of samples (median = 5.176, IQR range: 2.793 - 7.783 and 5.139, IQR range: 4.022 - 5.695 respectively for LCS and SLAESB survivors). May be the slight difference in anti-NP IgG titters in the two sets of survivors could be linked to host-virus interactions.

The absence of healthy control group constitutes a limitation to our study. It would have been interesting if we were able to differentiate household contacts who took care of survivors from those who only came in contact because they were sharing the same compound with the infected person. In addition, our study may have been enlightened if we did immunoglobulin G detection toward all the Ebola virus proteins. These gaps are mainly because we worked in resources constraint setting during emergency state where reagents and consumables acquisition was a challenge.

5. Conclusion

Our work, like previous published studies [11] [13] [14], shows heterogeneity in Ebola virus disease survivors developing humoral immunity against several Ebola virus proteins. Understanding the roles, each viral protein that plays in immune response could enhance the development of counter measures including vaccines and therapeutics. Our findings show that anti-EBOV IgG levels were higher in Ebola survivors than among household contacts. Immunity may be important for protection that allowed subclinical disease among asymptomatic household contacts. Nevertheless, it will be of great interest to study the interactions that might exist between the virus and the host together with transmission dynamics associated with minimally symptomatic Ebola virus (EBOV) infection. In addition, critical work is still needed to ameliorate our knowledge of Ebola virus disease infection in children through methodical collection of in-

formation on disease course and predictors of survival in infants of different ages. It could be possible that different pathophysiologic mechanisms of disease may be at work in paediatric patients, and children may benefit from different treatment than their adult counterparts.

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Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this article.

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