ORIGINAL ARTICLE

Basophils are seeking our attention in Multan region

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ABSTRACT

Basophils are a type of white blood cell. Though produced in the bone marrow, they are found in many tissues throughout our body. Basophils account for only 0.1-1% of all peripheral blood leukocytes and are rarely seen in routine blood smears. However, different ranges are set depending on the laboratory and also based on the local population. In Multan we noticed presence of basophil in almost every 100 cell count while examining peripheral smears of various patients with different diseases; this prompted us to examine basophils in blood of normal healthy individuals living in this region and to find out the reasons associated with it and whether the reference value of this specific cell should be changed here or not. Though brought to light long ago, basophils are still neglected for more than a century. They share features of innate and adaptive immunity and today basophils are well known for their obviously prominent effector role in the allergic reaction and functional roles in the context of the human immune system¹.

INTRODUCTION

Basophils help to get us healthy by fighting parasitic infections, by preventing blood clotting as they contain heparin and by mediating allergic reactions and inflammatory responses in the area of your body that was exposed to the allergen. Complete blood count is the common routine investigation carried out in medical practice². Reference interval is elementary for proper diagnosis of diseases. The establishment of normal laboratory values relies on the basis of various sociodemographic variables² and has prime importance in analysis of results³. The standard values implemented in Asia are acquired from populations of advanced countries and are not appropriate for most of the domestic setup⁴, so Clinical and Laboratory Standards Institute (CLSI) emphasized each region should create its own reference values5.

Basophil are the rare white blood cells circulating in blood⁶ and they elicit a particular response to IgE mediated challenge test⁷. Panels of cyto/chemokines secreted and receptors exhibited by basophils have been narrated. Besides, a substantial range of other molecules including immunoglobulins, factors of the complement system, bacterial compounds and growth factors appeared to trigger the basophils; along with their well-established role in allergy diagnosis8. The main role of basophils is to provide immunity against helminths infections, though its function in allergic reactions cannot be ignored⁹. There is flexibility in the function of basophils9. It participates in Th2 type immune response and immunoglobulin synthesis¹⁰⁻¹¹. Its role in autoimmunity cannot be pass over i.e. in lupus nephritis¹². The synthesis of chemokines in case of eosinophilic lung inflammation¹³. Last but not the least basophil contribution in irritant contact dermititis¹⁴ and in allergic inflammation¹⁵. Basophils are seen in patients with allergic rhinitis. A study conducted by Braunstahl and his fellows figure out that the number of basophils are increased after bronchoprovocation in allergic rhinitis patients⁷. The scarcity of this cell and its transient life span are also the limitation to study basophils¹⁶.

MATERIAL AND METHODS

Study design and study population: A cross sectional study was carried out on blood samples of 1400 blood donors and healthy residents of Multan city. The individuals were selected in time period (Sep 2019- Oct 2020) from Bakhtawar Amin Medical and Dental College/ Hospital. The inclusion and exclusion criteria for this study was based on the same questionnaire which was given to blood donors to assess their medical, demographic and life style information before collecting blood for donation. Additional exclusion criteria included i.e. drug/food allergies, hypersensitivity and smoking. As majority blood donors are male we selected female college and hospital staff, students, and paramedical staff to cover the healthy female population.

Blood collection and laboratory analysis: 2ml of venous blood was collected in EDTA tubes. After mixing the blood with the anticoagulant, test tubes were appropriately labeled. Hematological analysis was performed using Sysmex and Mindray analyzers which is 5 part differential platform. Differentiation into Eosinophils, Basophils and Monocytes were achieved by these analyzers. Total leukocyte count and differential leukocyte count were evaluated. We selected every sample for review of peripheral smear i.e with basophil count 0.1% or above mentioned on CBC electronically generated slip. Two to four slides were made from each sample. Peripheral smears were prepared following the staining protocols by Geimsa stain for microscopic analysis. All peripheral smears were scanned manually for basophils. At least 400 white blood cells were counted.

RESULTS

The age of the participants ranges between 18 and 55 years. Majority were between ages 18 years to 35 years.

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We deliberately selected equal number of males and females in this study. Every smear revealed 1 basophil in 100 cell differential count manually between ranges 0.1%-0.8% on CBC slip. In various smears with basophil %age 0.8 and above on the electronically generated CBC slip even 2 basophils were seen in 100 cells manual differential count. In the latter category eosinophils were also observed.

Gender distribution

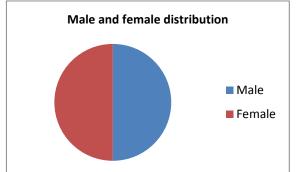


Figure 1 Basophil and neutrophil

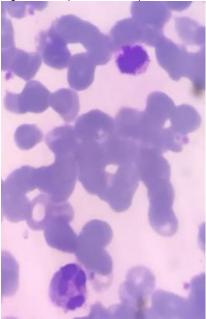


Figure 11 Basophil Giemsa stain

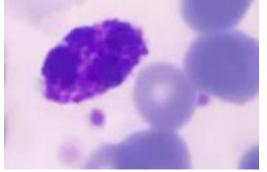
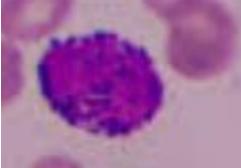


Figure 111 Basophil Giemsa stain



Age distribution according to gender

Age group	Males	Females	Total
18 to 25 years	204	381	585
26 to 35 years	391	208	599
36 to 45 years	80	74	154
46 to 55 years	25	37	62

Distribution of cases considering basophils on electronically generated slip and number of basophils per case verified manually

Basophil % on	Total number of	Basophils/100 cell
electronically	cases seen	count seen on
generated slips		microscope
0.1	112	1
0.2	140	1
0.3	252	1
0.4	266	1
0.5	154	1
0.6	154	1
0.7	84	1 to 2
0.8	70	1 to 2
0.9	42	1 to 2
1.0	126	1 to 2

DISCUSSION

Basophils are limited in peripheral blood but still catch the eye when peripheral smear are reviewed manually although in daily routine we used to ignore basophil. This study was conducted to note the presence of basophil in apparently healthy individuals of Multan and surrounding areas. The studies on hematological references values are rarely conducted in citizens of Asia especially in Pakistan¹⁷.

The age of our study participants ranges between 18-55 years which was consistent with the study conducted in Asmara i.e 18-49 years¹⁸. There were equal number of males (50%) and females (50%) in our study whereas a study conducted by Samaneka et al (2016) included 54% males and 46% females with age ranging 18-55 years which was almost consistent with our findings¹⁹. Another study conducted by Al-Jafar (2016) also revealed similar results, 50% males and females respectively³.

The main finding of this study was that we found basophil in 100 differential counts manually irrespective of the % basophil mentioned on electronically generated CBC slip. We found no gender discrepancy regarding finding of basophil in both male and female peripheral smear. A fairly consistent finding was demonstrated by Abbam et al (2021), they found no gender differences in % basophils²⁰. Whereas a study conducted by Addai et al (2019) concluded that the % basophil was higher in males as compared to females $^{21}\!\!\!$.

In various peripheral smear with % basophil 0.8 and above on the electronically generated CBC slip even 2 basophils were seen in 100 cells manual differential count along with bit high range eosinophils. The possible explanation of higher range eosinophils and presence of basophils in every healthy individual in our study without any symptoms could be associated to presence of aero allergens in this specific region of Punjab, Pakistan with high frequency of allergies and their propensity towards atopy along with extreme climate, dust storms, agricultural and and usage of pesticides and poor hygienic conditions of majority of population²¹.

Omuse et al (2018) stated in their study that vulnerability to environmental allergens and load of parasitic infection were most probable cause of high range eosinophils and basophils²²⁻¹. According to the study by Sabar MF et al Pakistani population has greater chances of asthma and allergic diseases. Almost 20% of overall pediatric population of Pakistan is affected by this disease; the probable reasons mentioned in their study were increasing population, economical instability, urbanization, poor health facilities, poor hygiene, increased pollutant exposures and consanguineous marriages²³. The province of Punjab is already known for its maximum incidence of allergies. Results of the study by Asim M recommend that aerosolic or aero allergens are the primary triggers (activators)²⁴.

Multan is oldest city of Southern Punjab and is the significant centre in the terms of flat and fertile land and is famous for cultivating plants and livestocks. Multan has extreme weather with hot summer and cold winters. The land cover and land surface temperature has revolutionized/shifted during 30 The past years. temperature rise due to change in the vegetation sequence in district Multan²⁵. Basophils are degranulated upon exposure to pollens and human immune system reacts to pollen with symptoms²⁶. The point to be mentioned here is that our subjects were symptomless and had no history of allergies. Pollen is the most likely environmental reason in this area, that provoked basophils²⁶⁻⁷. Multan city is well known for its blinding dust storms²⁷ and historically the role of dust storms in Asthma was well documented²⁶. To summarize in a nut shell the increased presence of basophils in the peripheral blood of resident of Multan can be due to extreme climate, dust storms, agricultural land and usage of pesticides. In a study carried out in Africa stated that basophil counts were higher in dry season as compared to rainy season²⁸.

The normal reference range of basophils in this region should be 1-2 rather than <1% that is being used by most of the laboratories.

Various studies were conducted on hematological reference value which showed % basophil in Kenya 0.30- 1.10^{22} , Zimbabwe 0.1- 1.3^{19} , Ghana 0.70- 2.60^{20} and Turkey $1.15\%^{29}$. A comparative study was conducted by Al-Jafar on adult hematology reference range of Kuwait and UK population. According to this study the % basophils reference value in Kuwait was 0.1-1 and in UK % basophils was < $1.0-2^3$.Most laboratories are using reference values from text book or follow the instructions provided by the

hematology analyzers. Consequently dissimilarity exists in various topographical regions and this specifies the requirement that suitable reference ranges must establish according to population³⁰. A study conducted in Malaysia in which hematological reference intervals in Multiethnic population was done. There data confirmed the differences of reference values in specific population hence emphasized the significance to adopt local guidelines rather than acquiring of generalized reference values³¹.

There are various approaches to count basophil³². The basophil count is the most overrated cell by hematology analyzers³³.Flow cytometry is the authentic technique for basophil recognition but inappropriate in regular use as it is expensive and not available here³².The examination of blood smear remains noteworthy compared with the advanced hematology analyzers³⁴. At least 400 white blood cells should be counted for manual basophil count to decrease statistical error³² so we followed this protocol in our study.

Limitations: Flow cytometry is the authentic method to count basophil but due to its non availability we did not use it. Another limitation of our study is that children and elderly are not included because of eligibility criteria for blood donation and being college and hospital staff. Last but not the least study should be done on large scale to cover maximum number of residents of this area for confirmation of our findings and before proceeding towards any change in normal reference range of basophils.

CONCLUSION

Our study indicates the presence of one to two basophils/100 differential count in blood samples of every healthy individual without any obvious sign and symptoms of allergies or parasitic infestation prompting us to suggest the change in normal reference range of basophils in this region from <1% to 1-2%. We believe this study will bring this cell into the spotlight and serve as a first step of ladder towards large scale studies.

Disclaimer: The abstract has not been previously presented or published in any conference, not a part of any research, PhD or thesis project.

Conflict of Interest: There are no financial, personal, or professional interests that could be construed to have influenced the work.

There are no funding sources to disclose

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