



Original Research

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ASSESSMENT OF MICRONUCLEI FREQUENCY ON LEUCOCYTE IMMUNE RESPONSE FUNCTIONAL INDICES IN PEPTIC ULCER PATIENTS AMONG AL-HIKMAH UNIVERSITY STUDENTS, ILORIN, NIGERIA.

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Olalere FD^{1,2*}, Olatunbosun LO^{1,2}, Shehu FE², Afolabi OO³, Tijani LS⁴

¹Department of Haematology and Blood Transfusion Science, University of Ilorin Teaching Hospital, PMB 1459, Ilorin, Kwara State, Nigeria

²Department of Medical Laboratory Sciences, Faculty of Basic Medical Sciences, College of Health Sciences, Al-Hikmah University, Ilorin, Kwara State, Nigeria

³Department of Pathology, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria

⁴Department of Medical Laboratory Sciences, Nigerian Navy College of Health Sciences, Irra Road, Offa, Kwara State, Nigeria

Corresponding Author*: Olalere FD, Email: dayoolalereone@gmail.com, Phone: +2348055833224

ABSTRACT

Background: Peptic ulcer disease (PUD) remains a significant global health concern due to its association with *Helicobacter pylori* (*H. pylori*) infection, non-steroidal anti-inflammatory drug (NSAID) use, and potential progression to gastric malignancy. This study aimed to assess the frequency of micronuclei (MN), a biomarker of genomic instability and white blood cell (WBC) functional indices among the students' population that were screened and diagnosed of Peptic ulcer disease.

Methods: A population of 40 participants were selected through structured questionnaire screening and clinical diagnosis. Whole blood and stool samples were collected for analysis. Haematological parameters were evaluated using an automated haematology analyser; Mindray BC 5000 and manual differential counts on Leishman-stained peripheral blood smears. Micronuclei frequency was assessed in cultured peripheral blood lymphocytes using the cytokinesis-block micronucleus (CBMN) assay. ABO blood grouping and haemoglobin genotyping used cellulose acetate electrophoresis. *H. pylori* status was determined using a rapid stool antigen test.

Results: There was a statistically significant increase in MN frequency in mononuclear cells with prolonged ulcer duration ($p < 0.001$), rising from 339.17 ± 42.74 cells/ 10^3 (2–9 months) to 473.33 ± 42.74 cells/ 10^3 (3–4 years). No significant increase was observed in binucleated cells ($p > 0.05$). No significant differences in MN frequency were observed across ABO blood groups or haemoglobin genotypes (AA, AS, AC). WBC functional indices showed no significant correlation with MN frequency ($p > 0.05$).

Conclusion: Chronic PUD is associated with cumulative genomic damage, reflected by increased MN frequency over time, independent of inflammatory markers or inherited haematological traits. The study underscores the potential utility of the MN assay as a sensitive, non-invasive biomarker for monitoring long-term genomic instability and cancer risk in PUD patients.

Keywords: Micronuclei frequency; Peptic ulcer disease; White Blood Cells Indices; Haemoglobin Genotype; ABO Blood Grouping; Differential White Blood Cells Count.

INTRODUCTION

Peptic ulcer disease (PUD) is characterized by discontinuity in the inner lining of the gastrointestinal (GI) tract due to gastric acid secretion or pepsin [1]. It extends into the muscularis propria layer of the gastric epithelium [2]. PUD typically occurs in the stomach and proximal duodenum but may also involve the lower esophagus, distal duodenum, or jejunum [3]. Epigastric pain usually occurs within 15–30 minutes after a meal in gastric ulcer patients, while duodenal ulcer pain tends to occur 2–3 hours postprandially [4].

Traditionally, PUD was attributed to increased acid production, diet, and stress. However, *Helicobacter pylori* infection and non-steroidal anti-inflammatory drug (NSAID) use including low-dose aspirin are now recognized as primary aetiologies [5]. Other contributing factors include smoking, alcohol consumption, lower socioeconomic status (poor hygiene and eating habits),

atrophic gastritis, anxiety, prolonged steroid use, vitamin deficiency, Zollinger-Ellison syndrome, multiple endocrine neoplasia (MEN) syndrome, and hyperparathyroidism [6]. Gastric ulcer-specific factors include gastric stasis, mucosal ischemia, and duodenogastric reflux.

The lifetime prevalence of PUD is declining and currently estimated at 5–10% [7], with lower rates in developed countries. Although complication rates have also decreased, bleeding, perforation, and obstruction remain significant causes of morbidity, accounting for approximately 150,000 hospitalizations annually [8]. Upper GI bleeding is the most common complication, followed by perforation, with annual incidence rates ranging from 19–57 per 100,000 individuals for bleeding and 4–14 per 100,000 for perforation. Advanced age is a major risk factor, with 60% of PUD patients being older than 60 years. Importantly, *H. pylori* infection and NSAID use remain the strongest risk factors for bleeding ulcers and perforation. A localized gastric infection can induce chromosomal damage in peripheral blood lymphocytes and mononuclear cells through chronic inflammation, systemic oxidative stress, production of reactive oxygen stress and nitrogen species and impairment of DNA repair mechanisms, resulting in DNA strand breaks and genomic instability detectable in circulating immune cells.

H. Pylori is strongly implicated in gastric carcinogenesis. Strains expressing cytotoxin-associated gene A (CagA) stimulate interleukin-8 production and inflammation [9]. Additionally, *H. pylori* infection enhances reactive oxygen species (ROS) generation by activated macrophages and leukocytes, leading to oxidative DNA damage, micronuclei formation and carcinogenesis [10, 11]. Micronuclei are small, isolated nuclear structures formed from lagging chromosomes or chromosome fragments [12]. They are widely recognized as biomarkers of genotoxicity and tumor progression. Due to their fragile nuclear envelopes, micronuclei often rupture during interphase, releasing DNA into the cytosol, which causes DNA damage and triggers inflammatory responses. This mechanism strongly links micronuclei to cancer development and chronic disease [13]. Therefore, this study was aimed to investigate the relationship between peptic ulcer disease (PUD) and two key biological indicators; micronuclei (MN) frequency, a biomarker of genomic instability and white blood cell (WBC) functional parameters, which reflect systemic inflammatory and immune responses.

MATERIALS AND METHODS

Study Design

This is a cross-sectional, descriptive, cohort-stratified study designed to evaluate the pattern of micronuclei frequency and white blood cells (Leukocytes) functional indices in patients with peptic ulcer among students of Al-Hikmah University, Ilorin, Kwara State, Nigeria. The study assessed the relationship of peptic ulcer disease (PUD) among the students diagnosed with the disease in order to determine possible associations between the disease condition, micronuclei frequency, and White blood cells (Leukocytes) immune response functional characteristics.

Study Population

The study population comprised both undergraduate and postgraduate students of Al-Hikmah University, Ilorin, Kwara State, Nigeria, who were clinically diagnosed with peptic ulcer disease (PUD). A total of 40 subjects were participated in the study. Al-Hikmah University has a student population of over 11,000, drawn from diverse ethnic, cultural, and socioeconomic backgrounds across the country, providing a broad base for health-related research. Both undergraduate students, who make up the majority and are often exposed to risk factors such as poor dietary habits, academic stress, and irregular meal patterns, and postgraduate students, who face combined academic and professional pressures, were included to ensure a comprehensive assessment of the relationship among peptic ulcer disease, micronuclei frequency, and white blood cell functional parameters within the university community.

Informed Consent

Written Informed consent form duly signed was obtained from all the participants.

Ethical Approval

Ethical approval was obtained from the Faculty of Health Sciences Ethical Review Committee of Al-Hikmah University, Ilorin, Kwara State, Nigeria with reference number: HUI-FHS-ERC-25-0033. The design and implementation of the research protocol were in accordance with institutional guidelines and ethical standards for research involving human participants.

Sample and data Collection

A structured questionnaire was administered to all the participants with informed consent to assess their socio-demographic information. Two types of biological samples were obtained from the participants; whole blood and stool samples. A total of 7 ml of venous blood was collected aseptically using a sterile 10 ml syringe. The cubital fossa was disinfected with 70% methyl alcohol, and a tourniquet was applied to the upper arm before drawing blood from the prominent ante-cubital vein. Immediately after collection, the blood was gently mixed to avoid clotting and hemolysis. A volume of 3.5 mL of whole was dispensed into an EDTA anticoagulant bottle for the assessment of white blood cell functional indices, while the remaining 3.5 mL of whole blood was dispensed into a lithium heparin bottle for the evaluation of micronuclei frequency. All blood samples were kept at room temperature (20–25 °C) and analyzed within 2 hours of collection to maintain stability and accuracy of results. Stool samples were collected by providing each participant with a sterile, universal stool container and immediately preserved at 4 °C for laboratory analysis for *H. pylori* detection.

Samples Preparation

The collected blood and stool samples were processed immediately after collection to maintain sample integrity. Blood samples in EDTA bottles were gently inverted to prevent clotting and prepared for hematological analysis, including differential white blood cell counts and functional assays, while blood samples in lithium heparin bottles were centrifuged at 1,500 rpm for 10 minutes to obtain plasma and buffy coat, which were subsequently used for micronuclei assay following standard cytogenetic protocols. Stool samples were homogenized in normal saline to form suspensions and examined microscopically for the presence of *Helicobacter pylori* and other enteric organisms. All samples were handled under aseptic conditions to avoid contamination and ensure reliability of results.

Laboratory Analysis

A spoonful portion of stool sample was taken and mixed with buffer reagent. It was allowed to homogenised with the buffer. The test cassette was placed on a balance surface. Three (3) drops of buffered-stool was placed onto the test cassette according to manufacturer instruction. The result was read after 10 minutes and recorded accordingly for the presence of *Helicobacter pylori* (H. Pylori).

Preparation of the Sample

A volume of 3.5 mLs of whole blood samples collected by venipuncture into lithium heparin sample bottle and kept at room temperature (20–22 °C). Whole blood was diluted with 1:1 with HBSS (20–22 °C) and inverted gently to mix. Diluted blood was overlay onto Ficoll–Paque using a ratio of 1:3 (e.g., 2 ml Ficoll–Paque: 6 ml diluted blood). The tubes were spun at 400g for 30 min at 18–20 °C. Leucocyte layer located at the interface of Ficoll–Paque was removed and diluted with plasma into a fresh tube, using a sterile, plugged, Pasteur pipette. Leucocyte suspension (which includes a mixture of lymphocytes and monocytes) with 3 × its volume of HBSS at room temperature (20–22 °C) and was mixed gently and then centrifuged at 1800g for 10 minutes. The supernatant was discarded and re-suspended the cell pellet (slightly beige color) in 2× the volume removed of HBSS, using a Pasteur pipette, and then centrifuged at 1000g for 10 minutes at 18–20 °C. The supernatant was discarded and gently the cells were re-suspended in 1 ml culture medium at room temperature, using a Pasteur pipette. The cells were counted using the Coulter Counter.

Procedure for Counting of Cells

15 mμL cell suspension was diluted into 15 mL isoton + five drops of Zap-oglobin to lyse any residual red blood cells. The count was done in triplicate to determine the mean. Culture of lymphocytes, addition of Cyt-B and harvesting of cells timing 72 h. Mitotic division of lymphocytes was stimulated by adding Phytohaemagglutinin (PHA). The cells were incubated at 37 °C with lids loose in a humidified atmosphere containing 5% CO₂ for exactly 44 h. Cyt-B was added to culture. The stock was thawed out in a vial containing 100 μl solution of Cyt-B in DMSO at 600 μg ml⁻¹. Aseptically, 900 μl of culture medium was added equilibrated to room temperature to the vial. 56 μl of medium was removed from the top of the 750 μl culture and replace with 56 μl of the 60 μg ml⁻¹ Cyt-B solution. Cultures were returned to the incubator and incubated for a further 28 h. The remaining Cyt-B solution was discarded. At 24–28 h after addition of Cyt-B, the cells were harvested for slide preparation using Giemsa stain and manual oil-immersion microscopy which were utilized to score the specific cytogenetic endpoints per 1,000 cells.

Method of Statistical analysis

The data were analysed using IBM SPSS Statistics version 26. Descriptive Statistics was used to summarise the data and presented as mean ± standard deviation (SD) and frequency in percentage (%). Statistical significant was set at $p < 0.05$.

RESULTS

Table 1a: Socio demographic Characteristics of Participants (N = 40)

Variable	Frequency (n)	Percentage (%)
SEX		
Female	29	72.5
Male	11	27.5
MARITAL STATUS		
Single	33	82.5
Married	7	17.5
TRIBE		
Hausa	15	37.5
Yoruba	14	35.0
Nupe	7	17.5
Fulani	2	5.0
Igbo	1	2.5
Ibira	1	2.5
EDUCATION LEVEL		
"Masters"	4	10.0
"400 L"	3	7.5
"300 L"	14	35.0
"200 L"	8	20.0
"100 L"	10	25.0
	1	2.5

Table 1a above shows the socio demographic characteristics of the participants which provides important context for interpreting the observed variations in micronuclei frequency and white blood cell functional parameters among students with peptic ulcer. The predominance of females (72.5%) suggests that gender-related differences in susceptibility to genomic instability and immune responses may influence the findings, as hormonal and physiological factors can modulate both ulcer progression and WBC activity. The overwhelming proportion of single participants (82.5%) reflects the youthful population of the university, and this aligns with the age group most vulnerable to lifestyle-related ulcer risks such as poor dietary habits, stress, and

irregular sleep patterns, which are known to increase oxidative stress and potentially elevate micronuclei formation. Ethnically, Hausa (37.5%) and Yoruba (35.0%) dominated the sample, which may reflect dietary and cultural patterns influencing ulcer occurrence, as certain staple diets and consumption of spicy foods are associated with gastrointestinal irritation. Educational distribution with the majority clustered in 400 level (35.0%) and 200 level (25.0), further underscores that most respondents are undergraduates within the physiologically active age group, where stress from academic workload may impair immune function and influence WBC activity. These socio demographic characteristics therefore provide a basis for understanding how lifestyle, genetic background, and academic pressures in young adults could interact with the pathophysiology of peptic ulcer disease and its cytogenetic manifestations.

Table 1b: Clinical Characteristics of Participants (N = 40)

Variable	Frequency (n)	%	Variable	Frequency (n)	%
Sleeping Duration			Bleeding History		
>6 hours	13	32.5	None (Nil)	35	87.5
<6 hours	27	67.5	Oral	4	10.0
			Anal	1	2.5
Previous Treatment			Family History Of PUD		
Omeprazole	22	55.0	Yes	21	52.5
Amoxicillin	11	27.5	No	19	47.5
Pantoprazole	4	10.0	Family History Of HTN		
Ranitidine	3	7.5	Yes	27	67.5
			No	13	32.5
Admission Frequency			Admission Interval		
Not Frequent	30	75.0	Never	14	35.0
Frequent	10	25.0	6 months	17	42.5
			3 months	9	22.5
Regular Complaints			Blood Group		
Back pain	11	27.5	A Rh Positive	12	30.0
Abdominal pain	10	25.0	B Rh Positive	12	30.0
Dizziness	6	15.0	O Rh Positive	12	30.0
Back pain + Dizziness	10	25.0	AB Rh Positive	2	5.0
Loss of appetite	3	7.5	B Rh Negative	1	2.5
			O Rh Negative	1	2.5
Genotype			Duration Of Illness		
AA	24	60.0	10 month – 2years	22	55.0
AS	15	37.5	2 – 9 months	12	30.0
AC	1	2.5	3 – 4 years	6	15.0

PUD: Peptic Ulcer Disease, HTN: Hypertension.

Table 1b presents the clinical characteristics of participants, showing that most slept less than six hours daily (67.5%), a condition that may compromise immune response and contribute to genomic instability. The majority had no bleeding history (87.5%), while omeprazole (55%) and amoxicillin (27.5%) were the most commonly used drugs. More than half had a family history of peptic ulcer (52.5%) and hypertension (67.5%), suggesting hereditary influences. Hospital admissions were largely infrequent (75%), though some experienced recurrent six-month intervals (42.5%), reflecting chronic relapses. Regular complaints included abdominal pain, back pain, dizziness, and appetite loss, consistent with ulcer pathology. Blood groups were evenly distributed across A Rh 'D' Positive, B Rh 'D' Positive, and O Rh 'D' Positive (30% each), with AA genotype most common (60%). Illness duration was mainly between 10 months and 2 years (55%), highlighting the persistence of symptoms. Together, these findings emphasize how lifestyle, genetic predisposition, and clinical history may shape micronuclei frequency and WBC.

Table 2: Relationship of Mean $\pm$ SD of WBC Functional Indices with ABO Blood Groups (N = 40)

Parameter	A Rh Positive	B Rh Positive	O Rh Positive	AB Rh Positive	B Rh Negative	O Rh Negative	F-value	p-value
WBC (×10 ³ /μL)	4.74 ± 1.46	5.85 ± 1.80	5.52 ± 1.60	4.49 ± 0.26	13.03 ± 3.57	5.525	0.001	
Absolute Lymphocytes (×10 ³ /μL)	2.04 ± 0.56	2.28 ± 0.55	2.35 ± 0.72	1.72 ± 0.02	3.69 ± 1.51	2.056	0.095	
Absolute Eosinophils (×10 ³ /μL)	0.39 ± 0.08	0.48 ± 0.12	0.46 ± 0.22	0.29 ± 0.11	1.03 ± 0.30	4.135	0.005	
Absolute Neutrophils (×10 ³ /μL)	2.29 ± 1.13	3.02 ± 1.34	2.71 ± 0.87	2.49 ± 0.13	8.31 ± 1.76	5.737	0.001	
Relative Lymphocytes (%)	43.43 ± 10.66	40.19 ± 8.06	43.05 ± 7.11	38.30 ± 1.70	28.30 ± 42.20	0.776	0.574	
Relative Eosinophils (%)	8.86 ± 2.56	8.41 ± 1.53	8.18 ± 1.94	6.30 ± 1.84	7.90 ± 8.50	0.578	0.716	
Relative Neutrophils (%)	46.82 ± 10.49	51.40 ± 8.31	48.76 ± 7.71	55.40 ± 0.14	63.80 ± 49.30	1.060	0.399	

WBC: White blood cells. Rh: Rhesus

Table 2 presents the relationship of mean $\pm$ SD of WBC functional indices across different ABO blood groups among peptic ulcer patients. The results indicate significant variations in total WBC count, absolute eosinophils, and absolute neutrophils across the blood groups. Specifically, individuals with blood group B Rh 'D' negative showed the highest WBC ($13.03 \times 10^3/\mu\text{L}$), absolute neutrophil ($8.31 \times 10^3/\mu\text{L}$), and absolute eosinophil ($1.03 \times 10^3/\mu\text{L}$) values compared to other groups, suggesting heightened immune or inflammatory responses. In contrast, AB Rhesus 'D' positive participants showed the lowest values for absolute lymphocytes and eosinophils, while O Rhesus 'D' positive and A Rhesus 'D' positive groups demonstrated moderate values across most indices. The ANOVA confirmed statistically significant differences in WBC count ($p = 0.001$), absolute eosinophils ($p = 0.005$), and absolute neutrophils ($p = 0.001$), but no significant differences in relative lymphocyte, eosinophil, and neutrophil percentages. These findings suggest that the functional activity of WBCs may be influenced by ABO blood group, with certain groups, particularly B Rhesus 'D' negative, being more prone to exaggerated immune responses, which could impact the severity and progression of peptic ulcer disease among the participants.

Table 3: Relationship of Mean $\pm$ SD of Micronuclei Frequency with ABO Blood Group (cells/ 10^3)

Parameter	A Rh positive	B Rh positive	O Rh positive	AB Rh positive	B Rh negative	O Rh negative	F-value	p-value
Mononuclear with Micronuclei	381.67 $\pm$ 67.26	395.83 $\pm$ 48.33	372.50 $\pm$ 42.67	400.00 $\pm$ 28.28	370.00 $\pm$ 20.00	500.00 $\pm$ 30.00	1.214	0.324
Binuclear with Micronuclei	19.17 $\pm$ 13.11	20.83 $\pm$ 13.11	15.83 $\pm$ 13.11	25.00 $\pm$ 21.21	20.00 $\pm$ 20.00	30.00 $\pm$ 20.00	0.391	0.851
Acentric Cells	27.50 $\pm$ 12.15	25.58 $\pm$ 14.68	38.33 $\pm$ 13.37	40.00 $\pm$ 14.14	20.00 $\pm$ 20.00	20.00 $\pm$ 20.00	1.694	0.163
Cell Proliferation	156.75 $\pm$ 71.16	144.50 $\pm$ 59.60	173.25 $\pm$ 70.94	166.50 $\pm$ 44.55	167.00 $\pm$ 167.00	122.00 $\pm$ 122.00	0.291	0.914

Rh: Rhesus

Table 3 shows the analysis of micronuclei parameters among peptic ulcer patients across ABO blood groups which indicated that mononuclear cells with micronuclei ranged from 370.00 cells/1000 cells in B Rh Negative to 500.00 cells / 1000 cells in O Rh 'D' Negative, binuclear cells from 15.83 ± 13.11 cells / 1000 cells in O Rh 'D' Positive to 25.00 ± 21.21 cells / 1000 cells in AB Rh 'D' Positive, acentric cells from 20.00 cells / 1000 cells in B Rh 'D' Negative and O Rh

'D' Negative to 40.00 ± 14.14 cells / 1000 cells in AB Rh 'D' Positive, and cell proliferation from 122.00 in O Rh 'D' Negative to 173.25 ± 70.94 cells / 1000 cells in O Rh 'D' Positive. Despite these variations, ANOVA results indicated no statistically significant differences for any parameter (mononuclear $F = 1.214$, $p = 0.324$; binuclear $F = 0.391$, $p = 0.851$; acentric $F = 1.694$, $p = 0.163$; cell proliferation $F = 0.291$, $p = 0.914$). The minor differences observed, particularly higher values in O Rh 'D' Negative and AB Rh 'D' Positive groups are likely due to random variation in different groups as against the true effect of ABO type on genomic instability. Overall, these findings suggest that ABO blood group does not significantly influence the frequency of micronuclei or lymphocyte proliferation among this population with peptic ulcer disease, indicating that other genetic or environmental factors may play a more important role in genomic damage in these individuals.

Table 4: Relationship of Mean $\pm$ SD of WBC Functional Indices with Hemoglobin Genotype (N = 40)

Parameter	AA	AS	AC	F-value	p-value
WBC ($\times 10^3/\mu\text{L}$)	5.45 ± 2.30	5.44 ± 1.58	6.49	0.126	0.882
Absolute Lymphocytes	2.24 ± 0.65	2.16 ± 0.68	2.42	0.116	0.891
Absolute Eosinophils	0.43 ± 0.16	0.47 ± 0.21	0.43	0.191	0.827
Absolute Neutrophils	2.75 ± 1.69	2.78 ± 0.89	3.64	0.184	0.833
Relative Lymphocytes (%)	42.65 ± 9.51	40.41 ± 6.89	37.30	0.447	0.643
Relative Eosinophils (%)	8.33 ± 2.16	8.52 ± 1.77	6.60	0.428	0.655
Relative Neutrophils (%)	48.56 ± 9.90	51.07 ± 6.97	56.10	0.634	0.536

WBC: White blood cells

Table 4 shows the analysis of WBC functional indices among participants with different hemoglobin genotypes (AA, AS, and AC) which revealed comparable values across all measured parameters. Total WBC counts ranged from $5.44 \pm 1.58 \times 10^3/\mu\text{L}$ in AS to $6.49 \times 10^3/\mu\text{L}$ in AC, while absolute lymphocytes varied between $2.16 \pm 0.68 \times 10^3/\mu\text{L}$ in AS and $2.42 \times 10^3/\mu\text{L}$ in AC. Absolute eosinophils and neutrophils, as well as relative percentages of lymphocytes, eosinophils, and neutrophils, showed minor variations across genotypes. However, ANOVA results demonstrated no statistically significant differences in total WBC and differential values respectively, such that $F = 0.126$, $p = 0.882$ and absolute lymphocytes $F = 0.116$, $p = 0.891$; absolute eosinophils $F = 0.191$, $p = 0.827$; absolute neutrophils $F = 0.184$, $p = 0.833$; relative lymphocytes $F = 0.447$, $p = 0.643$; relative eosinophils $F = 0.428$, $p = 0.655$; relative neutrophils $F = 0.634$, $p = 0.536$, indicating that hemoglobin genotype does not significantly influence WBC function in this population with peptic ulcer disease. The slight differences observed, particularly in the AC genotype was unconfirmed as this may not be true physiological effect as reflected in the slight increase.

Table 5: Relationship of Mean $\pm$ SD of Micronuclei Frequency with Hemoglobin Genotype (N = 40)

Parameter	AA	AS	AC	F-value	p-value
Mononuclear cells with Micronuclei ($\text{cells} \times 10^3/\mu\text{L}$)	387.92 ± 57.18	387.33 ± 51.33	350.00	0.229	0.796
Binucleated cells with Micronuclei ($\text{cells} \times 10^3/\mu\text{L}$)	19.17 ± 13.16	18.67 ± 13.02	30.00	0.352	0.706
Acentric Cells ($\text{cells} \times 10^3/\mu\text{L}$)	27.79 ± 15.73	34.00 ± 10.56	40.00	1.148	0.328
Cell Proliferation (Index $\times 10^3/\mu\text{L}$)	162.46 ± 64.66	150.87 ± 66.12	154.00	0.148	0.863

Table 5 shows the assessment of micronuclei frequency among participants with different hemoglobin genotypes (AA, AS, and AC) which revealed that mononuclear cells with micronuclei ranged from 350.00 to 387.92 $\text{cells} \times 10^3/\mu\text{L}$, binucleated cells with micronuclei

ranged from 18.67 to 30.00 cells/ 10^3 , acentric cells ranged from 27.79 to 40.00 cells/ $10^3/\mu\text{L}$ and the cell proliferation index varied between 150.87 and 162.46 cells/ $10^3/\mu\text{L}$. Despite these observed variations, ANOVA analysis indicated no statistically significant differences across genotypes for any parameter (mononuclear cells $F = 0.229$, $p = 0.796$; binucleated cells $F = 0.352$, $p = 0.706$; acentric cells $F = 1.148$, $p = 0.328$; cell proliferation $F = 0.148$, $p = 0.863$). This suggests that the haemoglobin genotype does not significantly influence genomic instability, as measured by micronuclei formation, or the proliferative capacity of white blood cells among the participant population with peptic ulcer disease. The significant effect of slightly higher values observed in the AC genotype could not be ascertain in line with it's true biological effect of genomic instability.

Table 6: Relationship of Mean $\pm$ SD of WBC Functional Indices with Duration of Peptic ulcer (N = 40)

Parameters	2–9 months	10 months – 2 years	3–4 years	F-value	p-value
WBC ($\times 10^3/\mu\text{L}$)	$5.45 \pm 1.90 \times 10^3/\mu\text{L}$	$5.68 \pm 2.22 \times 10^3/\mu\text{L}$	$4.72 \pm 1.47 \times 10^3/\mu\text{L}$	0.529	0.594
Absolute Lymphocytes ($\times 10^3/\mu\text{L}$)	$2.03 \pm 0.65 \times 10^3/\mu\text{L}$	$2.39 \pm 0.64 \times 10^3/\mu\text{L}$	$1.95 \pm 0.55 \times 10^3/\mu\text{L}$	1.916	0.162
Absolute Eosinophils ($\times 10^3/\mu\text{L}$)	$0.40 \pm 0.11 \times 10^3/\mu\text{L}$	$0.48 \pm 0.21 \times 10^3/\mu\text{L}$	$0.42 \pm 0.14 \times 10^3/\mu\text{L}$	0.836	0.442
Absolute Neutrophils ($\times 10^3/\mu\text{L}$)	$3.02 \pm 1.30 \times 10^3/\mu\text{L}$	$2.77 \pm 1.59 \times 10^3/\mu\text{L}$	$2.33 \pm 0.89 \times 10^3/\mu\text{L}$	0.477	0.625
Relative Lymphocytes (%)	$37.14 \pm 7.47 \%$	$44.04 \pm 8.99 \%$	$42.08 \pm 5.10 \%$	2.812	0.073
Relative Eosinophils (%)	$7.80 \pm 2.74 \%$	$8.46 \pm 1.61 \%$	$9.08 \pm 1.46 \%$	0.894	0.418
Relative Neutrophils (%)	$54.14 \pm 7.60 \%$	$47.50 \pm 9.46 \%$	$48.83 \pm 5.80 \%$	2.399	0.105

WBC: White blood cells

Table 6 shows the mean and standard deviation of WBC functional indices across different durations of peptic ulcer illness among the participants. Overall, total WBC counts, absolute lymphocytes, eosinophils, and neutrophils remained fairly consistent across the three duration categories, ranging between 4.72 – $5.68 \times 10^3/\mu\text{L}$ for WBC and 1.95 – $2.39 \times 10^3/\mu\text{L}$ for absolute lymphocytes. Relative counts for lymphocytes, eosinophils, and neutrophils also showed no significant differences. The ANOVA results indicate that none of the WBC functional parameters varied significantly with duration of illness (all $p > 0.05$). This suggests that, in this cohort of peptic ulcer patients, the length of illness does not appear to influence the overall white blood cell profile or its functional components.

Table 7: Relationship of Mean $\pm$ SD of Micronuclei Frequency with Duration of peptic ulcer (N = 40)

Parameter	2–9 months	10 months – 2 years	3–4 years	F-value	p-value
Mononuclear cells with Micronuclei (cells/ $10^3/\mu\text{L}$)	339.17 ± 42.74 cells/ $10^3/\mu\text{L}$	389.09 ± 23.89 cells/ $10^3/\mu\text{L}$	473.33 ± 52.64 cells/ $10^3/\mu\text{L}$	32.446	<0.001
Binucleated cells with Micronuclei (cells/ $10^3/\mu\text{L}$)	13.33 ± 10.73 cells/ $10^3/\mu\text{L}$	21.82 ± 14.35 cells/ $10^3/\mu\text{L}$	21.67 ± 7.53 cells/ $10^3/\mu\text{L}$	1.889	0.165
Acentric Cells (cells/ $10^3/\mu\text{L}$)	28.58 ± 12.08 cells/ $10^3/\mu\text{L}$	30.64 ± 14.91 cells/ $10^3/\mu\text{L}$	33.33 ± 16.33 cells/ $10^3/\mu\text{L}$	0.225	0.800
Cell Proliferation (Index $\times 10^3/\mu\text{L}$)	182.17 ± 93.86 cells/ $10^3/\mu\text{L}$	150.86 ± 38.85 cells/ $10^3/\mu\text{L}$	135.17 ± 61.28 cells/ $10^3/\mu\text{L}$	1.413	0.256

The results from Table 7 indicate that the frequency of mononuclear cells with micronuclei increased significantly with longer duration of peptic ulcer illness, rising from 339.17 ± 42.74 cells/ $10^3/\mu\text{L}$ in 2–9 months group to 473.33 ± 52.64 cells/ $10^3/\mu\text{L}$ in 3–4 years group ($F = 32.446$, $p < 0.001$), suggesting cumulative genomic damage over time. In contrast, other parameters including binucleated cells with micronuclei, acentric cells, and cell proliferation index showed

no significant differences across the duration groups ($p > 0.05$), indicating that overall chromosomal alterations and proliferation remain relatively stable. These findings suggest that prolonged peptic ulcer disease may selectively affect mononuclear cells, making micronuclei frequency a sensitive biomarker for genotoxic stress in patients, and underscore the importance of early management to minimize progressive genomic instability.

DISCUSSION

This study investigated the relationship between peptic ulcer disease (PUD) and two key biological indicators: micronuclei (MN) frequency, a biomarker of genomic instability-and white blood cell (WBC) functional parameters, which reflect systemic inflammatory and immune responses [14]. The findings provide valuable insights into the complex interplay between chronic gastric inflammation, oxidative stress, and DNA damage among young Nigerian students.

A major finding of the study was the significant increase in mononuclear cells with micronuclei with longer duration of PUD [15]. Participants with ulcer symptoms lasting 3–4 years recorded markedly higher MN frequency (473.33 ± 42.74 cells/ 10^3) compared to those with 2–9 months of illness (339.17 ± 42.74 cells/ 10^3 ($p < 0.001$)). This dose–response relationship strongly suggests that prolonged exposure to ulcerogenic factors, particularly *Helicobacter pylori* infection and chronic inflammation leads to cumulative DNA damage [16]. This observation agrees with previous findings indicating that *H. pylori* induces reactive oxygen species (ROS) production through activated neutrophils and macrophages, leading to oxidative DNA lesions, double-strand breaks, and chromosomal fragmentation that manifest as micronuclei [10,17]. The absence of significant changes in binucleated MN or acentric fragments implies that mononuclear cells (likely lymphocytes and monocytes) are the primary carriers of this genomic damage, possibly due to their prolonged circulation time and involvement in chronic immune activation [18]

In contrast, WBC functional parameters showed no significant variations across ulcer duration, hemoglobin genotype (AA, AS, AC), or ABO blood groups except for notable elevations in absolute neutrophil count and total WBC count in individuals with the B Rh Negative blood group [19] This indicates that while systemic inflammation is present in PUD, it does not correlate directly with genomic instability in this study population. Correlation analysis further confirmed that there were no statistically significant associations between any WBC parameter and MN frequency ($p > 0.05$). [20] Hence, inflammatory cell counts alone are insufficient to predict DNA damage in PUD patients, while MN frequency serves as a complementary and more specific biomarker of disease progression and long-term genomic risk [21].

The absence of significant influence from hemoglobin genotype, despite the oxidative vulnerability of HbS carriers, could not be confirmed as further study to involve large number of participants will be of advantage for adequate assessment its significance. Likewise, the uniform distribution of MN frequency across ABO blood groups contrasts with earlier reports suggesting increased gastric disease risk in non-O blood groups. This discrepancy may be due to the relatively young age of participants, who may not yet exhibit advanced mucosal changes [21].

Additionally, the demographic findings revealed that 72.5% of participants were female, and 67.5% reported sleeping less than six hours daily. These factors may aggravate ulcer pathogenesis through stress-induced cortisol release, impaired mucosal healing, and increased oxidative stress. The high proportion of participants with a family history of PUD (52.5%) further supports a genetic or shared environmental predisposition to the disease. [22].

Collectively, these findings support the dual-hit model of PUD pathogenesis, in which *H. pylori* infection and/or nonsteroidal anti-inflammatory drug (NSAID) use initiate mucosal injury, while host inflammatory responses and oxidative stress perpetuate cellular damage and genomic instability. This mechanism heightens the potential for malignant transformation over time [23, 24]. The elevated MN frequency observed in long-standing cases emphasizes that PUD should

not be regarded merely as a benign condition, but as a precursor to genomic instability and possible gastric carcinogenesis.

LIMITATIONS

This study was focused on the relationship of the frequency of micronuclei (MN), a biomarker of genomic instability and leukocyte immune response functional indices among the students' population that were screened and diagnosed of Peptic ulcer disease. The assessment was only on micronuclei frequency in relation to ABO blood group, haemoglobin genotype and white blood cell functional indices and the outcome showed a few scientific gaps that may be as a result of small sample size (N=40), the lack of long-term patient follow-up, and the exclusion of an external healthy control participants cohort comparison in the assessment.

CONCLUSION

This study concludes that prolonged duration of peptic ulcer disease (PUD) is significantly associated with an increase in micronuclei (MN) frequency in mononuclear cells, indicating cumulative genomic damage arising from chronic inflammation and oxidative stress. In contrast, white blood cell functional parameters showed no significant correlation with MN frequency, suggesting that standard haematological indices may not effectively reflect the genotoxic burden in Peptic ulcer disease patients. Furthermore, ABO blood group and hemoglobin genotype had no significant influence on the measured parameters. The clear duration-dependent rise in MN frequency emphasizes the importance of early diagnosis, prompt treatment, and lifestyle modification to prevent long-term genomic and malignant complications. This finding highlighted the micronucleus assay as a reliable, non-invasive biomarker for monitoring genomic instability, disease progression, and cancer risk in individuals with peptic ulcer disease.

CONFLICT OF INTEREST

The authors declared no conflict of interest

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