OXIDATIVE STRESS IN SCHOOL CHILDREN CO-INFECTED WITH SCHISTOSOMA MANSONI AND SOIL-TRANSMITTED HELMINTHS

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SUMMARY

Introduction: Oxidative stress has been implicated as a cause or as a complication of various chronic diseases such as atherosclerosis, diabetes, HIV and Hepatitic infections. Intestinal helminths rank first among chronic infections in developing countries where several foci of *Schistosoma mansoni* and soil transmitted helminths (STH) exist. Studies have shown that oxidative stress is involved in tissue damage in intestinal helminthiasis. Children of school age are usually the most infected with *Schistosoma mansoni, Ascaris,* and *Trichuris*. These insidious infections cause poor school performance, growth retardation, malnutrition and the loss of up to 4 Disability-Adjusted-Life-Years (DALY) amongst these children.

Objectives: The main objective of this study was to determine the effect of *S. mansoni* and STH infections on the levels of oxidative stress markers in school children. This was achieved by identifying children infected with *S. mansoni* and STH, and measuring the levels of their oxidative stress.

Methods: This was a transverse and comparative study carried out from November 2010 to March 2011. It involved healthy primary school pupils. Ethical Clearance was obtained from the National Ethics Committee of Cameroon. Administrative and local authorities of Makenene, together with the head teachers and parents of the pupils gave their consent on the study. Stool and blood samples were collected from the pupils. Helminth eggs were counted in the stool using the Kato technique, and oxidative stress markers measured in the serum samples The markers analysed included Malondialdehyde (MDA), Ferric Reducing Antioxidant Power (FRAP), Catalase and Superoxide dismutase (SOD) activities, as well as total protein concentration. Infected children were treated with praziquantel and albendazole. Statistical analyses were done using Microsoft Windows Excel 2007, Sigma Stat version 3.01A statistical analysis and SPSS version 17 software. A p value of <0.05 was considered statistically significant.

Results and discussion: One hundred ninety one (191) pupils, 85 males (44.5%) and 106 females (55.5%), of ages 7-15 years were identified. The parasite prevalence was: *S. mansoni* (42%), STH (40.8%,) *Ascaris* (18.8%), *Trichuris* (21%) and hookworm (1%). Eight point eight percent (8.8%) of the pupils were co-infected with *S. mansoni* and STH. There was a higher prevalence of helminthiasis in females (62%) compared to the males (55%). Those with co-infection had significantly higher MDA levels, together with lower FRAP, Catalase and SOD activity than the uninfected group ($p = 0$); they also had significantly lower FRAP ($p < 0.001$) and lower Catalase ($p < 0.001$) than those with STH, as well as lower SOD activity than those with only *S. mansoni*, though the difference was not significant ($p > 0.05$). Those infected with only *S. mansoni* had significantly higher MDA levels ($p = 0$), lower FRAP levels ($p < 0.001$), lower Catalase and SOD activities ($p < 0.001$) than the uninfected children; they equally had a significantly lower Catalase activity than the children infected with only STH ($p < 0.001$). Children infected with STH had a significantly lower SOD than the uninfected ($p < 0.001$). The variation of the oxidative stress markers did not correlate significantly with the intensity of infection. The decrease in FRAP and the elevated MDA are probably due to a decrease in SOD activity in the STH group, a decrease of catalase activity in the Schistosomiasis group, and a decrease of both catalase and SOD activities in those with co-infections.

Conclusion & recommendation: Makenene is still a focus of intestinal helminthiasis. The infected children had high oxidative stress status. The parasites probably induce oxidative stress through a fall in antioxidant mechanisms. Oxidative stress is more severe in individuals with co-infections. We recommend an intensification of the anti-helminth strategies already in place and an adjuvant antioxidant therapy in the management of intestinal helminthiasis.

Keywords: Soil transmitted helminths, *Schistosoma mansoni,* co-infections, primary school children, oxidative stress, anti-oxidants.

RESUME

Introduction : Le stress oxydatif est responsable des complications de diverses maladies chroniques à l'instar de l'athérosclérose, du diabète, du VIH-SIDA et des infections hépatiques. Les helminthes intestinaux occupent la première place parmi les infections chroniques, en particulier dans les pays en voie de développement, où des nombreux foyers de *Schistosoma mansoni* et des géohelminthes (STH) existent. Des études ont révélé que le stress oxydatif entraine des dommages des tissus au cours des infections par des helminthes intestinales. Les enfants d'âge scolaire sont généralement les plus infectés par *S. mansoni*, *Ascaris* et *Trichuris*. Ces infections insidieuses sont à l'origine des mauvaises performances scolaires, des retards de croissance, de la malnutrition et d'une perte jusqu'à 4 DALY (disability adjusted life years) chez ces enfants.

Objectifs : L'objectif principal de ce travail était de déterminer les effets des infections causées par *S. mansoni* et STH sur les marqueurs du stress oxydatif. Pour se faire, nous avons identifié des enfants infectés par *S. mansoni* ou par les STH à Makenene et déterminé le taux des marqueurs du stress oxydatif chez les enfants infectés et non-infectés.

Méthodes : Ce travail est une étude transversale et comparative réalisée de novembre 2010 à mars 2011, sur des enfants de l'école primaire apparemment sains. La clairance éthique a été obtenue du Comité Nationale d'Ethique du Cameroun. Les autorités administratives et locales de Makenene, ainsi que les Directeurs des écoles et les parents des enfants ont donné leur consentement pour cette étude. Les échantillons de selles et de sang ont été collectés chez les enfants. Les œufs des helminthes ont été comptés dans les selles en utilisant la technique de Kato, tandis que les marqueurs du stress oxydatif ont été mesurés dans le sérum. Les marqueurs du stress oxydatif étaient de la malone dialdehyde (MDA), du pouvoir réducteur antioxydant ferrique (FRAP), de la catalase, de la superoxide dismutase et des protéines totales. Les enfants infectés ont été traités avec le praziquantel et l'albendazole. Les analyses statistiques ont été réalisées grâces aux logiciels : Microsoft Windows Excel 2007, Sigma Stat statistical analysis et SPSS version 17. Les différences ont été considérées significatives à $p<0,05$.

Résultats & discussion: L'étude a été réalisée sur 191 enfants, soient 85 males (44,5 %) et 106 femelles (55,5 %) de 7 à 15 ans. La prévalence des infections parasitaires était de 42 % pour *S. mansoni,* 40,8 % pour les STH, 18,8 % pour Ascaris, 21 % pour *Trichuris* et 1% pour les vers à crochets. 18,8 % des enfants étaient co-infectés par *S. mansoni* et par les STH. La prévalence des helminthiases était plus élevée chez les femelles (62 %) que chez les mâles (55 %). Les enfants qui présentaient une co-infection ont eu un taux de MDA significativement élevé ($p = 0$), une faible activité de la catalase ($p < 0,001$) et de la SOD ($p < 0,001$), ainsi qu'un faible FRAP ($p < 0,001$) que les enfants non-infectés. Ils ont également présenté une faible activité de catalase ($p < 0,001$) que les enfants infectés par les STH, et une faible activité de la SOD que les enfants infectés uniquement par *S. mansoni*, mais la différence n'était pas significative ($p > 0,05$). Les enfants infectés uniquement par *S. mansoni* ont eu un taux significativement élevé de MDA ($p = 0$), un faible FRAP ($p < 0,001$), une faible activité de catalase ($p < 0,001$) et de la SOD ($p < 0,001$) que les enfants non-infectés. Ils ont également présenté une activité de catalase significativement faible ($p < 0,001$) que les enfants infectés uniquement par les STH. Les enfants infectés par les STH ont présenté une activité significativement faible de SOD que les non infectés ($p < 0,001$). La variation des marqueurs du stress oxydatif n'était pas significativement corrélée à la charge parasitaire. La diminution du FRAP et le taux élevé de MDA sont probablement dus à la baisse de l'activité de la SOD telle qu'observée chez les enfants infectés par les STH, à la baisse de l'activité de catalase comme chez les enfants infectés par S. *mansoni,* ou encore à la baisse de l'activité de ces deux enzymes comme chez les enfants qui présentent une co-infection.

Conclusion & recommandations : Makenene est jusqu'alors un foyer des helminthiases intestinales. Les enfants infectés présentent un stress oxydatif élevé. Les parasites induisent probablement le stress à travers la baisse des mécanismes antioxydants. Le stress oxydatif est plus sévère chez les individus qui présentent une co-infection. Nous recommandons de ce fait une intensification des stratégies anti-helminthes déjà en place, et une thérapie antioxydante adjuvante dans la prise en charge des helminthiases intestinales.

Mots clés : Geohelminthes, *Schistosoma mansoni,* co-infections, élèves du primaire, stress oxydatif, antioxydantes.

INTRODUCTION:

One of the key mechanisms by which the human body defends itself against invading parasites is by the production of reactive oxygen species (ROS) by immune cells. In an inflammatory environment, activated macrophages can produce these ROS to a concentration of up to 100uM in the vicinity of these cells (1). These ROS serve to destroy the pathogens, but uncontrolled production leads to the oxidisation of self lipids, proteins, and DNA, thereby stimulating apoptosis. The human body has regulatory mechanisms which maintain equilibrium between the production and elimination of ROS. An increase in ROS production or decrease in its elimination will lead to oxidative stress (2). Oxidative stress has been evoked as both a cause and consequence of various pathologies as diabetes, atherosclerosis, HIV-AIDS, cancers and even in helminth infection (3-6).

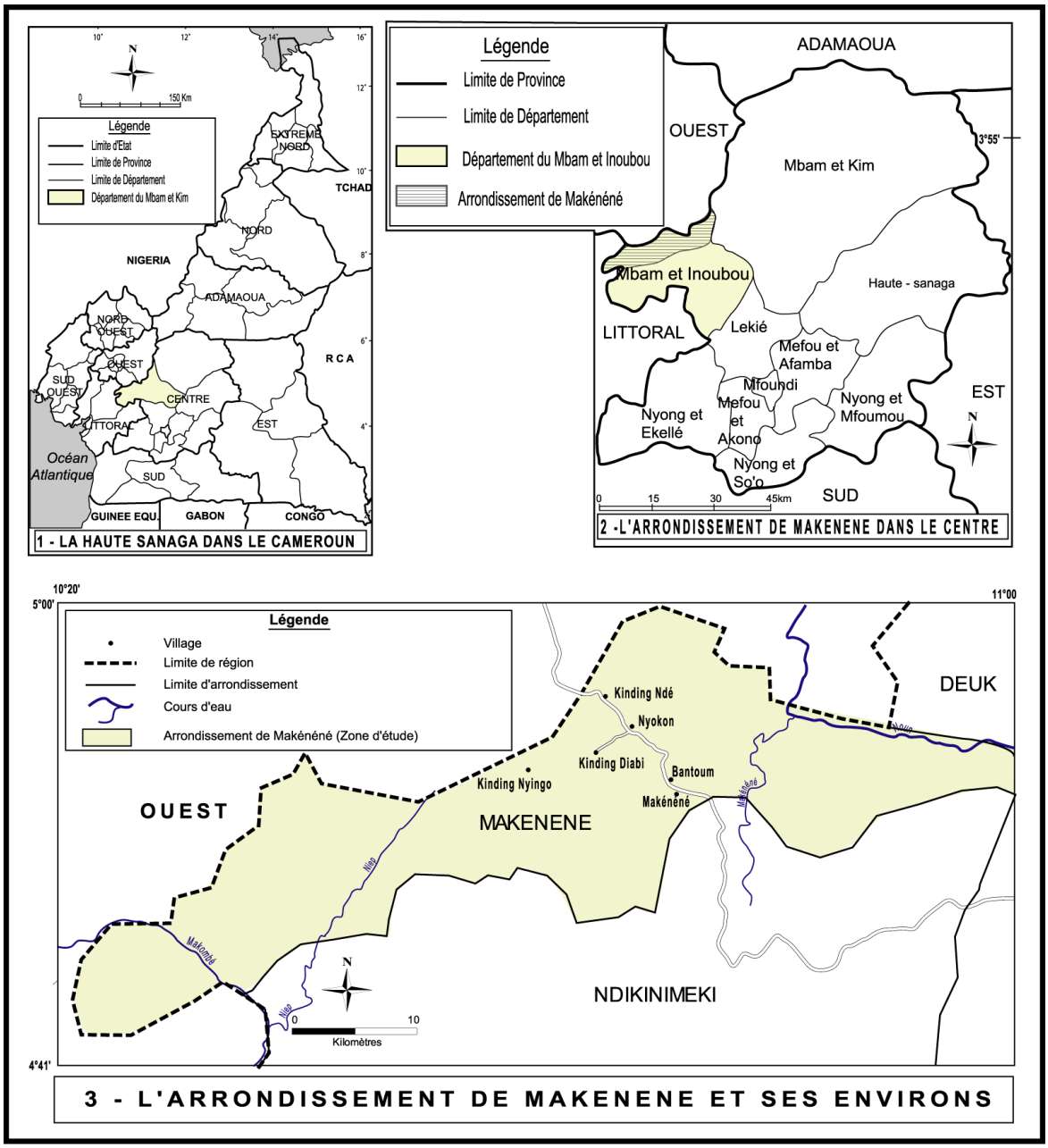
Intestinal helminths remain the most common chronic organisms plaguing mankind (7), especially in developing countries, where the sanitary conditions are poor. Of the 20 helminths of public health importance, the most common are the soil-transmitted helminths (7), which are *Ascaris, Trichuris* and *Ankylostoma*. They together affect over 2 billion humans, with Ascaris infecting a quarter of the world's population; *Trichuris* infects over 500 million people, while the hookworm infects an estimated 740 million people (8). Schistosomiasis is endemic in 76 countries, with up to 700 million people at risk, 200 million infected, 120 million symptomatic, and 20 million presenting severe complications (9).

Children of school age are at particular risk of helminth infection. *Ascaris* and *Trichuris* infections peak between the ages of 5-10 years and *Schistosoma* at 10-14 years of age (7). Oxidative stress has been implicated as a mediator of tissue damage in each of these helminth infections (3, 4, 10, 11). Helminths equally are known to release anti-oxidative molecules, which enables them to manage the host's oxidative defence (12).

Polyparasitism between Schistosomiasis and geohelminths is a well known phenomenon (13, 14). This is the case in Makenene, a rural town of the Centre Region of Cameroon. In this study, our research hypothesis was that there is an increase in oxidative stress markers in school children with co-infections of *Schistosoma mansoni* and STH. To test this hypothesis, we set out to determine the effect of *S. mansoni* and STH infections on the levels of oxidative stress markers.

METHODOLOGY:

Study site: Makenene is a rural town of the Mbam and Inoudou Division of the Centre Region. It is situated about 85km North-west of Yaounde. It has a population of about 16.000 inhabitants, most of whom are of the Yambassa tribe. It belongs to the Bafia Health District. The main crops grown here are cassava, corn, groundnuts, yam, cocoa and palm nuts. The main occupation of its inhabitants is farming and petty trading (see Figure 1). This region has an equatorial climate, with 4 seasons: two dry seasons, a long one from November to March and a short one from mid-May to mid-August; two rainy seasons, a long one from August to November and a short one from March to July.



Source : Carte topographique IGN au 1 / 200. 000ème et carte administrative du Cameroun, 1990.

Figure 1: Map of Makenene

Type of study: It is a transverse and comparative study that lasted from November 2010 to March 2011.

Sample size: Using an estimated prevalence of helminth infections of 23.6% (15), the sample size was calculated using Lorentz formula to give 272. The study subjects were chosen from primary school children of Classes 4, 5 and 6 that had resided in Makenene for over a year. Children that had recently been treated for helminthiasis were excluded from the study.

Collection and analysis of stool sample: The material needed included sterile transparent plastic containers, wooden spatulas, examination gloves, white tissue paper, Kato set, template with hole, screen, Microscope, microscope slides, cellophane cover slips. The pupils were given labelled, sterile plastic containers together with a wooden spatula to collect morning stool. The stool samples were transported to the Makenene District Hospital for analysis using the Kato-Katz technique (16). A small amount of faeces was placed on filter paper and pressed through a mesh (150µm pore size) sieve. The sieved stool sample was scraped off the wire surface of the sieve with the edge of a wooden spatula and placed on microscope slide, with the aid of a calibrated board. The stool sample was spread over the slide with the aid of a test tube applied on the cellophane paper. The prepared slides were stored in closed boxes in a refrigerator at 4 °C to 8 °C, and examined less than 48 hours from preparation. The slides were examined under the

optical microscope with 10X objective, and suspected parasite forms confirmed with the 40X objective.

Collection of blood samples: About 5 ml of blood was collected from each pupil at the health centre using a G-20 needle into dry tubes and allowed to clot. The blood was then centrifuged at 3000g for 10 minutes, and 5 aliquots of 0.3ml each were made from each sample, transported in flasks filled with ice blocks to the IMPM Pharmacology and Toxicology Laboratory and stored at -20^{o}C until used.

Assay of samples: Superoxide Dismutase Determination (SOD) was assayed by the method of Misra and Fridovich (17). The method is based on the fact that SOD inhibits the oxidation of adrenaline to adrenochrome. Adrenochrome formed is detected spectrophotometrically at 480nm. One unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during 1 minute. The results were expressed as SOD units/mg of protein/min. Catalase activity was determined using the method of Sinha, 1972 (18). It is based on the fact that catalase breaks down H_2O_2 to water, but its activity is inhibited by dichromate/acetic acid mixture which acts as a "stop bath". The H_2O_2 left in the mixture reacts with the dichromate/acetic acid to give an unstable blue precipitate of perchromic acid. This precipitate is decomposed to the green solution whose absorbance is read at 570nm, and is proportionate to the amount of H_2O_2 left in the mixture. The results were expressed as units/mg of protein/minute. Malondialdehyde (MDA) was determined by the method of Buege and Aust (19), which is based on the fact that heating 1mole of MDA and 2 moles of Thiobarbituric Acid (TBA) in acidic conditions gives a pink colour whose absorbance can be measured at 532nm. Using the law of Beer-Lambert: OD=εcl concentrations were calculated and expressed as nmol/l of serum. Ferric Reducing Antioxidant power (FRAP) was determined by the method of Benzie and Strain, 1996 (20) measures the anti oxidant reducing power by the transfer of electrons. FRAP measures the capacity of a sample to reduce iron (Fe^{3+}) at acidic pH (3.6). The intense blue colour formed when ferric-tripyridyltriazine (TPTZ.Fe^{3+}) complex is reduced to ferrous-tripyridyltriazine (TPTZ.Fe^{2+}) is measured at 593nm. The results are expressed in µmol/l. Total Protein was determined by the method of Lowry, 1951 (21). The results are expressed in mg/ml of protein.

Statistical Analysis: Statistical analyses were done using Microsoft Windows Excel 2007, Sigma Stat version 3.01A statistical analysis and SPSS version 17 software. Dunn's test for unequal groups was used to detect significant differences between groups. A p value of < 0.05 was considered statistically significant. Groups with less than 5 cases were not included in the statistical analyses.

RESULTS:

Subjects: One hundred ninety one (191) senior primary school pupils of both sexes were identified. There were 85 males (44.5%) against 106 females (55.5%), giving a male to female ratio of 0.8. The age range of the school children was from 7 to 15 years. The mean age was 10.5 ± 2.0 years and the median age 10 years. The pupils were recruited from 3 different primary schools: G.P.S Baloua (78 pupils), Ecole Publique Carrière (46 pupils), and Ecole Publique Mock sud (71 pupils).

Prevalence of helminths: Eighty-one (42%) pupils were infected with *S. mansoni,* while 78 pupils (40.8%) had STH. Ascaris infections constituted 18.8%, Trichuris 21% and Hookworm 1%. A total of 79 pupils were free from all the helminths. The overall prevalence of helminth infections was 58.6%. Thirty six pupils (18.8%) were co-infected with STH and S. mansoni (Table I).

Table I: Prevalence of helminth infections in 191 school children

	Number infected	% infected
S. mansoni	81	42.41
Ascaris	36	18.85
Trichuris	40	20.94
Hookworm	2	1.05

There was a higher prevalence of the various helminths in the female children (62%) as opposed to the males (55%).

Co-infections were as follows: between *S.mansoni* and *Ascaris* (n = 13 pupils), *S.mansoni* and *Trichuris* (14 pupils), and *Ascaris* and *Trichuris* (1 pupil). Nine pupils had triple infections with *Ascaris*, *Trichuris* and *S.mansoni*. Two pupils had moderate *Ascaris* infection; 16 pupils had light *Trichuris* infection and 2 pupils had light hookworm infection. No pupil with a single infection had heavy STH infection nor moderate *Trichuris* or moderate hookworm infection.

École Publique Carriére recorded the highest prevalence of infection with 32 of its 46 pupils (69%) having infections with helminths, while GPS Baloua and École Publique Mock Sud had prevalences of 55%.

Biochemistry: Four markers of oxidative stress were analysed in the serum samples collected from the pupils: FRAP, MDA, catalase and SOD.

Ferric Reducing Antioxidant Power (FRAP):
There was no statistically significant difference between the FRAP levels of both sexes ($P > 0.05$). Table II summarises FRAP levels in different kinds of infection.

Table II: Levels of FRAP in pupils with different infection status

Type of infection	Mean ± Std Deviation (µmol/l)	n
Uninfected	512.08 ± 80.48	79
STH only	366.35 ± 42.15	31
S. mansoni only	306.84 ± 24.64	45
Co-infection	228.88 ± 25.30	36

There were differences in the mean values of FRAP amongst the different groups (Table II). Although the uninfected group had higher FRAP values than the group infected with STH only, the difference was not statistically significant ($p > 0.05$). However, the uninfected group had statistically significantly higher FRAP levels than those who were infected with *S. mansoni* only ($p < 0.05$) or who had co-infections ($p < 0.05$). Though the differences between FRAP values in the STH and co-infected groups were statistically significant ($p < 0.05$), there was neither a statistically significant difference between the STH infected group and those infected with *S. mansoni* ($p > 0.05$), nor between those infected with *S. mansoni* only and the co-infected groups ($P > 0.05$).
There was a significant difference ($p < 0.05$) of FRAP levels between all the combinations of multiple infection and the uninfected group (Table III) but there were no significant differences between the combinations of multiple infections ($p > 0.05$).

Table I: Effect of co-infection on the level of FRAP

Infection combinations	Mean ± SD (µmol/l)	n
Uninfected	512.08 ± 80.48	79
Ascaris + Trichuris	363.00	1
S.Mansoni + Ascaris	226.07 ± 23.80	13
S.mansoni + Trichuris	232.35 ± 28.25	14
S.mansoni + Ascaris + Trichuris	227.55 ± 24.87	9

There was no statistically significant difference between the groups of different intensities of infection ($P > 0.05$) (Table IV).

Table IV: Effect of intensity of infection on FRAP levels

S.everity of infection	Mean ± SD (µmol/l)	n
Uninfected	512.08 ± 80.48	79
Light *S.mansoni*	313.12 ± 21.96	25
Moderate *S.mansoni*	303.87 ± 23.38	16
Heavy *S.mansoni*	279.50 ± 30.62	4
Light *Ascaris*	356.00 ± 24.16	10
Moderate *Ascaris*	309.50 ± 108.18	2
Light *Trichuris*	382.93 ± 35.40	16
Light Hookworm	344.00 ± 67.88	2

Malondialdehyde (*MDA*): MDA is a product of lipid peroxidation, therefore it serves in estimating the degree of oxidation.

Table V: Levels of MDA in pupils with different infection status

Type of infection	Mean ± SD (nmol/l)	n
Uninfected	0.625 ± 0.111	79
STH only	0.842 ± 0.134	31
S. mansoni only	1.168 ±0.247	45
Co-infection	1.156 ±0.368	36

There was a statistically significant difference between the uninfected group and those with *S. mansoni* only ($p < 0.05$) and co-infections ($p < 0.05$). The difference in the means between the uninfected and STH groups was not statistically significant ($p > 0.05$) (Table V).

Table VI: Effect of co-infection on MDA levels.

Infection combinations	Mean ± SD (nmol/l)	n
Uninfected	0.625 ± 0.111	79
Ascaris + Trichuris	0.849	1
S.mansoni + Ascaris	1.635 ± 0.541	13
S.mansoni + Trichuris	1.496 ± 0.203	14
S.mansoni + Ascaris + Trichuris	1.570 ± 0.265	9

Table VI shows the effect of multiple infections on MDA levels. There were statistically significant differences in the means between the uninfected group and the group with *S.mansoni + Ascaris* ($p < 0.05$), *S.mansoni + Trichuris* ($p < 0.05$), and *S.mansoni + Ascaris + Trichuris* ($p < 0.05$), but there was no statistically significant difference between the uninfected group and the *Ascaris + Trichuris* group ($p > 0.05$). However, there was no statistically significant difference between the *S. mansoni + Ascaris, S. mansoni+ Trichuris* and *S. mansoni + Ascaris + Trichuris* ($p > 0.05$).

Table VII: Effect of intensity of infection on MDA levels.

Severity of infection	Mean ± SD (nmol/l)	n
Uninfected	0.625 ± 0.111	79
Light *S.mansoni*	1.148 ± 0.278	25
Moderate *S.mansoni*	1.174 ± 0.205	16
Heavy *S.mansoni*	1.276 ± 0.218	4
Light *Ascaris*	0.890 ± 0.112	10
Moderate *Ascaris*	0.862 ± 0.323	2
Light *Trichuris*	0.812 ± 0.130	16
Light Hookworm	0.820 ± 0.263	2

Table VII shows the effect of intensity of infection on MDA levels. Light, moderate and heavy infection did not cause statistically significant changes in the levels of MDA. However, the groups with *S. mansoni* infection had significant changes in levels of MDA with respect to the uninfected group ($p < 0.05$) and but not with respect to the other groups infected with other parasites ($p > 0.05$).

Catalase: Catalase is one of the major anti-oxidant enzymes. It breaks down hydrogen peroxide to water and oxygen. There were no significant differences in the levels of catalase between the two sexes for different parasite groups. Table VII summarises the effect of type of infection on the activity of catalase.

Table VIII: Catalase activity in pupils with different infection status

Type of infection	Mean ± Std Deviation (units/ mg of protein/ minute)	N
Uninfected	2.58 ± 0.74	79
STH only	2.64 ± 0.73	31
S. mansoni only	0.99 ± 0.35	45
Co-infection	0.85 ± 0.27	36

There was no significant difference in the levels of catalase between the uninfected and the group infected with STH ($p > 0.05$), and between the group infected with *S. mansoni* compared to the co-infected group ($p > 0.05$). However, there was a statistically significant difference between the group infected with *S. mansoni* and the uninfected group ($p < 0.05$) together with the STH group ($p < 0.05$).

When the parasites in multiple infections were identified, groups that were co-infected with *S. mansoni*, had significantly lower catalase activities than the *Ascaris* + *Trichuris*, and uninfected groups ($p < 0.05$) (Table IX).

Table IX: Effect of co-infection on Catalase activity

Infection combinations	Mean ± std Deviation (units/mg of protein/minute)	n
Uninfected	2.58 ± 0.74	79
Ascaris + Trichuris	2.11	1
S.mansoni + Ascaris	0.83 ± 0.33	13
S.mansoni + Trichuris	0.85 ± 0.17	14
S.mansoni + Ascaris + Trichuris	0.90 ± 0.33	9

When catalase activities were compared with respect to the intensity of infection, there was a decrease in the activity of catalase with increasing intensity of infection, but these differences were not statistically significant ($p > 0.05$) (Table X). The uninfected, light Ascariasis and light Trichuriasis groups had a statistically significant higher Catalase activity than those with moderate *S. mansoni* infection. ($P<0.05$).

Table X: Effect of intensity of infection on Catalase activity

Severity of infection	**Mean ± Std Deviation** (units/mg of protein)	**n**
Uninfected	2.58 ± 0.74	79
Light *S.mansoni*	1.02 ± 0.37	25
Moderate *S.mansoni*	1.00 ± 0.35	16
Heavy *S.mansoni*	0.71 ± 0.18	4
Light *Ascaris*	2.52 ± 0.56	10
Moderate *Ascaris*	2.16 ± 1.15	2
Light *Trichuris*	2.90 ± 0.77	16
Light Hookworm	1.89 ± 0.37	2

Superoxide Dismutase: There were no significant variations of SOD levels between the sexes in the different groups infected with the uninfected as well as those infected with STH and *S. mansoni* ($p > 0.05$).
There was a significant decrease of SOD activity in all those with helminthiasis compared to the uninfected group ($p < 0.05$) (Table XI). Those infected with *S. mansoni*-only had a higher SOD activity than those with STH and Co-infections, but the differences were not statistically significant ($p > 0.05$) (Table XI).
When the parasites were identified, there were no statistically significant differences in the level of SOD in pupils infected with different parasite combinations (Table XI).

Table XI: Level of SOD activity in pupils with different infection status

Type of infection	**Mean ± Std Deviation** (units/mg of protein/minute)	**n**
Uninfected	4.98 ± 0.59	79
STH only	2.49 ± 0.44	31
S. mansoni only	3.27 ± 0.47	45
Co-infection	2.08 ± 0.45	36

The SOD activity of pupils infected with light *Ascaris* was significantly lower than that of the uninfected pupils ($p < 0.05$), and pupil with light *Trichuris* ($p < 0.05$) (Table XII).

Table XII: Effect of intensity of infection on SOD activity

Severity of infection	**Mean SOD ± Std Deviation** (units/mg of protein/minute)	**N**
Uninfected	4.98 ± 0.59	79
Light *S.mansoni*	3.35 ± 0.44	25
Moderate *S.mansoni*	3.26 ± 0.39	16
Heavy *S.mansoni*	2.74 ± 0.68	4
Light *Ascaris*	2.35 ± 0.32	10
Moderate *Ascaris*	2.01 ± 0.91	2
Light *Trichuris*	2.59 ± 0.46	16
Light Hookworm	2.57 ± 0.11	2

The effect of multiple infections on the levels of SOD activity is summarised in Table XIII. There were no significant differences between the different groups.

Table XIII: Effect of co-infection on SOD activity

Infection combinations	**Mean ± std Deviation** (units/mg of protein/minute)	**N**
Uninfected	4.98 ± 0.59	79
Ascaris + Trichuris	2.93	1
S.mansoni + Ascaris	2.10 ± 0.43	13
S.mansoni + Trichuris	2.18 ± 0.49	14
S.mansoni + Ascaris + Trichuris	1.89 ± 0.42	9

Correlations of oxidative stress markers:

Table XIV: Pearson correlation of tested markers of oxidative stress

	CATALASE	SOD	FRAP	MDA
CATALASE	1	0.582	0.825	-0.753
SOD	0.582	1	0.887	-0.744
FRAP	0.825	0.887	1	-0.842
MDA	-0.753	-0.744	-0.842	1

There was a significant positive correlation at 0.01 level between catalase, SOD and FRAP ($p < 0.001$), and a negative correlation between MDA and the other markers ($p < 0.001$) (Table XIV).

DISCUSSION:

Limitations of the study

The study had the following limitations: 1) since oxidative stress is not specific to helminths, there may have been need to determine the complete infectious state of the children studied; due to material factors, we assumed that these factors would cancel out in the children that were from the same community; 2) there may be need to determine the individual anti-oxidants that contribute to the FRAP; 3) the sensitivity of the parasitological methods is low; 4) the inefficacy of the Kato-katz faecal technique used here for the diagnosis of *N. americanus* (hookworm) as discussed by Glinz et al.(22).

Study population:

We had 191 participants from 3 primary schools in Makenene. The population consisted of 85 males to 106 females, with a male to female ratio of 0.8. This variation was because there were more female children in these schools than males. The age ranges in the study subjects were between 7-15 years with a mean and median age of 10 years. The range coincides with that of Akufongwe *et al*, 1995 (15). Most of the participants were final year primary school pupils, thus more than half of the pupils were between 10-12 years of age. Senior primary school pupils were preferred because they would better understand the manipulations and precautions to take concerning the stool samples.

The study was carried out in 3 schools: 40% from GPS Baloua, 36% from EP Mock Sud and only 24% from EP Carrière. This difference was due to poor compliance of the EP Carrière pupils.

Infections with Helminths:

The overall prevalence of helminth infections was 58.6%. This is significantly lower than the 90.3% recorded in Loum by Tchuem Tchuente *et al* in 2003 (13). This is probably a reflection of the control programmes carried out by the Ministry of Public Health. The prevalence of *S. mansoni* was 42%. Ripert et *al* in 1982 (14)reported a prevalence of 18.6% in Bafia, while Akufongwe (1995) (15) reported a prevalence of 23.6% in Makenene in 1995, and Moyou-Somo *et al* (2003) (23) reported a prevalence of 54.4% in Yorro. These differences could be due to increase in human activity and environmental changes, which usually lead to an increase in the transmission of

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infection of treated persons in endemic zones of Schistosomiasis.

The prevalence of STH was 40.8%; 18.8% were infected with *Ascaris*, 21% with *Trichuris*, and 1% with Hookworm. The Hookworm prevalence was lower than the 6.7% reported by Moyou *et al* (23) but similar to the prevalence reported by Tchuem Tchuente (13). These values are relatively lower compared to Akufongwe's (15) 98% prevalence of STH in Makenene and its environs. The Kato-katz faecal technique used here for the diagnosis of *N. americanus* (hookworm) is not very sensitive (22).

Moyou-Somo et *al* reported a prevalence of 20.3% for *Ascaris* in Yorro, while Tchuem Tchuente (13) reported 65% Ascaris in Loum and Ripert *et al* (14) reported 69% in Bafia. Our prevalence of 21% for *Trichuris* was lower than the 50-70% reported in other studies (13, 15, 23). These differences in prevalence in our results and previous reports could be attributed to improving hygiene amongst senior school children and the National de-worming program of the Ministry of Public Health.

Polyparasitism was present with 18.8% of pupils excreting eggs of both *S. mansoni* and STH. The peak ages of infection with *Ascaris* and *Trichuris* were

between 5-15 years, while that of *Schistosoma* was 10-15years. This provides long time span for an interaction of infection. The absence of co-infection with Hookworm could be explained by the fact that Hookworm infestation is common in early adulthood (> 21 years), although the KLato-Katz method used for the diagnosis of hookworm is not very sensitive (22).
Ecole Publique Carrière had the highest prevalence of helminthiasis of 69% compared to 55% for the other schools; probably a reflection of differences in hygienic conditions in the schools.

Biochemical Assays:

There is need for the human host to maintain a critical balance between generation of reactive oxygen species and other free radicals, and antioxidant defense. Oxidative stress occurs when this balance between pro-oxidative and anti-oxidant is upset. In the present study, we measured the anti-oxidant capacity using the levels of SOD and Catalase, the level of oxidative destruction using MDA, and the ability of the host to resist antioxidant damage using FRAP.

Superoxide Dismutase serves as the first line of defence against oxidative stress (25). We observed a decrease in the activity of SOD in the serum of the infected patients compared to the uninfected group. The pupils with Ascariasis had significantly lower SOD activity than those with mild Schistosomiasis and Trichuriasis. Helminth infections stimulate the immune system to produce H_2O_2 which is usually a product of SOD enzymatic activity; accumulation of H_2O_2 during oxidative metabolism may lead to a decrease in the activity of SOD probably through feedback inhibition (26).
The enzyme catalase breaks down hydrogen peroxide to water and molecular oxygen. Our results show a significantly lower catalase activity in the serum of all those who had *S. mansoni*, compared to those with STH and the uninfected group. STH infection does not seem to affect the activity of catalase. In contrast Schistosomiasis seems to cause a decrease of catalase activity. This is possibly due to the effect of Schistosomiasis on the liver, which is a major source of catalase; Schistosomiasis is known to induce liver fibrosis. Schistosomes are also known to feed on red blood cells that are a source of catalase. There was no difference in catalase activity between those with Schistosomiasis and those with co-infections. This suggests that the decrease in catalase activity of those with co-infections may be mainly due to Schistosomiasis. The decrease in catalase activity correlated with the parasite load.

*Malondialdehyde (MDA) is a product of lipid peroxidation, and a measure of the state of oxidative stress. We had significantly higher MDA levels in those with Schistosomiasis. Those with STH had higher MDA levels than the uninfected group, but the difference was not significant. Various studies have reported an increase in MDA levels in helminthes infections (3, 4, 27), and in other infectious diseases which affect the liver like Hepatitis C (**28**).*

Ferric Reducing Antioxidant Power (FRAP) is a method that measures the combined anti-oxidant effect of non-enzyme defences in the serum. It measures the ability of the host to resist oxidative damage. The FRAP levels were lowest in those with co-infections, followed by the Schistosomiasis group, then the STH group. All pupils infected with *Schistosoma* had significantly lower FRAP levels than the uninfected group. Those with co-infection had significantly lower FRAP values than those with STH, but not those with Schistosomiasis only. The degree of variation did not correlate with parasite load.
In general the levels of oxidative stress marker (MDA) was higher in infected pupils than in uninfected, while the level of anti-oxidants (SOD, catalase and FRAP) were higher in uninfected pupils than in the infected.
The immediate impact of parasitic tissue penetration is free radical generation by the host's leucocytes (29, 30). Indeed, when the B cell is presented with the antigen of a pathogen, it is activated and divides to increase its population, and becomes a plasma cell that produces large amounts of antibody specific to the antigen. The antibody so produced may bind to the pathogen and neutralize it; binding to the pathogen triggers the complement system which uses neutrophils and activated macrophages to identify pathogen to attack and eliminate it. Reactive oxygen species are produced during these processes.
Reactive oxygen species (ROS) and free radicals that cause oxidative stress play both a positive and negative role in the host (31, 32). This is because the same reactive species that are used by white blood cells to destroy pathogens in a controlled manner can also damage host lipids, proteins, and nucleic acids. However, the host's extensive antioxidant enzyme system acts to scavenge and to eradicate free radicals released during the host's defense by the immune system (30, 33). We show in this study that the host's enzyme system seems to be negatively affected in helminthes infections, thus exposing the host to the damaging effects of oxidative stress.
Parasitic co-infections are common in persons that live in poor sanitary conditions in developing countries (13,34). Since helminthes produce chronic infections, sequential, acute phases for each parasite probably results in concurrent chronic phases that produce simultaneous immune actions in the host. The host immune response against the parasites probably adapts to the host immune response to permit such co-parasitism, because such hosts have been shown to mount immune responses that differ from the responses observed during single parasite infections (35).
S. mansoni, *A. lumbricoides*, and *T. Trichiura* are known to be strong T helper-type 2 (Th2) cytokine inducers and produce similar immunoglobulin responses and expression of the Th2 cytokines (interleukin-4, interleukin-5, and interleukin-10) (36, 37, 38, 39). It has been suggested that damage to the liver during *S. mansoni* murine Schistosomiasis results

from oxidative stress produced in the liver (40); IL-4 seems to play a protective role by controlling the tight regulation of generation of such oxygen and nitrogen intermediates in the liver (41). The high oxidative stress levels shown by our results are probably already downgraded by the IL-4 produced in reaction to the infections. Further, humans co-infected with *A. lumbricoides* and *S. mansoni*, have been reported to have lower intensities for each parasite than in individuals with mono-infections (39), probably suggesting a positive effect of higher oxidative stress status of co-infected individuals.

In studies in Nyomo village, and Ayéné village in the Centre Region of Cameroon where *T. trichiura* and *A. lumbricoides* are endemic, immune responses were shown to be Th2 mediated, but the two parasites were shown to be differently affected by Th2 immune responses (37, 42, 38, 43, 44). It is possible that differences in the oxidative stress generated by the infections as reported in our results could account for these differences. Further studies are needed to examine the consequences of the oxidative stress status reported in our results on the handling of parasites by the human host.

CONCLUSION:

Our research hypothesis was that: "There is an increase in oxidative stress markers in school children with co-infections of *Schistosoma mansoni* and STH." We have shown that helminth infections seem to result in the production of oxidative stress. This probably occurs through the induction of a decrease in antioxidant defense mechanisms of the human host. The level of oxidative stress seems to depend on the type of parasitic infection. Children with co-infections produce more oxidant-antioxidant imbalance. There is usually cooperation between cytoprotective enzymes and antioxidants for scavenging of reactive oxygen species. Disturbance of this cooperation by helminthes may involve many different biochemical and immunological factors. In co-infections, the immune system probably mounts specific responses that differ from those observed in single parasitic infections, resulting in differing levels of oxidative stress. More studies are required to delineate the mechanisms involved, including determining the effect of treatment of the helminthes on the oxidative stress parameters.

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