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Assessment of Metabolic Functions of Liver Through Total Serum Protein in Choleric & Phlegmatic Individuals

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ABSTRACT

BACKGROUND: A large number of metabolic processes take place in the liver which is aimed at benefiting the liver cells themselves as well as the entire body. Therefore, Ibn Sina (980-1037) says: the power of transformation (Quwat-e-mughayirah) found in the liver performs such vast and extensive function by which the entire body is benefited.

OBJECTIVE: To assess the metabolic functions of liver by the estimation of total serum protein in Choleric and Phlegmatic temperament individuals through Biuret Method.

DESIGN, SETTING, PARTICIPANTS: Volunteers were selected randomly from students of Aligarh Muslim University and Aligarh city, preference was given to students residing in hostels because their environment and nutritional status remain almost static and constant. 110 male volunteers in the age group of 20–35 years, having Choleric and Phlegmatic temperament were selected for the study.

RESULTS: The mean and standard deviation were calculated. It was revealed after this study that the mean total serum protein was 7.51 (g/dl) with SD 0.83 and 7.05 (g/dl) with SD 0.63 of choleric and phlegmatic subjects respectively. And there is significant difference ($p<0.001$) between both groups after applying Student t-test.

CONCLUSION: The individuals having Choleric temperament have higher levels of total protein than the Phlegmatic individuals. The metabolic activities of liver in choleric individuals were found to be more handsome than phlegmatic ones within normal values.

KEYWORDS Serum protein, Temperament, Choleric, Phlegmatic, Liver.

Introduction:

The concept of temperament is a pillar of Unani Medicine on which health or disease condition of human being and the entire Unani therapeutics including diagnosis, treatment and prevention of diseases are based. The liver has, since the times of recording of medical history, been considered as one of the principal vital organs governing health and disease and liver (*Kabid*) known as the seat of life since ancient times [1]. Liver is involved in the metabolism of almost all the physiological important substances and is also independently involved in many other important biochemical functions. Liver is the most important organ which is responsible for generating heat and blood, qualities essential for life and soul. Hippocrates (460-377 BC) considered that liver was the root of the veins and the source of blood and heat. Plato (428-347 BC) considered liver as the manager for the body and the origin of physical appetites [2]. Raban Tabri said that Liver is the storehouse of blood [3]. It is a large, expandable venous organ capable of acting as a valuable blood reservoir and capable of supplying extra blood when diminish blood volume. Abu Sehal Masihi observes in this regard liver prepares most of the constituent of blood and sends them in the vessels for circulation among the organs.

A large number of metabolic processes take place in the liver which is aimed at benefiting the liver cells themselves as well as the entire body. Therefore, Ibn Sina says: the Quwat-e-mughayirah (power of transformation) found in the liver performs such vast and extensive function by which the entire body is benefited [4].

According to the Unani physician and philosophers, the power of assimilation, retention, digestion and excretion occurs in liver. The liver is one of the principal vital organs of the body and source of origin of Quwat-e-tabiyah (physical or somatic powers) and the seat of digestion of chyme [5].

Majoosi (930-994 AD) while explaining the reason of hot and moist (haar-ratab) temperament of liver says that various metabolic processes and formation of blood take place in the liver [6].

Kabiruddin while quoting the views of Avicenna writes "Liver is a huge factory where due to digestive and metabolic changes the various humors of the body are formed in plenty".

Major metabolic processes of the body are performed through the liver metabolism (hazm kabidi) and most of the constituents of blood are formed through this metabolism. It is why the liver is said to be the "seat production of Akhlat".

Production of Blood (Taulid al-Dam): Unani physicians considered that the Akhlat (Dam, Balgham, Sauda and safra) is formed in the liver i.e. liver is the main seat for synthesis of blood. According to Ibn Sina (980-1037); the chyle from stomach reaches the liver where it is cooked and some substance are formed. One of them is foam (rughwah) that is yellow bile (safra); the other is black bile (sauda); the incomplete digested matter is phlegm (balghm) and the fully digested and filtered matter is blood (dam) [4].

Nafis says that normal (tabai) blood is that which is formed in the liver. But the most part of the blood is produced in the liver such as plasma protein and in the foetal life red blood corpuscles are also produced in liver. Liver is the primary site of the synthesis of plasma proteins.

According to Ibn Rushd liver have five faculties so the ailments are according to them, Disorder in the faculty of digestion (quwwate hadhma) leads to production of phlegmatic blood which provide nutrition to all body parts changing their temperament to phlegmatic

temperament which in turn creates ascites (istisqia e lahmi).

When the blood flows through the liver amino acids are partly retained and a "reserved" protein, which can easily utilized by the body during limited protein consumption, is synthesized from them in the liver. A small reserve protein can, apparently, also be stored in the muscle. Proteins are probably also formed in the liver. Thus after loss of blood the normal concentration of albumins and globulins in the blood plasma is rapidly restored. But if the function of the liver is impaired by phosphoric poisoning the restoration of the normal protein composition of blood is greatly impeded. The formation of albumins in the liver has been demonstrated in experiments with it's finely ground tissue. The liver also plays the leading part in intermediate protein metabolism. Process of deamination and the synthesis of urea take place in the liver on a large scale [7].

Ideally, the total protein will be approximately 7.5 g/dl.

Optimal Range: 6.6-8.3 g/dl [8].

Total protein may be elevated due to:
Liver dysfunction, Chronic infection (including tuberculosis), Adrenal cortical hypofunction, Collagen Vascular Disease (Rheumatoid Arthritis, Systemic Lupus, Scleroderma), Hypersensitivity States, Sarcoidosis, Dehydration (diabetic acidosis, chronic diarrhea, etc.), Respiratory distress, Hemolysis, Alcoholism, Leukemia, Upright position for several hours after rising (highest level at mid-morning), Venous stasis, Short-term high-protein diet, Strenuous exercise [9].

Total protein may be decreased due to:
Malnutrition and malabsorption (insufficient intake and/or digestion of proteins), Liver disease (insufficient production of proteins), Diarrhea (loss of protein through the GI tract), Severe burns (loss of protein through the skin), Loss through the urine in severe kidney disease (proteinuria), Pregnancy (dilution of protein due to extra fluid held in the vascular system), Overnight recumbent position, Prolonged bed rest [9].

Temperament of the liver:
Unani physicians have described the temperament of the organs in relation to the four qualities (Kaifiat) i.e. heat, cold, moist and dry and thereby classified organs of the body in four categories accordingly. Before discussion of temperament of the liver we discussed the temperament. Abu Sehal Masihi describes temperament as: "Because there are so many primary components (*ustuqussat*) of the body which are mixed together not in close proximity, it is necessary that the qualities of primary components must be mixed as a whole new qualities arise from inter-mixing of primary components which will be in between the previous qualities, called mizaj"[10].

IbnSina says:
"The temperament is a quality resulting from the interaction of opposite qualities present in elements consisting of minute particles so that most of the particles of each of the elements may touch most of the others. Thus when these particles act and react on one another with their properties, there emerges from their total properties, a uniform quality which is present in all of them [11].

Galen says that:
"Temperament is a quality produced by action and reaction of opposite qualities of body fluids (*Akhlat*). When these components interact by virtue of their respective powers (qualities) a condition is achieved which is found in equal proportions in all the components of that intermixture; this is called temperament" [12].

Similarly the normal Temperament of an individual is defined as "a condition in which he survives comfortably with all systems of healthy life", which varies widely as per composition and as well as other surrounding factors and circumstances in which he resides.

Therefore, in this universe all animates or inanimate, however alike in origin and structure does not resemble one another perfectly. The Temperament fluctuates in particular normal range of maximum and minimum limits. Whenever it crosses its normal limit, it leads to the abnormality (Su-e-Mizaj) within the body [13].

Ibn-e-Rushd holds the view that temperament of the liver is hot and moist (haar-ratab). The reason behind this is that substance of liver is fleshy. It is rich in vessels and contains most of the constituents of blood [14].

Majoosi (930-994 AD) while explaining the reason of haar-ratab (hot and moist) temperament of liver says that various metabolic processes and formation of blood take place in the liver.

According to Abu Sehl Masihi those organs whose blood supply is very rich are hot and whose blood supply is poor are cold. Unani physicians maintains the view that hot organs are those in which heat is produced comparatively more and this is possible only in those organs which have a rich blood supply. Liver has, rich blood supply and a large amount of heat production due to metabolic process, so it temperament should be hot [4].

Kabiruddin asserts that "liver is the great chemical factory (matbakh) of the body where food is produced for the organs [15].

Ibn-e-Nafis give the reason of liver being hot in the following manner: The function of liver is to convert chyle into humors. This function of the liver is a type of harkat kaifia and aainia and harkat (movement) always produced heat [16].

According to Azmi the organs which contain considerable quantity of blood or water or fat, are considered moist. Hence the temperament of liver must be moist because this organ is the store house of blood and is nourished by blood [17].

Ibn Rushd says temperament of liver is har ratab (hot and wet) because it contains more lahm (flash) and more blood contents and blood vessels [14].

Characteristic of choleric and phlegmatic temperament individual [11, 12].
According to Unani classical literature, the choleric (Safravi) individuals are energetic and hyperactive. They possess hot and dry temperament, a medium stature, thin and hairy body, moderate musculature, deficient fat, good digestion, increased appetite and thirst, well-formed and prominent joints, yellowish complexion, thick, curly, black, rough and abundant hairs, prominent Veins, strong and rapid pulse. They pass fiery and yellow urine. Sometimes, they feel the sensation of pain and pricks over the body. The temperament of phlegmatic (Balghami) individual is cold and wet (barid-ratab). They have flaccid and obese body built with soft and flabby muscles. Their movements and activities are sluggish. Their functions like digestion, appetite and thirst are poor, sleep is excessive.

Objective of the study:
The present study has been attempted with two different categories Choleric and Phlegmatic temperament individuals because these two categories show different types of signs and symptoms as their physiological, physical, and psychological features and are opposite to each other on the basis of their

Hot and Cold temperament respectively. Basically these two main states of hotness and coldness play vital role in determination of Temperament.

The study was started with following objectives.

1. To analyze the value of total serum protein in Choleric and Phlegmatic Temperament to assess the metabolic function of liver through Biuret Method.
2. To assess whether function of liver in Choleric and Phlegmatic Temperament are same or different within normal healthy state.

Hypotheses of the study:
Total serum protein would be relatively higher in Choleric than Phlegmatic because as per Unani literature, the choleric (Safravi) individuals are energetic and hyperactive, a medium stature, thin and hairy body, moderate musculature, deficient fat, good digestion, increased appetite and thirst and they have excellent metabolism activities. In comparison to Choleric, Phlegmatic individuals are flaccid and obese body built with soft and flabby muscles and they possess more fat in their body. Their functions like digestion, appetite and thirst are poor, sleep is excessive. Their movements and activities are sluggish as stated by few studies that total protein may be decreased due to prolonged bed rest and may be elevated due to Upright position for several hours after rising, Strenuous exercise [9].

According to the classic literature of Unani Medicine the temperament of choleric individuals are hot & dry (haar yabis) and those of phlegmatic is cold & moist (barid ratab). The liver in choleric is comparatively more (hot) har and contain more blood contents in relation to phlegmatic people; and choleric individuals have less ratubat than phlegmatic i.e. this might be the reason behind higher plasma protein concentration in choleric temperament individuals when compared to those of phlegmatic temperament.

Method of the study: ***Selection of volunteers, Sample Size, and Inclusion criteria:*** This study was carried out in PG Lab, Department of Kulliyat, Ajmal Khan Tibbiya College, Aligarh Muslim University, Aligarh, during the period from 2010-2013. Volunteers were selected randomly from students of the Ajmal Khan Tibbiya College and some other faculties of Aligarh Muslim University and Aligarh city, preference was given to students residing in hostels because their environment and nutritional status remain almost static and constant. One hundred ten (110) male volunteers in the age group of 20–35 years, having Choleric and Phlegmatic temperament were selected for the study. The features and characteristics of these temperaments differ from each other because of the domination of bile and phlegm in the body. Therefore, for this study Bilious and Phlegmatic individuals were selected and the reason for selecting this age group is that the volunteers in this age group are mostly healthy and disease free.

Exclusion Criteria:
Female volunteers were excluded because of hormone estrogen which is secreted from ovary, estrogen induces anabolism of proteins, by which it increases the total body protein and volunteers below 18years and above 35 years were excluded, the persons of Melancholic and Sanguine temperament were excluded from the study, the persons of the athletic activities, heavy exercise and gymnastics were excluded from the study because the level of serum protein fluctuate with exercise, the volunteers having a history of Radiotherapy, Chemotherapy, major accident and surgery or taking steroids for long periods were excluded from this study and the volunteers suffering from Malnutrition, Diabetes Mellitus, Poliomyelitis, Tuberculosis, Hyperthyroidism, Hypothyroidism or any other chronic diseases, were excluded from the study.

For the selection of healthy volunteers, detailed clinical history, physical, general and local examinations were done.

Determination of Mizaj:
The assessment of *Temperament* of the volunteers was made on the basis of ten determinants *(Ajnas-e-Ashra)*, mentioned in classical Unani literature.

Categorization of individual:
After determination of the mizaj, selected volunteers were divided into two groups according to their mizaj.

Group A: *Choleric Mizaj*

Group B: *Phlegmatic Mizaj*

After determination of Choleric and Phlegmatic temperament, the volunteers were randomly called for venipuncture. All volunteers were advised to remain at fast overnight before their blood samples were drawn. The blood specimens were allowed to clot at room temperature and then centrifuged within an hour of venipuncture. The sera obtained were assayed within two hours of venipuncture.

Estimation of total protein:
BIURETE METHOD was adopted in order to estimate the total serum protein. The most widely used method of measuring serum protein is the Biuret Reaction. The Principle of this reaction is that Peptide bonds of protein form a blue-violate colored complex with cupric ions an alkaline medium. The intensity of the color is proportional to the number of peptide bonds and the color is read at 546 nm (530-570 nm).

Total protein + Biuret reagent ----->purple colored Cu-peptide bond complex

(alkaline Cu++)

Reagents:
(They were supplied by Siemens ltd**)**

Calculation:
T(Test)

Total serum protein (g/dl) = ——————— × concentration of standard

S(Standard)

Normal Values:
Total Serum Protein: 6.6-8.3 g/dl [8].

Observations & results:
Out of 110 volunteers, 65 were Choleric temperament and 45 Phlegmatic and their percentage of 59.10% & 40.90% respectively see Table-01and Graph-01.

The mean and standard deviation of serum total protein levels of Choleric and Phlegmatic subjects were calculated. It was revealed after this study that the mean serum total protein of Choleric subjects was 7.51 (g/dl) with standard deviation of 0.83 while the mean total serum protein of Phlegmatic subjects was 7.05 (g/dl) with standard deviation of 0.63. In this way it can be said that the average serum total protein of the healthy Choleric subject is higher than that of a Phlegmatic subject see table-02.

The mean and standard deviation of age, height, weight, and total protein of Choleric and Phlegmatic temperament subjects were calculated. It was revealed after this study that the mean of age, height, weight and total protein of Choleric subjects was 25.2 (years), 169.69 (cm), 60.12 (kg), and 7.51 (g/dl) with standard deviation of 2.5, 4.4, 5.8, and 0.83 respectively, while the mean of age, height, weight and total protein of Phlegmatic subjects was 27.8 (years), 168.68 (cm), 67.75 (kg) and 7.05 (g/dl) with standard deviation 3.0, 3.08,

5.6 and 0.63 respectively, as shown in table 03.

Total serum protein was higher in Choleric volunteers as compared to Phlegmatic subject and the difference was statistically significant as shown in the graph-03.

Present study after statistical analysis showed that the difference of total serum protein level of Choleric and Phlegmatic temperament subjects was highly significant as shown in Table 04. The level of total serum protein was higher in the Choleric temperament volunteers as compared to volunteers of Phlegmatic temperament.

Result:
The mean and standard deviation of total serum protein levels of Choleric and Phlegmatic subjects were calculated. It was revealed after this study that the mean total serum protein of Choleric subjects was 7.51 (g/dl) with standard deviation of 0.83 while the mean total serum protein of Phlegmatic subjects was 7.05 (g/dl) with standard deviation of 0.63. In this way it can be said that the average serum total protein of the healthy Choleric subject is higher than that of a Phlegmatic subject.

The T-test was applied to test the significance of the results and it was found that the difference of total serum protein level of Choleric and Phlegmatic subjects was highly significant ($p<0.001$).

Discussion and Conclusion:
Choleric individuals are energetic and hyperactive than Phlegmatic individuals, they have excellent metabolism activities, hence they have physiologically higher level of total protein because liver plays an important role in metabolism and the metabolic rate of Choleric individuals are rapid than phlegmatic, because of there temperamental differences. As indicated in the classic literature of Unani Medicine the temperament of choleric individuals are hot & dry (haar yabis) and those of phlegmatic is cold & moist (barid ratab). The liver in choleric is comparatively more har and contain more blood contents in relation to phlegmatic people; and choleric individuals have less ratubat than phlegmatic i.e. this might be the reason behind higher plasma protein concentration in choleric temperament individuals when compared to those of phlegmatic temperament. Therefore the level of total protein should be higher in Choleric than Phlegmatic individuals.

From the findings of this study following inference can be drawn.

The individuals having Choleric temperament have higher levels of total protein than the Phlegmatic individuals and the metabolic activities of liver in choleric individuals were found to be more handsome than phlegmatic ones within normal values.

In our study, it is clearly evident that temperament of an individual is influenced by his blood composition and its constituents. Therefore, the blood compositions play an important role in creation of Temperament of an individual. The need of the hour is to understand the concept & importance of temperament and its application in Unani as well as other health care.

Table–01: Showing Distribution of Volunteers according to the Mizaj

Mizaj	Frequency	Percentage
Choleric	65	59.10
Phlegmatic	45	40.90
Total	110	100

Graph-01: Showing Distribution of Volunteers according to the Mizaj

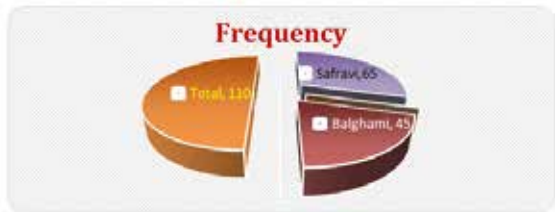


Table-02: Indicating Total Serum protein of volunteers expressed as Mean ± SD*

Mizaj	Number of Volunteers	Mean ± SD*
Choleric	65	7.51 ± 0.83
Phlegmatic	45	7.05 ± 0.63
Total	110	

Table-03: Showing Characteristics of Volunteers Expressed As Mean ± SD

Mizaj	Choleric	Phlegmatic
No. of Subjects	65	45
Age (Mean ± SD)	25.2±2.5	27.8±3.0
Heigh(Mean ± SD)	169.69±4.4	168.68±3.08
Weight (Mean ± SD)	60.12±5.8	67.75±5.6
TP (Mean ± SD)	7.51±0.83	7.05±0.63

SD: Standard deviation.

Graph-03: Showing Total serum protein of Volunteer



Table-04: Showing the Results in Volunteers according to their Mizaj

Parameters	Mizaj	Number of Volunteers	Mean± S.D	Significance
Total Serum Protein	Choleric	65	7.51±0.83	P<0.001
	Phlegmatic	45	7.05±0.63	

Significant at the level of p< 05.

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