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Oxidative stress–driven red blood cell damage underlies anemia in typhoid fever patients

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Abstract: Typhoid fever is a systemic infectious disease associated with considerable morbidity and occasional mortality. Although *Salmonella* toxins are recognized as key determinants of disease pathology, the contribution of oxidative stress to red blood cell damage and hematological abnormalities in typhoid fever has not been fully elucidated. This study evaluated oxidative stress status and its association with hematological parameters in patients with typhoid fever. Patients with clinical suspicion of typhoid fever were screened using the Typhidot IgM/IgG qualitative enzyme immunoassay. Serum samples from one hundred and two (102) confirmed cases were analyzed for lipid peroxidation using malondialdehyde enzyme immunoassay and for total antioxidant potential using enzyme immunoassay. Full blood count analysis was performed for all participants, and thirty apparently healthy individuals served as controls. Typhoid patients showed significantly reduced mean values of red blood cell count, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, and lymphocyte percentage compared with controls (all $p < 0.05$). In contrast, white blood cell count and granulocyte percentage were significantly elevated ($p < 0.05$). Circulating free radical levels were markedly higher in typhoid patients than in controls (8.14 ± 1.00 vs 3.31 ± 0.44; $p < 0.001$), while total antioxidant potential was significantly lower ($p < 0.001$). Several hematological indices demonstrated strong negative correlations with free radical levels, indicating an association between oxidative stress and red blood cell impairment. These findings suggest that typhoid fever is characterized by a pronounced oxidative imbalance, reflected by increased lipid peroxidation and reduced antioxidant defenses. Beyond the established pathogenic effects of *Salmonella* toxins, oxidative stress appears to be an important contributory factor to red blood cell damage and hematological disturbances associated with typhoid-related morbidity. Further studies are warranted to clarify the mechanistic role of oxidative injury and to assess the potential benefit of antioxidant-based adjunctive therapeutic strategies in typhoid fever.

Keywords: Typhoid fever; Oxidative stress; Red blood cell damage; Malondialdehyde; Antioxidant status.

INTRODUCTION

Typhoid fever, caused by Salmonella typhi, is widely recognized as a major public health problem in many developing countries [1]. Perforation of the affected persons' intestinal walls by Salmonella typhi organisms is one of the most critical manifestations of typhoid fever [2]. While there are many serovars of Salmonella, the disease in humans is caused by a group of related serovars commonly referred to as the 'typhoidal Salmonellae', of which Salmonella typhi is the chief cause [3,4].

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Globally, typhoid cases have become astronomical with 9 million illnesses and 110,000 deaths per year [5]. Typhoid incidence varies by place and time, between and within countries; and although the disease cuts across all ages, severe cases are more rampant among children below 2 years of age [6]. It is endemic in Africa, Sub-Saharan Africa (especially Nigeria) [1]. In Nigeria, typhoid fever positivity rates were put at 2.4%, 10.4% and 18.6% in Abuja, Kano and Lagos respectively; among patients with febrile illness [1]. Relatively lower figures were found in Ethiopian and Ghanaian cities [7]. Several factors including inadequate access to safe water supply and safe sanitation contributed to these alarming figures. Added to this are lifestyle patterns of patronizing unhygienic street food vendors by people living in some developing countries [7].

S. typhi, the causative organism of typhoid may be found in the bloodstream and intestinal tract of sufferers [8]. Although there are many Salmonella strains, S. typhi is the most lethal, as it possesses the Vi antigen, a virulence factor that enables it to survive within the host cells [9]. S. typhi is uniquely adapted to humans and infection may be propagated through carriers. A group of carriers are known as convalescent carriers serving as the sole source of these organisms for a short period of time, while the chronic carriers are the ones who shed the organism for longer than 1 year [8]. Clinical diagnosis of typhoid fever based on signs and symptoms may be misleading since these are nonspecific [10]. In fact, the clinical features of typhoid fever may vary markedly in different geographic locations and hosts [11] and may simulate those of other diseases. There is thus no consistency in its clinical features [12]. As a result of this, classical descriptions may be difficult to apply to all cases, which vary from the mildest to the most critical cases [13].

Consequently, clinical suspicion of typhoid fever requires confirmation by appropriate laboratory investigations. Cultures of blood and bone marrow aspirate can provide the definitive diagnosis of typhoid fever while cultures of rectal swabs, stools or urine are less definitive, although these may also be positive in chronic carriers [13,1]. The recovery rate of Salmonella in blood culture is however low. As a result of this, serological investigations have often been carried out for laboratory diagnosis, although many have questioned the reliability of these methods. Serological tests for typhoid are not only cheap and easily accessible, evidence shows that they are relatively sensitive and reliable, especially when facilities in poor resource nations are considered [14]. Dissemination of the disease is often facilitated by the ability of the organism to replicate within macrophages [15,16].

In addition, Zwadyk, (2012) postulated that typhoid is often established in many cases as a result of the oxidative stress caused by the imbalance between the pro-oxidant and antioxidant states in the body of ill persons at any point in time [8]. When there is enhanced generation of free radicals, toxic products of natural cell metabolism or the poor elimination of these byproducts, commonly consequent to scavenging in the body, the pro-oxidant state may dominate. Radicals like superoxide anion and nitric oxide which together form peroxynitrite, a strong biological oxidant, are produced during the entry of the organism. These oxidative and nitrosative stresses influence the outcome of Salmonella infections. Salmonella organisms have a number of inducible proteins that are able to detoxify these highly reactive species, thus enabling them to survive within the host and allowing proliferation within immune cells such as macrophages [17,18].

The process of obtaining energy by the oxidation of carbohydrates, fats and proteins generally leads to the generation of free radicals. Since red blood cell membranes are made up of unsaturated lipids, their membrane molecules are easily prone to free radical damage. The presence in blood of methylmalonyl acid (MDA), an end product of lipid peroxidation caused by free radicals, limits the function of red cell membrane phospholipids [19]. RBC membrane protein damage is therefore responsible for the consequential impaired cellular deformability associated with oxidative stress. Aside this, typhoid fever patients placed on antibiotic drugs may have enhanced free radical generation that elevates the level

of oxidant parameters, since the drugs are concerned with the enzymes of oxidative stress-xanthine oxidase (XO), glutathione reductase (GR) and glutathione peroxidase (GPX) [10].

The imbalance caused by overproduction of free radicals is responsible for cellular damage and tissue injury in affected persons, particularly in those instances where the level of free radicals exceeds the available antioxidant levels. The molecular consequences include damage to the cell's DNA and improper delivery of message signals to the body's cells in various disease conditions. Although only bone marrow precursor erythrocytes have nuclei, such damage may lead to maturation arrest in some of the precursor cells, thereby decreasing the net number of erythrocytes available for release to the peripheral circulation. Some of the damage caused by free radicals lead to pathologic states among which is cellular damage of mature red blood cells [20]. Naturally, healthy individuals are capable of limiting the effect of reactive oxygen species (ROS)- and reactive nitrogen species (RNS)-induced free radicals from causing cell damage when anti-oxidants neutralize or inhibit the free radicals, thus preventing the destructive activity of oxidants on intact cells [21,22].

By molecular analysis, Galan (2016), proved that typhoidal toxins play a central role in the pathology of typhoid disease. However, there appears to be other factors involved in the morbidity seen in this debilitating disease. For instance, some studies have shown decreases in blood cellular counts and evidence of bone marrow suppression in typhoid cases [23,24,25]. Despite the continuous exposure of red blood cells (RBCs) to both endogenous and exogenous sources of free radicals like superoxide anion and hydrogen peroxide [26,27], the RBC is powered with an antioxidant system consisting of both non-enzymatic and enzymatic antioxidants [10,27] able to mop up the free radicals constantly being generated. Increased levels of heme degradation products (HDP) which some workers have used as a measure of RBC oxidative stress are detected during cellular aging and in various diseases [28]. HDP and RBC deformability studies have established the contribution of RBC oxidative stress to cellular stiffness and impaired deformability [28].

Inability of the RBC to deform and easily squeeze itself through the microcapillary vessel leads to further damage to its membrane as it traverses the narrow blood vessels of the microcirculation. The same oxidative impact is also responsible for the uptake of RBCs by macrophages that remove RBCs from circulation, following calpain activation, a process that also contributes to the level of oxidative stress. It is therefore obvious that RBC oxidative stress is significantly involved in inducing RBC aging [29] which also reduces the cells' life span. It can then be suggested that this process depreciates the RBC in quality and quantity.

While it is established that bacterial factors and especially Salmonella toxins are responsible for morbidity in typhoid disease [3], there is reason to believe that the organism's toxin alone is not responsible for all the pathological consequences commonly observed in sufferers and particularly those that impact red blood cellular damage; hence the motivation to examine other determinants. In this study, our goal is to assess the level of red blood cell oxidative stress and its impact on erythrocyte health in typhoid disease.

MATERIAL AND METHOD

Study Design and Ethical Approval

This hospital-based cross-sectional study was conducted in Ilorin metropolis, Kwara State, Nigeria. A total of 132 participants were enrolled, comprising 102 patients with clinically suspected typhoid fever and 30 apparently healthy controls. Ethical approval was obtained from the Health Research Ethics Committee of the Kwara State Ministry of Health, Ilorin (Approval No. MOH/KS/EU/777/214). The study was conducted in accordance with the Declaration of Helsinki, and written informed consent was obtained from all participants prior to enrollment.

Study Population

Patients of both sexes presenting with symptoms suggestive of typhoid fever at three major hospitals in Ilorin were recruited consecutively and confirmed serologically at presentation. Individuals with known chronic diseases, inflammatory conditions, or disorders associated with oxidative stress were excluded.

The control group consisted of age- and sex-matched apparently healthy adults recruited from hospital staff, all of whom tested negative for typhoid fever using the same diagnostic method. Individuals with any form of acute or chronic illness were excluded from the control group.

Sample Size Determination

Sample size was calculated using the formula described by Araoye[30] for populations exceeding 10,000, based on an estimated typhoid prevalence of 7% in Kwara State as reported by the Kwara State Community Health Insurance Scheme[31], a confidence level of 95%, and a margin of error of 5%. The minimum sample size obtained was 100 participants. To improve statistical power and account for possible attrition, a total of 132 participants were recruited. *The detailed sample size formula and computation are provided in Supplementary Material S1.*

Sample Collection and Hematological Analysis

Venous blood samples (5 mL) were collected into plain tubes for serological and oxidative stress assays, and 2 mL into EDTA tubes for hematological analysis. Serum was separated by centrifugation at 3000 rpm for 5 minutes. Thin blood films were examined microscopically for red blood cell morphology as a quality control measure. Full blood count parameters were analyzed using a Sysmex automated hematology analyzer.

Typhoid Serology

Typhoid fever was assessed using the TYPHIDOT Rapid IgG/IgM (Combo)™ test (Malaysian Biodiagnostics research Sdn. Bhd., Malaysia), an immunochromatographic assay for the qualitative detection of IgM and IgG antibodies against *Salmonella enterica* serovar Typhi outer membrane protein antigens. Test procedures and interpretation were performed in accordance with the manufacturer's instructions.

Assessment of Oxidative Stress and Antioxidant Status

Lipid peroxidation was evaluated by measuring malondialdehyde (MDA) using the thiobarbituric acid reactive substances (TBARS) method[32]. Total antioxidant potential (TAP) was determined using the ferric reducing antioxidant power (FRAP) assay as described by Benzie and Strain[33]. *Detailed reagent composition, assay procedures, calibration standards, and calculation formulas for MDA and FRAP analyses are provided in Supplementary Material S2.*

Statistical Analysis

Statistical analysis was performed using SPSS version 16.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation. Independent-sample *t* tests were used to compare parameters between typhoid cases and controls. Linear regression analysis was applied to assess associations between hematological indices and oxidative stress markers. A p value < 0.05 was considered statistically significant.

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RESULTS AND DISCUSSION

Table 1 showed the socio-demographic attributes of the study participants. The age distribution exhibited no significant difference between typhoid cases and controls (χ^2 test, p = 0.726), and the mean age was similar across groups (23.8 ± 13.9 vs 26.4 ± 15.8 years; independent-sample t test, p = 0.462). The gender distribution was comparable (47.1% vs 46.7% male; χ^2 test, p = 0.973), and no statistically significant difference in ethnicity was seen between cases and controls (χ^2 test, p = 0.457). Conversely, socioeconomic level exhibited a significant

disparity between the groups (p = 0.031), with a markedly greater percentage of public servants in the control group (80.0%) and a predominance of craftsmen among typhoid cases (59.8%). Table 1 demonstrates that cases and controls were generally comparable in essential demographic variables (age, gender, and ethnicity), although socioeconomic level varied between the groups.

Table 1. Socio-demographic characteristics of study participants

Variable	Category	Typhoid cases (n = 102)	Controls (n = 30)	*p* value
Age group (years)	≤10	21 (20.6)	5 (16.7)	0.726
	11–20	20 (19.6)	6 (20.0)	
	21–30	41 (40.2)	12 (40.0)	
	31–40	12 (11.8)	2 (6.7)	
	>40	8 (7.8)	5 (16.6)	
	Mean ± SD	23.8 ± 13.9	26.4 ± 15.8	0.462
Gender	Male	48 (47.1)	14 (46.7)	0.973
	Female	54 (52.9)	16 (53.3)	
Socioeconomic status	Civil servants	18 (17.7)	24 (80.0)	0.031*
	Artisans	61 (59.8)	0 (0.0)	
	Traders	20 (19.6)	1 (3.3)	
	Students	3 (2.9)	5 (16.7)	
Ethnicity	Yoruba	72 (70.6)	21 (70.0)	0.457
	Fulani	20 (19.6)	5 (16.7)	
	Nupe	5 (4.9)	2 (6.7)	
	Ebira	3 (2.9)	1 (3.3)	
	Igbo	2 (2.0)	0 (0.0)	
	Mixed parentage	0 (0.0)	1 (3.3)	

The demographic similarity among groups indicates that the observed differences in hematological and oxidative stress markers are likely due to disease-related processes rather than age, sex, or ethnicity. The identified socioeconomic disparity may indicate inherent variations in exposure risk (e.g., access to water and sanitation or food hygiene) and should be regarded as a possible source of selection bias when analyzing subsequent correlations. Having established baseline comparability for major demographic variables (Table 1), we next compared hematological parameters between typhoid cases and controls to characterize disease-associated blood profile alterations (Table 2).

Table 2. Comparison of hematological parameters between typhoid cases and controls

Parameter	Typhoid cases (n = 102) Mean ± SD	Controls (n = 30) Mean ± SD	*p* value
RBC (×10¹²/L)	3.50 ± 0.52	4.65 ± 1.06	<0.001
Hemoglobin, HGB (g/dL)	10.11 ± 0.87	12.47 ± 2.15	<0.001
Hematocrit, HCT (%)	32.81 ± 5.16	39.49 ± 7.38	<0.001
Mean corpuscular volume, MCV (fL)	60.50 ± 7.16	75.75 ± 13.59	<0.001
Mean corpuscular hemoglobin, MCH (pg)	24.10 ± 1.59	25.99 ± 2.41	<0.001
Mean corpuscular hemoglobin concentration, MCHC (g/dL)	25.63 ± 1.23	27.37 ± 2.18	<0.001
Red cell distribution width, RDW (%)	13.08 ± 2.54	15.96 ± 2.72	<0.001
White blood cell count, WBC (×10⁹/L)	9.94 ± 2.71	8.12 ± 4.26	0.039
Absolute lymphocyte count, LYM (×10⁹/L)	2.05 ± 0.63	2.59 ± 1.31	0.038
Mixed leukocytes, MID (×10⁹/L)	1.19 ± 0.50	1.04 ± 0.72	0.300
Granulocyte count, GRA (×10⁹/L)	2.75 ± 0.46	2.48 ± 1.82	0.431
Lymphocyte percentage, LY (%)	38.34 ± 11.75	36.44 ± 9.29	0.423
Mixed leukocyte percentage, MI (%)	12.33 ± 4.06	9.77 ± 1.74	0.002

Granulocyte percentage, GR (%)	48.04 ± 11.73	40.42 ± 11.28	0.005

Note: *Data are presented as mean ± standard deviation. Independent-sample* **t** *test was used for all comparisons.* A p value < 0.05 was considered statistically significant.

Table 2 presents a comparison of hematological parameters between typhoid patients and seemingly healthy controls. Patients with typhoid demonstrated markedly reduced mean values for red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and red cell distribution width in comparison to controls (all $p < 0.001$).

Conversely, the total white blood cell count, mixed leukocyte percentage, and granulocyte percentage were markedly elevated in typhoid cases ($p < 0.05$). The absolute lymphocyte count was dramatically reduced in typhoid patients, although no significant variations were noted in the absolute counts of mixed leukocytes and granulocytes.

The observed decreases in erythrocyte-related parameters signify anemia in typhoid patients. Considering the absence of notable disparities in age, gender, and ethnicity between patients and controls (Table 1), these hematological abnormalities appear improbable to be ascribed to demographic factors.

The conjunction of diminished red cell indices and heightened inflammatory leukocyte proportions indicates that typhoid fever correlates with both compromised erythrocyte integrity and a vigorous inflammatory response. The data establish a hematological foundation for investigating oxidative stress as a possible contributing mechanism, further analyzed by the evaluation of malondialdehyde and total antioxidant capability in Table 3.

Given the significant alterations observed in erythrocyte indices among typhoid patients, oxidative stress markers were subsequently evaluated to explore their potential role in red blood cell damage and disease-related morbidity (Table 3).

Table 3. Comparison of oxidative stress and antioxidant parameters between typhoid cases and controls

Parameter	**Typhoid cases** (n = 102) Mean ± SD	**Controls** (n = 30) Mean ± SD	***p* value**
Malondialdehyde, MDA	8.14 ± 1.00	3.31 ± 0.44	<0.001
Total antioxidant potential, TAP	448.35 ± 95.72	752.77 ± 52.29	<0.001

Data are presented as mean ± standard deviation. Independent-sample **t** *test was used for comparisons.*A p value < 0.05 was considered statistically significant.

Table 3 contrasts indicators of oxidative stress and antioxidant levels between typhoid patients and seemingly healthy controls. The mean serum malondialdehyde (MDA) content was considerably elevated in typhoid patients compared to controls ($p < 0.001$), suggesting enhanced lipid peroxidation. Total antioxidant potential (TAP) was considerably reduced in typhoid patients compared to controls ($p < 0.001$).

The average MDA level in typhoid patients was over twice that of the control group, although TAP readings decreased by roughly 40%, indicating a significant systemic oxidative imbalance. The concurrent increase of MDA and reduction of antioxidant capacity indicates a significant transition to a pro-oxidant state in typhoid illness. When analyzed in conjunction with the hematological data in Table 2, this oxidative imbalance offers a credible molecular rationale for the noted declines in erythrocyte parameters, considering the vulnerability of red blood cell membranes to lipid peroxidation. These findings advocate for additional investigation into the association between oxidative stress and hematological changes, as analyzed through the relationships among MDA, TAP, and blood parameters presented in Table 4.

Table 4. Correlation between malondialdehyde levels and hematological parameters in typhoid cases and controls

Parameter	Typhoid cases (n = 102) *r*	*p* value	Controls (n = 30) *r*	*p* value
RBC (×10¹²/L)	−0.431	0.002	−0.450	0.013
Hemoglobin, HGB (g/dL)	−0.274	0.002	−0.107	0.573
Hematocrit, HCT (%)	−0.379	0.006	−0.357	0.053
Mean corpuscular volume, MCV (fL)	−0.167	0.237	−0.194	0.305
Mean corpuscular hemoglobin, MCH (pg)	−0.293	0.037	−0.379	0.039
Mean corpuscular hemoglobin concentration, MCHC (g/dL)	−0.282	0.045	−0.083	0.665
Red cell distribution width, RDW (%)	0.092	0.520	−0.044	0.816
White blood cell count, WBC (×10⁹/L)	0.040	0.781	0.220	0.244
Absolute lymphocyte count, LYM (×10⁹/L)	−0.226	0.111	0.126	0.508
Mixed leukocytes, MID (×10⁹/L)	0.092	0.520	0.174	0.356
Granulocyte count, GRA (×10⁹/L)	−0.174	0.223	0.049	0.798
Lymphocyte percentage, LY (%)	0.096	0.501	−0.198	0.293
Mixed leukocyte percentage, MI (%)	−0.003	0.984	0.041	0.828
Granulocyte percentage, GR (%)	0.187	0.189	0.181	0.339

Note: Pearson correlation coefficients (***r***) are shown.*A p value < 0.05 was considered statistically significant.*

Table 4 illustrates the connection between serum malondialdehyde concentrations and hematological markers in typhoid cases compared to controls. In typhoid patients, five erythrocyte-related parameters RBC count, hemoglobin concentration, hematocrit, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration exhibited statistically significant negative relationships with MDA levels ($p < 0.05$).

Conversely, in the control group, only the red blood cell count and mean corpuscular hemoglobin exhibited significant negative connections with malondialdehyde, and these relationships were less pronounced than those found in typhoid cases. The notable negative relationships between MDA and essential erythrocyte markers in typhoid cases suggest that heightened oxidative stress correlates with the deterioration of red blood cell integrity. The higher MDA levels in Table 3, combined with the anemia-related hematological abnormalities in Table 2, substantiate a disease-specific role of oxidative stress in red blood cell destruction in typhoid fever.

Table 5. Correlation between total antioxidant potential and hematological parameters in typhoid cases and controls

Parameter	Typhoid cases (n = 102) *r*	*p* value	Controls (n = 30) *r*	*p* value
RBC (×10¹²/L)	−0.232	0.101	−0.140	0.461
Hemoglobin, HGB (g/dL)	−0.238	0.093	−0.166	0.381
Hematocrit, HCT (%)	−0.156	0.274	−0.211	0.263
Mean corpuscular volume, MCV (fL)	−0.152	0.286	−0.250	0.180
Mean corpuscular hemoglobin, MCH (pg)	−0.192	0.176	−0.152	0.424
Mean corpuscular hemoglobin concentration, MCHC (g/dL)	−0.109	0.448	−0.507	0.004
Red cell distribution width, RDW (%)	−0.200	0.159	−0.217	0.249
White blood cell count, WBC (×10⁹/L)	−0.011	0.938	−0.076	0.690

Absolute lymphocyte count, LYM (×10⁹/L)	−0.098	0.495	0.078	0.683
Mixed leukocytes, MID (×10⁹/L)	−0.319	0.022	0.115	0.544
Granulocyte count, GRA (×10⁹/L)	−0.220	0.120	0.325	0.079
Lymphocyte percentage, LY (%)	0.566	<0.001	0.228	0.226
Mixed leukocyte percentage, MI (%)	−0.165	0.246	0.147	0.438
Granulocyte percentage, GR (%)	−0.212	0.135	−0.263	0.160

Note: Pearson correlation coefficients (***r***) are shown. *A p value < 0.05 was considered statistically significant.*

Table 5 illustrates the connection between total antioxidant potential and hematological markers in typhoid cases and controls. In typhoid patients, the absolute mixed leukocyte count exhibited a substantial negative connection with TAP ($r = -0.319$, $p = 0.022$), whereas the lymphocyte percentage revealed a robust positive correlation ($r = 0.566$, $p < 0.001$). No substantial associations were detected between TAP and erythrocyte-related indicators in cases with typhoid.

Among the controls, only the mean corpuscular hemoglobin concentration exhibited a significant negative connection with TAP ($r = -0.507$, $p = 0.004$), whereas other hematological measures did not reveal statistically significant relationships. The restricted quantity of notable relationships between TAP and erythrocyte indices indicates that antioxidant depletion alone insufficiently accounts for the red blood cell abnormalities seen in typhoid illness. When analyzed in conjunction with the pronounced negative correlations between malondialdehyde and erythrocyte parameters (Table 4), these results suggest that excessive pro-oxidant activity, rather than inadequate antioxidant capacity alone, is the primary factor contributing to red blood cell damage in typhoid disease.

The robust positive correlation between TAP and lymphocyte percentage may indicate a compensatory immune response or a selective maintenance of antioxidant capacity in lymphocyte-dominant profiles during infection. The association between oxidative stress and antioxidant status was illustrated by a scatter plot, revealing a weak yet statistically significant positive correlation between malondialdehyde and total antioxidant potential in typhoid cases (Figure 1).

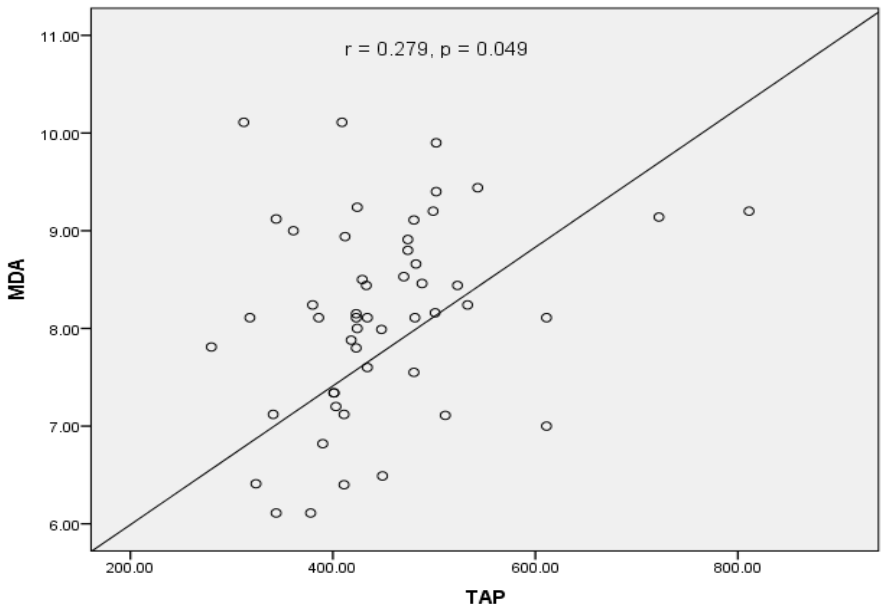

Figure 1. Relationship between malondialdehyde and total antioxidant potential in typhoid cases.

Scatter plot illustrating the relationship between malondialdehyde (MDA) and total antioxidant potential (TAP) among typhoid cases (n = 102). A weak but statistically significant positive correlation was observed ($r = 0.279$, $p = 0.049$). The solid line represents the linear regression fit.

Enteric fever, caused by Salmonella spp., including typhoid fever, is a human-specific infection that can advance to severe illness with considerable morbidity and mortality. This study establishes that, in addition to typhoid toxin's role in disease pathology through bone marrow suppression and direct cellular toxicity, oxidative stress is a significant and clinically relevant factor contributing to red blood cell damage and anemia in typhoid fever.

The study population consisted of 102 typhoid patients and age- and sex-matched controls exhibiting similar demographic features. No statistically significant variations were detected in age, gender, or ethnicity across the groups. Socioeconomic status exhibited significant variation, with a majority of artisans and traders among typhoid cases, while civil servants constituted the control group, possibly indicating disparities in exposure risk associated with sanitation, water quality, and food hygiene rather than biological vulnerability.

Socioeconomic status differs between the test and control subjects. Most of the control subjects are literate civil servants who are middle-income earners. On the other hand, the majority of test subjects are artisans and traders whose income is dependent on level of patronage, although a small percentage of them are also civil servants. Both the test and control subjects have diverse ethnic backgrounds since the hospitals serve all citizens of the state. The staff who constituted the control subjects similarly come from diverse ethnic origins. There was no significant difference in the ethnicity of participants.

Typhoid cases exhibited significantly lower values in several red blood cell parameters and higher values in certain white blood cell parameters compared to controls. The red blood cell parameters include: Red blood cell (RBC) count, haemoglobin estimation (HGB), haematocrit (HCT) and the red cell indices – Mean Cell volume (MCV), Mean Cell Haemoglobin (MCH) Mean Cell Haemoglobin Concentration (MCHC) and Red Cell Diameter Width (RCDW). Similar results were obtained by Ndako et al., (2020) and Anusuya and Sumathi (2021). Virtually all these red blood cell diagnostic parameters reflect the haemoglobin status of the erythrocytes in the individual at any point in time. The differences observed in these parameters suggest potential impacts of typhoid fever on hematological profiles. Decrease in red blood cell related values in our study may be due to increased removal of damaged cells from the circulation. Ndako et al., (2020) as well as Etouke et al., (2023) have also reported decreased levels of certain red blood cell parameters among typhoid patients.

The altered haematological parameters have been attributed to bone marrow suppression and hemophagocytosis in typhoid patients in these studies. Bone marrow suppression affects blood cell numbers in that it is accompanied by reduced haemopoiesis while haemophagocytosis results in the destruction of erythrocytes. While these are the established mechanisms by which red cell parameters are decreased in this condition, it is also known that fatty acid-rich red blood cells with their reactive methylene groups are susceptible to hydrogen atom abstraction; and when these fatty acid molecules on RBCs are affected by lipid peroxidation, MDA levels increase, resulting in decreased erythrocyte deformability and membrane fluidity [19]. It is worthy of mention that once RBCs lose their structural integrity, they are prone to early removal from the circulation. The results here show depreciation in the values of both the haematocrit and haemoglobin.

The mean values of most of the parameters indicate that there is anaemia in the affected patients. Anaemia in this case would have been induced by (i), ineffective bone marrow function and (ii), persistent haemolysis of available erythrocytes by haemophagocytosis. In addition to these, there is free radical-induced damage to red blood cells. Cells are damaged when free radicals interact with cell membranes. Pizzino et al. (2017) posited that red blood cells (RBCs) are exposed to endogenous and exogenous oxidants, commonly referred to as

reactive oxygen species (ROS) and reactive nitrogen species (RNS). Normally, reactive oxygen species (ROS) generated from different sources are neutralized by the endogenous RBC antioxidant system consisting of both enzymatic and non-enzymatic antioxidants including catalase, glutathione, peroxidase and peroxiredoxin-2 [35]. Unfortunately, the increased affinity of partially oxygenated hemoglobin for the red cell membrane inhibits the essentially cytosolic anti-oxidant system from effectively neutralizing the free radicals formed at the red cell membrane. The remaining un-neutralized ROS in the RBC has been shown to damage the RBC membrane [36,35]. Damage to RBC's membrane contributes to RBC uptake by macrophages and to its subsequent removal from circulation, thus resulting in low values of red blood cell parameters.

Typhoid cases showed significantly higher levels of malondialdehyde (MDA), a marker of oxidative stress, and lower levels of total antioxidant potential (TAP) compared to controls. The findings suggest an imbalance between pro-oxidants and antioxidants in typhoid fever patients, potentially contributing to oxidative damage. From the data presented here, it is obvious that most RBCs do not live up to their average 120 days life span once they are affected by free radical formation in typhoid disease. Thus, a significant loss of RBCs is due to the presence of excessive reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated by the typhoidal disease process, as can be seen from our results. Since only MDA was assayed among many potential oxidants in the subjects, the level of oxidants may even be more than is presented in this report. Rajakeshariah, (2022) stated that a major contributing factor to the decreasing lifespan of the erythrocytes could be a decrease in the antioxidant defence system or an increase in oxidative stress. In this study, both conditions are present, showing that the damage induced by oxidants has affected all aspects of red cell health.

Significant correlations were observed between free radical levels and certain haematological parameters in typhoid cases and controls, indicating potential associations between oxidative stress and hematological alterations. Out of the seven red blood cell parameters, five (RBC count, HGB, HCT, MCH and MCHC) showed significantly strong negative correlation with the level of free radicals in blood, indicating that changes in the erythrocyte values seen between the typhoid cases and the normal group are attributable to free radical-induced damage to red blood cells. This implies that as the free radicals level increases, the five red cell parameters decrease, culminating in anaemia and complicating the disease condition. Thus, the anaemia witnessed in typhoid disease is partly due to free radical damage to red blood cells. Free radicals affect erythrocytes by diffusing across the lipid-rich fraction of cellular membranes into the cytosol and altering the homeostasis [22].

Three mechanisms are involved in the process of free radical damage to cells: alteration of calcium homeostasis, caspase-3 activation and band-3 clustering. However, in the case of erythrocytes, it has been found that calpain, not caspase, is activated. Following free radical diffusion across the erythrocyte membrane, altered calcium homeostasis begins with increased membrane permeability, leading to intracellular calcium ion accumulation, progression to red cell fragility and subsequent clearance of affected cells from the circulation [37,38]. Intracellular calcium activates the Gardos channels resulting in the loss of cell content by osmosis and thereafter, cell shrinkage occurs [38].

In addition, increased concentration of free intracellular calcium activates calpains (calcium-activated proteases). Calpains target cytoskeleton proteins for degradation, a phenomenon that leads to membrane blebbing and subsequent removal of affected cells. Clustering of band 3 enhances deposition of complement C3 and subsequent binding of autoantibodies that may be present in serum. Antibody-coated red cells are then cleared by macrophages[39,22]. These

phenomena result in haemolysis with a consequence that red blood cell parameters are reduced.

For red blood cells to function effectively, they must be flexible enough to deform and squeeze themselves through the narrow blood capillary vessels. Oxidative damage has been known to contribute to decreased deformability and consequently, impaired oxygen delivery as well as red cell accelerated aging [29,22]. Hemoglobin itself is a key generator of oxidative stress within the erythrocyte. Partially oxygenated haemoglobin molecules that have altered conformation in the RBCs in microcirculation while transporting oxygen to the tissues, have an elevated affinity for the RBC membrane; and potentially can cause increased auto-oxidation, a condition that is enhanced by hypoxia, consequently producing ROS that are not completely neutralized by the RBC antioxidant system. Hb autoxidation leads to the generation of a superoxide radical, catalyzed further to produce hydrogen peroxide, both of which induce haemichrome formation, haem degradation, and release of free iron [29].

Aside from auto-oxidation, the increased lipid peroxidation as depicted by the high concentration of MDA in this study, may arise from a variety of factors. Since both toxins and oxidative stress from infection are exogenous factors that depreciate the RBC count and other red cell associated values, a gradual reduction in functional haemoglobin level is invariably the consequence, with resultant hypoxia. Some persistent Salmonella strains make use of unique niches called the Salmonella-containing vacuole (SCV) of haemophagocytic cells of the host to survive, as they become immune priviledged within these cells and grow inside of them [40].

The affected host cells then produce reactive oxygen species (ROS, including superoxide anion, hydrogen peroxide, and hydroxyl radical) and reactive nitrogen species (RNS), including nitric oxide and peroxynitrite, so as to limit the microbe's growth and multiplication [41]. This mechanism, in addition to the other processes, may have greatly enhanced the serum concentration of malondialdehyde (MDA) to that level seen in this study. Additionally, the reaction of nitric oxide from endothelial spaces results in the production of peroxynitrite, a highly reactive free radical that can bring about damage to cellular constituents.

The peroxynitrite stress occasioned by the presence of NOS and ROS may be amplified by the flavohemoglobin-induced oxidative stress that is common to certain strains of Salmonella [42,18]. This mechanism is utilized by these organisms to bypass the phagocytes and establish themselves in the host. It can then be inferred that directly or indirectly, the free radicals produced in typhoid disease contribute to the severity of red blood cell damage observed during the infection.

An additional factor that may contribute to oxidative stress in typhoid patients is antibiotic therapy, which has been shown to induce redox-related metabolic perturbations. However, the extent to which antibiotic exposure independently amplifies oxidative damage in this cohort could not be directly assessed and warrants further investigation, to the level of oxidative stress among those typhoid patients undergoing treatment. Dwyer et al. (2014), Belenky et al. and Orrici et al. (2023) showed that antibiotic treatment leads to a build-up of toxic metabolic by products which could impact oxidation of cellular elements.

The cumulative effect of damage from these sources of free radicals is among the factors that contribute to RBC aging and the premature removal of RBCs from the circulation; with a consequent reduction in red blood cell number. It is also pertinent to add that infection with Salmonella and exposure to the by products of its metabolism tends to stimulate erythrophagocytosis of non-senescent RBCs in the host's system [16], a factor that may further lead to decrease in red blood cell parameters. Alterations in the two other RBC parameters not associated with oxidative stress (MCV and RCDW) might be due to the effect of toxins on the bone marrow since toxins are the major factor in typhoid disease manifestation. The typhoid toxin produced by intracellular S.typhi is trafficked out

of the infected host cell to the extracellular milieu to intoxicate other target cells [2]. Thus when Salmonella organisms invade the bone marrow, it would be easy for the entire marrow cells to be colonised via this mechanism. Anusuya and Sumathi, (2021) reported in their study that anaemia in cases of typhoid disease was due to toxin effect and bone marrow suppression.

Our study has also shown that alterations seen in red cell parameters in typhoid cases may not be remedied significantly by the level of antioxidant present in the system since none of the red cell parameters showed any correlation with the level of antioxidant (TAP), an approximate estimate of all the antioxidants in serum. One remarkable observation in the typhoid disease process is that the antioxidant level is decreased among sufferers, thus creating an imbalance between systemic pro-oxidant and antioxidant levels; with consequent anti-oxidant shortfall. The reduction in the level of serum anti-oxidants cannot but be attributed to the recurrent utilization of these antioxidants to mop up free radicals as frequently as they are generated in typhoid disease. This confirms the position of Zwadyk (2012) that the imbalance between pro-oxidants and anti-oxidants is responsible for establishing the disease among sufferers. There are three categories of antioxidants those that prevent the formation of ROS, those that intercept free radical formation and those that repair the damage caused by these free radicals [20].

Although total antioxidant potential was significantly reduced among typhoid cases, its limited correlation with erythrocyte indices suggests that antioxidant depletion alone does not sufficiently counteract excessive free radical generation. The weak but significant positive correlation between malondialdehyde and total antioxidant potential may reflect a compensatory but inadequate antioxidant response under sustained oxidative stress.

An additional finding in our study is the increase in the total leukocyte number among typhoid cases compared to the control, whereas the absolute lymphocyte count is depressed. This finding suggests that while the inflammatory effect of typhoid disease brings about an increase in the total white blood cell count, the absolute numbers of granulocytes and those of mixed leukocytes are not significantly altered; indicating that the lymphocyte subset is the one most impacted by the oxidative damage inflicted on the leukocytes.

This observation points to a situation in which either the production of lymphocytes is suppressed in the bone marrow due to the presence of Salmonella toxins, or attrition of lymphocytes in circulation is enhanced by the damaging effect of free radicals in the same manner that it affects the red blood cells. However, since no correlation exists between MDA level and absolute lymphocyte number, the former is likely to be the case.

Typhoid toxin has selective affinity for cells having Neu5Ac-decorated glycans [34]. Incidentally, lymphocytes and red blood cells are among this category of cells to which it binds. It is therefore safe to assume that the low level of haemoglobin indicators is influenced more by free radical damage to red blood cells in typhoid patients resulting in haemolysis, while bone marrow suppression due to toxins may result in reduced haemopoiesis across board, among its other effects. It is important to state that MDA as a biomarker of inflammation has its limitations because pre-analytical factors may affect its measurement [43].

In addition, the use of total antioxidant potential for assessing the level of antioxidants in food and body fluid may have its flaw in that different available assays used in measurement provide completely different results [44]. More studies with larger enrolment of subjects should establish the exact level of impact that free radical damage in typhoid disease has on red blood cells, especially in causing anaemia among sufferers.

This research possesses numerous limitations. Oxidative stress was evaluated using malondialdehyde as a sole biomarker, which may inadequately represent the intricacies of redox imbalance. Total antioxidant potential offers a

cumulative assessment of antioxidant capacity without differentiating among individual antioxidant constituents. The cross-sectional design prohibits causal inference, and the possible confounding effects of antibiotic medication could not be quantitatively managed.

CONCLUSION

This study illustrates that typhoid fever is linked to pronounced oxidative stress and notable hematological changes, especially for erythrocyte-related parameters. Increased malondialdehyde levels and diminished total antioxidant capacity were correlated with significant negative relationships between oxidative stress and essential red blood cell parameters, suggesting that oxidative damage plays a considerable role in anemia among typhoid patients.

In addition to the recognized function of typhoid toxin in disease morbidity, the findings underscore oxidative stress as a significant and clinically pertinent aspect in the pathophysiology of typhoid fever. The dominance of pro-oxidant activity over antioxidant capacity indicates that red blood cell damage is mostly caused by excessive free radical production rather than solely by antioxidant depletion.

These findings highlight the potential benefit of integrating measures to reduce oxidative stress into the clinical treatment of typhoid fever. Clinicians must recognize the role of oxidative injury in anemia, especially in patients undergoing extended or severe antibiotic treatment, and take this mechanism into account when assessing disease severity and therapeutic efficacy.

AUTHORS' CONTRIBUTIONS

AOI conceptualized and designed the study. ASA conducted the fieldwork and data collection. ASA and KTO performed the data analysis and methodological procedures. RII reviewed and validated the microbiological aspects of the study. All authors (AOI, ASA, KTO, and RII) contributed to the final review of the manuscript and approved the submitted version.

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DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request for academic and research purposes.

DISCLOSURE STATEMENT

The views and opinions expressed in this article are solely those of the authors and do not necessarily reflect the official policies or positions of their affiliated institutions. The data presented in this study are original, were generated by the authors, and have not been previously published or submitted for publication elsewhere.

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