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Estimation of Erythrocyte and Leucocyte Counts in Individuals of *Mizaj-e-Damwi*

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ABSTRACTS

Erythrocytes and leucocytes are the cells present in blood. Erythrocytes are concerned with the transport of oxygen and carbon dioxide while the leucocytes are concerned with body immunity. The total counts of erythrocytes and leucocytes changes in both physiological as well as pathological conditions. Total counts changes from person to person and place to place. A number of environmental factors may also cause variation in total counts of erythrocytes and leucocytes. As per Unani literature, Mizaj is characterized by physiological, physical and psychological parameters. Keeping in mind the physiological parameters, this study was designed to find out the relation of total erythrocytes and total leucocytes with Mizaj-e-Damwi. For this study, 88 volunteers were randomly selected from Ajmal Khan Tibbiya College and other colleges of Aligarh and total erythrocytes and total leucocytes were estimated by using Neubaur's Chamber.

Key Words: Erythrocytes, Leucocytes, Mizaj, Mizaj-e-Damwi, Neubaur's Chamber.

INTRODUCTION

Blood is composed of blood cells which accounts for 45% of the blood tissue by volume, with the remaining 55% of the volume composed of plasma, the liquid portion of the blood. Blood cells are the cells which are produced during hematopoiesis and found mainly in the blood. There are three types of blood cell; i.e. red blood cells (erythrocyte), white blood cells (leucocytes) and platelets (thrombocytes). (Guyton, 1996)

Erythrocytes (RBC) are most abundant cells in blood which account for approximately 40-45 percent of the blood. They are non-nucleated biconcave disc like having mean diameter of about 7.8 micrometers and a thickness of 2.5 micrometers at the thickest point and 1 micrometer or less in the center. The shapes of RBC can change remarkably as the cells squeeze through capillaries. The major function of RBC is to transport hemoglobin which in turn carries oxygen from the lungs to tissues. Besides transport of hemoglobin, RBC have other functions like transport of carbon dioxide from tissues to lungs and hemoglobin of RBC acts as strong acid-base buffer. In the early weeks of embryonic life, primitive, nucleated red blood cells are produced in the yolk sac. During the middle trimester, the liver is the main organ for production of RBC, but reasonable numbers are also produced in the spleen and lymph nodes. Then during the last month or so of the gestation and after birth, RBC are produced exclusively in bone marrow. In normal men, the average number of RBC per cubic millimeter of blood is 5200000; in normal female it is 4700000. (Guyton, 1996)

Leucocytes (WBC) account for only 1% of the blood. They are the cells that make up the majority of the immune system they are formed partially in the bone marrow (granulocytes and monocytes and few lymphocytes) and partially in lymph tissue (lymphocytes and plasma cells). After formation they are transported in the blood to different part of the body where they are needed. Six types of WBC are normally present in the blood. They are neutrophils, eosinophils, basophils, monocytes, lymphocytes and occasionally plasma cells. The adult human being has about 7000 (4000-7000) WBC per cubic millimeter of the blood. (Guyton, 1996)

The concept of mizaj (temperament) is a pillar of Unani Medicine on which the states of health and disease of human being and the entire Unani therapeutics including diagnosis, prevention and treatment of diseases are based (Galen, 2008). According to Unani system of medicine, there are four types of temperament viz. safrawi (bilious), damwi (samguine), balghami (phlegmatic) and saudawi (melancholic) indicating the dominance of safra (bile), dam (blood), balgham (phlegm)

and sauda (black bile), respectively (Majoosi, 1902; Maseehi, 1963). For the diagnosis of the temperament of an individual, a number of parameters have been devised by different Unani physicians. However, Ajnas-e-Ashrah (ten parameters) suggested by Ibn Sina (Ibn Sina, 1993) to diagnose the temperament of an individual are widely accepted. It includes psychological (infia'lat-e-nafsaniyah), morphological and physiological parameters. Unani physicians have been applying the theory of mizaj in their practice with a fair degree of success since hundreds of years. They did their best despite having limited instrumental and technical facilities, to imply the theory of temperament in clinical and therapeutic practices but at present an evidence based data is required to prove its validity and relevance (Ahmad, 1980).

The present study was conducted on individuals of *DamwiMizaj*. The study was started with following objectives:

1. To find the correlation between erythrocytes and leucocytes count and temperament, if any.
2. To find out physiological limits of Erythrocyte and Leucocytes count for individuals of *DamwiMizaj'*
3. To institute erythrocytes and leucocytes count as a parameter of temperament assessment.

MATERIAL AND METHODS

This study was carried out in the Department of Kulliyat at Ajmal Khan Tibbiya College, Aligarh Muslim University, Aligarh Selection of Volunteers Eighty eight (88) healthy male and female volunteers of *Mizaj-e-Damwi* were selected randomly from the student's fraternity of Ajmal Khan Tibbiya College and some other departments of studies of Aligarh Muslim University and colleges of Aligarh city. Preference was given however, to the students of Ajmal Khan Tibbiya College as they were easily available for the study.

Inclusion Criteria

Only healthy volunteers of *Mizaj-e-Damwi* of both the sexes in the age group of 15–45 years were included in this study. Exclusion Criteria

Following volunteers were excluded from the study:

- Those having *Balghami*, *Safrawi* and *Saudawi* temperament.
- Volunteers below 15 years and above 45 years of age.
- Volunteers suffering from any physical, mental or psychiatric disorder.

For the selection of healthy volunteers, detailed clinical history, physical, general and local examinations were done (Annexure I)

Informed Consent

A multi-lingual informed consent form was provided to all the subjects included in the study(Annexure-II). The purpose of the informed consent form was to obtain permission from each of the volunteers and confirm their willingness to take part in the study. The form indicated exactly what the study demands, what the volunteers expect from the study, the risks and benefits of their participation, and guaranty of confidentiality. It stated clearly that a volunteer may withdraw himself/herself from the study at any time without citing any reason.

Determination of *Mizaj*

The assessment of temperament (mizaj) of the volunteers was made on the basis of *Ajnas-e-Ashra* (ten determinants), mentioned in classical Unani literature. A tabular proforma designed to assess the temperament was given to the volunteers (Annexure-III). The assessment was made on the basis of information they filled in the proforma.

After determination of *Mizaj*, individuals of *Mizaj-e-Damwi*were selected for the study and rest were rejected. Also after determination of *Mizaj*, erythrocyte and leucocyte counts were estimated.

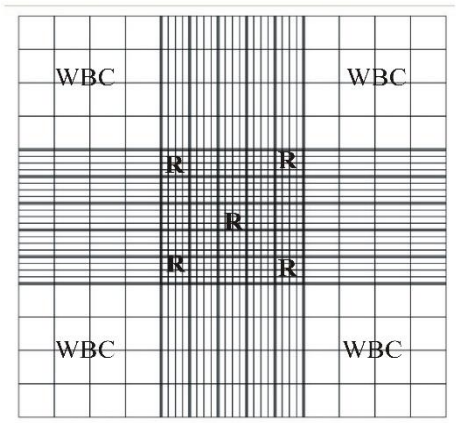
Estimation of Erythrocyte Counts (Absher, Marlin: 1973)

Materials Required:

0.9%NaCl (normal saline), RBC pipette, EDTA blood, Microscope, Cover Slip, Neubauer's chamber.

Neubaur's Chamber: Neubauer's chamber is a thick glass plate with the size of a glass slide (30x70x4mm). The counting region consists of two square shaped ruled areas. There are depressions or the moats on either side or in between the areas on which the squares are marked thus giving an "**H**" shape.

The ruled area is 3mm2 divided into **9 large squares** each with a 1 mm^2 area. The large central square (which can be seen in its entirely with the 10X objective), is divided into **25 medium squares** with double or triple lines. Each of these 25 squares are is again divided into **16 small squares** with single lines, so that each of the smallest squares has an area of 1/400 mm^2.



Method:
1. Make a 1:200 Dilution using EDTA Whole blood and saline
2. Put one drop of the solution in the Neubauer's chamber, using RBC pipette
3. Count the cells in Central Square (R marked) of the chamber.

Calculation:

$$RBC\ per\ Cubic\ mm\ of\ Blood = \frac{N\ \times Dilution\ Factor \times 5}{Depth\ of\ Fluid}$$

Where,

N= $Number\ of\ Cells\ Counted$

Dilution Factor = 200

Depth of Fluid = 0.1

Therefore,

RBC per Cubic mm of Blood = Number of cells counted x 10000

Normal ranges:

Male: about 5.4 million per µl or cubic mm of blood

Female: about 4.8 million per µl or cubic mm of blood

Estimation of Leucocyte Counts (Absher, Marlin: 1973)

Requirements: Neubauer's Chamber, WBC pipette, WBC diluting fluid, EDTA blood, Cover slip, Microscope

Procedure:

1. The blood specimen is diluted 1:20 in a WBC pipette with the diluting fluid.
2. Put one drop of the solution in the Neubauer's chamber, using a pipette
3. The cells are counted under low power of the microscope (10X) by using a counting chamber. The cells are counted in all four "W" marked corners.

The glacial acetic acid lyses the RBC while the gentian violet slightly stains the nuclei of the leukocytes to locate the WBC under microscope.

Calculations:

$$WBC\ per\ Cubic\ mm\ of\ Blood = \frac{N\ \times Dilution\ Factor}{Area\ Counted\ \times Depth\ of\ Fluid}$$

Where,

N= $Number\ of\ Cells\ Counted$
Dilution Factor = 20
Area Counted = 4
Depth of Fluid = 0.1

Therefore,

WBC per Cubic mm of Blood = Number of cells counted x 50

Normal ranges: 4000-11000 per cu mm of Blood

OBSERVATIONS AND RESULTS

This study was carried out in PG Lab, Department of Kulliyat, Ajmal Khan Tibbiya College, Aligarh Muslim University, Aligarh. One hundred twenty male and female volunteers in the age group of 15-45 years of *Mizaj-e-Damwi*were selected for the study, out of which thirty two volunteers could not fulfill the inclusion criteria and hence were left out of the study and 88 volunteers were finally included in the study. The volunteers, suffering from Malnutrition, Diabetes Mellitus, Hyperthyroidism, Hypothyroidism, and Tuberculosis or other psychological diseases were excluded from the study. The assessment of temperament of the volunteers was made on the basis of *Ajnas-e-Ashra*, mentioned in classical Unani literature.

Data obtained in this study was statistically evaluated which are as follows:

Table 01: Age Distribution

Age Group	Frequency	Percentage	Cumulative Frequency	Percentage
15-20	11	12.50	11	12.50
20-25	28	31.80	39	44.30
25-30	22	25.00	61	69.30
30-35	12	13.30	73	82.90
35-40	09	10.20	82	93.20
40-45	06	6.80	88	100.00
Total	88	100		

	Mean Age	**Median Age**
From Raw Data	28.09	27.00
From Frequency Table	27.82	27.14

In the present study, 88 volunteers of age 15- 45 years were selected randomly. Out of which maximum (28) were of the age group of 20-25 while minimum (6) were of the age group of 40-45 (table o1). From the raw data the mean age was 28.09 while the median age was 27. Also from Frequency table the mean age was 27.82 while the median age was 27.14.

Table 02: Sex Distribution

Sex	Frequency	Percentage
Male	81	92.05
Female	07	7.95
Total	88	100

In the present study, 81 volunteers (92.05%) were male while 07 7.95% were female. (Table-02)

Table 03: Average Mean Value of Erythrocytes/cubic mm of Blood

Age Group	Frequency	Leucocytes/cu mm
15-20	11	5130000
20-25	28	5090000
25-30	22	4970000
30-35	12	4530000
35-40	09	4510000
40-45	06	4450000
Total	88	4830000

In the present study, mean total erythrocytes count was maximum 5130000 in the age group of 15-20 while the mean total erythrocytes count was minimum 4450000 in the age group 40-45-30. The total mean of total erythrocytes count in all individuals o*f Damwi Mizaj*was found to be 4830000. (Table-03)

Table 04: Average Mean Value of Erythrocytes/cubic mm of Blood in two Genders

Gender	Frequency	Leucocytes/cu mm
Male	81	4930000
Female	07	4310000
Total	88	4830000

Also the total mean of total erythrocytes count in male volunteers was higher (4930000) than female volunteers (4310000). (Table-04)

Table 05: Average Mean Value of Leucocytes/cubic mm of Blood

Age Group	Frequency	Leucocytes/cu mm
15-20	11	6594.54
20-25	28	6322.75
25-30	22	6311.92
30-35	12	7560.00
35-40	09	7977.00
40-45	06	7861.66
Total	88	7104.65

In the present study, mean total leucocytes count was maximum 7977.00 in the age group of 35-40 while the mean total leucocytes count was minimum 6311.92 in the age group 25-30. The total mean of total leucocytes count in all individuals o*f DamwiMizaj*was found to be 7104.65. (Table-05)

Table 06: Average Mean Value of Leucocytes/cubic mm of Blood in Two genders

Gender	Frequency	Leucocytes/cu mm
Male	81	7304.75
Female	07	6976.58
Total	88	7104.65

Also the total mean of total leucocytes count in male volunteers was higher (73.0475) than female volunteers (6976.58). (Table-06)

DISCUSSION

*Mizaj*occupies a very important place in Unani Medicine. It forms the basis in the pathology, diagnosis and treatment of the diseases. The temperament of the person to be treated is expressed by the Galenic concept of its being *Damvi*(Sanguine), *Balghami*(Phlegmatic), *Safravi*(Bilious) and *Saudavi* (Melancholic) according to respective preponderance of the humour.

The most distinguishing feature of the *mizaj*is its approach of individuality. The temperament of an individual is a morpho-physio-psychological state that comes in to existence as a consequence of a dynamic interplay between his genes and the environment. Actually temperament is an empirical expression describing the humoural composition that governs and regulates the physiological, psychological and pathological changes in human body.

Keeping in mind the features of individuals of*DamwiMizaj*this study entitled "Estimation of Erythrocyte and Leucocyte Counts in Individuals of *Mizaj-e-Damwi*" was designed. In this study, total erythrocytes and total leucocytes count was estimated by using Neubaur's Chamber.

Mean total erythrocytes count in all individuals of *Mizaj-e-Damwi* was 4830000 and mean total erythrocytes count in males of *Mizaj-e-Damwi* was4930000 while mean total erythrocytes count in females of *Mizaj-e-Damwi* was4310000. In this study it was also found that mean total erythrocytes count was maximum in age group of 15-20 years and mean total erythrocytes count was minimum in age group of 40-45 years.

Mean total leucocytes count in all individuals of *Mizaj-e-Damwi* was 7104.65 and mean total erythrocytes count in males of *Mizaj-e-Damwi* was 7304.75while mean total leucocytes count in females of *Mizaj-e-Damwi* was 6976.58. In this study it was also found that mean total leucocytescount was maximum in age group of 35-40 years and mean total leucocytescount was minimum in age group of 25-30 years.

CONCLUSION

From the above study it can be concluded that total counts of erythrocytes and also of leucocytes in individuals of *DamwiMizaj*is near upper limit of normal range of both erythrocytes and leucocytes. The study validated the Unani concept that individuals of *Damwi Mizaj*are rich in components of *Khilt-e-Dam.*

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ANNEXURE: I
ProFORMA FOR HEALTH ASSESSMENT

NAME OF VOLUNTEER:_____FATHER'S NAME:_____
AGE/ GENDER:_____ OCCUPATION:_____
ADDRESS: _____

Recent history of:

Sickle Cell Anaemia	Sarcoidosis	Any Psychiatric Disorder
Bacterial Endocarditis	Leukaemia	Pernicious
Anaemia	Leishmaniasis	Hodgkin Disease
Banti's Disease	Tumours	Glandular Fever
Hereditary Spherocytosis	Gaucher'sDisease	Malaria

Past history

Diabetes mellitus	Hyperthyroidism	Hypothyroidism
Tuberculosis	Hiatus hernia	Poliomyelitis
Accident	Gastric Ulcer	Bronchial Asthma

Treatment history

Radiotherapy	Chemotherapy	Surgery
Prednisone	$NSAID_S$	Steroids

ANNEXURE-II
CONSENT FORM

Thank you for your interest in this project. Just to remind you, the data you provide in the course of this project will be treated in the strictest confidence and will be used for research purposes only. Furthermore, as a participant in this research you will never be identified in any outputs (e.g., reports, research articles) that arise from this project and your data will never be identifiable or viewed by any other party outside the research team.

CONSENT OF THE VOLUNTEER

(The participant should complete the whole of this sheet himself/herself)

Title of Experiment: A Comparative Study of Anxiety in Persons of Bilious and Phlegmatic Temperament

Name of Experimenter: Sartaj Ahmad

Please tick boxes

1. I confirm that I have read and understand the information sheet for the above experiment. ☐
2. I have had opportunities to ask questions and my questions have fully been answered. ☐
3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason. ☐
4. I have received enough information about the experiment. ☐
5. I agree to take part in the above experiment. ☐

"This experiment has been explained to me to my satisfaction, and I agree to take part. I understand that I am free to withdraw at any time."

_____	_____	_____
Name of Participant	Date	Signature

I have explained the experiment to the above participant and he/she has agreed to take part.

_____	_____	_____
Name of Experimenter	Date	Signature

ANNEXURE-III

MIZAJ ASSESSMENT PROFORMA

NAME OF VOLUNTEER:_____ FATHER'S NAME:_____
AGE: _____ GENDER: _____
HEIGHT: _____ WEIGHT: _____
OCCUPATION: _____ MOBILE NO: _____
ADDRESS: _____

Parameter (Evidence)	**Damwi (Sanguineous)**	**Balghami (Phlegmatic)**	**Safrawi (Bilious)**	**Saudawi (Melancholic)**
MORPHOLOGICAL				
1.**Skin texture/ Temperature Score:01**	Warm and smooth ☐	Soft and moist ☐	Hard and hot ☐	Rough and cold ☐
2.**Complexion Score:0.5**	Reddish ☐	Whitish ☐	Pale ☐	Blackish ☐
3.**Body built Score:5**	Muscular ☐	Fatty ☐	Moderate ☐	Lean and thin ☐
4.**Texture of hairs Score:0.5**	Thick and lusty ☐	Thin and smooth ☐	Curly ☐	Straight ☐
5.**Growth and distribution of hairs Score:0.5**	Rapid, Average ☐	Slow, Scanty ☐	Moderate/ Profuse ☐	Excessive ☐
6.**Colour of hairs Score:0.5**	ackish ☐	ownish ☐	Yell… black (Golden) ☐	Black and white (Mixed) ☐

PHYSIOLOGICAL				
7. **Urine Score:01**	Moderate in quantity	White, more in quantity	Yellow ,less in quantity	Turbid, less in quantity
8.**Tolerate Well Score:01**	Dryness	Summer	Cold	Dampness
9.**Remains well in Score:03**	Spring	Summer	Winter	Autumn
10.**Appetite Score:01**	Strong appetite(can skip a meal)	Less appetite(feel heaviness after eating)	Strong appetite (can't skip a meal)	Irregular appetite
11.**Thirst Score:01**	Average (+++)	Poor (+)	Increased (++++)	Low (++)
12.**Digestion Score:01**	Average	Slow	Strong	Irregular
13.**Movements and activities Score:03**	Average in physical activity	Dull laziness	Brisk, Hyper active	Less

14.**Sleep** **Score:01**	Average ☐	Excess sleep ☐	Disturbed sleep ☐	Insomnia ☐
PSYCHOLOGICAL				
15.**Dream** **Score:01**	Blood, Red objects ☐	Water, Snow ☐	Fire, Yellow objects ☐	Black, Fearful dreams ☐
16.**Anger/Joy** **Score:01**	Comes on easily and easily lost ☐	Comes on hardly ☐	Frequent, Severe and persists for long ☐	Infrequent but persist ☐
17.**Responsee to external stimuli in adverse condition** **Score:01**	Aggressively respond ☐	Weakly respond ☐	Bravely respond ☐	Cowardly respond ☐
18. **Decision taking power** **Score:01**	Take boldly ☐	Hesitate in taking decisions ☐	Take quickly ☐	Afraid in taking decisions ☐
19.**Memory** **Score:01**	Good retention also good ☐	Not good ☐	Good but can't retain for long ☐	Don't learn easily but excellent retention ☐

TOTAL COLLECTION:
DAMWI: _____ SAFRAWI: _____
BALGHAMI: _____ SAUDAWI: _____
DIAGNOSED TEMPERAMENT: _____

SIGNATURE OF INVESTIGATOR: _____