

## **Inflammatory Markers and Some Haematological Profiles in Apparently Healthy Residents of Adeyanba Village and Egor Urban Communities in Edo State**

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### **ABSTRACT**

**Introduction:** Differences in lifestyle, access to healthcare and type of food consumed may cause variation in inflammatory biomarkers among persons in different location and ethnic background. There is paucity of data on the effect of location on inflammatory markers in Nigerian population. **Aim:** This is a cross-sectional study aimed at determining the effect of location on status of some inflammatory markers among residents of Adeyanba village and Egor urban communities of Edo State. **Methodology:** A total of 200 participants (consisting of 100 from each community) were recruited. Whole blood sample was collected from each participant and analysed for albumin, complete blood count (FBC) and erythrocyte sedimentation rate (ESR). Platelet lymphocyte ratio (PLR), Neutrophil lymphocyte ratio (NLR) were calculated from complete blood count parameters. Serum C-reactive protein (CRP) was determined using enzyme linked immunosorbent assay technique. Data obtained were compared between rural and urban by students' t- test, using the statistical software INSTAT®. **Results:** Comparatively, the serum albumin concentration was slightly higher in urban dwellers than in the village dwellers ( $p=0.5$ ). Whereas there was statistically higher Ferritin concentration ( $p=0.0001$ ) among Egor urban dwellers than the Adeyanba village dwellers. Adeyanba rural dwellers had a significantly higher total White Blood Cell counts ( $p=0.001$ ), ESR ( $p=0.001$ ), PLR ( $p=0.001$ ), NLR ( $p=0.001$ ) and CRP ( $p=0.01$ ) than their urban counterparts. **Conclusion:** This study suggests that rural residents may have a higher risk for inflammatory conditions than urban population. This underlines the need for regular screening of rural populations for markers of inflammatory disorders and provision of intervention strategies for rural residents of Edo State.

**Keywords:** Adeyanba, Egor, inflammatory markers, Neutrophil Lymphocyte Ratio

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## **INTRODUCTION**

In recent years, inflammation as a risk factor and indicator for various chronic illnesses, including cardiovascular disease, cancer, diabetes and various illness has been studied and described (1,2). Inflammation is regarded as a complex system of host systemic and local responses to injury and infection (3). Inflammation plays a role in almost all disease processes, including immunological and vascular complications, metabolic injuries, sepsis and cases of cancer. The role of the inflammatory process in disease development has been accompanied by efforts to identify dietary factors that may promote or inhibit the inflammatory process, thereby affecting disease risk and severity (2). Inflammation is characterized as acute or chronic. (4,5)

Acute inflammation is a normal and comparatively short-lived physiologic response (lasting few minutes to days) to injury, irritation, or infection (4). The physiologic processes responsible for acute inflammation (increased blood flow, greater blood vessel permeability, and accumulation of white blood cells), lead to redness, swelling, heat, and pain at the affected site (4). The same process plays a role in the synthesis of the collagen matrix which helps to promote healing of the damaged tissue (5).

Chronic inflammation is a long-term physiologic response (lasting weeks, months, or years) to one or more factors, including exposure to environmental toxins, microbial or viral infection, poor nutrition, stress, and processes related to aging (6). Chronic inflammation is activated when the mechanisms of acute inflammation fail to arrest infection or heal an injury. When unchecked, prolonged chronic inflammation generates a series of destructive reactions that damage cells and eventually lead to the clinical symptoms of

disease. Ultimately, chronic inflammation is a failure of the body's immune system to maintain a healthy homeostatic state (6). During Gram negative bacterial infections, lipopolysaccharide (LPS), a highly proinflammatory endotoxin is released from the surface of replicating Gram-negative bacteria into the circulation, where it is recognized by a variety of circulating cell types including macrophages, triggering gene induction of pro-inflammatory cytokines, such as tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 (IL-1), and biosynthesis of prostaglandins (PGE2) (3). These and other cytokines act in an autocrine or paracrine manner to induce and amplify the host cell response and defence systems that help to eliminate the bacterial infection (7).

However, uncontrolled and excessive cytokine expression can cause or induce acute or chronic inflammatory responses. It is well established that increased expression of cytokines elicits the cytotoxic actions in many inflammatory disease conditions. Moreover, cytokines play a critical role in several cardiovascular and neurological degenerative diseases as well as cancer (7). Hence the elucidation of mechanisms that mediate and regulate cytokine signals is of profound importance to understanding and managing a very large array of disease processes. Furthermore, recent studies have provided important links between elevated cytokines such as TNF- $\alpha$  and IL-1 with oxidative stress during initial inflammatory processes (8), and have shown to alter redox equilibrium through a thiol-dependent mechanism. Interestingly, antioxidants have been shown to down-regulate cytokine transcription and biosynthesis (9). Conversely, increased oxidative stress, e.g., depletion of glutathione (GSH), can augment pro-inflammatory signals by up-regulating Reactive Oxygen Species (ROS). Hence, levels of reduced glutathione are a critical

determinant of ROS signalling. Under physiological conditions, there is a balance between generation of ROS and their removal by antioxidant systems (9). In general, oxidative stress occurs when this balance is disrupted, either directly by infectious agents or by cytokines released from inflamed cells that may lead to increased ROS generation and/or decreased antioxidant defence. Normally, ROS are involved in some of the essential cellular functions such as host cell defence, mitochondrial respiration, cytokine generation and cell proliferation/apoptosis (10). There are several potential sources of ROS in inflammation, one of which is the activation of NADPH oxidase in phagocytes, monocytes and most other inflammatory cell types. In fact, activation of NADPH oxidase has been observed upon exposure of various cell types to cytokines, growth factors and hyperglycaemia (10). Thus, NADPH oxidase is a key player in signal transduction. The NADPH oxidase (a membrane bound holoenzyme with different flavocytochrome subunits) catalyses the one electron reduction of oxygen, using NADPH or NADH as the electron donor and leading to the production of superoxide anions (11). The superoxide anions thus generated can form other ROS (such as hydroxyl radicals and hydrogen peroxide) and cause tissue injury and alter gene expression. Several investigators have shown in multiple cell types that NADPH oxidase, rather than xanthine oxidase and other mitochondrial enzymes, is the main source of ROS (11). LPS and various inflammatory cytokines such as TNF- $\alpha$ , IL-1 and IL-6 can activate NADPH oxidase to generate significant, sometimes toxic, amounts of ROS (initially O<sub>2</sub><sup>-</sup>) which propagate their signals that activate transcription factors (12). Besides cytokines, growth factors and hyperglycaemia can also activate NADPH oxidase to propagate their

signals. This is supported by the observations that cytokines, growth factors and hyperglycaemic signals are interrupted by such antioxidants as N-acetyl cysteine and  $\alpha$ -tocopherol and glutathione (GSH), which can attenuate inflammation (13). Therefore, when oxidants overwhelm the antioxidative capacity, lipid peroxides and toxic lipid-derived aldehydes (LDAs) such as 4-hydroxy-trans-2-nonenal (HNE) are formed by the process called lipid peroxidation. Lipid peroxidation is known to be a causative factor that contributes to the pathophysiology of inflammation (12).

Furthermore, some lipid peroxidation products may stimulate leukocyte recruitment to the site of inflammation (13).

Urban and rural areas are two distinct settlements which may have varying influence on the health status on its dwellers due to differences in lifestyle, types of food consumed, exposure to environmental hazards, socio-economic status of individuals in these areas (14). These may impact on the metabolic activities, differential production of oxidative stress and inflammatory biomarkers among urban and rural residents.

Hence, this study aims to evaluate some inflammatory markers among apparently healthy residents in Adeyanba and Egor communities in Edo State.

## METHODS

**Study Participants:** The study was a cross-sectional survey, comprising of adults from the age of 18-60 years, residing in Adeyanba and Egor communities of Edo State. A total number of 200 adult participants were included for study which comprises of 100 participants each from the rural and the urban communities. Subjects with any form of chronic sickness or any medication were excluded.

**Ethical Consideration:** The approval for ethical consideration for the study was sought and obtained from the Edo state ministry of Health.

**Specimen Collection and Laboratory Analyses:** Ten millilitres of fasting blood was collected from each participant into a plain and EDTA containers. Serum was harvested from the plain container for biochemical profiles; albumin, C-reactive protein and ferritin with the aid of Xelectra Pro Chemistry Analyser® (15, 16, 17). The EDTA sample was used for the complete blood count (18) with the aid of Mythic 18 Automated Haematology analyser® (Orphee SA Switzerland). The Neutrophil-Lymphocyte and platelet-lymphocyte ratio was calculated from the complete blood count while the erythrocyte sedimentation rate (ESR) was estimated accordingly (19).

**Statistical Analysis:** Data were analysed using Statistical package for social science (SPSS). Values obtained were expressed as mean  $\bar{x} \pm \sigma$  standard deviation and compared between the groups using the independent student T- test. A p-value of less or equal to 0.05 was considered statistically significant

## RESULTS

Table 1 shows the mean values of biochemical indices among Adeyanba and Egor residents in Edo State. Serum ferritin was observed to be significantly higher ( $p<0.05$ ) among the Egor (urban) residents compared to the Adeyanba (rural) residents.

The concentration of albumin between the two settlers was not significantly different ( $p> 0.05$ ).

**Table 1: Albumin and Ferritin levels of Adeyanba and Egor residents in Edo State.**

PARAMETERS	ADEYANBA (N=100) $\bar{x} \pm \sigma$	EGOR (N=100) $\bar{x} \pm \sigma$	P - VALUES
ALB (g/L)	3.92 $\pm$ 1.13	4.17 $\pm$ 1.37	0.5
FERRITIN (ng/ml)	57.53 $\pm$ 3.63	89.24 $\pm$ 6.45	0.0001

**KEY:** ALB =Albumin

Table 2 shows the mean values of inflammation markers such CRP, PLR, NLR, MPV, ESR and some haematological indices of the residents of Adeyanba and Egor in Edo State. There is a significant difference ( $p<0.05$ ) in some profiles such as WBC, Neutrophil,

Monocyte, Basophil, Eosinophil, ESR, PLR, NLR and CRP, of Adeyanba and Egor residents. There is no significant difference in the Absolute lymphocytes, platelets count and mean platelets volume (MPV) values of Adeyanba and Egor residents ( $p>0.05$ ).

**Table 2:** Comparison of some markers of inflammation and haematological indices among residents of Adeyanba and Egor in Edo State.

PARAMETERS	ADEYANBA (N=100) ( $\bar{x} \pm \sigma$ )	EGOR (N=100) ( $\bar{x} \pm \sigma$ )	P – VALUES
ESR (mmhr <sup>-1</sup> )	28.3±5.21	20.16±2.31	0.001
PLR	99.6±8.94	89.82±5.54	0.001
NLR	1.62±0.85	0.63±0.78	0.001
MPV (fl)	10.01±0.84	10.02±0.92	0.5
CRP (mg/l)	0.101±0.5	0.077±0.04	0.01
White blood cells( $\times 10^9 l^{-1}$ )	9.14±12.14	4.81±2.28	0.001
Neutrophils ( $\times 10^9 l^{-1}$ )	3.53±0.73	1.58±0.78	0.001
Lymphocytes ( $\times 10^9 l^{-1}$ )	2.49±0.72	2.50±0.85	0.50
Monocytes ( $\times 10^9 l^{-1}$ )	0.64±0.35	0.39±0.24	0.001
Basophils ( $\times 10^9 l^{-1}$ )	1.21±0.15	0.13±0.05	0.001
Eosinophils ( $10^9 l^{-1}$ )	1.27±0.48	0.21±0.06	0.001
Platelets ( $10^9 l^{-1}$ )	157.75±43.71	156.06±48.25	0.164

**KEY**

ESR	=	Erythrocytes Sedimentation Rate
PLR	=	Platelets Lymphocytes Ratio
NLR	=	Neutrophils Lymphocytes Ratio
CRP	=	C – Reactive Protein
MPV	=	Mean Platelet Volume

**DISCUSSION**

Numerous studies exist on markers of inflammation and lipid peroxidation among Nigerians with special reference to the sick and/or hospitalized patients. To our knowledge no study has focused on apparently healthy Nigerians especially with special consideration on location of residence. The serum ferritin level was significantly higher in Egor urban residents than in their Adeyanba village counterparts. The significantly higher

ferritin concentration observed in Egor population disagreed with an earlier report which showed a higher ferritin concentration among rural residents than urban residents (20).

Ferritin acts as a buffer against Iron deficiency and iron overload. It is an acute phase reactant and a marker of acute phase and chronic inflammation which is non-specifically elevated in a wide range of inflammatory conditions (21). It also has the ability to modulate immune function by inhibiting lymphocyte function (22).

The variation observed in the serum ferritin concentration in the two populations may be due to differences in geographical location, socio-economic status, dietary habit and life style of study population.

The significantly higher, neutrophil, monocytes, basophils and eosinophils counts observed in the rural population than their urban counterparts were similar to several studies of which, elevated neutrophil count is a reflection of inflammatory response (24). The basophil, monocytes and eosinophils are white blood components that are responsible for the release of histamine into the bloodstream to combat allergy or bacterial/parasitic attack (25).

In this study we also observed a significantly higher Neutrophil lymphocyte ratio (NLR) and Platelets lymphocyte ratio (PLR) in rural Adeyanba residents than Egor residents. To our knowledge no study has compared NLR and PLR among apparently healthy individual in rural and urban communities. Studies have shown that NLR and PLR are good predictors of systemic inflammatory response (23, 24, 26). A study by Alexander in 2016, reported reference value of 1.2-4.4 and 75-199 for NLR and PLR respectively in apparently healthy adults from a tertiary health facility in North Central Nigeria. There is preponderance of disease (malaria and intestinal parasitic disease) which could be asymptomatic in a rural population and may precipitate changes in the expression of inflammatory markers such as NLR and PLR. This may not be the case in urban population where such infections are not common due to increase access to health institution, clean drinking water and knowledge of mode of prevention and transmission of disease agents. Further research on factors affecting increased expression of NLR and PLR among rural population is warranted.

Rural and urban population vary by their socio-economic status, dietary habits, physical activities and means of livelihood (27).

Regular exercise has been reported to be a major influencing factor that regulates the body anabolic and catabolic functions including metabolism of carbohydrate and fat (28). The biochemical and physical changes in a body that are initiated by the response to tissue damage or foreign organism are termed inflammatory response. These changes begin the moment an intruder is detected and expand exponentially to meet the perceived threat. However, evidence of inflammation may be covert if the infection is minor or the body's immunity is particularly effective in preventing the disease and removing the cause of the infection (29).

## **CONCLUSIONS**

The rural population of Adeyanba presented a higher value in some inflammation markers such as ESR, PLR, NLR and CRP and as well as higher white blood cell, platelet, Neutrophil, Monocyte, Basophil and Eosinophil counts compared to the Egor urban dwellers' similar profiles. Surprisingly, there was significantly higher concentration of serum ferritin level in the urban than rural dwellers.

## **Recommendations**

Individuals, most especially from the rural communities should be encouraged to cultivate the habit of regular physical exercise. Government should create an enabling environment for rural populations to access prompt health service. Regular health campaign on the need for clean and healthy environment should be done regularly in the rural communities.

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**Conflict of Interest:**

The authors have no conflict of interest.

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