The Dynamics Of Intestinal Helminthes And Malaria Co-Infection: Impact On Cytokine Responses And Malaria Severity Among School Children In KISII County, Kenya

Elijah Mogoi Matiabe, Dr.Stanslaus Kiilu Musyoki, Dr.Benson Nyanchong’i

Abstract Background: Intestinal helminthic infections are common among children especially in sub-Saharan Africa where they may co-infect with malaria. In co-infections synergistic effects affects severity. Therefore, there is need to understand immune dynamics and modulations in dual infections. Materials and Methods: Specimens from 168 children aged between 6-14 years with malaria were obtained of whom 84 (50%) had intestinal helminthes. Blood specimen was obtained and malaria parasites were demonstrated using Giemsa staining technique and cytokines were evaluated by flow cytometry technique. Results It was observed that pro-inflammatory IL2 and IL6 cytokine responses were more elevated in severe cases as compared to uncomplicated malaria (P< 0.001); levels of TNFα (pro-inflammatory) and IL10 (anti-inflammatory) response was directly proportional to malaria severity. There was an increased IL10 and TNFα response in Hookworm infection compared with malaria infection only (P=0.009); and (p=0.042) respectively; while IL2 and IL6 were reduced (p=0.001 and p<0.001) respectively. S.mansoni significantly increased IFNγ response compared with malaria infections only (P=0.046) whereas T.trichuria and S.sercularis reduced IL6 response in comparison with malaria cases only (P =0.047) and T.trichuria reduced IL6 response (P=0.001). A.lumbricoides reduced IL2 and IL6 (P<0.001) responses. E.vesiculosa; H.nana; and T.saginata did not affect response of cytokines under this study. Hookworm and A.lumbricoides modulate cytokine responses during co-infections, with the former increasing both IL10 and TNFα levels while the later decreased IL2 and IL6 which was positively associated with an increased risk of malaria severity. From the findings, IL6 may be used as biomarker to differentiate between uncomplicated and severe malaria.

Key words: Cytokine; Helminthes; Malaria

1. INTRODUCTION

Intestinal helminthes are among the common infectious agents that have afflicted human populations, particularly in marginalized, low-income and resource-constrained regions of the world(Nobre et al., 2013). The effects of such infections cut across the health; academic; and economic viability of the affected individuals. Besides the helminthic infections; malaria on the other hand; causes co-morbidities and mortalities in the same individuals. This malarial infections have been postulated to be killing 1 child per minute within the overlapping poly-parasitism regions especially in Africa,(Cohen, 1988) (Y Titanic et al., 2010). Globally; the prevalence of intestinal helminthic infections have been documented to occur in children especially those that live in endemic areas such as sub-Saharan Africa, followed by Asia and then Latin America and the Caribbean (Nii, Osakunor, Sengeh, & Mutapi, 2018). According to Brooker (2010) in sub-sub-Saharan Africa, it was estimated that around a quarter of the total population was infected with one or more helminthes, typically the nematodes worms which are the most prevalent of all gastrointestinal parasites(Kamara, 2013). The 2010 estimates suggest that of the then 181 million school-aged children in sub-Saharan Africa, almost half (89 million) were affected by one more of these parasitic worm. This study is supported by studies in other literature(Bailey et al., 2013), (Njua-Yafi et al., 2016), (Kamara, 2013) that concludes on the vulnerability of the populations. There is a greater risk for malaria-associated co-infections in morbidity and mortality among vulnerable groups especially that of children compared to adults according to recent studies(Marcelline et al., 2016). During an infection; there is an induced immune reaction in young children that contrasts profoundly from that in adults that are not too old, and these dissimilarities most likely have been associated with increased vulnerability of children to severe malaria and their hindered immune development(Moreau & Chauvin, 2010). In this regard, multi-infection of this magnitude remains single handedly the most deadly of young children in sub-Saharan Africa (Nobre et al., 2013) where Kisii is a center of focus. The foundation of the interaction between intestinal helminthes and malaria co-infection within a host is attributed to be an immunological interplay(F. C. Hartgers & Yazdanbakhsh, 2006), with the helminthes altering the immune response to favor their survival(Hassan, 2018) and this severely affects the ensuing immune response to malaria infection thus leading to increased pathological consequences(Pietkiewicz, 2005). It has increasingly been demonstrated through research that intestinal helminthic infections may modify vulnerability to pathological development in clinical Plasmodium malari infection(Nacher, 2011) and there is now an upward increase in interest to investigating the implications associated with co-infections on the cytokine response during an active infection(Farrington et al., 2017; Franca C. Hartgers et al., 2009; Nacher, 2011) of the affected individuals. Results of analysis from one of the findings; broadly support the idea that intestinal helminthic co-infections increases chances of mortality(Mulu et al., 2013) and amplified peak parasitaemia within ordinarily resolving malaria infections as demonstrated in animal models(Knowles, 2011) especially in lethal prototypes and precisely CM models. Remarkably, notwithstanding the limitations of the prevailing studies in human populations, contrary findings from a study in Thailand are in support of this immunological pattern(Salazar-castañon, Legorreta-herrera, & Rodriguez-sosa, 2014). In a series of studies from this region, there is a suggestion that individuals infected with soil-transmitted helminths (predominantly Ascaris lumbricoides) had a greater risk of mild incidents of malaria infection compared with those with malaria infection only. These individuals were also at reduced risk of mounting various forms of severe malarial disease(Nobre et al., 2013). Nevertheless, despite all this findings, it appears likely that different effects of helminthic co-infection
in resolving and lethal malaria models arise from an immune discrepancy in how parasites and their hosts interact (Knowles, 2011) though it cannot be ruled out that some other unknown differences between Plasmodium species may underlie these patterns. Additional co-infection experimentations with lethal and nonlethal emulations of the same Plasmodium species has been explored on the possibility of this occurrence (van Riet, Hartgers, & Yazdanbakhsh, 2007). It has also been observed in comparable studies that effects of helminthic co-infection on Plasmodium multiplication are diverse and that the overall development observed from this helminthic co-infections is the upsurge Plasmodium parasitaemia (Franca C. Hartgers et al., 2009). This effect has been observed to be stronger in trials involving clearing malaria infections, particularly those of P. yoelii, and H. polygyrus helminth in animal models (Gonçalves, Lima, & Ferreira, 2014). Further elucidations from this findings showed an increased mortality in this co-infection in resolving malaria models was accompanied by an amplified peak malaria parasitaemia. Further studies have established that such effects can be reversed upon application of anti-helminthic drugs (N. et al., 2014). This indicates the inevitability of an ongoing helminthic infection for these interactions to occur (Kepha et al., 2016). In immunological study findings obtained from some of these experiments, there is a suggestion that helminthic infections induce decreased pro-inflammatory immune responses which is a significant mechanism underlying these effects. The most significant role of this primary inflammatory response in an infection has been demonstrated in a variety of ways, comprising of manifold studies that have demonstrated that peripheral blood mononuclear cell IFN-y production in reaction to ex vivo malaria antigen stimulation is associated with malaria infection protection (Ezenwa & Jolles, 2011; Perez-mazliah & Langhorne, 2015; Pietkiewicz, 2005). Conversely, a number of this pro-inflammatory cytokines have also been associated in the expansion of severe disease, including IFN-y, TNF, IL-1α, IL-1β, IL-6 and IL-2 receptor (Nasr, Allam, Hamid, & Al-ghamdi, 2014; Torre et al., 2002). These immunological reactions are equally suggested to be likely responsible for a number of the assimilated symptoms and pathological variations associated with malarial infection (Nasr et al., 2014). Experimental statistics have indicated that balancing of both pro and anti-inflammatory signals ignites a central role in determining the outcome of an infection (Moreau & Chauvin, 2010), i.e. whether it leads to body defense or accelerated immunopathology (van Riet et al., 2007). Subsequently, clinical immunity might also be contingent with its ability to adequately moderate pro-inflammatory responses. Consequently it has been implored in other literature that by reconstituting the immune responses towards Th2-type effect or mechanisms, helminthes may weaken the pro-inflammatory Th1-type mechanisms required to kill malaria parasites (Frosch & John, 2012). On the other hand, helminthes are depicted to provoke a potent regulatory T cell (T reg) reactions (Ezenwa & Jolles, 2011), which pacify completely the types of cellular effects for this mechanisms that encompass those against malaria parasites (Farrington et al., 2017). On this background; the present study explores how intestinal helminthes affects malaria outcome and their role in cytokine response in children infected with malaria attending Kisii referral hospital; Kenya. The elucidation of the impact of malarial-intestinal helminthic co-infections on cytokine profiles will clearly be helpful in understanding how the body responds during dual malaria and helminthic infections with the aim of establishing integrated malaria and helminth control strategies. The interventions may provide insights into vaccine development or recruiting part of the cytokines as a candidate in vaccine development targeting children hence securing future generations.

2. MATERIALS AND METHODS

Study Population: The study was conducted in Kisii County; Kenya. Malarial infection in this area is endemic with a prevalence of 28 per 1000 against the country’s 20 per 1000 persons during peak seasons (Division of Malaria control Ministry of Public Health and Sanitation, 2010). In case control study design in which participants were consecutively recruited until the sample size was achieved as per the Hederke; Gibbons; & Watenux,1999 formula. Both boys and girls aged between 6-14 years were recruited. Only school going children confirmed positive for either malaria or malaria helminthic co-infection upon consent from custodians were included in the study.

Sample collection and analysis: During recruitment; the physical examination and symptom evaluation was done by a clinician. If the examination and evaluation indicated a malaria case; a blood sample was collected in the laboratory by initial sterilization of the fourth finger using alcohol soaked cotton swab. The finger was then pricked using a sterile disposable lancet. A drop of capillary blood was obtained and a smear prepared on a microscope slide and stained with Giemsa to examine for presence of malaria parasites using WHO recommended technique (Eight, 2016) . If positive; the malaria was categorized as either uncomplicated or severe based on the number of parasitized red blood cells in the film according to CDC classification. The total numbers of infected and uninfected erythrocytes from - 10 fields (Mg x 100) were counted and parasitaemia levels calculated. If the malaria slides tested positive for malaria a pea size stool specimen was also obtained from the same patient in a poly pot upon consent to examine for presence of intestinal helminthes using formal ether concentration technique. One (1) ml of venous blood was collected using venipuncture technique from all eligible study participants for cytokine profiling. Cytokine analysis was done using the flow cytometer technique by BD biosciences (Human, Th, & Li, 2010). The BD™ CBA human Th1/Th2 cytokine kit II ( catalogue No. 551809) was used. The cytokine analysis involved obtaining serum from the sample and compared with standards provided. Levels of Th1 cytokines (IL2; TNFα and IFNγ ) and Th2 cytokines (IL4; IL6 and IL10) in serum were determined by utilizing cytometric bead array technology (BD) and fluorescent detection by flow cytometry according to manufacturer’s instructions. The technique allows detection by flow cytometry of multiple cytokines in samples. In summary; 50μl of bead population with discrete fluorescent intensities of peridin chlorophyll protein (perCP) cy5.5 coated with cytokine specific capture antibodies were added to 50μl of phycoerythrin conjugated anti-human inflammatory cytokine bodies. Standards for each cytokine (20-5000pm/ml) were mixed with cytokine capture beads
and phycoerythrin-conjugated reagent. The vortex mixtures were allowed to incubate and flow cytometric analysis performed and analyzed for cytokine concentration values. The data obtained was read as pg/ml. The gating strategy used was forward scatter (FSC) and side scatter where viable single cell events are evaluated. Any debris, dead cells and clumps or doublets are eliminated in this gating technique.

**Data Management Statistical analysis:** In data analysis, categorical variables were described as proportions and percentages whereas continuous variables were described as mean; median and standard deviations (SD). Multivariable analysis (ANCOVA) was used to compare the median of cytokine levels between the two study groups with reference to the type of intestinal helminth infection and co-infection status.

**Ethical Consideration:** Ethical approval was granted by the National Council for Science and Technology office (permit number NACOSTI/17/98963/18112) and ethical clearance from Baraton University Ethical committee. Permissions were also obtained from Kisii University Research and Extension office; Ministry of Education and Kisii County. Both written and oral consent was sought from the patient custodian. The information obtained was confidential and private.

### 3. RESULTS

The present study investigated on the dynamics of cytokine response in malaria severity and conjoint infection of intestinal helminthes among school going children in Kisii County, Kenya. The study compared the levels of cytokine response between the uncomplicated and severe malaria cases. The study observed that there was significantly higher levels of IL-2 (P < 0.001) and IL6 (P < 0.001) of cytokine levels in the severe cases of malaria compared to the uncomplicated cases of malaria. The other cytokines did not show any significant difference between the two study groups as shown in table 1 below.

<table>
<thead>
<tr>
<th>Type of cytokine response (pg/ml) x 10³</th>
<th>Uncomplicated Malaria MDN (IQR)</th>
<th>Severe Malaria MDN (IQR)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL2</td>
<td>2.120(5.53)</td>
<td>8.770(13.11)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TNFα</td>
<td>3.600(4.10)</td>
<td>3.400(6.10)</td>
<td>0.149</td>
</tr>
<tr>
<td>IL4</td>
<td>2.600(3.75)</td>
<td>2.600(4.25)</td>
<td>0.522</td>
</tr>
<tr>
<td>IL6</td>
<td>8.400(4.50)</td>
<td>10.200(9.00)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IL10</td>
<td>2.030(1.10)</td>
<td>2.010(1.47)</td>
<td>0.544</td>
</tr>
<tr>
<td>IFNƔ</td>
<td>3.800(6.00)</td>
<td>3.600(4.30)</td>
<td>0.181</td>
</tr>
</tbody>
</table>

**Table 1: Comparison of Cytokines Levels between Uncomplicated and Severe Malaria**

**Table 1 legend:** The study observed that there was significantly higher levels of difference in both IL-2 (P < 0.001) and IL6 (P < 0.001) of cytokine levels in the severe cases of malaria compared to between the uncomplicated cases of malaria. The other cytokines did not show any significant difference between the two study groups. The study further investigated on trends of cytokine levels in malaria only cases compared with malaria and helminthic co-infection in relation to malaria severity. In regards to IL-2, The IL-2 cytokine levels in cases of helmith co-infection are observed to be concentrated in the uncomplicated cases of malaria but in malaria cases only the IL-2 levels seem to be higher in severe cases of malaria infection compared to the uncomplicated. These trends are as shown in Figure 1.

**Figure 1:** Trends of IL-2 (pg/ml x 10³) response in Malaria only cases compared with Malaria and Helminthic Co-Infection in relation to Malaria severity

**Figure 2 Legend:** TNFα was shown to be produced in both cases of uncomplicated and severe malaria however an increasing trend of the cytokine levels was also observed with severity of malaria cases in both malaria infections only and malaria helminthic co-infection study groups. Therefore increase in malaria severity seems to increases TNFα concentration.

When the TNFα levels were compared with malaria severity, TNFα was shown to be produced in both cases of uncomplicated and severe malaria however an increasing trend of the cytokine levels was also observed with severity of malaria cases in both malaria infections only and malaria helminthic co-infection study groups. Therefore increase in malaria severity seems to increases TNFα concentration.

**Figure 2:** Trends of TNFα (pg/ml x 10²) response in Malaria only cases compared with Malaria and Helminthic Co-Infection in relation to Malaria severity

In regards to IFNƔ response when compared with Malaria severity an inconsistent trend was observed with malaria severity however, the malaria only cases seem to continue to produce IFNƔ in the most severe cases of malaria compared to the malaria helminthic co-infection.
Trends of IL-10 (pg/ml × 102) response in Malaria only cases compared with Malaria and Helminthic Co-Infection in relation to Malaria severity

Figure 3 Legend: An inconsistent trend was observed with malaria severity however; the malaria only cases seem to continue to produce IFNγ in the most severe cases of malaria compared to the malaria helminthic co-infection.

IL-4 levels were observed not to have a well-defined trend in the cytokine concentration with malaria severity. However the IL-4 production seems is produced in low levels in both severe and uncomplicated cases among the malaria helminth co-infections compared to malaria only cases which seem to have higher concentration levels in both severe and uncomplicated malaria cases.

Figure 4: Trends of IL-4 (pg/ml × 102) response in Malaria only cases compared with Malaria and Helminthic Co-Infection in relation to Malaria severity

Figure 4 Legend: InterLeukin-4 levels are observed not well defined trend in the cytokine concentration with malaria severity. However the IL-4 production seems is produced in low levels in both severe and uncomplicated cases among the malaria helminth co-infections compared to malaria only cases which seem to have higher concentration levels in both severe and uncomplicated malaria cases.

When IL-10 was investigated, the results indicate that IL-10 increase with malaria severity in both malaria only cases and Malaria Helminth Coinfection as also shown by TNFα levels. However, malaria only cases seem to reach higher concentrations in the most severe cases of malaria compared to the helminthic infection.

Figure 5: Trends of IL-10 (pg/ml × 102) response in Malaria only cases compared with Malaria and Helminthic Co-Infection in relation to Malaria severity

Figure 5 Legend: The current study indicates that production of IL-10 increase with malaria severity in both malaria only cases and Malaria Helminthic Coinfection as also shown by TNFα levels. However, malaria only cases seem to reach higher concentrations in the most severe cases of malaria compared to the helminthic infection. When Interleukin-6 was studied, it was shown that the concentration of IL-6 is higher in malaria only cases in both severe and uncomplicated cases of malaria compared with malaria helminthic co-infection which showed lower levels of IL-6 in both cases of severe and uncomplicated malaria infection.

Figure 6: IL6 (pg/ml × 102) Mean Response in Malaria Only Compared with Malaria and Helminthic Co-Infection In Relation To Malaria Severity

Figure 6 Legend: Interleukin-6 concentration is shown to be higher in malaria only cases in both severe and uncomplicated cases of malaria compared with malaria helminthic co-infection which showed lower levels of IL-6 in both cases of severe and uncomplicated malaria infection. The study also evaluated how various intestinal helminthes may affect cytokines response compared to malaria only cases. When cytokine levels was analyzed among the malaria and A. lumbricoides co-infection cases IL-2 and IL-6 was observed to have a significant difference when compared with malaria only cases, however all other cytokines were comparable between the two study groups. It was observed that IL-2 and IL-6 had significantly (P<0.001) lower levels among the Malaria and A. Lumbricoides conjoint infections cases when compared to the malaria only cases. This is as shown in table 2.

<table>
<thead>
<tr>
<th>TYPE OF CYTOKINE</th>
<th>MALARIA ONLY INFECTION [N=84;MDN (IQR)]</th>
<th>MALARIA WITH HELMINTHIC INFECTION (A. lumbricoides; N=46; MDN (IQR))</th>
<th>P-VALUE</th>
</tr>
</thead>
</table>


The present study observed that all the other cytokines except Interleukin-6 did not show any significant difference when malaria with T. Trichura coinfection was compared with malaria only cases. Interleukin- 6 had significantly (P= 0.047) higher levels among the malaria with S.stercolaris co-infection compared with malaria only cases. This is as shown in table 5.

Table 5: Cytokine Response in Malaria- S. Stercolaris co-infection compared to malaria only cases of infection

Table Legend: All the other cytokines except IL-6 did not show any significant difference when malaria with S.stercolaris co-infection was compared with malaria only cases. Interleukin- 6 had significantly (P= 0.046) lower levels among lower among the malaria with S.stercolaris co-infection compared with malaria only cases. When Malaria- S.mansoni co-infection was investigated, all the other cytokines except IFNγ did not show any significant difference when malaria with S.mansoni co-infection was compared with malaria only cases. Interferon gamma (IFNγ) had significantly (P= 0.047) higher levels among the malaria with S.mansoni co-infection compared with malaria only cases. This is as shown in table 6.

Table 6: Cytokine Response in Malaria- S.mansoni co-infection compared to malaria only cases of infection

Table Legend: All the other cytokines except IFNγ did not show any significant difference when malaria with S.mansoni co-infection was compared with malaria only cases. Interferon gamma (IFNγ) had significantly (P= 0.047) higher levels among the malaria with S.mansoni co-infection compared with malaria only cases. The study also investigated the malaria co-infections with E.vermicularis, H.nana and T.saginata and how they may affect cytokine levels when compared to malaria only cases. The study observed that all the cytokines were comparable between the two study groups. This is as shown in table 7.
This finding contradicts raújo, & Pearce, 2002. An correlation with malaria severity. However, sed in malaria cases with co- infections did not contribute to severity of malaria infection. Other types of co-infections were comparable between the two study groups.

**DISCUSSION**

The present study evaluated dynamics of cytokine response in conjoint cases of intestinal helminthes and malaria infection among school children in Kisii County, Kenya. At first the study established that helminthic co-infection contributed to severity of malaria when compared with malaria only cases. In this regard, the study observed that the proportion of severe cases of children with malaria only was significantly lower compared to the uncomplicated malaria (Mogoi Elijah et al 2019; unpublished results). Significant higher numbers of uncomplicated malaria cases was observed in malaria co-infections with A.lumbricoides and hookworm compared to the severe cases (Mogoi Elijah et al 2019; unpublished results). These worms have been shown to reduce IL2 and IL6 response which has been associated with malaria severity. Findings by (Farrington et al., 2017) indicate that when IL2 and IL6 levels are increased in malaria infection; severity tends to increase. Other types of co-infections did not show any significant difference between the uncomplicated and severe malaria infection. These worms did not affect IL2 and IL6 response concurrently. These results indicate that helminthic co-infection did not contribute to severity of malaria infection, in the contrast may have reduced the severity of malaria with significant proportion of uncomplicated malaria being observed in the cases of A.lumbricoides and hookworm co-infections (Mogoi Elijah et al 2019; unpublished results). This findings are similar to those done in Cameroon (Kwenti, Nkume, Tanjeko, & Kwenti, 2016) in which there was a significantly lower numbers of uncomplicated cases of malaria in Hookworm co-infections with malaria with malaria cases only. Therefore, A.lumbricoides and hookworm co infections may have an underlying effect on IL2 and IL6 that may induce decrease in malaria severity among children. In support of the present study another study showed that these individuals with helminthic co-infections had reduced risk of mounting various forms of severe malarial disease (F. C. Hartgers & Yazdanbakhsh, 2006).On the other hand, this finding contradicts another study that observed that there was no significant relationship between malaria infections only and those with helminthic co-infections (Kwenti et al., 2016). (Knowles, 2011) in his findings on mice model; concluded that helminthes may not be directly implicated in malaria severity. The current study also determined whether there was a significant difference in cytokine production in severe cases of malaria infection compared to the uncomplicated cases of malaria. The study observed significantly higher levels of IL-2 and IL6 cytokine levels in severe cases of malaria compared to the uncomplicated cases of malaria. These findings are in unison with one of (Farrington et al., 2017) who found that IL2 and IL6 had a positive correlation with malaria severity. The other cytokines did not show any significant difference between the two study groups. These results therefore give an indication that malaria severity may be increased due to increased IL-2 and IL6 concentration. These results compares with previous studies that observed children with severe malaria having higher levels of these cytokines (Frosch & John, 2012). On the contrary other studies observed that a number of pro-inflammatory cytokines have been associated in the expansion of severe disease, including IFN-γ, TNF, IL-1α, IL-1β, IL-6 and IL-2 receptor (Nasr, Allam, Hamid, & Al-ghamdi, 2014; Torre et al., 2002). In a different study IL4 pro-reactivation phenotype was shown to play an important role in a latent malaria infection without helminthic infection (Perez-mazliah & Langhorne, 2015). This observation has been associated with the role IL4 plays in response and amplification of Th2 (Macdonald, Araujo, & Pearce, 2002).The current study further established the trends of the cytokine levels in relation to the severity of malaria in both malaria only infections and malaria-helminthic co-infections cases. In respect to the pro-inflammatory cytokines; the trends showed that IL-2 levels seem to be higher in severe cases of malaria infection compared to the uncomplicated in malaria only cases while malaria severity seems to increases TNFα concentration in both cases. An inconsistent trend was observed with malaria severity however; the malaria only cases seem to continue to produce and increase IFNγ in the most severe cases of malaria compared to the malaria helminthic co-infection. These results indicate that pro-inflammatory cytokine are more suppressed in malaria cases with co-infection compared to malaria only cases except for TNFα which is observed to have an increasing trend with malaria severity. These results concur with previous studies which observed that IFNγ cytokine is associated with malaria infection protection (Helmsby, 2015; Nolund, Urban, Fried, & Kumar, 2008; Torre et al., 2002; Zhang & Cao, 2014).However, these results contrast with other studies that showed that

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Control (MDN/IQR)</th>
<th>Malaria (MDN/IQR)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα</td>
<td>3.500(1.400)</td>
<td>3.350(0.500)</td>
<td>0.905</td>
</tr>
<tr>
<td>IL4</td>
<td>2.250(1.450)</td>
<td>2.160(0.000)</td>
<td>0.287</td>
</tr>
<tr>
<td>IL10</td>
<td>2.005(1.340)</td>
<td>1.975(0.050)</td>
<td>0.869</td>
</tr>
<tr>
<td>IFNγ</td>
<td>3.800(4.000)</td>
<td>2.180(0.020)</td>
<td>0.068</td>
</tr>
<tr>
<td>IL2</td>
<td>8.765(15.750)</td>
<td>8.770(0.740)</td>
<td>0.708</td>
</tr>
<tr>
<td>IL6</td>
<td>10.350(8.200)</td>
<td>9.800(8.000)</td>
<td>0.571</td>
</tr>
</tbody>
</table>

**Table 7: Cytokine Response in Malaria co-infections with E.vermicularis, H.nana and T.saginata compared to malaria only cases of infection**

**Table Legend:** The study also investigated the malaria coinfections with E.vermicularis, H. Nana and T. Saginata and they may affect cytokine levels when compared to malaria only cases. The study observed that all the cytokines were comparable between the two study groups.
IFNγ is predominant in severe malaria cases an indication it contributes to its severity (Prakash, Fesel, Jain, Mishra, & Pied, 2006). In regards to Th2 related cytokines; interleukin-4 production seemed to be in low levels in both severe and uncomplicated cases among the malaria helminth coinfections compared to malaria only cases. Interleukin-10 cytokine was shown to increase with malaria severity in both malaria only cases and Malaria cases with helminth co-infection. This results are comparable to (Farrington et al., 2017) in which IL10 response was strongly associated with severity of malaria due to its regulatory role. However, malaria only cases seem to reach higher concentrations in the most severe cases of malaria compared to the helminthic infection. Interleukin-6 concentration is shown to be higher in malaria only cases in both severe and uncomplicated cases of malaria compared with malaria helminthic co-infection which showed lower levels of IL-6 in both cases of severe and uncomplicated malaria infection. These results indicate that IL-4 and IL-6 may be suppressed in cases of malaria with co-infection compared to malaria only cases which is different in regard to IL-10 which indicate that it is continuously produced as malaria severity advance. These results are similar to previous studies which observed that Th2 plays a regulatory role in malarial infections (Perez-mazliash & Langhorne, 2015). Further, this study is contrasted by another finding in which IL 6 cytokine were increased in helminthic co-infections (Prakash et al., 2006). The present study also investigated how the specific helminthic co-infection may affect cytokine response. The study observed that only IL-2 and IL-6 had significantly lower levels while among the Malaria and A. lumbricoides conjoint infections cases when compared to the malaria only cases. When malaria coinfection with Hook worm was analyzed, significantly lower levels of IL-2 and IL-6 while higher levels of TNFα and IL-4 were observed in cases of hookworm coinfection when compared with the malaria only cases. Only interleukin-6 had significantly lower levels among the malaria with T. Trichura co-infection compared with malaria only cases. Further only Interleukin-6 had significantly lower levels among the malaria with S. stercolaris co-infection compared with malaria only cases. All the other cytokines except IFNγ did not show any significant difference when malaria with S. mansoni co-infection was compared with malaria only cases. Interferon gamma (IFNγ) had significantly higher levels among the malaria with S. mansoni co-infection compared with malaria only cases. The study also investigated the malaria coinfections with E. vermicularis, H. Nana and T. Saginata and it was observed that all the cytokines were comparable between the two study groups. These results clearly indicate that IL-2, IL-6 and IFNγ are the only cytokine that were influenced by the specific helminthic infection in respect to A. lumbricoides, Hookworm, T. trichura, S. stercolaris and S. mansoni. The results are similar to other previous studies which showed that intestinal helminthes alters IL2 levels (Arinola et al., 2014) by increasing their concentration. Overall; the present study doesn’t support hypothesis that helminthic co-infection may advance malaria severity on the contrary it supports the hypothesis that helminthic co-infection may alter immune response to favor children survival from malaria severity and is supported by a previous study (Hassan, 2018) and as demonstrated in animal models (P. chabaudi and P. yoelli, 2009). Experimental studies have also indicated that balancing of Th1 and Th2 cytokines play a key role in determining the progression of an infection (Moreau & Chauvin, 2010) That is, if will result accelerated immunopathology or otherwise. On the contrary, it has also been implied that reconstituting towards Th2-type of immune response by helminthes coinfection the Th1-type required to fight malaria parasites (Frosch & John, 2012) The results provided by this study therefore significant in that they provide information on the most significant worms that attract attention in their elimination for they have been associated to co-infect with malaria. On cytokines; it has been indicated that both IL2 and IL6 have profound effect on malaria severity. Results from this can be inferred on control strategies that mitigate malaria episodes especially among the vulnerable groups.

CONCLUSION AND RECOMMENDATION
The study concludes that helminthic con-infection especially A. lumbricoides and Hookworm may not contribute to severity of malaria infection, in the contrast may significantly reduce the chances of malaria infections advancing to severe malaria. Malaria severity may increase the production of IL-2 and IL6 regardless of helminthic co-infection. The study further concludes that both Th1 and Th2 cytokines are more suppressed in malaria cases with co-infection as compared to malaria only cases except for TNF- alpha and IL-10 which seem to increase in both case malaria only cases and coinfection. These results also clearly indicate that IL-2 is significantly suppressed by A. lumbricoides, while IL-6 is reduced by A. lumbricoides, S. stercolaris hookworm and T. Trichura co-infections while IFNγ is increased in S. mansoni co-infections. Further hookworm may significantly increase levels of TNFα and IL-4 compared with the malaria only cases. A key finding is that levels of IL6 may be used as a biomarker to differentiate between severe and uncomplicated malaria. The present study therefore recommends that it’s worth noting that findings from dysregulated immune systems involved in malaria and helminthic cannot be explained simply by Th1/Th2 dichotomy alone and therefore further robust studies may give more in sight on these interactions.

ETHICAL APPROVAL
The study protocol used in this research complied with the guidelines of the Baraton University and was approved by National council for science and technology (NACOSTI/17/98963/18112).

CONSENT FOR PUBLICATION
Not applicable

AVAILABILITY FOR SUPPORTING DATA
Data used and analyzed during current study is available from corresponding author on reasonable request.

COMPETING INTERESTS
The authors declare that they have no competing interests.

FUNDING
This work was funded by the authors.
AUTHORS’ CONTRIBUTION

Elijah Mogoi Matiabe wrote the manuscript; Dr. Stanslaus Musyoki designed the experiments and Dr. Benson Nyanchong’ designed and illustrated figures and tables. All authors contributed equally and approved the final manuscript.

ACKNOWLEDGEMENT

We acknowledge the KTRH Laboratory staff in their effort to ensure we had required access to required information even in odd times. We acknowledge contributions by Philip Mosioma on data analysis.

REFERENCES


