IBOM MEDICAL JOURNAL
Vol.14 No.4 Oct, 2021. Pages 455 - 460
www.ibommedicaljournal.org

Hematological Profiles of Nigerian Patients with Asthma on Inhaled Corticosteroids

Saidu H[1,4], Iyanda RO[2], Garba N[1], Danladi SB[1], Aliyu IA[1,4], Bala JA[1,4], Saidu A[3]*

[1]*Department of Medical Laboratory Science, Bayero University, Kano, Nigeria*
[2]*Department of haematology, Ladoke Akintola University teaching hospital, Osogbo, Nigeria*
[3]*Department of Pharmacology, Bayero University Kano, Nigeria*
[4]*Center for Infectious Disease Research, Bayero University Kano, Nigeria*

Abstract

Background: Bronchial asthma is a disease characterized by aberrant leukocyte trafficking and allergic inflammation. This study investigated the distribution of the various leukocyte subsets and some erythropoietic indices in asthma patients on inhaled corticosteroids.
Methodology: This is a comparative cross–sectional study involving 108 adult asthmatic patients on inhaled corticosteroids and 108 healthy adults. Socio–demographic and clinical data were collected using a structured questionnaire. Four milliliters of blood sample was collected from each study participant for hematological analysis. Full blood count and differential leukocyte count were done using an automated hematology analyzer. The mean and standard deviation of the measured parameters were calculated and inter-group comparison was made. The significance level was set at p–value ≤ 0.05.
Result: The absolute and relative counts of neutrophils, eosinophil and, basophil were significantly elevated in asthmatic patients compared to control group. On the other hand, relative and absolute counts of monocytes and lymphocytes were significantly lower in asthmatic patients compared to the normal healthy control. The packed cell volume (PCV), red blood cell count (RBC) and hemoglobin concentration (Hb) were significantly low in asthmatic patients compared to the control group.
Conclusion: Asthma patients on corticosteroids have altered hematological parameters compared to apparently healthy controls

Keywords: Asthma, inhaled corticosteroids, Hematological parameters

Introduction

The nature of the leukocytes recruited to the lungs in asthma, its dynamics, and the extent of their activation confer tremendous diversity to asthma disease; this is made evident by the various phenotypes and endotypes observed.[1] The profound inflammation can have effects on the entire hematopoietic tree including red cell production.[2]

Corresponding Author: Hayatu Saidu

Department of Medical Laboratory Science & Center for Infectious Disease Research, Bayero University, Kano, Nigeria.
Email: hayatuddeensaeed@gmail.com, hsaidu.mls@buk.edu.ng

Classically, the allergic inflammation in asthma is believed to be orchestrated by basophils, mast cells and, later eosinophil.[1] Progress in the pathobiology of asthma has revealed a variant of the disease that is characteristically driven by neutrophilic inflammation.[3] The hallmark of atopic and non-atopic asthma is a T-helper 2 lymphocyte immune response characterized by the production of allergen-specific immunoglobulins (IgE and IgG).[1] A T-helper 17 cell-mediated inflammation is also seen in asthma and it contributes significantly to the pathogenesis of the disease.[4] Persistent waves of tissue destruction and repair courtesy of the inflammatory cells typify the asthmatic respiratory tract.[1] As highlighted above, the major cellular

drivers of asthma pathogenesis are peripheral blood-derived formed elements; even mast cells are believed by many experts to have aroused from a distant progenitor up the hematopoietic hierarchy; this progenitor exits the bone and takes residence in body tissues.[5] Knowing that the peripheral blood serves as the interim reserve of these effector cells from where they are mobilized to the lungs, it is logical to expect some dynamics in their quantity as they are selectively trafficked to and from the asthmatic lungs.

Management of bronchial asthma with corticosteroids minimizes the inflammatory reaction in the lungs.[6] Specifically, glucocorticoids reduce vascular permeability and hence laryngeal edema.[6] They also actively minimize the egress of leukocytes from the peripheral blood to the lungs by down-regulating the expression of the cellular adhesion molecules selectins and integrins.[7] Maturation and survival of leucocytes are also significantly impaired by corticosteroids, thus depleting the leucocyte reserve.[6,8] As such, supposedly management of asthma with corticosteroids is expected to normalize the distribution of leukocytes in the peripheral and the lungs thus ensuring disease control. Whether the clinical remission achieved as made evident by a reduction in the frequency of acute attacks following asthma management with inhaled corticosteroids correlates with normalization of blood counts is not adequately investigated among Nigerians. Such information could prove useful to physicians in striking a balance between the anti-inflammatory and the immunosuppressive effects of corticosteroids in asthma management in the region.

Method

Study design and participants recruitment

The study design was a comparative cross-sectional hospital-based, performed in Aminu Kano teaching hospital, Kano of Nigeria (AKTH). One hundred and eight adult patients [20-60 years of age] with bronchial asthma met in the pulmonology clinic of AKTH were enrolled for the study. The participants were diagnosed to have persistent asthma of either mild or moderate grade as determined using the global initiative on asthma network guideline (GINA). Simple random sampling technique was used in deciding who to enroll in the study. At the time of recruitment, they were on maintenance therapy with low dose inhaled corticosteroids for at least one month. As part of the exclusion criteria, patients with known respiratory comorbidities were not recruited. Participants with other inflammatory diseases like human immunodeficiency virus (HIV) infection and, diabetes were also excluded. Healthy adults with no current history of respiratory diseases and seronegative for HIV, hepatitis C virus (HCV), hepatitis B virus (HBV) and, Syphilis were used as control. The controls were recruited from the blood donor bay of AKTH.

Sample collection, management and laboratory analyses

Four milliliters of venous blood were collected from the participants in a resting position between 10:00 am and 12:00 noon (to minimize diurnal variations in cell count) using a 5ml syringe. The samples were quickly and gently emptied into dipotassium ethylene diamine tetra-acetic acid (K2 EDTA) bottle with the needle removed. The blood was mixed with the anticoagulant by gentle inversion 5 times. Samples were inspected for the presence of nucleated red blood cells which can cause errors in white blood cell count by examining a Leishman-stained thin blood film. Full blood count and differential were performed using an automated hematology analyzer [Cobac Swelab] to obtain the red cell count, hematocrit, hemoglobin concentration, red cell indices, total white blood cell count and, absolute and relative counts of neutrophils and lymphocytes. The instrument manufacturer's instruction was strictly adhered to while operating the machine. Because degranulated eosinophils and basophils are usually missed by the automated hematology analyzer, the eosinophil, monocyte and, basophil count was done manually using a thin blood film stained with Leishman stain. Only short thin films with even thickness in which the leukocytes were evenly distributed and could easily be identified even at the head end of the film were used. The cells were counted by inspecting strips of the film running from head to tail using X40 objective lens. A 500-cell count was done as recommended by Briggs and Bain, (2019).[9] The eosinophil and basophil counts were obtained from the total white blood cell count expressed in counted cells per liter. Where the automated count is flagged, a manual evaluation was done using peripheral

Table 1: Participants' socio bio data

	Adult asthmatics n (%)	Adult non-asthmatics n (%)
Gender		
Male	32 (29.6)	32 (29.6)
Female	76 (70.4)	76 (70.4)
Total	108 (100)	108 (100)
Age		
21-30 years	29 (26.9)	29 (26.9)
31-40 years	33 (30.6)	33 (30.6)
41-50 years	23 (21.3)	23 (21.3)
51-60 years	23 (21.3)	23 (21.3)

Key: n = Frequency, % = percentage, p value determined using chi square for prospective data

Table 2: Comparison of hematological parameters among patientswith asthma and normal healthy control

	Asthmatic patients(mean ±SD)	Control group (mean ±SD)	t-statistic	*P*-value
PCV (%)	36.78 ± 6.00	38.56 ±3.27	2.71	<.05
RBC (10^{12}/L)	4.34± 0.80	4.51 ±0.41	1.97	.05
Hb (g/dl)	12.17 ± 2.05	13.54±0.86	6.40	<.05
MCV (fl)	84.92 ± 5.59	91.29 ±1.91	11.21	<.05
MCH (pg)	28.09 ± 2.18	31.09 ±3.04	8.33	<.05
MCHC (g/dl)	33.14 ± 1.23	35.26±1.66	10.66	<.05
WBC (10^9/L)	8.15±1.42	6.33 ± 2.27	7.06	<.05
Neutrophil (10^9/L)	3.89 ±1.79	2.6 ± 2.2	4.73	<.05
Eosinophil (10^9/L)	0.3 ± 0.17	0.20 ±0.12	5.00	<.05
Basophil (10^9/L)	0.12± 0.09	0.06±0.05	12.27	<.05
Lymphocyte (10^9/L)	2.90 ±1.14	3.00± 1.27	0.63	.05
Monocyte (10^9/L)	0.3± 0.27	0.85 ±0.27	15.00	<.05
Neutrophil (%)	38.68± 10.90	55.54 ±8.68	12.60	<.05
Eosinophil (%)	5.16± 2.60	3.25 ±1.69	6.40	<.05
Basophil (%)	1.41±1.08	0.39 ±0.28	9.50	<.05
Lymphocyte (%)	54.57±9.59	35.24 ±8.16	16.00	<.05
Monocyte (%)	4.37±1.54	5.54 ±2.12	4.64	<.05

Key: PCV, packed cell volume, HB, hemoglobin, RBC, red blood cell, MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC: mean cell hemoglobin concentration, WBC: white blood cell.

blood film examination.

Clinical data and drug use information

A structured interviewer-administered questionnaire was used to gather information on the current clinical status of the participants and the drug(s) therapy they were receiving. The folders of the patients were also accessed retrospectively to get additional data on the diagnosis, presence of comorbidities and management details of the disease.

Results

A total of 108 asthmatic patients and 108 controls were included in this study. Seventy-six (70.4%) of the asthmatics were female and the rest (29.6%) were males. Similar gender distribution was used in recruiting the control to ensure matching (Table 1). The age of the participants ranged from 20 to 60 years and was divided into four age groups: 21-30 years, 31-40 years, 41-50 years and 51-60 years with frequencies and percentages 29 (26.9%), 33 (30.6%), 23 (21.3%) and, 23 (21.3%) (Table 1). Twenty-one (19.4%) and 30 (27.8%) of the asthmatics and the healthy control were singles while, 87 (80.6%) and 78 (72.2%) were married respectively.

The mean and standard deviation of the packed cell volume, red cell count, hemoglobin concentration, mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) of both the asthmatics and normal healthy control were 36.78±6.00% versus 38.56±3.27%, $4.34\pm0.80\times10^{12}$/L versus $4.51\pm0.4\times10^{12}$/L, 12.17±2.05g/dl versus 13.54±0.86g/dl, 84.92±5.59fl versus 91.29±1.91fl, 28.09±2.18pg versus 31.09±3.04pg, and 33.14±1.23g/dl versus 35.26±1.66g/dl respectively. The corresponding P values are: 0.007, 0.056, 0.001, 0.001, 0.001 and 0.001 respectively.

The mean and standard deviation of total WBC counts, absolute neutrophil, lymphocyte, monocyte, eosinophil and basophil counts for the asthmatic participants and normal healthy control were $8.15\pm1.42\times10^{9}$ /L versus $6.33\pm2.9\times10^{9}$/L, $3.89\pm1.79\times10^{9}$/L versus $2.6\pm2.2\times10^{9}$/L, $2.90\pm1.14\times10^{9}$/L versus $3.00\pm1.27\times10^{9}$/L, $0.3\pm0.27\times10^{9}$/L versus $0.85\pm0.27\times10^{9}$/L, $0.3\pm0.17\times10^{9}$/L versus 0.20±0.12×109/L, and $0.12\pm0.09\times10^{9}$/L versus $0.06\pm0.05\times10^{9}$/L respectively. The relative counts of neutrophil, lymphocyte, monocyte, eosinophil and basophil for the asthma patients and normal healthy control in % were 38.68±10.90% versus 55.54±8.68%, 54.57±9.59% versus 35.24±8.16%, 4.37±1.54% versus 5.54±2.12%, 5.16±2.60% versus 3.25±1.69%, and 1.41±1.08% versus 0.39±0.28% respectively (Table 2).

Discussion

The red cell counts, packed cell volume, hemoglobin concentration and, all the red cell indices are reduced in the asthmatics on inhaled corticosteroids compared to normal healthy control. This occurs even though enhanced erythropoiesis is the norm in the vast majority of patients with asthma as a sympathetic response to counter the effects of the arteriohypoxemic hypoxia seen in the patients.[2,10-12] Hailemaryam et al. (2016),[13] reported a different finding from a cohort of asthma patients in Ethiopia; however, whether or not the participants were on corticosteroids was not made known. Ejaz et al (2017),[14] reported an elevation in the haemoglobin concentration and hematocrit in a group of 51 Pakistani asthmatics. Corticosteroids are found to affect the maturation of erythroid cells through transcriptional mechanisms and by the direct effect on erythropoietin signaling.[15] The magnitude of the effect on erythropoiesis is not clinically significant, this may not be unconnected to the route of administration of the drug; the systemic effects of corticosteroids are most profound when administered parenterally.[6] From the result, the asthmatic patients have slightly lower MCV, MCH and MCHC; though the decrease is not of clinical significance, it may however be suggestive of the early phase in the development of hypochromic microcytic anemia; though other causes of low MCV, MCH and MCHC have not been ruled out. Corticosteroid have effects on iron metabolism[15], as impaired iron metabolism leads to the development of hypochromic microcytic anemia.[16]

There is an overall increase in the mean WBC count among the asthmatics compared to normal healthy control; and the increase is largely accounted by a rise in absolute neutrophil, eosinophil and basophil counts respectively. Paradoxically however the mean lymphocyte and monocyte counts are lower in

the asthmatic compared to normal healthy controls. These findings are in line with the report of Evans et al. (1993),[17] John et al. (1998),[18] Zhang et al (2014),[19] Nadif et al. (2016),[20] and Hailemaryam et al (2018).[13] Corticosteroids are known to cause neutrophilia.[7] The neutrophilia occurs as a consequence of L-selectin shedding by neutrophils and down-regulation in the expression of P-selectin ligand (PSL) on the vascular endothelium. This hampers the extravasation of neutrophils into the interstitial environment.[7] As opposed to rising neutrophil count, corticosteroids are known to induce instant and persistent eosinopenia and basopenia.[17,21] These occur by suppression of the production of granulocyte monocyte colony-stimulating factor and interleukin [5], both of which are eosinophilopoietic. Redistribution of the cells is also suspected to play a role as altered expression of adhesion molecules has been reported.[17,22] Corticosteroids also decrease the survival of eosinophils via apoptosis.[22] Similarly, basophil production and function are also retarded by corticosteroids.[21] The persistence of eosinophilia and basophilia in the asthmatic group despite treatment with inhaled corticosteroids in effect reflects the central role of these leucocyte sub-populations in maintaining the allergic inflammation. However, a better appreciation of their trafficking dynamics would have been achieved had pre-treatment measurement been made. A slight reduction in lymphocyte count and a marked reduction in monocyte count were observed among asthmatic patients. Suppression of lymphopoiesis and monocytopoiesis by corticosteroids has been reported.[23,24] Down-regulated granulocyte monocyte colony-stimulating factor and enhanced IL-10 production by macrophages may be the mechanisms for such changes.[18]

Conclusion

Asthmatic patients on inhaled corticosteroids have reduced red cell indices, monocyte and lymphocyte count. However, the neutrophil, eosinophil and, basophil counts are still higher despite therapy with inhaled corticosteroids.

Ethical consideration

Ethics clearance from the ethics committee of Aminu Kano teaching hospital Kano was obtained before commencing recruitment [AKTH/MAC/SUB/12A/P-3/VI/1930]. The participants were made aware of the study details as enshrined in the Helsinki Declaration, 1964 and its modifications; they all consented to participate before enrollment.

References

1. Carrel KD. Asthma Variants: Allergic Asthma. In: Mackay IR and Rose NR (Editors). Encyclopedia of Medical Immunology-Allergic Disease. Ist Ed. New York: Springer; 2014.
2. El Gazzar AG, Al Mehy GF, Gouda TM, El-Shaer OS, Mohammed SH. Evaluation of erythropoietin hormone in chronic obstructive pulmonary disease patients during exacerbation and after remission. Egypt Journal of Chest Diseases and Tuberculosis 2017; 66 (1):27-32.
3. Ray A, Kolls JK. Neutrophilic Inflammation in Asthma and Association with Disease Severity. Trends in Immunology 2017; 38(12): 942–954; doi:10.1016/j.it.2017.07.003.
4. Zhou T, Huang X, Chen W. Associations between Th17 related inflammatory cytokines and asthma in adults: A case-control study. Nature scientific reports 2017; Doi: 10.1038/s41598-017-15570-8.
5. Befus AD, Mcnagny KM, Denburg JA. Mast Cells and Basophils: Ontogeny, Characteristics, and Functional Diversity. In: Greer JP, Rodgers GM, Glader B, Daniel A. Arber DA, et al. (Editors). Wintrobe's clinical hematology. 14th Ed. p739. Philadelphia: Wolters Kluwer; 2019.
6. Ericson-Neilsen W, Kaye AD Steroids: Pharmacology, Complications, and Practice Delivery Issues. Ochsner Journal 2014; 14(2): 203–207.
7. Ronchetti S, Ricci E, Migliorati G, Gentili M, Riccardi C. Review: How glucocorticoids affect the neutrophil life. International Journal of Molecular Science 2018; (19)4090; doi: 10.3390/ijms19124090.
8. Coutinho EA, Chapman KE. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. Molecular and Cellular Endocrinology 2011; 335 (2011) 2–13.

9. Briggs C, Bain BJ. Basic Hematological Techniques. In: Bain BJ, Bates I, Laffan MA, Lewis ML (Editors). Dacie and Lewis Practical Hematology. Philadelphia: Elsevier; 2017.
10. Wedzicha JA, Rudd RM, Apps MC, Cotter FE. Erythropoiesis in patients with polycythemia secondary to hypoxic lung disease. British journal of medicine 1983; 286 (1983), pp. 511-514.
11. Liverko IV. The blood oxygen-transport system and oxygen tissue balance in patients with bronchial asthma (BA) concurrent with carbohydrate metabolic disturbances. Probl Tuberk Bolezn Legk. 2005; (2):26-8.
12. Haase VH. Regulation of erythropoiesis by hypoxia-inducible factors. Blood Reviews 2013; 27(1): 41–53. doi: 10.1016/j.blre.2012.12.003.
13. Hailemaryam T, Adissu W, Gedefaw L, Asres Y. Hematological profiles among asthmatic patients in southwest Ethiopia: a comparative Cross-sectional study. hematology and transfusion international journal 2018; 6(2):77–82.
14. Ejaz S, Nasim F-H, Ashraf M, Ahmad S. Hematological and Biochemical Profile of Patients Suffering from Non-Atopic Asthma. Indian journal of chest diseases 2017; 2 (2: 6) DOI: 10.21767/2577-0578.10006.
15. Stellacci E, Di Noia A, Di Baldassarre A, Migliaccio G, Battistini A, Migliaccio AR. Interaction between the glucocorticoid and the erythropoietin receptors in human erythroid cells. Journal of Experimental Hematology 2009; 37(5): 559–572. doi: 10.1016/j.exphem.2009.02.005.
16. Britteham GM. Disorders of iron homeostasis: Iron deficiency and overload. In: Hoffman R, Benz EJ, Silberstein LE, Heslop HE, Weitz JI et al. (Editors). Hematology basic principles and practice. p563. Philadelphia: Elsevier; 2018.
17. Evans PM, O'Connor BJ, Fuller RW, Barnes PJ, Chung KF. Effect of inhaled corticosteroids on peripheral blood eosinophil counts and density profiles in asthma. Journal of allergy and clinical Immunology 1993; 91:643-50.
18. John M, Lim S, Seybold J, Jose P, Robichaud A, O'connor B, et al. Inhaled Corticosteroids Increase Interleukin-10 but Reduce Macrophage Inflammatory Protein-1a, Granulocyte-Macrophage Colony-stimulating Factor, and Interferon g Release from Alveolar Macrophages in Asthma. American journal of respiratory and Critical care medicine 1998; 157:256 262.
19. Zhang XL, Simpson JL, Powell H, Yang IA, Upham JW. Full blood count parameters for the detection of asthma inflammatory phenotypes. Clinical & Experimental Allergy 2014; 44, 1137–1145.
20. Nadif R, Siroux V, Boudier A, le Moual N, Just J, Gormand F, et al.. Blood granulocyte patterns as predictors of asthma phenotypes in adults from the EGEA study. European Respiratory Journal 2016; 48: 976–978.
21. Dunsky EH, Zweiman B, Fischler E, Levy DA. Early effects of corticosteroids on basophils, leukocyte histamine and tissue histamine. Journal of allergy and clinical Immunology 1978; 63 (6). 426-432.
22. Schleimer RP, Bochner BS. The effects of glucocorticoids on human eosinophils. Journal of allergy and clinical Immunology 1994; 94:1202-13.
23. Dygai AM, Shakov VP, Mikhlenko AV, Goldberg ED. Role of glucocorticoids in the regulation of bone marrow hematopoiesis in stress reaction. Biomedicine & Pharmacatherapy 1990; 45.9 - I4.
24. Courties G, Frodermann V, Honold L, Zheng Y, Herisson F, Maximilian J. et al. Glucocorticoids Regulate Bone Marrow B Lymphopoiesis After Stroke. Circulation research 2018; 124(9).