**Impact of Malaria and intestinal Parasitic infections on inflammatory cytokines amongst armed forces of the North West and South West, Cameroon**

**Abstract**

Malaria and intestinal helminthic infections present a significant public health challenge, particularly in tropical regions. The study was designed to determine the impact of Malaria and Intestinal helminthic infections on inflammatory cytokines amongst Armed Forces in the North West and South West Cameroon. This was a cross-sectional study carried out between May 2022 to April 2024. A total of 812 stool and venous blood samples were collected for the identification of malaria and intestinal parasites and for evaluation of inflammatory cytokines. The study used Giemsa staining and Kato-Katz methods to identify malaria and intestinal parasites, while Enzyme-linked immunosorbent assay (ELISA) was used to evaluate inflammatory cytokine concentrations and statistical comparisons were performed using SPSS. Results obtained showed that the prevalence of Malaria, intestinal helminths and co-infections were 13.6%, 22.8% and 3.2% respectively. TNF-α, INF-γ**,** and IL-1β concentration mean levels were significant in *Plasmodium falciparum* infected participants (*p = 0.000*). *Trichuris trichiura* infected participants showed a positive significant impact on TNF-α (*p = 0.000*); IL-1β (*p = 0.000*) and IL-6 (*p = 0.035*) while *Schistosoma mansoni* showed a significant impact on TNF-α (*p =* *0.044*) and IL-1β (*p = 0.037*). *Schistosoma intercalatum* infected participants showed a significant value of IL-1β (*p = 0.004*). Also, co-infection with these parasites significantly impacted TNF-α (*p* = 0.002), INF-γ (*p* = 0.001)**,** and IL-1β (*p* =0.000). The study provided evidence that *Plasmodium* spp., *Trichuris* *trichiura*, *Schistosoma* *mansoni*, *Schistosoma* *intercalatum* and co-infections negatively impacted pro-inflammatory cytokines. This confirms that Malaria and Intestinal helminthic infections engage immunity responses.

**Keywords:** Malaria; Intestinal Parasites; Inflammatory cytokines; Armed-forces

**Introduction**

Malaria and Intestinal helminth infections are major public health issues worldwide [1,2,3], affecting millions of people and leading to serious health problems and even mortality in Africa, particularly in resource-limited tropical and sub-tropical regions in sub-Saharan Africa [4]. These diseases are influenced by socio-economic problems such as poverty [5], behavioural attributes [6], environmental factors like stagnant water and poor sanitation, which may favour the transmission of parasites [7, 8]. In regions where these parasites are co-endemic, especially in the Western region of Cameroon, co-infections result in severe morbidity and mortality [9; 10]. The Armed Forces are a mobile population group which are often checked to determine if they are physically apt and given appropriate preventive measures (vaccines, impregnated mosquito nets etc) before being deployed to their various operational zones (forest). These operational zones are characterised by high transmission rates of infections with Malaria and Intestinal Helminths [11].

Immunity against malaria and intestinal helminthic parasites involve a cascade of immune responses, including pro and anti-inflammatory markers: Tumor Necrosis factor-alpha (TNF-α), Interferon-gamma (INF-γ), Interleukine-1beta (IL-1β), Interleukine-6 (IL-6), and Interleukine-10 (IL-10). [10]. Cytokines, acting as homeostatic regulators, control infection by inhibiting parasite proliferation. However, if they are not down-regulated, immune-related pathologies can develop, leading to complications [12,13]. Disruptions in cytokine expression networks can cause abnormal conditions like anaemia [13].

The distinction between protective and pathological roles of inflammatory mediators is not well-established and challenging to measure, as seen in human malaria [13]. Immunity against malaria is influenced by both cellular and humoral mechanisms [14]. Cytokines, such as TNF-α, IFN-γ, IL-1β, IL-6 and IL-10, are crucial for suppressing parasitaemia and regulating the macrophage response to inflammation [14]. IL-1β is a potent pyrogen that triggers an inflammatory response, acting as a primary defence mechanism against pathogens [15]. It enhances the production of IFN-γ in murine malaria models, but prolonged elevated levels can lead to various haematological disorders, including anaemia [15, 16]. IL-6 is essential for the activation of leukocytes and endothelial cells and stimulates the synthesis of hepatic acute-phase reactants (Woods et al., 2000). IL-10 inhibits the production of cytokines, including IFN-γ and TNF-α, and may confer protective benefits in managing parasitaemia and preventing severe malaria anaemia [17].

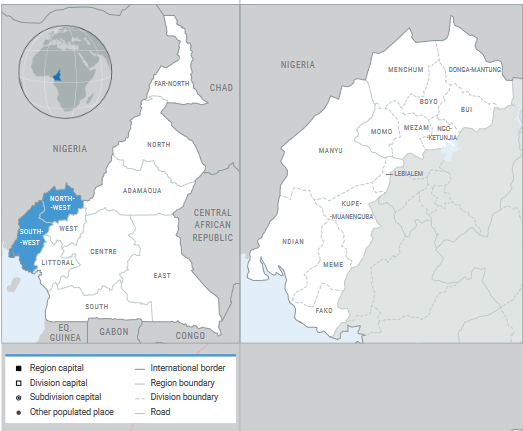
Inflammation entails the activation of immune cells, including monocytes and T-cells, which secrete pro-inflammatory cytokines [18].

Does Malaria and/or Intestinal helminthic co-infected impact inflammatory cytokines? This study investigated the impact of Malaria and intestinal helminthic infections on inflammatory cytokines among the armed forces in the North and South West regions of Cameroon.

**Materials and methods**

**Study area**

This study was military-based conducted in the North West and South West regions of Cameroon, focusing on the Legions, Air Force Military Units, Rapid Intervention Units, and amphibians military base Tiko. The North West Region is located between longitude 5˚56’ N and latitude 10˚10’E, with a cosmopolitan population of 2 million inhabitants and a surface area of 17910 Km2 [19]. It is characterized by cold, tropical climatic conditions, with an annual rainfall of about 2,145mm and an annual temperature range of 16˚C–25˚C [20]. The South West Region is located between located between longitude 4°01′N and latitude 9°13′E [20], has a surface area of 25.410 km2 and a population of 1.153.125 inhabitants [21]. The North West and South West Regions of Cameroon fall in the large equatorial forest, where malaria and soil-transmitted helminthic parasites transmission are high and perennial [22]. The study was conducted in Bafut, Buea, Limbe, Idenao, and Tiko, due to participants being redeployed to different divisions and sub-divisions.



**Figure 1:** Map of North West and South West regions of Cameroon adapted from open-source web image

**Study design, study population and selection criteria**

This study was a Military-based cross-sectional study, conducted in the North West and South West Regions of Cameroon, which involved the collection of samples from May 2023 to April 2024, to include a good number of participants since they are redeployed every three months.

The population sample size was estimated at a 50% prevalence rate since the prevalence was unknown in the calculation [23]. The minimum sample size was determined and adjusted by 11%. as described by Cochran [24] below. Only the Armed Forces coming immediately from an operational service, did not take anthelminthic or antimalaria drugs and who consented to take part in the study were recruited. Participants who were unable to provide stool or blood samples were not included.

n = Z2 P [1-P] / D2

Where D = Margin of error between the sample and the population (5%), n = Sample size, Z = 95% confidence interval (1.96 at 95% confidence interval), P = Prevalence rate based on previous study

N = (1.96)2×0.5 (1­0.5) ÷ (0.05)2 = 384

N = 384 + 384 = 768

A total of eight hundred and twelve (812) participants were recruited for the study.

**Collection of blood samples**

Approximately 5mL of venous blood sample was collected from each participant into labelled Ethylenediamine tetraacetate (EDTA) test tubes using a vacutainer. The Blood samples were centrifuged and Plasma was collected and stored at 20oc in a fridge for the evaluation of pro and anti-Inflammatory cytokines.

**Collection of Stool Samples**

Fresh stool specimens were collected into dry labelled stool containers with tight covers bearing serial numbers from each participant. The stool samples were examined using the Kato-Katz technique [25, 26] for the detection and quantification of soil-transmitted helminths.

**Malaria parasite detection**

Thick and thin blood films were prepared on a grease free slide labelled with the participant’s code following the technique recommended by Cheesbrough [27]. Both thick and thin blood films were air dried and the thin films fixed using methanol for 10 s seconds. Both the thin and thick films were later stained with 10% Giemsa for 10 minutes. [27; 28]. The slides were carefully washed, dried and observed under the x100 (oil immersion) objective of a compound microscope (Olympus CX22, Olympus Corporation, Tokyo, Japan). The World Health Organization bench aid for the diagnosis of malaria parasite [28] was used to identify any of the *Plasmodium* (*P.*) *spp*. Thin films were used for the identification of the malaria parasitic species and the thick films used to quantify the malaria parasite density per microliter (μL) of blood. This was done by counting the asexual stages (trophozoites) against 200 leukocytes assuming Total White Blood Cell (WBC) count of 8000 leukocytes/ μL of blood [28]. Slides with no asexual or sexual stages of malaria parasite were reported as negative after observing up to 100 high power fields.

**Biochemical Analysis:**

The remaining parts of the blood samples in the EDTA test tubes were centrifuged at 3 000 revolutions per minute (rpm) for 15 minutes (Horizon centrifuge, Drunker Diagnostics, USA) and the plasma was pipetted into labelled Eppendorf tubes and stored at -200C before immunological analysis. These parameters were analysed in different samples using ELISA techniques according to the manufacturer’s instructions (Quantikine Colorimetric ELISA Kits (Quantikine®) obtained from R&D Systems Biotech, USA).

#### **Evaluation of Plasma inflammatory cytokines**

The levels of tumour necrosis factor-alpha (TNF-α), interferon-gamma(INF-γ), interleukin 1-beta (IL-1β), interleukin 6 (IL-6),and interleukin 10 **(**IL-10)in plasma were determined in different samples using ELISA techniques according to the manufacturer’s instructions (Quantikine Colorimetric ELISA Kits (Quantikine®) obtained from R&D Systems Biotech, USA). The intensity of the colour measured was proportional to the amount of the inflammatory cytokine bounded in the initial step. The values for TNF-α > 10 pg/ml were considered abnormal as those used by Achidi *et al*., [29], for IFN-γ > 50 pg/mL were considered abnormal as those used by Monastero and Pentyala, [30], for IL-1β > 12pg/mL were considered abnormal, as those reported by Lyke *et al*., [29], for IL-6 > 54.6 pg/ml were considered abnormal, as those used by Lyke *et al*., [31], and for IL-10 >20 pg/ml were considered abnormal similar as those used by Lyke *et al*., [31] and Achidi *et al*., [29].

**Stool samples and intestinal parasitic infections determination**

Five grams of stool were collected from each participant into air tight labelled sterile stool bottles. Collected stool samples were processed using the Kato-Katz technique following the protocol of the manufacturer (Kato-Katz kit) to diagnose intestinal helminths [32, 26]. The slides were read within sixty minutes to avoid missing eggs of hookworms and other helminths that may disintegrate during longer clearing time. The egg burden for each species of intestinal helminths was calculated as egg per gram (epg) of stool by multiplying total eggs of each helminth species on the slide by 24 following recommended stool templates of 41.7mg [26]. Slides that did not have any helminth eggs or larvae were reported as negative. The egg burden of the intestinal parasites were further classified as light, moderate or heavy as defined by Montresor *et al*, [33]. Only single independent stool samples were collected from each participant and examined for the presence of intestinal parasites without post treatment diagnosis or assessment of the patients.

**Data analysis**

Data collected from the field was entered and analysed using the Statistical Package for Social Scientists (SPSS) version 16. Mean differences between groups for normally distributed variables was assessed using the student t-test and One-Way ANOVA (analysis of variance). Multiple comparisons within groups were computed using the Tukey Multi comparison Test. The binary logistic regression was used to assess level of association between variables. The malaria parasite density and helminth egg count in co-infected samples was log transformed in base 10. The cut off point for assessing all statistical significance between groups was set at probability level (p) < 0.05.

**Results**

**Baseline characteristics of the study population**

In this study, a total of 812 participants were recruited all of single-sex (males) which were sub-divided into three (3) age groups. The age group with the highest number of participants was the 18 -30years age group (425/812; 52.3%) while those of the 41 -50years age group had the least number of participants (147/812; 18.1%). In addition, the participants’ marital status was taken into consideration of which 54.2% (440/812) were married and 45.8% (372/812) were single as shown on Table 1.

Table 1: Population demographic information

|  |  |  |  |
| --- | --- | --- | --- |
| Parameters | | Frequency | Percentage (%) |
| Age group | 18 – 30 | 425 | 52.3 |
| 31 – 40 | 240 | 29.6 |
| 41 – 50 | 147 | 18.1 |
| Total | 812 | 100.0 |
| Marita status | Married | 440 | 54.2 |
| Single | 372 | 45.8 |
| Total | 812 | 100.0 |

**Prevalence of parasitic infections among the study group.**

According to Table 2, Out of the 812 participants, the prevalence of *Plasmodium falciparum,* was observed to be 11.9% (97/812) Five (5) different species of intestinal helminths were identified which included *Ascaris* (*A*.) *lumbricoides*, *T. trichiura*, Hookworms, *S. mansoni*, and *S. intercalatum*,of which *Ascaris lumbricoides* was most prevalent (4.7%; 38/812) and the least prevalent species was *S. intercalatum* (1.2%; 10/812) as indicated on the Table .

Table 2: Prevalence of parasitic infections among study population

|  |  |  |  |
| --- | --- | --- | --- |
| Parasitic species | Number examined | Number infected | Prevalence  (%) |
| *P. falciparum* | 812 | 97 | 11.9 |
| *P. vivax* | 14 | 1.7 |
| Overall malaria infection | 111 | 13.7 |
| *Loa-loa* | 2 | 0.2 |
| *A. lumbricoides* | 38 | 4.7 |
| *Hookworms* | 11 | 1.4 |
| *T. trichiura* | 20 | 2.5 |
| *S. mansoni* | 18 | 2.2 |
| *S. intercalatum* | 10 | 1.2 |
| *E. coli* | 55 | 6.8 |
| *E. histolytica* | 33 | 4.1 |
| Overall intestinal parasites | 185 | 22.8 |

**Prevalence of the co-infection of parasitic infection:**

Out of the 812 participants recorded, the overall prevalence of co-infection of parasitic infection was 3.2% (26/812). The most prevalent co-infections were *Plasmodium falciparum/Ascaris lumbricoides* and *Plasmodium falciparum/Trichuris trichiura* (0.9%; 7/812) and the least prevalent were *Plasmodium vivax/ Trichuris trichiura*; *Ascaris lumbricoides/ Trichuris trichiura* and *Ascaris lumbricoides/Schistosoma mansoni* (0.1%; 1/812) as shown in table 3.

**Table 3: Co-infection prevalence of parasitic infections**

|  |  |  |
| --- | --- | --- |
| Co-infection | Number examined | Co-infection prevalence n(%) |
| *P. falciparum/P. vivax/S. mansoni* | 812 | 4 (0.5) |
| *P. vivax/T. trichiura* | 812 | 1 (0.1) |
| *P. falciparum/A. lumbricoides* | 812 | 7 (0.9) |
| *P. falciparum/T. trichiura* | 812 | 7 (0.9) |
| *A. lumbricoides/T. trichiura* | 812 | 1 (0.1) |
| *P. falciparum/S. intercalatum* | 812 | 2 (0.2) |
| *P. vivax/S. mansoni* | 812 | 3 (0.4) |
| *A. lumbricoides/S. mansoni* | 812 | 1 (0.1) |
| Total | 812 | 26 (3.2) |

**Effect of Malaria parasites (*Plasmodium falciparum and Plasmodium vivax*)on cytokines:**

In this study, results show that the mean Tumor necrosis factor-alpha (TNF-α), Interferon-gamma **(**INF-γ**)** and Interleukin 1 beta (IL1 β) concentration mean levels were higher and significantly positive in participants infected with *P. falciparum* (12.74±4.43 pg/mL, *p = 0.000*); (57.64±53.73pg/mL, *p=0.000*) and (15.66±7.07pg/mL, *p=0000*) respectively compared the negatively infected participants although the value for INF-γ was normal while those for TNF-α and IL1 β were abnormal as shown in Table 4. Moreover, the mean Interleukin 6 concentration and Interleukin 10 concentration were higher and abnormal in infected participants (15.01±4.34pg/mL, *p=0.071*) and (84.50±129.62 pg/mL, *p= 0.058*) respectively though not significant. In addition, we observed that patients infected with *Plasmodium vivax* were significantly positive with mean concentration levels of TNF-α (*p = 0.012*; 12.68±5.34pg/mL) and INF-γ (*p=0.016*; 54.00±27.25pg/mL) than in non-infected participants even though the value INF-γ was normal (table 4).

**Influence of intestinal helminths on cytokines:**

This study reports that, participants infected with *T. trichiura* had higher and statistically significant levels of TNFα (13.74±8.46pg/mL, *p = 0.000*); IL1β (15.78±9.23pg/mL, *p = 0.000*) and IL 6 (17.81±15.05pg/mL, *p = 0.035*) as compared to the uninfected participants (table 4). Also, the mean concentration values for TNFα (11.78±9.48pg/mL, *p =* *0.044*) and IL1β concentration level (14.34±3.61, *p = 0.037*) was statistically significantly higher and positive among the participant infected with *S. mansoni* (table 5). Equally a statistically higher and positive significance was recorded for IL1β (15.83±9.52, *p = 0.004*) in participants positive to *S. intercalatum* compared to the negative cases (12.53±3.50pg/mL). Even though the mean IL6 concentration level is higher in participants infected with *S. mansoni* (16.27±10.12pg/mL) compared to those that were negatively infected (13.31±9.48pg/mL) it is insignificant (table 5).

Table 4: Variations of Pro, anti-inflammatory based on Malaria parasites infection status

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Parasites** | | **Cases** | **Mean ± Standard deviation (SD) Cytokines levels** | | | | |
| **TNF-α (pg/mL)** | **INF-γ (pg/mL)** | **IL1β (pg/mL)** | **IL6 (pg/mL)** | **IL10 (pg/mL)** |
| *P. falciparum* | Positivea | 97 | 12.74±4.43 | 57.64±53.73 | 15.66±7.07 | 15.01±4.34 | 84.50±129.62 |
| Negativeb | 715 | 9.19±4.43 | 24.51±36.18 | 12.15±2.62 | 13.16±9.98 | 69.25±63.31 |
| Total | 812 | 9.61±4.61 | 28.47±40.12 | 12.57±3.64 | 13.38±9.50 | 71.07±74.45 |
| Range | | 1.53 – 46.20 | 4.93 – 509.12 | 5.89 – 51.84 | 3.28 – 92.55 | 13.53 – 995.52 |
| *p-value* | | *0.000* | *0.000* | *0.000* | 0.071 | *0.058* |
| *P. vivax* | Positivea | 14 | 12.68±5.34 | 54.00±27.25 | 13.75±5.11 | 15.44±5.00 | 72.71±23.72 |
|  | Negativeb | 798 | 9.56±4.58 | 28.02±40.17 | 12.55±3.61 | 13.35±9.56 | 71.05±75.04 |
| Total | 812 | 9.61±4.61 | 28.47±40.12 | 12.57±3.64 | 13.38±9.50 | 71.07±74.45 |
| Range |  | 1.53 – 46.19 | 4.93 – 509.12 | 5.89 – 51.84 | 3.28 – 92.55 | 13.53 – 995.52 |
| *p-value* |  | *0.012* | *0.016* | 0.222 | 0.412 | 0.934 |
| Note:  \*Student t-test used to assess the mean difference in means at 95% C. I with equal variances in mean assumed (2-tailed)  \*Statistical significance set a p < 0.05 | | | | | | | |

Table 5: Variations of Pro, anti-inflammatory based on intestinal helminthic parasites infection status

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Parasites** | | **Cases** | **Mean ± Standard deviation (SD) Cytokines levels** | | | | |
| **TNF-α (pg/mL)** | **INF-γ (pg/mL)** | **IL1β (pg/mL)** | **IL6 (pg/mL)** | **IL10 (pg/mL)** |
| *A. lumbricoides* | Positivea | 38 | 10.81±8.87 | 37.38±81.27 | 11.78±2.56 | 11.84±3.35 | 60.80±23.26 |
| Negativeb | 774 | 9.55±4.29 | 28.03±36.99 | 12.61±3.68 | 13.46±9.69 | 71.58±76.05 |
| Total | 812 | 9.61±4.51 | 28.47±40.11 | 12.57±3.64 | 13.38±9.50 | 71.07±74.45 |
| Range |  | 1.53 – 46.19 | 4.92 – 509 | 5.89 – 51.84 | 3.28 – 92.55 | 13.53 – 995.52 |
| *p-value* |  | 0.100 | 0.161 | 0.170 | 0.307 | 0.384 |
| Hookworms | Positivea | 11 | 8.36±2.78 | 21.99±25.92 | 12.34±2.57 | 11.94±2.54 | 64.68±24.94 |
| Negativeb | 801 | 9.63±4.63 | 28.56±40.29 | 12.57±3.35 | 13.39±9.56 | 71.16±74.90 |
| Total | 812 | 9.61±4.61 | 28.47±40.12 | 12.57±3.64 | 13.38±9.50 | 71.07±74.45 |
| Range |  | 1.53 – 46.19 | 4.93 – 409.12 | 5.89 – 51.84 | 3.28 – 92.55 | 13.53 – 995 |
| *p-value* |  | 0.64 | 0.590 | 0.833 | 0.613 | 0.775 |
| *T. trichiura* | Positivea | 20 | 13.74±8.46 | 4.23±33.66 | 15.78±9.23 | 17.81±15.05 | 58.95±25.88 |
| Negativeb | 792 | 9.51±4.43 | 28.07±40.21 | 12.49±3.36 | 13.27±9.31 | 71.38±75.25 |
| Total | 812 | 9.61±4.61 | 28.47±40.11 | 12.57±3.64 | 13.38±9.50 | 71.07±74.45 |
| Range |  | 1.53 – 46.19 | 4.92 – 509.12 | 5.89 – 51.84 | 3.28 – 92.55 | 13.53 – 995.52 |
| *p-value* |  | *0.000* | 0.075 | *0.000* | *0.035* | 0.461 |
| *S. mansoni* | Positivea | 18 | 11.78±9.48 | 26.61±32.37 | 14.34±3.61 | 16.27±10.12 | 68.68±22.01 |
| Negativeb | 794 | 9.56±4.44 | 28.44±40.29 | 12.54±3.63 | 13.31±9.48 | 71.13±75.23 |
| Total | 812 | 9.61±4.61 | 28.47±40.12 | 12.57±3.64 | 13.38±9.50 | 71.01±74.45 |
| Range |  | 1.52 – 46.20 | 4.93 – 509.12 | 5.89 – 51.84 | 9.50 – 3.28 | 13.53 – 995.52 |
| *p-value* |  | *0.044* | 0.903 | *0.037* | 0.192 | 0.890 |
| *S. intercalatum* | Positivea | 10 | 8.71±3.02 | 32.47±30.29 | 15.83±9.52 | 16.96±13.35 | 59.53±18.84 |
| Negativeb | 802 | 9.62±4.63 | 28.42±40.24 | 12.53±3.50 | 13.34±9.45 | 71.22±74.87 |
| Total | 812 | 9.61±4.61 | 28.47±40.12 | 12.57±3.64 | 13.38±9.50 | 71.07±74.45 |
| Range |  | 1.52 – 46.19 | 4.93 – 509.12 | 5.89 – 51.84 | 3.28 – 92.55 | 13.53 – 995 |
| *p-value* |  | 0.534 | 0.752 | *0.004* | 0.231 | 0.622 |
| Significancea | | | F=2.310  p=*0.037* | F=1.510  p=0.179 | F=3.186  p=*0.006* | F=2.070  p=0.060 | F=0.496  p=0.811 |
| Significanceab | | | F=45.513  *p=0.000* | F=41.007  *p=0.000* | F=42.966  *p=0.000* | F=3.577  p=0.059 | F=0.459  p=0.498 |
| Note:  \*Student t-test used to assessment meant difference in means at 95% C.I with equal variances in mean assumed (2-tailed)  \*Statistical significance set a p < 0.05 | | | | | | | |

***Plasmodium*/ Intestinal Helminths co-infection and cytokines variations**

Results from Table 6 shows that participants infected with *Plasmodium* spp.alone had elevated levels of TNF-**α** (12.69 ± 4.51), IFN-**γ** (57.14 ± 60.89), IL-1**β** (15.04 ± 5.56), IL-6 (14.65 ± 4.19), and IL-10 (91.37 ± 149.34) pg/mL compared to the un-infected participants that served as the control group while participants infected with Intestinal Helminths alone had an elevated value of IFN-**γ** (31.22 ± 69.97pg/mL) only. The co-infection of *Plasmodium*/STHs had elevated values of TNF-**α** (13.56 ± 4.99), IFN-**γ** (62.97 ± 25.10), IL-1**β** (17.31 ± 11.02), and IL-6 (16.31 ± 4.82) pg/mL compared to the control group, these values were higher than those infected with *Plasmodium* alone even though the mean value of IL-6 was statistically not different from those of the un-infected group (13.22 ± 9.95). The one-way ANOVA used to evaluate the impact between groups shows that, infected participants with *Plasmodium* alone, intestinal helminths alone, and co-infections had an extremely significant influence on TNF-**α** (F=16.799, p=0.000), IFN-**γ** (F=19.251, p=0.000), and IL-1**β** (F= 22.913, p= 0.000). Also, the Tukey HSD used for multiple comparisons within groups shows that, co-infected participants had a significant impact on TNF-**α** (p = 0.002), IFN-**γ** (p = 0.001), and IL-1**β** (p=0.000) against the non-infected participants (Table 6).

**Table 6: *Plasmodium*/Intestinal Helminthic co-infection and cytokines variations**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Infection status** | **Cases** | **Mean ± Standard deviations of cytokines levels** | | | | |
| **TNF-α (pg/mL)** | **INF-γ (pg/mL)** | **IL-1β (pg/mL)** | **IL-6 (pg/mL)** | **IL-10 (pg/mL)** |
| *Plasmodium* onlya | 72 | 12.69 ± 4.51 | 57.14 ± 60.89 | 15.04 ± 5.56 | 14.65 ± 4.19 | 91.37 ± 149.34 |
| STH onlyb | 53 | 9.45 ± 6.09 | 31.22 ± 69.97 | 11.94 ± 2.16 | 12.94 ± 9.65 | 59.23 ± 22.64 |
| Co-infectionc | 14 | 13.56 ± 4.99 | 62.97 ± 25.10 | 17.31 ± 11.02 | 16.31 ± 4.82 | 63.23 ±28.87 |
| No infectiond | 673 | 9.61 ± 4.61 | 24.47 ± 32.09 | 12.57 ± 3.64 | 13.22 ± 9.95 | 71.07 ±74.45 |
| ANOVA (one way) – between groups | | F=16.799  **p=0.000** | F=19.251  **p=0.000** | F= 22.913  **p= 0.000** | F= 0.978  p= 0.403 | F= 2.340  p= 0.072 |
| Tukey HSD multiple comparison within groups  **a = infected with *Plasmodium* spp only, b = infecte with STHs only, c = co-infections and d = no infection (control)** | | **p=0.000**ab , p=0.910ac, **p=0.000ad**, **p=0.012bc**, p=0.982bd, **p=0.002cd** | **p=0.001**ab , p=0.956ac, **p=0.000ad**, **p=0.034bc**, p=0.615bd, **p=0.001cd** | **p=0.000ab**, p=0.120ac, **p=0.000ad**, **p=0.000bc**, p=0.922bd, **p=0.000cd** | p=0.753ab , p=0.934ac, p=0.614ad, p=0.641bc, p=0.997bd, p=0.624cd | p=0.080ab , p=0.567ac, p=0.094ad, p=0.998bc, p=0.740bd, p=0.987cd |

**Discussion**

In developing countries, notably in Sub-Saharan Africa, where a higher prevalence of parasitic diseases has been observed, parasitic illnesses especially intestinal parasites and malaria remain a serious public health concern [34]. People of all ages are susceptible to infection, although those living with HIV, pregnant women, and children under five years continue to have a higher burden of intestinal parasitic infections and malaria. [35, 36]. This study was a community-based cross-sectional study carried out among the Armed Forces of the North West and South West of Cameroon to determine the impact of Malaria and Soil-Transmitted Helminths on inflammatory cytokines.

In this study*, Plasmodium falciparum* malaria-infected participants expressed significantly higher levels of TNF-α, IFN-γ, and IL-1β (*p=0.000*) as compared to health controls. This is in agreement with some studies observed by Doolan *et* *al*., [37], and Bwanika *et al*., [38] that *Plasmodium falciparum* malaria expressed higher IFN-γ levels. These increases in IFN-γ levels have been linked to the cytokine’s protective function against *P*. *falciparum* malaria [39, 37]. It has also been suggested that IFN-γ can activate macrophages and monocytes in the blood stage of malaria parasites which is responsible for their quick death by reactive oxygen and nitrogen intermediates [40]. Further evidence linking elevated IFN-γ production to improved disease outcomes has been found in animal models of malaria [41]. Also, malaria infection increased serum IL-6 and IL-10, though not significant, this could be because IL-6 is a candidate marker for severe malaria [42, 43]. This study was carried out on asymptomatic community malaria cases. The mean TNF α level was significantly higher in participants infected with *Plasmodium* spp. (*P. falciparum*:12.74±4.43 pg/mL, *p = 0.000*; and *P*. *vivax*: 12.68±5.34pg/mL *p = 0.012*) compared to those not infected (9.19±4.43 pg/mL and 9.56±4.58 pg/mL respectively). This is contrary to studies reported by Hartgers *et al*., [44] and Bwanika *et al*., [38] that TNF-α showed no significant difference. Since TNF-α acts systemically and has been shown to have a pro-inflammatory cytokine role during the acute phase stress response [45], our study population is under constant stress and carries out a lot of physical activities which is the reason behind the increase and insignificant results obtained in this study. Additionally, TNF-α has two focal effects: immunomodulation and increased inflammation, both of which may be harmful to host survival as reported by Venkatraman and Pendergast, [46]. Also, the mean INF-γ concentration was statistically significantly higher in positive participants with *Plasmodium falciparum* (57.64±53.73pg/mL, *p=0.000*) and *P*. *vivax* (54.00±27.25pg/mL, *p=0.016*) compared the negative participants (24.51±36.18 pg/mL) and (28.02±40.17pg/mL) respectively. In addition, the Interleukin 1 beta (IL1β) concentration mean level was significantly positive and higher in malaria-*falciparum* participants (15.66±7.07pg/mL, *p=0000*) as for the non-malaria *P. falciparum* participants contrary the *P*. *vivax* infected participants that was higher but insignificant (72.71±23.72 pg/mL, *p= 0.934*). this finding was in agreement with that conducted by Ntonifor *et al*., [10]. The mean Serum levels of IL-10 and IFN-γ were markedly elevated in participants with malaria. This was in line with that conducted in Bangkok, Thailand [47]. These findings highlight the multifactorial network between host and parasite in malaria immunology and imply that stimulatory and inhibitory cytokines for macrophage activation and/or antibody production (i.e., TH1- and TH2-type immunoreaction, respectively) are co-expressed during acute *P*. *falciparum* infection.

This study reports that, participants infected with *T. trichiura* had significantly higher mean levels of TNFα (13.74±8.46pg/mL, *p = 0.000*), IL1β (15.78±9.23pg/mL, *p = 0.000*) and IL 6 (17.81±15.05pg/mL, *p = 0.035*) compared to non-infected participants. This was in concordance with that reported by Juliana *et al*., [48]. Although the mean TNFα (10.81±8.87pg/mL, *p = 0.100*) and Interferon gamma (37.38±81.27, *p = 0.161*) concentrations were higher in cases infected to *A. lumbricoides* against the non-infected cases, the results were insignificant. This is in agreement with that reported by Shalaby and Shalaby [49], there was no impairment of TNF-α expression in the infected children which may explain a mixed Th1/Th2 response. It has been documented the critical role of TNF-α is in regulation of Th2 cytokines [50]. This result was different from the observation of Nmorsi *et* *al* [51] who revealed a significantly high TNF-α concentration among their investigated subjects. This was equally contrary to that reported by Mugob *et al*., [22]. Also, the mean TNFα (11.78±9.48pg/mL, *p =* *0.044*) concentration was statistically significantly higher among the infected participants to *S. mansoni*. This is identic to that reported by Booth *et al*. [52]. The mean IL1β concentration level (14.34±3.61, *p = 0.037*) was higher and significant in the infected participants with *S. mansoni*. This was contrary to that reported by Ntonifor *et* al., [10]. Equally a statistically higher and significant value was recorded for the mean concentration of IL1β (15.83±9.52, *p = 0.004*) in participants positive for *S. intercalatum*. Even though the mean IL6 concentration is higher in participants infected with *S. mansoni* (16.27±10.12pg/mL) it was insignificant. This finding was in concordance with that reported by Mutengo *et al*., [53]. This increase in level could suggest that IL-6 modulates *S. mansoni*-related immune responses.

*Plasmodium falciparum* malaria and helminth-infected individuals both expressed higher levels of IL-10 though those infected with *plasmodium spp*. were greateras compared to healthy individuals. This was contrary to that reported by Bwanika *et al*., [38].This is probably because IL-10 is a key anti-inflammatory cytokine protective against helminth infections that cause inflammation [54].

Analysing the variation in cytokines response to Malaria and soil-transmitted helminthic parasites in infected and non-infected participants, the significantly elevated levels of TNF-α, IFN-γ, and IL-1β and non-significantly elevated levels of IL-6 and IL-10 could be attributed to many other factors other than a balanced immune response. This population of the study is constantly doing physical exercise, and exercise-induced stress response, which includes an increase in TNF-α, has been demonstrated to be significantly influenced by inflammatory cytokines [55, 56]. The participants are equally under stress, sustained stress triggers a series of changes in the brain and the body [57]. At the early stage of stress, the activated hypothalamus-pituitary-adrenal axis and sympathetic nervous system axis can upregulate the levels of glucocorticoid and catecholamines, respectively, and then they, in turn, inhibit the secretion of proinflammatory cytokines directly or indirectly while promoting the secretion of anti-inflammatory cytokines. [58].

**Conclusion**

Malaria and intestinal helminthic infections are still public health concern in Cameroon. These parasites have an impact on some cytokines. Analysing the variation of cytokines response to Malaria and intestinal helminthic parasites in infected and non-infected participants, the significantly elevated levels of these inflammatory could be attributed to many other factors other than a balanced immune response such as physical exercise, stress, trauma, fear etc. Pro-inflammatory cytokines play pivotal roles in initiating and amplifying the immune response against parasites. These cytokines are essential for inhibiting parasite development, stimulating parasite clearance and interacting with macrophages, which amongst other processes control parasitic infections through antibody-dependent and independent phagocytosis

The pivotal role of cytokines in immunomodulation during malaria and intestinal parasitic clearance therefore underscores their significance as potential targets for therapeutic interventions, offering promising prospects in the global fight against these infectious diseases.

Hence it is important for this population of study to be predisposed to good conditions of service.

**Ethical Approval and Consent:**

The study was approved by the University of Bamenda's Faculty of Health Sciences' Ethical Review Committee (2022/0786H/Uba/IRB) and administratively authorized by the Minister of Defence (06068/DV/MINDEF/024/4), the North West and South West Regional Delegations of Public Health (R11/MPH/SWR/RDPH/PS/680/520). Participants were informed about the study's aim and purpose through an information sheet written in English and French. Blood and stool samples were collected after participants gave written informed consent, the results were communicated to the participants, and those who tested positive were referred to medical professionals for appropriate management and treatment. Additionally, participants received education on self-protection against intestinal helminths and malaria as well as preventive measures. All procedures were respected following guidelines. In addition, all methods were performed in accordance with the relevant guidelines and regulations of the ethical review board.

**Disclaimer (Artificial intelligence)**

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

**References**

1. WHO (2006). Constitution of the World Health Organization–Basic documents
2. WHO (2018). World Malaria Report 2018. Cameroon, African Region. Accessed 04 Mar 2020.
3. Brooker S, Akhwale W, Pullan R, Estambale B, Clarke SE, Snow RW (2007). Epidemiology of Plasmodium- helminth co-infection in Africa: populations at risk, potential impact on anaemia and prospects for combining control. *Am J Trop Med Hyg*.; **77** (6S):88–98.
4. Bangert M (2017). The cross-cutting contribution of the end of neglected tropical diseases to the sustainable development goals. *Infectious Diseases of Poverty*. **6**(1):73.
5. Bethony J, Simon B, Marco A, Stefan M G, Alex L, David D, Peter JH (2006). Soil-transmitted helminth infections: *ascariasis*, *trichuriasis*, and hookworm*.* *Lancet*. **367**(9521):1521-32.
6. Karan A, Chapman, GB, Galvani A. 2012. The influence of poverty and culture on the transmission of parasitic infections in rural Nicaraguan Villages. *J Parasitol Res*. doi.org/10.1155/2012/478292.
7. Nkuo-Akenji TK, Chi PC, Cho JF, Ndamukong KK, Sumbele I (2006). Malaria and helminth co-infection in children living in a malaria endemic setting of Mount Cameroon and predictors of anemia. *J Parasitol*. **92**:1191-1195.
8. Messina JP, Taylor SM, Meshnick SR, Linke AM, Tshefu AK (2011). Population, behavioural and environmental drivers of malaria prevalence in the Democratic Republic of Congo. *Malar J*. **10** :161.
9. Sumbele IUN, Nkemnji GB, and Kimbi HK, (2017). Soil-transmitted helminths and Plasmodium falciparum Malaria among individuals living in different Agroecosystems in two Rural Communities in the Mount Cameroon Area: a Cross-Sectional Study. *Infectious Diseases of Poverty*. **6** (67):28302147.
10. Ntonifor HN, Chewa JS, Oumar M, Mbouobda HD (2021). Intestinal helminths as predictors of some malaria clinical outcomes and IL-1β levels in outpatients attending two public hospitals in Bamenda, North West Cameroon. *PLoS Negl Trop Dis*. **15**(3): 0009174.
11. Vilay P, Nonaka D, Senamonty P. *et al.* Malaria prevalence, knowledge, perception, preventive and treatment behavior among military in Champasak and Attapeu provinces, Lao PDR: a mixed methods study. *Trop Med Health* **47**, 11 (2019). https://doi.org/10.1186/s41182-019-0138-9
12. Mawson AR. 2013. The pathogenesis of malaria: a new perspective. *Pathog Glob Health*.;**107**(3):122-9. doi: 10.1179/2047773213Y.0000000084. PMID: 23683366; PMCID: PMC4003589 (2013).
13. Perkins D J, Were T, Davenport GC, Kempaiah P, Hittner JB, Ong'echa JM (2011). Severe malarial anemia: innate immunity and pathogenesis. *Int J Biol Sci*.;**7**(9):1427-42. doi: 10.7150/ijbs.7.1427. PMID: 22110393; PMCID: PMC3221949.
14. Wyler DJ. 1983. The spleen in malaria. In: Evered D, Whelan J, editors. Ciba Foundation symposium on malaria and the red cell. London: Pitman; pp. 98–116. [DOI] [PubMed] [Google Scholar]
15. Dinarello CA, Simon A, van der Meer JW (2012). Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nat Rev Drug Discov*.;**11**(8):633-52. doi: 10.1038/nrd3800. PMID: 22850787; PMCID: PMC3644509.
16. Lakkavaram A, Lundie RJ, Do H, Ward AC, de Koning-Ward TF (2020). Acute Plasmodium berghei Mouse Infection Elicits Perturbed Erythropoiesis With Features That Overlap With Anemia of Chronic Disease. Front. *Microbiol*. **11**:702. doi: 10.3389/fmicb.2020.00702.
17. Naka T, Nishimoto N, Kishimoto T (2002). The paradigm of IL-6: from basic science to medicine. *Arthritis Res Ther* **4** (Suppl 3), S233. <https://doi.org/10.1186/ar565>.
18. Oyegue-Liabagui SL, Bouopda-Tuedom AG, Kouna LC, Maghendji-Nzondo S, Nzoughe H, Tchitoula-Makaya N, Pegha-Moukandja I, Lekana-Douki JB (2017). Pro- and anti-inflammatory cytokines in children with malaria in Franceville, Gabon. *Am J Clin Exp Immunol.*;**6**(2):9-20. PMID: 28337387; PMCID: PMC5344990.
19. Letouzey R (1980). Phytogeographic map of Cameron. In: JA, editor. Les Atlas Jeune Afrique : Paris, France ; 20-24.
20. Mojoko FM (2011). Urban Development in Fako Division (Unpublished M.Sc. Thesis). Department of Geography, University of Buea .
21. Council Development Plan. TIKO (2011). - communes et Villes unies du Cameroun, cvuc.cm/national/index.php/fr/carte-communale/region-du-sud/142.../575-Tiko.
22. Mugob BB, Ngum NH, Boubga C, Ngwenah EF, Mahamat O (2024). Analysis of oxidative status, inflammatory cytokines, and Ascaris lumbricoides infection in women at a health district in Bamenda, Northwest, Cameroon. *Egypt J Intern Med* **36**, 42. <https://doi.org/10.1186/s43162-024-00306-8>.
23. Pourhoseingholi MA, MohsenV, Mitra R (2013). Sample size calculation in medical studies. *Gastroenterol Hepatol Bed Benchv*.**6**(1); Winter PMC4017493.
24. Cochran GW (1977). Sampling techniques. 3rd Edition. Wiley, New York. ISBN: 047116240X .
25. Katz N, Chaves A, Pellegrino J (1972). A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. *Rev Inst Med Trop Sao Paulo*. **14**: 397–400.
26. WHO (2019). Bench aids for the diagnosis of intestinal parasites, second edition. Geneva (2019).
27. Cheesbrough M (2009) District Laboratory Practice in Tropical Countries, 3Rd ed., part 1. United Kingdom: *Cambridge University Press*; p. 178–215.
28. WHO (2010) Basic Malaria Microscopy: World Health Organization.
29. Achidi EA, Apinjoh TO, Yafi CN, Besingi R, Anchang-Kimbi JK, Awah NW, Troye-Blomberg M (2013). Plasma Levels of Tumour Necrosis Factor-Alpha, Interleukin-10, Interleukin-12, Macrophage Inhibition Factor and Transforming Growth Factor-Beta in Children with Severe and Uncomplicated Falciparum Malaria. *J Trop Dis* **1**: 103.
30. Monastero RN, Pentyala S (2017). Cytokines as Biomarkers and Their Respective Clinical Cutoff Levels. *Int J Inflam*.:4309485. doi: 10.1155/2017/4309485. PMID: 28487810; PMCID: PMC5401738 .
31. Lyke KE, Burges R, Cissoko Y, Sangare L, Dao M, Diarra I, Kone A, Harley R, Plowe C V, Doumbo, OK, Sztein MB (2004). Serum levels of the proinflammatory cytokines interleukin-1 beta (IL-1beta), IL-6, IL-8, IL-10, tumor necrosis factor alpha, and IL-12(p70) in Malian children with severe Plasmodium falciparum malaria and matched uncomplicated malaria or healthy controls. *Infect Immun*. ;**72**(10):5630-7. doi: 10.1128/IAI.72.10.5630-5637.2004. PMID: 15385460; PMCID: PMC517593.
32. John DT, Petri WA, Markell EK, Voge M. 2006. Markell and Voge's *Med Parasitol*, Elsevier Health Sciences.
33. Montresor A, Crompton DWT, Hall A, Bundy DAP, Savioli L (1998). Guidelines for the evaluation of soil-transmitted helminthiasis and schistosomiasis at community level : WHO/CTD/SIP/98.1.Vol,**1** <https://iris.who.int/handle/10665/63821> ‎.
34. UNAIDS (2018). *Undetectable = untransmittable: public health and HIV viral load suppression*, Joint United Nations Programme on HIV/AIDS (UNAIDS). [Geneva]: <https://coilink.org/20.500.12592/69p8ff1>. [COI: 20.500.12592/69p8ff1](https://coilink.org/20.500.12592/69p8ff1).
35. Imboumy-Limoukou RK, Maghendji-Nzondo S, Sir-Ondo-Enguier PN, Niemczura De Carvalho J, Tsafack-Tegomo NP, Buekens J, Okouga AP, Mouinga-Ondeme A, Kwedy Nolna S, Lekana-Douki JB (2020). Malaria in children and women of childbearing age: infection prevalence, knowledge and use of malaria prevention tools in the province of Nyanga, Gabon. *Malar J*.;**19**(1):387. doi: 10.1186/s12936-020-03411-5. PMID: 33138819; PMCID: PMC7607695.
36. Mekachie SS, Sumbele IUN, Tasah MM, Kimbi HK (2021). Malaria and intestinal parasite co-infection and its association with anaemia among people living with HIV in Buea, Southwest Cameroon: A community-based retrospective cohort study. *PLoS One*. 22;**16**(1).
37. Doolan DL, Dobaño C, Baird JK (2009). Acquired immunity to malaria. *Clin Microbiol*.22:13–36.
38. Bwanika R, Kato CD, Welishe J (2018). Cytokine profiles among patients co-infected with *Plasmodium falciparum* malaria and soil borne helminths attending Kampala International University Teaching Hospital, in Uganda. *Allergy Asthma Clin Immunol*. **14**:10.
39. Cheesebrough M (2002). District Laboratory Practice in Tropical Countries 1 & 2. Cambridge University Press, Cambridge, 614-661.
40. Taylor-Robinson AW, Looker M (2013). Sensitivity of malaria parasites to nitric oxide at low oxygen tensions. *Lancet*. **351**:1630.
41. Riley EM, Wahl S, Perkins DJ, Schofield L (2006). Regulating immunity to malaria. *Parasite* *immunology*; **28**: 1-2.doi.org/10.1111/j.1365-3024.2006. 00775.x.
42. Wilairatana P, Masangkay FR, Kotepui KU, Milanez GDJ, Kotepui M (2022). Prevalence and risk of Plasmodium vivax infection among Duffy-negative individuals: a systematic review and meta-analysis. *Sci Rep* 12, 3998. <https://doi.org/10.1038/s41598-022-07711-5>.
43. Hamilton F, Mitchell RE, Constantinescu A, Hughes D, Cunnington A, Ghazal P, Timpson NJ (2023). The effect of interleukin-6 signaling on severe malaria: A Mendelian randomization analysis. *Int J Infect Dis*.; **129**:251-259. doi: 10.1016/j.ijid.2023.02.008.
44. Hartgers FC, Obeng BB, Kruize YC, Dijkhuis A, McCall M, Sauerwein RW, Luty AJ, Boakye DA, Yazdanbakhsh M (2009). Responses to malarial antigens are altered in helminth-infected children. *J Infect Dis*. **78**:760–9.
45. Moldoveanu AI, Shephard RJ, Shek PN (2000). Exercise elevates plasma levels but not gene expression of IL-1beta, IL-6, and TNF-alpha in blood mononuclear cells. *J Appl Physiol*. **89**(4): 1499–504.
46. Venkatraman JT, Pendergast D (1998). Effects of the level of dietary fat intake and endurance exercise on plasma cytokines in runners. *Med Sci Sports Exerc*. **30**(8): 1198–204.
47. Wenisch C, Parschalk B, Narzt E, Looareesuwan S, Graninger, WenischC (1995).Elevated serum levels of IL-10 and IFN-gamma in patients with acute *Plasmodium falciparum* malaria *Clin Immunol Immunopathol*.**74**(1) :115-117.
48. Juliana PG, Cassia GON, Wheverton RCN, Virginia MBL, Décio MP, Vlaudia MAC, Constança SB, Dirceu S, Emanuel SCS, Valdênia MOS (2020).Cytokine production in allergic and Trichuris trichiura-infected children from an urban region of the Brazilian northeast, *Parasitology International,* **74:** 101918, ISSN 1383-5769, doi.org/10.1016/j.parint.2019.04.015.
49. Shalaby NM, Shalaby NM (2016). Effect of Ascaris lumbricoides infection on T helper cell type 2 in rural Egyptian children. *Ther Clin Risk Manag*.**12**:379-85. doi: 10.2147/TCRM.S94019. PMID: 27022269; PMCID: PMC4790525.
50. Artis D, Humphreys NE, Bancroft AJ, Rothwell NJ, Potten CS, Grencis RK (1999). Tumor necrosis factor alpha is a critical component of interleukin 13-mediated protective T helper cell type 2 responses during helminth infection *J. Exp. Med*., **190**, pp. 953-962.
51. Nmorsi OPG, Irior DO, Abu M (2010). Serum cytokines profiles in Nigerian children with Ascaris lumbricoides infection, *Asian Pacific Journal of Tropical Medicine,* **3,** Issue 4, Pages 288-291, ISSN 1995-7645, doi.org/10.1016/S1995-7645(10)60070-3 .
52. Booth M, Vennervald BJ, Butterworth AE, Kariuki H C, Amaganga C, Kimani G, Mwatha JK, Otedo A, Ouma JH, Dunne DW (2004). Exposure to malaria affects the regression of hepatosplenomegaly after treatment for Schistosoma mansoni infection in Kenyan children. *BMC Med*. **2**:36. doi: 10.1186/1741-7015-2-36. PMID: 15450118; PMCID: PMC522803.
53. Mutengo MM, Mduluza T, KellyP, Mwansa JCL, Kwenda G, Musonda P, Chipeta J (2018). Low IL-6, IL-10, and TNF-α and High IL-13 Cytokine Levels Are Associated with Severe Hepatic Fibrosis in Schistosoma mansoni Chronically Exposed Individuals. *J Parasitol Res.*: doi: 10.1155/2018/9754060. PMID: 29610679; PMCID: PMC5828471.
54. O'Garra A, Vieira P (2004). Regulatory T cells and mechanisms of immune system control. *Nat Med*.; **10**(8):801-5. doi: 10.1038/nm0804-801. PMID: 15286781.
55. Bernecker C, Scherr J, Schinner S, Braun S, Scherbaum WA, Halle M (2013). Evidence for an exercise induced increase of TNF-α and IL-6 in marathon runners*. Scand J Med Sci Sports* **23**(2): 207–14.
56. Peake JM, Della GP, Suzuki K, Nieman DC (2015). Cytokine expression and secretion by skeletal muscle cells: regulatory mechanisms and exercise effects. *Exerc Immunol* **21** : 8–25.
57. Tong RL, Kahn UN, Grafe LA, Hitti FL, Fried NT, Corbett BF (2023). Stress circuitry: mechanisms behind nervous and immune system communication that influence behavior. Front. Psychiatry. **14**:1240783. doi: 10.3389/fpsyt.2023.1240783.
58. Tian LG, Wang TP, Lv S, Wang FF, Guo J, Yin XM (2014). HIV and intestinal parasite co-infections among a Chinese population: an immunological prole. *IDP*.; **2**:18.