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Analysis of oxidative status, inflammatory cytokines, and *Ascaris lumbricoides* infection in women at a health district in Bamenda, Northwest, Cameroon

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Abstract

Background Clinical outcomes of ascariasis, one of the most common parasitic infections, are remarkably variable ranging from asymptomatic infection to death. Ascariasis can pair absorption of fats, vitamin A, iodine, and lactose digestion and destroys the villi, with significant consequences in pregnancy outcomes, leading to growth retardation, and cognitive impairment, decreased work capacity, and adverse pregnancy outcomes. One of the crucial factors driving the clinical outcomes of ascariasis is the immune response and associated oxidative stress. This study therefore examined the prevalence of ascariasis and associated immune response dysfunction by measuring four inflammatory cytokines alongside with the oxidant and antioxidant biomarkers in women of reproductive age in a health center in Cameroon.

Results Three-hundred and fifty-five women (pregnant and non-pregnant) were examined for the parasite. Because some participants did not donate blood and due to low volume of blood available, only 127 individuals (62 non-pregnant women and 65 pregnant women) were included for analysis of oxidative biomarkers, and 90 samples were used for the evaluation of inflammatory biomarkers (40 non-pregnant and 50 pregnant). The prevalence of *Ascaris lumbricoides* was of 13.23%. Ascariasis was associated with high levels in inflammatory cytokines (IL-1β, IL-12, IL-10, and TNF-α) and oxidative markers (TOS, OSI, MDA, and CAT) in both pregnant and non-pregnant women, while high level of NO was only seen in pregnant women. A significant relation was observed between some cytokines and oxidant markers: IL-10 and OSI and IL-12 and NO and between IL-1β and MDA in pregnant women, while in non-pregnant, significant relation was found between Il-10 and NO as well as IL-1β and OSI and NO. Principal component analysis (PCA) underlined a pro-inflammatory cytokine signature (with strongest contributions from IL-1β, IL-10, TNF-α). PCA also highlighted an oxidative stress with strongest contributions from TOS, OSI, SOD, NO, and CAT in pregnant and from OSI, TOS, NO, CAT, and TAC in non-pregnant.

Conclusion These findings demonstrate elevated cytokines (IL-1β, IL-12, IL-10, and TNF-α) and high oxidative stress imbalance, adding further evidence for the role of a pro-inflammatory cytokine signature of oxidative stress in women with *A. lumbricoides*.

Keywords Women, *A. lumbricoides*, Inflammation, Cytokine, Oxidant, Antioxidant

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Background

Intestinal parasitic infections (IPIs) affecting humans are still a major public health burden worldwide, responsible for a major problem in countries with poor hygienic conditions [1]. They constitute a global health burden causing clinical morbidity in 450 million people. Many of these are women of reproductive age and children in developing countries [2]. IPIs are more common in underdeveloped countries, as a result of lack of proper sanitation, poor hygienic conditions, and lack of appropriate health education [3]. IPIs are known to be one of the major causes of digestive disorders, especially among high-risk groups, namely, children, pregnant women, and immunocompromised patients [4]. In pregnancy, IPIs are associated with serious adverse outcomes, both for the mother and the unborn baby. Ascariasis, one of the common IPIs, can cause a myriad of symptoms ranging from asymptomatic disease to death. It impairs absorption of fats, vitamin A, iodine, and lactose digestion and destroys the villi [5], with significant consequences in public health, leading to growth retardation, and cognitive impairment, decreased work capacity, and adverse pregnancy outcomes [6, 7]. Manifestations will depend on the stage of the larvae, the load of the parasite, and the age and the immune system of the affected individual.

Ascariasis or parasitic infections are thought to increase oxidative stress, through the release of reactive oxygen species (ROS) in patients [8]. ROS as well as other substances are produced internally as by-products of basal metabolism and nonspecific physiological responses [9, 10]. In the development of an infection, infectious agents develop inflammatory responses during which some cells of the immune system produce cytotoxic compounds that act like ROS to attack the pathogen but that can also harm the host [11, 12]. Inflammatory responses can be costly, but individuals unable to produce them are more likely to suffer an increased infection-induced mortality [12]. Among other parasites, intestinal parasites (IPs) may have harmful effects on the physiological condition and on the life-history traits of women. Both clinical and experimental studies have confirmed that parasites are an important source of excessive ROS production [13] with negative impacts on health [14], reproductive success [15], or hormone modulation [16]. In pregnant women, it can result in abortion, recurrent pregnancy loss (RPL), preeclampsia, and intrauterine growth restriction (IUGR) [17]. The capacity of women to modulate the physiological responses to fight off the infections and the potential costs of these responses may be dependent on the intrinsic factors of the individual, notably pregnancy. But, up to date, the results obtained in the different studies regarding the relationship between oxidative stress and IPs infection are contradictory [18]. Despite these controversies, reports presenting the differences in oxidative stress and antioxidant levels among women with IPs are lacking. The shortage of information on this issue of great epidemiological importance fashioned the background against which this study was conducted in Bambili, Cameroon. In this study, it was hypothesized that *A. lumbricoides* is present, and it is associated with an increase in inflammatory cytokines and oxidative stress markers in women of reproductive age. To test this hypothesis, the aim of this study was to determine the level of oxidative stress biomarkers and some inflammatory cytokines in women of reproductive age both pregnant and non-pregnant women with *A. lumbricoides*.

Methods

Ethical consideration

Ethical clearance was obtained from the Ethical Review Board of the Faculty of Health Sciences, University of Bamenda (ref. no. 2022/0701H/UBa/IRB). Administrative clearance was obtained from the Regional Delegation of Public Health, Northwest Region Bamenda, Cameroon, as well as from the district hospital, Bambui. The purpose of the study was made known to every participant, stating the need to participate.

Study area

Women were sampled in Tubah subdivision, Northwest Region, Cameroon at Bambili. The village is situated some 10 km east of Bamenda, and its geographical coordinates are latitude 5° 59′ 0″ N and longitude 10° 15′ 0″ E with an elevation of 1350 m. Tubah is characterized by two seasons: rainy season from March to October with heavy rain falls and temperatures which are relatively warm. The dry season begins from November to February with strong sunshine during the day and very cold nights. Agriculture is the main occupation of the people in Tubah and to an extent animal husbandry [19]. Bambili is divided into 28 quarters, grouped into 6 health zones

Criteria for selection and study population

This study is cross-sectional, involving patients presenting themselves at the hospital for different purposes. Only apparently healthy women of 12 to 45 years (pregnant and non-pregnant women) were approached for this study. Participants were submitted to the complete infectious state screening. Patients with health issues such as diabetes, HIV, hypertension, and kidney problem were excluded from this study. Also, patients currently taking antihelminthic/antiprotozoan drugs (metronidazole, albendazole, etc.) or in the last 3 weeks as well as those positive for any bacterial, fungal, or viral infections were excluded (all participants who were newly diagnosed of

malaria and bacterial or fungal infections). Thus, a total of 355 patients were randomly selected.

The sample size was determined using the Cochran's formula for sample size determination reported by Naing et al. [20] for the calculation. The 95% confidence level (standard value of 1.96) and 5% sampling error (standard value of 0.05) were considered.

$$n = Z^2 p(1-p)/d^2$$

where n is the projected sample size; Z is the statistic for level of confidence = (1-α); p is the priori estimate of the prevalence, and the overall prevalence rate of intestinal parasites of 34.5% obtained in Bamenda by Bissong et al. [21] was used; and d is the sampling error.

By applying the above formula, the sample size was calculated as presented below:

$$n = (1.96)^2 \times 0.345(1-0.345)/(0.05)^2$$

Therefore, $n = 347$

A total of 355 patients were randomly chosen irrespective of age, social class, marital status, and cultural or religious affiliation

Stool sampling and parasitological analysis

Fresh stool specimens were obtained from women by providing them with stool bottles. Each study participant was requested to bring about 3–4 g of stool. All stool specimens collected were subjected different parasitological tests depending on the nature of the stool. Solid stool was subjected to formol-ether concentration technique [22], while watery diarrheic stool was subjected to acid fast staining (to detect the presence of *Cryptosporidium parvum*) and also direct wet mount (for other intestinal protozoans).

In direct wet mount analysis, stool specimens were first observed macroscopically for the presence of adult helminth worms. Later, a drop of normal saline was placed on a slide, and a drop of lugol was placed on same slide at another edge. A small amount of stool was picked using a wooden applicator stick and mixed with the drop of saline water and lugol. The slide was covered with a coverslip and read under a microscope with ×10 objectives and then at ×40 [23].

For the formol-ether concentration technique, 1 g of stool specimen was placed in a centrifuge tube containing 7 ml of 10% formalin. The sample was suspended and mixed thoroughly with an applicator stick. The resulting suspension was filtered through a sieve (cotton gauze) into a beaker, and the filtrate was poured back into the same tube. Later, 3 ml of diethyl ether was added to the mixture, and the tube was closed and shaken vigorously. The mixture was centrifuged at 1500 rpm for 2 min. After centrifugation, the supernatant (layers of ether, debris, and formalin) was discarded, and the sediment containing the parasites at the bottom of the test tube was resuspended. The sediment obtained was again transferred to a slide using a pipette and examined microscopically under 10× and 40× objective lenses for the presence of intestinal parasites [23, 24]. The parasites were identified based on the features of decorticated egg, fertilized egg, and unfertilized egg as described by Soham and Gadadhar [25].

Blood collection and biochemical analyses

Physiological blood measurements may provide an overall assessment of the organism's oxidative status and its variability. Blood was collected from the patients into dry tubes (without anticoagulant) and kept at the room temperature for 1 h. The serum was then separated by centrifugation at 1000 rpm for 10 min and stored at −20 °C until analysis.

Oxidative status of participants was determined by the total oxidative stress (TOS) of their samples and the assessment of thiobarbituric acid reactive substances (TBARS) as MDA and also the assessment of nitric oxide (NO) levels. The breakdown of hydroperoxides was catalysed by iron to alkoxyl (RO) and peroxyl radicals oxidative characterizing the total oxidative status (TOS). To quantify it, TOS was measured (μmol H_2O_2 equiv./L) as they react with the chromogen (N, N-dimethyl-phenylenediamine sulfate) forming a colored compound measured at a wavelength of 505 nm. The intensity of the color correlates directly with the quantity of radical compounds, according to the Beer-Lambert's law, and it can be related to the oxidative status of the sample [26]. Thiobarbituric acid reactive substances were measured (nmol MDA/ml) as they react with MDA producing a red-pink product measured at a wavelength of 535 nm [27]. Nitric oxide was measured by determining the nitrite, the stable end product of nitric oxide, using the Griess reagent [28].

The antioxidant capacity of the patients was determined by measuring the total antioxidant capacity (TAC) of the samples and the activity of the two enzymes that take part in the redox system: the superoxide dismutase (SOD) and the catalase (CAT). TAC (μmol Trolox equiv./L) is estimated by measuring the reduction of the radical cation of the chromogen (N, N-dimethyl-phenylenediamine sulfate) develops in an acidic medium (pH = 5.2) and a suitable oxidant (FeCl3) by the antioxidant compounds of the sample, quenching the color and producing a discoloration of the solution measured at a wavelength of 505 nm [26]. The SOD (IU) was determined with the inhibition rate of the oxidation of adrenaline to adrenochrome as the source of peroxide radicals, which is proportional to the activity of SOD and absorbs

at a maximum wavelength of 480 nm [29]. The catalase (μmol/L) was quantified using the reduction of dichromate in acetic acid in the presence of H_2O_2 to chromic acetate and measured colorimetrically at 570 nm [30].

The oxidative stress and redox imbalance, OSI, were calculated as a ratio between TOS and TAC. The results were presented as a percentage ratio, based on the following formula: OSI = TOS [μmol/L]/TAC [μmol/L] × 100. Higher ratios in the samples are a sign of predominance of oxidation processes over antioxidant activity [31].

Analysis of immune markers using enzyme-linked immunosorbent assay

To investigate the effect of *A. lumbricoides* on the inflammatory immune response in women, four inflammation-related cytokines were assessed in serum. They included Th1-related (IL-12 and TNF-α), Th2-related (IL-10), and IL-1 family (IL-1β). Sandwich enzyme-linked immunosorbent assay (ELISA) kits (Quantikine, France) were used for the analysis. Assays were carried out as per the manufacturers' instructions, and plates were read using a microplate reader set to 450 nm.

Statistical analysis

The data are express as mean ± standard deviation. For the differences between oxidative and immune marker levels in infected patients and healthy controls, ANOVA test was used with a post hoc Student's test. To evaluate the suitability of the dataset for PCA, data was first scrutinized using the Kaiser-Meyer-Olkin measure and the Bartlett test of sphericity. A principal component analysis (PCA) was done using the correlation matrix to recapitulate the oxidative biomarkers and cytokines into two principal components (PC1 and PC2). Multiple linear regressions were used to assess the relationship between the four immune markers and all oxidative biomarkers. Backward stepwise selection procedure was used to show either the variable or the interaction with the highest *p*-values. A *p*-value of < 0.05 was considered statistically significant across all analysis. PCA and multiple linear regressions analysis were performed on STATAv17, while ANOVA and Student's test were performed using Prism 8 (GraphPad Software).

Results

Participant cohort

In this study, 355 individuals (pregnant and non-pregnant) living in the study area were recruited. All participants were examined for the infection, and 47 (13.23%) of them were found with the parasite. With respect to the age of the participants, 6 (1.7%) of them were less than 18 years out of which 1 (16.67%) was positive. There were 241 (67.9%) participants of 18 to 30 years, and 38 (15.77%) were positive. There were also 108 (30.4%) participants of 31 to 40 years among which 8 (7.41%) were positive. A total of 224 (63.1%) were non-pregnant women among which 25 (11.20%) were those with parasite, while 131 (36.9%) were pregnant women with 22 (16.8%) which were positive. There was no significant difference in the prevalence of the parasite within the age groups or following the pregnancy status (Table 1). But not all participants donated a blood sample. Thus, for serological analyses, 127 individuals (62 non-pregnant women and 65 pregnant women) were included for oxidative biomarkers ($n = 127$), but due to low volume of blood available for some individuals, 90 samples were used for the evaluation of inflammatory biomarkers ($n = 90$; 40 non-pregnant and 50 pregnant).

Effect of A. lumbricoides infection on oxidant and antioxidant biomarkers and associated major oxidative features in pregnant women

As shown in Fig. 1, PW with *A. lumbricoides* showed a significant ($p < 0.0001$) high level of total oxidative stress (TOS) values than parasites-free PW (Fig. 1E). There was difference in the mean values of total antioxidant capacity (TAC) of infected PW and parasite-free PW ($p = 0.35$; Fig. 1F), but the calculated OSI value obtained for the infected PW was higher than for the parasite-free PW group ($p = 0.0001$; Fig. 1G). MDA value (Fig. 1A) as well as NO value (Fig. 1B) for PW measured as oxidant biomarkers was significantly higher in infected than parasite-free groups ($p = 0.003$ and $p < 0.0001$, respectively).

Table 1 Characteristics of participants by age, sex, and prevalence of *A. lumbricoides*

Variable	Category	Total examined	Prevalence	X2, *p*-value
Total	-	355 (100)	47 (13.23)	
Age group (years)	[12–18]	6 (1.70)	1 (16.67)	
	[18–30]	241 (67.9)	38 (15.77)	4.60; 0.10
	[31–40]	108 (30.4)	8 (7.41)	
Pregnancy status	Non-pregnant	224 (63.1)	25 (11.2)	2.28; 0.049
	Pregnant	131 (36.9)	22 (16.8)	

Values in bracket are the percentage (%). *p*-value in bold corresponds for each variable to the factor for which the *p*-value is significant

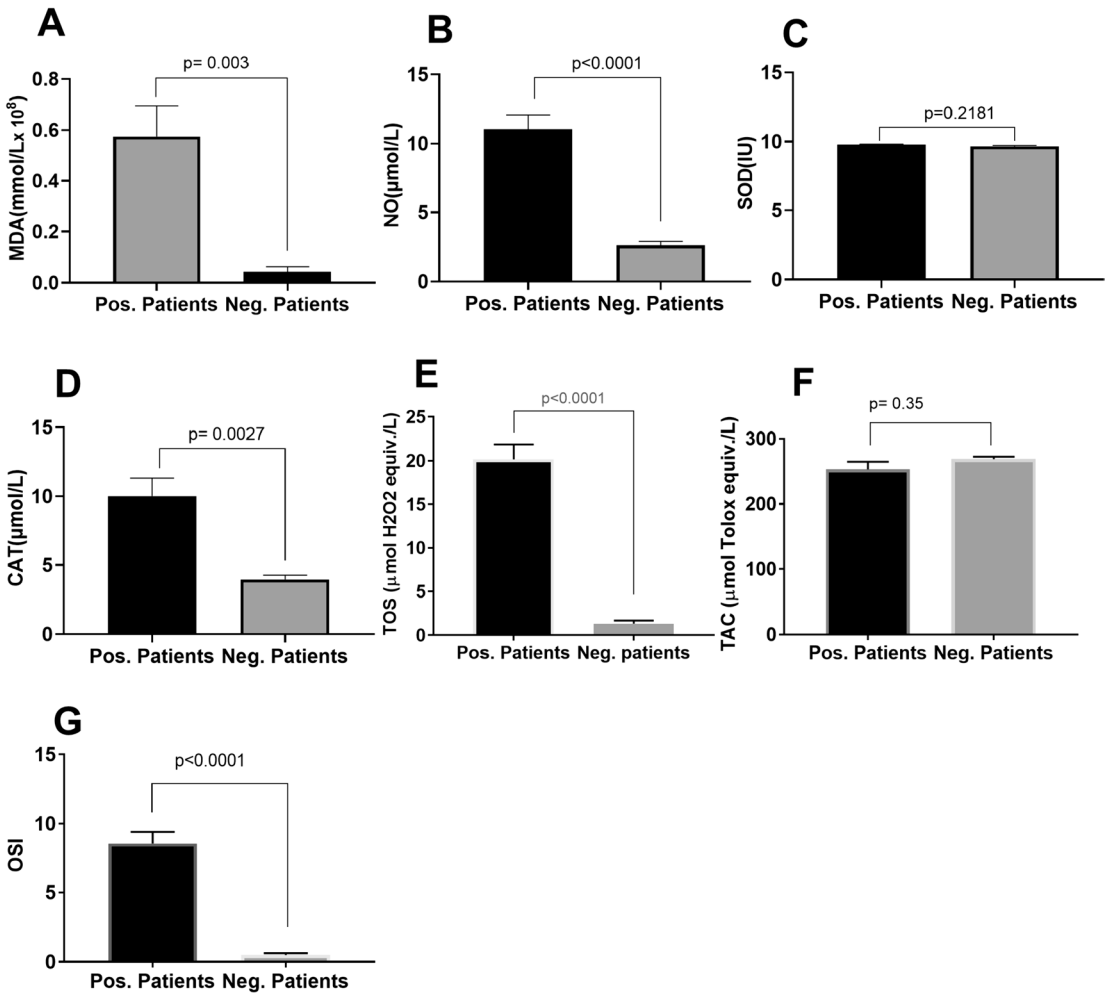

Fig. 1 Serum values of oxidant and antioxidant biomarkers in parasite-free pregnant women and infected pregnant women with *A. lumbricoides*. Pos. patients, patients with *A. lumbricoides*. Neg. patients, patients free from any parasites

For the antioxidant biomarkers, there was no significant difference between SOD in the infected PW and their parasite-free counterparts ($p = 0.2181$; Fig. 1C), whereas there was significantly higher CAT level in infected PW than the parasite-free group ($p = 0.0027$; Fig. 1D).

Analysis of principal factor of the different oxidative status markers in PW with *A. lumbricoides* (Fig. 2) indicated that PCA resulted in two components F1 and F2 explained for 60.72% of the variance in the oxidative and antioxidants biomarkers in pregnant women with *A. lumbricoides* for a Kaiser-Meyer-Olkin measure of sampling adequacy of $KMO = 0.385$ and a significant Bartlett test of sphericity ($p < 0.0001$). PC1 explained for 33.90% the variance, and it was largely driven by the positive loading scores for TOS ($r = 0.732$), OSI ($r = 0.906$), and SOD ($r = 0.679$) and with the contributions of 22.55%, 34.61%, and 19.40%, respectively, while PC2 explained for 26.82% of the variance and was largely driven by the positive loading scores for NO ($r = 0.781$) and CAT ($r = 0.799$) which contributed for 32.50% and 33.99%, respectively (S1).

Effect of A. lumbricoides infection on cytokine response and identification of highly associated cytokines in pregnant women

Pregnant women with *A. lumbricoides* had significantly elevated levels of the four cytokines studied, namely TNF-α, IL-10, IFN-γ, and IL-12 in comparison to healthy controls (all $p < 0.001$, Fig. 3). Principal component analysis was used to assess the association of inflammatory cytokines with *A. lumbricoides* in pregnant women (Fig. 4). It was found that both PC1 and PC2 explain for 63.14% of the variance across the data sets. In this contribution, PC1 contributed for 33.34% and PC2 for 29.79%. PC1 is largely driven by positive loading score for the TNF-α ($r = 0.759$) and by negative

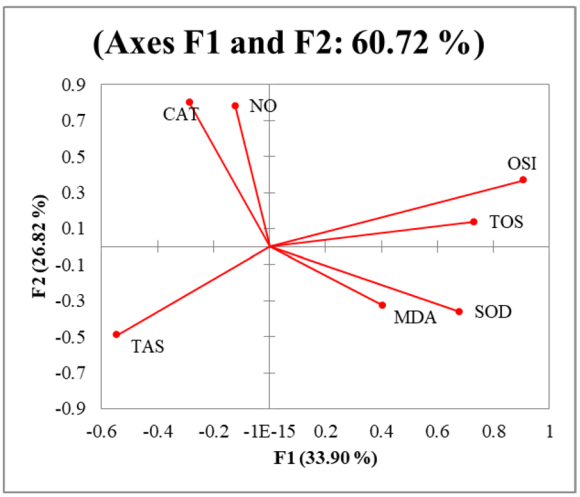

Fig. 2 Principal component analysis performed on the oxidants (TOS, OSI, NO, and MDA) and antioxidants (TAC, CAT, and SOD) potential determinants of *A. lumbricoides* infection in pregnant women

loading scores for IL-1β ($r = -0.617$), and they contributed for 43.19% and 28.51%, respectively. The variance in PC2 was largely due to positive loading scores for IL-10 ($r = 0.565$) and IL-12 ($r = 0.736$), with contribution of 26.75% and 45.44%, respectively (S2).

Relationship between oxidant and antioxidant biomarkers and cytokines in pregnant women with A. lumbricoides

As shown by Table 2, after forward elimination of non-significant variables, the results showed that IL-10 negatively related with TOS and OSI and positively with TAC. But these relations were not significant. IL-12 related only to NO, showing a negative and significant relation. TNF-α was related only to SOD, obtaining a nonsignificant negative coefficient. IL-1β related to CAT and MDA but displayed significant and positive relation only with MDA. Between values of the functional variables (oxidative status biomarkers) obtained in pregnant women with *A. lumbricoides* and serum TNF-α, IL-1β, IL-10, and IL-12 values, results showed that NO is significantly explained by IL-12 ($R^2 = 0.176$; $p = 0.048$), while MDA was significantly explained by IL-1β ($R^2 = 0.195$; $p = 0.045$).

Effect of *A. lumbricoides* infection on oxidant and antioxidant biomarkers and associated major oxidative features in non-pregnant women

As it can be seen in Fig. 5, the total oxidative stress (TOS) values obtained for the infected NPW were higher than of parasite-free NPW ($p = 0.001$; Fig. 5E), while the total antioxidant capacity (TAC) did not differ in infected NPW and the parasite-free group ($p = 0.08$; Fig. 5F). The OSI level calculated for the infected NPW was higher

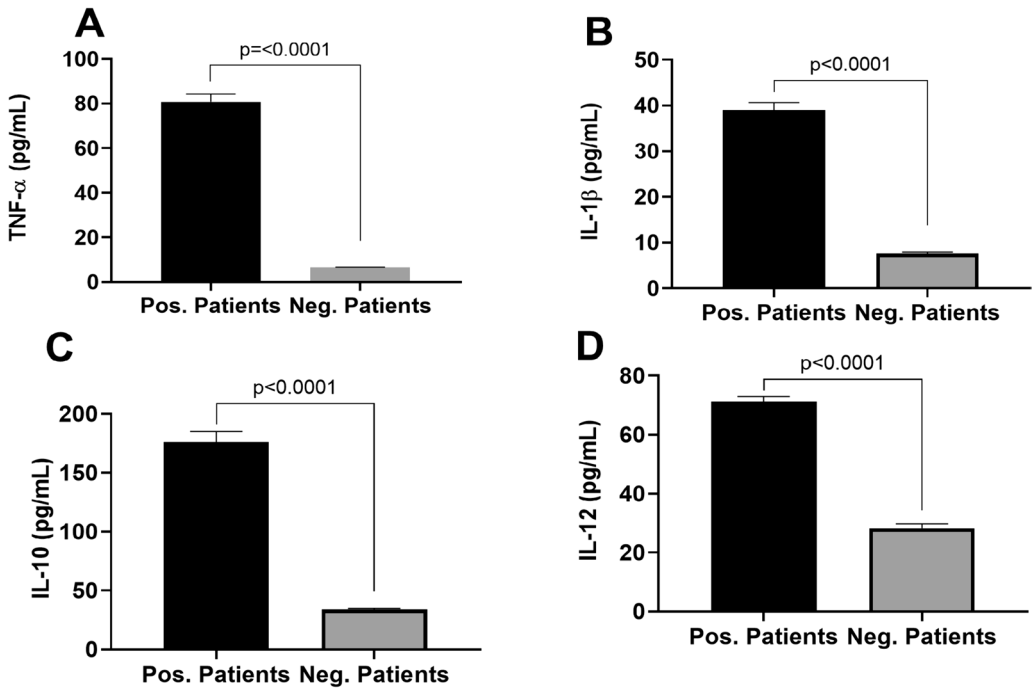

Fig. 3 Mean values of TNF-α (**A**), IL-1β (**B**), IL-10 (**C**), and IL-12 (**D**) in non-infected pregnant women and pregnant women with *A. lumbricoides*. Pos. patients, patients with *A. lumbricoides*. Neg. patients, patients free of any parasites

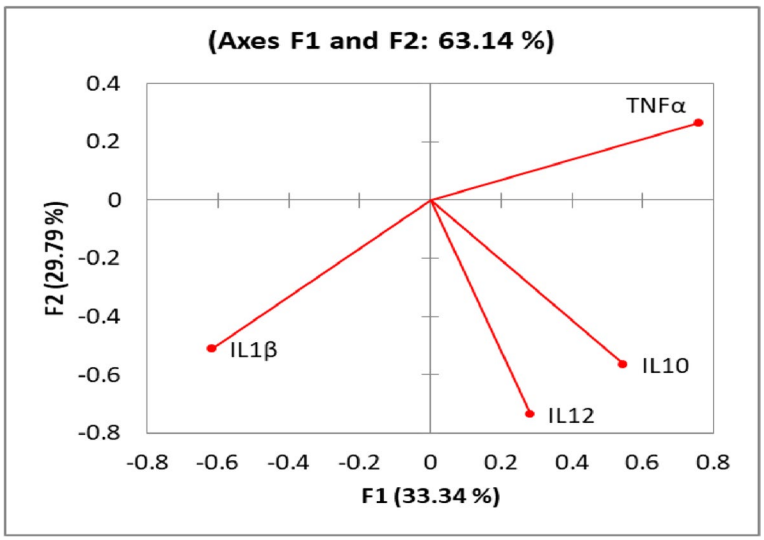

Fig. 4 Principal component analysis performed on IL-1β, TNF-α, IL-10, and IL-12 as potential determinants of *A. lumbricoides* infection in pregnant women

Table 2 Parameters of model explaining oxidative status biomarkers in pregnant women with *A. lumbricoides* using cytokines (with *SD* = standard deviation of the slope)[a]

Independent variables	Dependent variables	Slope		R^2		
		Value (SD)	95% *CI*	Value	*F*	*P*
IL-10	TOS	−0.060 (0.04)	−0.156 to 0.036	0.083	1.719	0.205
	TAC	0.524 (0.33)	−0.167 to 1.214	0.117	2.520	0.129
	OSI	−0.042 (0.02)	−0.089 to 0.005	0.156	3.500	0.077
IL-12	NO	−0.264 (0.13)	−0.538 to 0.010	0.176	4.062	**0.048**
TNFα	SOD	−0.006 (0.00)	−0.013 to 0.002	0.113	2.413	0.137
IL-1β	CAT	−0.168 (0.18)	−0.562 to 0.226	0.040	0.797	0.383
	MDA	0.039 (0.01)	0.001 to 0.076	0.195	4.615	**0.045**

[a] Only significant variables remaining after backwards elimination are shown. Values in bold correspond for each variable to the factor for which the *p*-value is significant. Probability for entry: 0.05/probability for removal: 0.1

than for the parasite-free NPW ($p = 0.0001$; Fig. 5G). Results also showed that the mean MDA value for the infected patients was higher than that of the parasite-free group ($p = 0.008$; Fig. 5A), but there was no difference in mean NO value ($p = 0.719$; Fig. 5B). Moreover, infected non-pregnant women showed higher CAT values than the parasite-free group ($p = 0.002$; Fig. 5D); conversely, there was no difference in the mean SOD values ($p = 0.2$; Fig. 5C).

In principal components analysis, the Kaiser-Meyer-Olkin measure of sampling adequacy (KMO = 0.45) and the Bartlett test of sphericity ($p = 0.000$) were obtained in non-pregnant women with *A. lumbricoides*. PCA resulted in two components which explained 65.42% of the variance in oxidative and antioxidant biomarkers in non-pregnant women with *A. lumbricoides* (Fig. 6). The PC1 explained for 43.56% the variance, and it was driven by positive factor loading scores of OSI ($r = 0.914$) and negative loading scores of NO ($r = -0.786$) and CAT ($r = -0.769$) for which the variables OSI, NO, and CAT, respectively, contributed to 27.41%, 20.25%, and 19.37% of the variation, while PC2 explained 21.85% of the variance, for which TOS and TAC were the variables with great contributions (39.91 and 24.85, respectively). Both TOS and TAC had negative loading scores ($r = -0.781$ and $r = -0.617$, respectively) (S3).

Evaluation and multivariate analysis of the effect of A. lumbricoides on serum cytokines in non-pregnant women

Individuals with *A. lumbricoides* had significantly elevated levels of four cytokines studied, namely TNF-α, IL-12, IL-10, and IL-1β in comparison to healthy controls (Fig. 7). The analysis of principal components showed that PCA resulted in two components (PC1 and PC2)

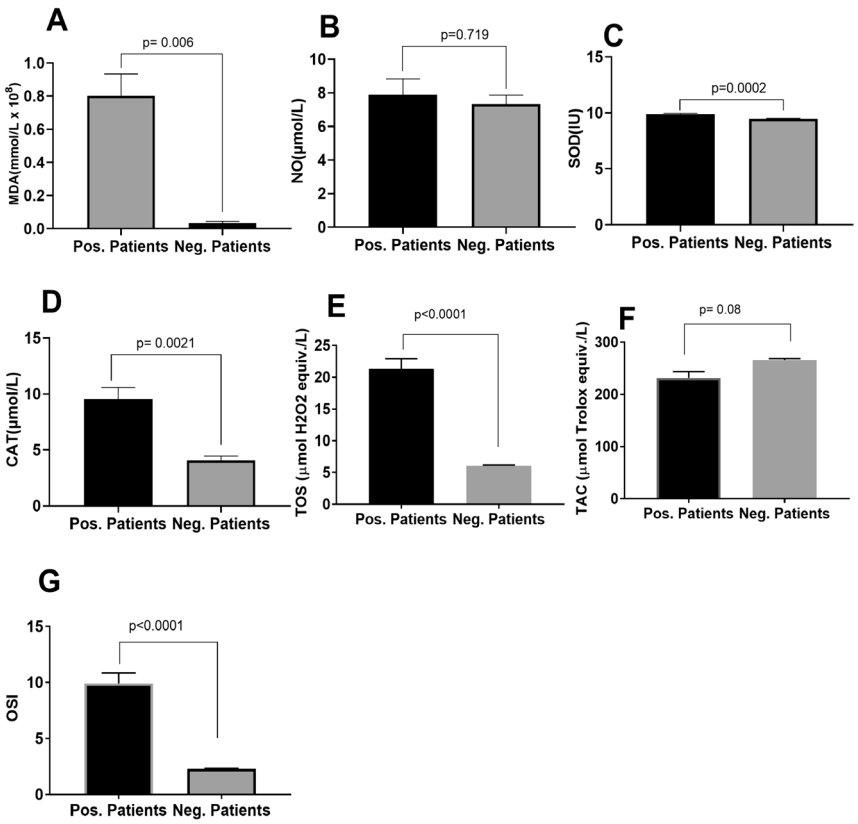

Fig. 5 Serum values of oxidant and antioxidant biomarkers in parasite-free non-pregnant women and infected non-pregnant women with *A. lumbricoides*. Pos. patients, patients with *A. lumbricoides*. Neg. patients, patients free of any parasites

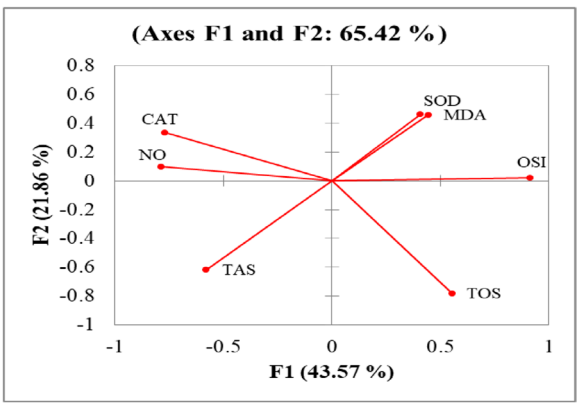

Fig. 6 Principal component analysis performed on oxidant (TOS, OSI, NO, and MDA) and antioxidant (TAC, CAT, and SOD) biomarkers as potential determinants of *A. lumbricoides* infection in non-pregnant women

which explained the variance of the four cytokines in non-pregnant women with *A. lumbricoides* of 76.53%. PC1 accounted for 41.15%, while PC2 contributed for 35.38% (Fig. 8). The component loadings for each variable showed that the variance in PC1 was largely driven by positive loading scores for TNF-α ($r = 0.923$) with a contribution of 51.74%. PC2 was largely driven by positive loading scores for IL-12 ($r = 0.617$) and IL-10 ($r = 0.776$) and by negative loading scores for IL-1β ($r = -0.647$). The contribution of IL-1β, IL-10, and IL-12 to PC2 was respectively 29.60%, 42.56%, and 26.93% (S4).

Relationship between oxidant and antioxidant biomarkers and cytokines in non-pregnant women with A. lumbricoides

Table 3 presented parameters of the model explaining oxidative status biomarkers including TOS, TAC, OSI, NO, SOD, CAT, and MDA by cytokines in pregnant women with *A. lumbricoides*. It shows that IL-1β is related with TOS, OSI, and NO, but the relation is significant with OSI and NO and not with TOS. The relationship between IL-1β and OSI was negative ($R^2 = 0.30$; $p = 0.04$), while it was positive with NO ($R^2 = 0.57$; $p = 0.008$). IL-10 was positively related with TAC, NO, and SOD, but the relationship was significant only with NO ($R^2 = 0.512$; $p = 0.000$). TNF-α was positively related with

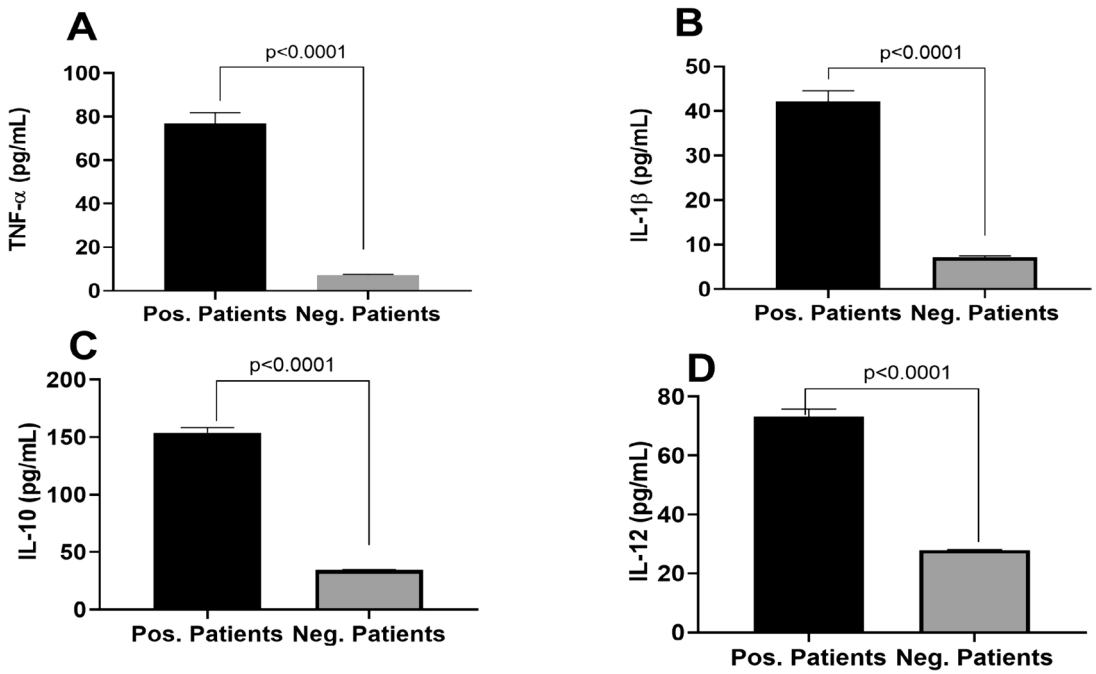

Fig. 7 IL-1β, TNF-α, IL-10, and IL-12 concentrations in pregnant and non-pregnant infected with *A. lumbricoides* and analysis of principal components

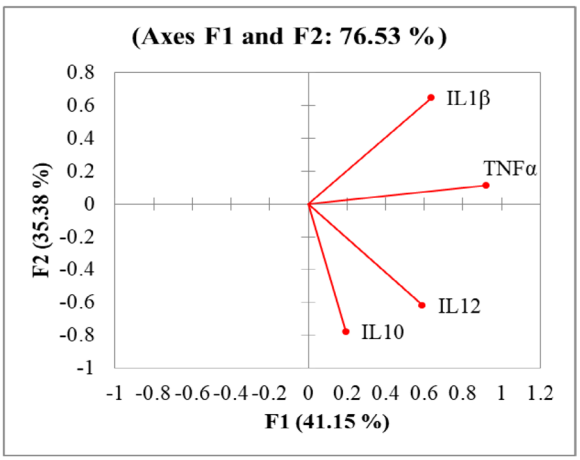

Fig. 8 Principal component analysis performed on IL-1β, TNF-α, IL-10, and IL-12 as potential determinants of *A. lumbricoides* infection in non-pregnant women

CAT and positively related with MDA, but it was not significant.

Discussion

This study sought to determine the prevalence of *A. lumbricoides* and the level of oxidative stress biomarkers bearing in mind the role of inflammatory immune response which was examined in pregnant and non-pregnant women. Various oxidative stress-related biomarkers were measured: TOS, TBARS or MDA, NO, TAC, CAT and SOD, and OSI. Besides, four cytokines: TNF-α, IL-1β, IL-10, and IL-12 were assessed.

The overall prevalence of *A. lumbricoides* infection was 13.23%. This is significantly lower than the 18.8% recorded in children in Yaounde by Aloho et al. [32], and this is probably a reflection of the control programs put in place by the Ministry of Public Health. Also, the difference observed in the prevalence could be due to decrease in human activity and environmental changes, which usually lead to an increase in the transmission. Moreover, the difference in prevalence in this study and previous reports of Aloho et al. [32] in Yaounde could be attributed to improved hygiene among adults and the National Deworming Program of the Ministry of Public Health.

In pregnant women, the results indicated that *A. lumbricoides* infection significantly affected the oxidative status of this group of women, influencing the concentration of free radical compounds, lipids oxidative damage, nitrogen reactive species, and CAT, giving a significant oxidative stress imbalance. *A. lumbricoides* infection also significantly increased serum TNF-α, IL-1β, IL-10, and IL-12 levels in pregnant women. The use of multivariate analysis indicated that 2 components (PC1 and PC2) reflected 60.72% of oxidative status biomarkers which were positively driven by TOS, OSI, SOD, NO, and CAT. This indicated that the augmented oxidative response

Table 3 Parameters of model explaining oxidative status biomarkers in non-pregnant women with *A. lumbricoides* using cytokines (with *SD* = standard deviation of the slope)

Independent variables	Dependent variables	Slope		R^2		
		Value	95% CI	Value	*F*	*p*
IL-1β	TOS	−0.366 (0.27)	−0.961 to 0.230	0.13	1.791	0.206
	OSI	−0.372 (0.16)	−0.725 to −0.019	0.305	5.272	**0.040**
	NO	0.313 (0.09)	0.101 to 0.524	0.752	16.65	**0.008**
IL-10	TAC	0.044 (0.04)	−0.047 to 0.136	0.085	1.117	0.311
	NO	0.011 (0.002)	0.006 to 0.015	0.512	4.912	**0.000**
	SOD	0.000 (0.00)	−0.001 to 0.000	0.083	28.387	0.319
TNF-α	CAT	0.131 (0.07)	−0.038 to 0.299	0.191	2.836	0.118
	MDA	−0.012 (0.00)	−0.032 to 0.008	0.123	1.68	0.219

Only significant variables remaining after forwards elimination are shown. Values in bold correspond for each variable to the factor for which the *p*-value is significant. Probability for entry: 0.05/probability for removal: 0.1

displayed by *A. lumbricoides*-infected subjects was represented by higher TOS and a trend towards higher NO production. An augmented antioxidant response, with a trend towards higher CAT activity, which is one of the main components of the antioxidant system, was seen; this can be understood as a trial physiological response to counteract a chronic overload of free radicals. But this physiological response was found insufficient in case of *A. lumbricoides* as it was demonstrated by high level of OSI which was positively and strongly correlated with the PC1, which may reflect the role of *A. lumbricoides* in modulating the process of systemic oxidative stress.

The results of this study also showed that pregnant women infected with *A. lumbricoides* showed a significant increase in TNF-α, IL-1β, IL-10, and IL-12 levels. Using the PCA analysis, it was found that PC1 and PC2 together explained 63.14% of the variance across the data sets for cytokines. Factor 1 was made up predominantly of the pro-inflammatory cytokines, TNF-α, which have a positive loading score and IL-1β, while in factor 2, there was a pattern of pro-inflammatory cytokine, IL-12, with an addition of IL-10. These findings demonstrate that immunity to *A. lumbricoides* involves the four inflammatory cytokines (namely TNF-α, IL-1β, IL-10, and IL-12). The increase in IL-10 simultaneously with the increase in IL-12 and TNF-α can be taken as a normal physiological process to ensure immune homeostasis [33] in pregnant women in the presence of *A. lumbricoides*. All the four studied cytokines were associated with inflammation; this suggests that *A. lumbricoides* may be associated with an inflammatory response in pregnant women. With high levels of IL-10, *A. lumbricoides* can polarize the immune response towards the Th2 immune response, thus modulating the oxidative damage caused by the pregnancy state. This may probably be an explanation to the increase in oxidative biomarkers in *A. lumbricoides*-infected pregnant women. In this study, the analysis of the relationship between cytokines and oxidative or antioxidant biomarkers showed that IL-10 inversely influenced TOS and OSI, IL-12 is positively associated with NO, while IL-1β is negatively associated with MDA. The fact that a negative, strong, and direct relationship exists between IL-10 and TOS or OSI as well as IL-1β and MDA buttresses the fact that *A. lumbricoides* is capable of suppressing the production of type 1 cytokines which promote oxidative stress. Therefore, IL-10 is being elevated in pregnant women with *A. lumbricoides* to combat the effect of oxidative stress [34]. The positive relationship between IL-12 and NO may suggest the consumption of IL-12 in fighting *A. lumbricoides* infection.

In non-pregnant women, the mean MDA and CAT titer values were higher among *A. lumbricoides*-infected patients compared to non-infected patients, while infected patients and the parasite-free group have a similar total antioxidant capacity (TAC). This might indicate an increased generation of H_2O_2 as ROS by the *A. lumbricoides* in the host's plasma against which CAT is produced to counteract and may be justified by the increase of the total oxidative stress marker [35, 36]. A compromised antioxidant defense mechanism, alongside increased oxidant levels and OSI values in patients with *A. lumbricoides*, might indicate that oxidative stress plays an important role in the pathogenesis and severity of the infection in non-pregnant women. The PCA analysis showed that the first two components explained 65.42% of the variance in oxidative and antioxidant biomarkers, which were largely driven by positive factor loading scores of OSI, which simply shows that *A. lumbricoides* is responsible of the oxidative stress imbalance in non-pregnant. Importantly NO, CAT, and TOS and TAC may be the important biomarkers of oxidative status which were found to be the marker most significantly

associated with *A. lumbricoides* in non-pregnant women and has a negative relationship. Analyzing the variation in cytokines response to *A. lumbricoides* in non-pregnant women, it was found that individuals with *A. lumbricoides* had significantly elevated levels of pro-inflammatory (TNF-α and IL-12) and anti-inflammatory (IL-10 and IL-1β) cytokines. In factor loading analyses, factors 1 and 2 explained 76.53% of the variance of cytokines, which were driven positively by TNF-α (factor 1) and IL-10 and IL-12, along with a negative loading of IL-1β (factor 2). These findings may indicate that *A. lumbricoides* infection in non-pregnant could be characterized by an elevated TNF-α, IL-12, and IL-10 and a decrease in IL-1β and further evidence to the role of IL-10 and TNF-α in the cytokine milieu associated with *A. lumbricoides* infection. The analysis of the relationship between oxidative biomarkers and cytokines showed a significant positive relationship between IL-10 with CAT, which may suggest that IL-10 is responsible for the high catalase level shown in non-pregnant women.

In pregnant and non-pregnant, the data demonstrated that some of the Th 1 and adaptive immunity (Th 2) cytokines present in *A. lumbricoides* infection work towards a more Th2 than Th1 response to the group of biomarkers that are positively affected during the pro-inflammatory stress response event. Parasite infections harm their hosts directly by draining their resources and indirectly through the costs of producing inflammatory and immune responses against infections that increase the presence of ROS [8]. Moreover, during parasitic diseases, phagocytic cells produce a high ROS and reactive nitrogen species (RNS) that contribute to the pathogenesis [37]. This suggests that oxidant-producing cellular immune responses may be involved in the control of *A. lumbricoides* infection [38].

In both pregnant and non-pregnant, the level of MDA, characterizing the lipids oxidative damage, was higher in infected individuals than in uninfected women similar to the findings of study reported by Kilic et al. [39]. The high MDA level in infected individuals could be due to decreased activity of defense system protecting tissues from free radical damage as a result of parasitic infections [40]. The host defense system against oxidative stress can be accomplished with the help of enzymatic and nonenzymatic molecules which makes up the antioxidant defense mechanism. In pregnant women as well as in non-pregnant, CAT was found as one of the variables driven the *A. lumbricoides* infection, demonstrating its importance during the infection. However, OSI was high in infected individuals suggesting that the antioxidant mechanisms do not seem sufficient enough to completely remove all ROS produced during *A. lumbricoides* infection.

Conclusions

The results of this study put into manifest the importance of *A. lumbricoides* when controlling the parasites in the area of study. Moreover, the findings of this study indicated that immune response to *A. lumbricoides* may be dominated by inflammatory response, which consequently results in high oxidative stress imbalance exposing the women to the oxidative stress damage. However, future studies should consider the intensity of infection as well as do experimental infections for a better elucidation of the host-parasites interactions during *A. lumbricoides* infection in women.

Abbreviations

MDA	Malondialdehyde
CAT	Catalase
SOD	Superoxide dismutase
A. *lumbricoides*	*Ascaris lumbricoides*
ROS	Reactive oxygen species
OSI	Oxidative stress index
RNS	Reactive nitrogen species
NO	Nitric oxide
Th	T helper
PW	Pregnant women
TAC	Total antioxidant capacity
TOS	Total oxidative stress
IL	Interleukins
TNF	Tumor necrosis factor

Supplementary Information

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Additional file 1: S.1. Correlations between variables and factors explaining the relationship between ascaris lumbricoides infection and oxidative stress status in pregnant of women.

Additional file 2: S.2. Correlations between variables and factors explaining the relationship between ascaris lumbricoides infection and cytokine response in pregnant of women.

Additional file 3: S.3. Correlations between variables and factors explaining the relationship between ascaris lumbricoides infection and oxidative stress status in non-pregnant of women.

Additional file 4: S.4. Correlations between variables and factors explaining the relationship between ascaris lumbricoides infection and cytokine response in pregnant of women.

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Authors' contributions

BBM, conceptualization, data collection, data analysis, and writing of the original draft. CB and FEN, data collection and data analysis. OM and NHN, conceptualization, review, editing, and supervision.

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Availability of data and materials

Data will be made available on request to the corresponding author.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethical Review Board of the Faculty of Health Sciences, University of Bamenda (ref. no. 2022/0701H/UBa/IRB).

Competing interests

The authors declare that they have no competing interests.

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