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Original Research

Biochemical Approach to the Hotness and Coldness of Mizaj in Persian Medicine: A Cross-Sectional Survey in the Healthy Population

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Abstract

An individualized viewpoint in Persian Medicine is introduced with the concept of Mizaj (temperament). Accordingly, every individual is placed in a unique point among a spectrum ranging from extreme hotness to extreme coldness. The whole-body Mizaj is mainly determined by three organs of heart, brain, and liver. This study investigates the relationship between whole-body and three main organ Mizajes with some hormonal and biochemical factors. In 324 healthy volunteers between the ages of 20 and 40, whole-body Mizaj and Mizajes of heart, brain, and liver were determined in two sessions with an expert panel method. Any disagreement between experts and the moderate diagnosis was excluded. Finally, 72 healthy volunteers (including 40 hot Mizaj individuals and 32 cold Mizaj) entered the second phase. In this phase, some hormonal and biochemical factors were evaluated. In whole-body Mizaj, FBS, Hgb, Hct, MCHC, WBC, and Monocytes, were higher in people with hot Mizaj than cold Mizaj. Besides, relating to the Mizaj of the liver, FBS, cortisol, TG, Hgb, Hct, MCH, MCHC in hot Mizaj were higher than cold Mizaj. Also, in the heart, MCV and MCH were higher in hot individuals and in the brain FBS, Hgb, Hct, MCV, MCH, lymphocytes were significantly higher in hot Mizaj. It seems that FBS and Hgb as the factors involved in metabolism have the most important role in the whole-body Mizaj. Also, the association between cortisol with the hotness of the liver can be suggested by increasing sympathetic activity as one of the hotness/coldness theories.

Keywords: Temperament; Precision medicine; Medicine; Traditional; Complementary therapies

Introduction

The use of complementary and alternative medicine (CAM) is increasing worldwide and today they are a part of the health care system in many countries [1]. So much researches have been done in different areas of therapeutic effects of CAM ideas [2-4].
As the lack of standardized diagnostic tools [5] makes a variety of diagnostic differences between therapists [6,7], many CAM paradigms are trying to develop their diagnostic tools [8,9], nevertheless few studies in diagnosis methods have been completed, yet.
One of the CAM paradigms is traditional Persian Medicine (PM) [10]. The most important principle of PM is an individualized viewpoint called Mizaj (or tem-

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perament).
Mizaj is assessed by 10 criteria [11] and used to save health and get treatment [12] in an individualized manner. Based on these criteria the whole-body Mizaj of every person is determined in a wide spectrum, with the two extremities of hotness or coldness and a middle range of moderate Mizaj [13]. This range is also defined for the wetness-dryness of each person based on the 10 criteria [14]. Based on this concept the whole-body Mizaj is mainly influenced by Mizaj of the three important organs including heart, brain, and liver [15].
In recent years, efforts have been made to standardize diagnostic criteria in PM. However, most of these standards are questionnaires and subjective [16].
Discovering the relationship between Mizaj and the biological system has been an interesting topic in PM to researchers both in the past and now [17]. It is believed that Mizaj has a biological and genetic basis [18,19]; however, environmental factors, and changes during puberty can also affect the Mizaj [20].
Although few studies on the relationship between Mizaj and hormonal and biochemical factors were found [17], some studies investigate the relationship between Mizaj and malignancies [19], cardiovascular and hematologic [21,22], psychological [23], and neuroendocrine system [24]. But as a major weakness and pitfall, each of them have their own limitations in Mizaj assessment, so their results may be unreliable.
This study aimed to investigate the correlation between some hormonal and biochemical indices with the best available manner to assess the hotness/coldness of Mizaj.

Materials and Methods

Study design

This cross-sectional study was conducted at Babol University of Medical Sciences (BUMS), Mazandaran province, North of Iran, from January to April 2020. It was approved by the Research Ethics Committee of BUMS with the code of HRT.REC.1396.172. All participants were volunteers who undersigned written informed consent and all their identities remained confidential.

Participants

All healthy individuals could include in the study based on inclusion and exclusion criteria.
Inclusion criteria:
Men or women aged 20 to 40 years could enter the study
Exclusion criteria:
All volunteer with chronic illness, continues drug use including levothyroxine and corticosteroids, etc., and pregnancy were excluded. Women did not enter the study during the menstruating period.
Patients recruitment:
By advertisement on the university campus and PM clinic at BUMS, the volunteers were invited to participate in the study. Each volunteer who fulfilled the inclusion criteria entered the study.

Assessment

This study was performed in two phases:
Phase 1: Mizaj assessment
Each participant was visited twice. In each visit, volunteers were interviewed and examined separately by three PM experts, without any discussion between them. The whole-body Mizaj and Mizaj of brain, heart, and liver of each participant were determined based on the experience of experts. Then in an expert panel, the diagnosis was evaluated.
If at least two of the three experts proposed a similar diagnosis for the whole-body Mizaj of an individual and the third expert did not disagree, it would be accepted as an agreement.
This expert panel was done in two sessions with average two weeks intervals for each person.
If the final diagnosis of whole-body Mizaj of each person in two sessions of Mizaj assessment were the same, this volunteer could enter the next phase of the study.
If there was no agreement in at least one of the sessions, or the diagnoses of the two sessions were not the same, or volunteers whose final whole-body Mizaj were moderate, were excluded from the study.
This step continued until we reached at least 32 individuals in each hot or cold Mizaj diagnosis of whole-body Mizaj.
Phase 2: biochemical and hormonal assessment
Sampling: All individuals were invited for blood sampling after 12 hours of fasting. Blood samples of all subjects were taken by an expert nurse in the PM clinic in two consecutive days. Volunteers were forbidden to take any medications from the day before the sampling.
The amount of 10 mL of intravenous blood was divided into two parts as follows: First, supplied with Ethylenediaminetetraacetic acid (EDTA) as an anticoagulant for cell blood counting (CBC) and blood type determination and the other was dedicated for hormonal and biochemical evaluation which had no anticoagulant. The EDTA-supplied tube was mixed and stored in the refrigerator for less than 4 h until further experiment. The last tube was delivered to the biochemistry laboratory and was centrifuged in 2500 g for 15 min, to separate the supernatant fluid as serum. The serum was aliquoted in new microtubes and stored at -80 ˚C until further analyses.

Methods of biochemical and hormonal assessment: CBC and ABO/Rh typing
A Sysmex cell counter was used to perform CBC. Direct cell typing assay was applied to determine the ABO/Rh blood types of individuals. Specific antibodies to blood ABO/Rh antigens were provided from the Iran Blood Transfusion Organization, Tehran, Iran.
Measurement of biochemical parameters

FBS measurement
Serum Fasting Blood Suger (FBS) levels were measured in the colorimetric assay using Pars Azmoom (Pars Azmoon Co, Tehran, Iran) commercial kit based on glucose oxidase (GOD_PAP) method.
Lipid profile assessment
Serum Triglyceride (TG) and total cholesterol (Chol) levels were measured in the colorimetric assay using Pars Azmoom (Pars Azmoon Co, Tehran, Iran) commercial kit based on glycerophosphate-oxidase (GPO/PAP) and cholesterol oxidase (CHOD-PAP) methods respectively. After precipitating the other chylomicrons with magnesium chloride, serum high-density lipoprotein cholesterol (HDL) levels were measured by enzymatic techniques (Pars Azmoon Co, Tehran, Iran). Low-density lipoprotein (LDL) was estimated by Friedewald equation as below [25]
LDL = CHOL- (HDL + TG / 5)

Albumin measurement
A biochemistry commercial kit was used for the measurement of Albumin. The experiment was based on the bromocresol green method (Pars Azmoon Co, Tehran, Iran).

Blood Urea Nitrogen (BUN) measurement
To measure BUN, serum urea levels were measured by colorimetric assay using a urea commercial kit (Pars Azmoon Co., Tehran, Iran). BUN was calculated as follow:
BUN (mg/dl) = UREA (mg/dl) / 2.14

Measurement of hormonal parameters
Serum Thyroid Stimulating Hormone (TSH) was measured using an sandwich ELISA, antigen captured (antibody sandwich), commercial kit (Pishtaz Teb Zaman Co., Tehran, Iran). The normal range of the hormone TSH was 0.52-32 mIU/L. Serum T4 and T3 were measured using competitive ELISA. The normal range of T4 was 4.7-12.5 µg/dL and for T3 was 0.1-2.6 ng/mL. The IBL ELISA kit (IBL GmbH, Hamburg, Germany) was used to measure the amount of cortisol based on Competitive ELISA. The normal range was considered as 635-138 ng/mL.

Data analysis
To analyze the data the statistical software SPSS (Statistical Package for special science) version 22 was used. For reporting quantitative data, mean (±standard deviation) and for qualitative data, number (percentage) was used. To compare the frequency of qualitative variables (e.g. sex) between groups, we used chi-squared test. To compare the means in the two groups, a t-test was used, and also for more than two groups we used ANOVA, and Bonferoni post hoc tests.
A statistically significant level of 5% was considered in the study.

Results
In this clinical study after advertisement, 324 volunteers were referred to the clinic. After entering the study based on inclusion and exclusion criteria and after the first step of the study (Mizaj assessment) 72 people entered the second phase and blood samples were prepared. Of these, 40 (54.8%) had a hot, and 32 (44.4%) had a cold whole-body Mizaj. The mean age of participants was 36.11±8.7 years old with no significant difference between groups. The Mizaj of the heart, brain, and liver were also assessed. Details are given in table 1. The demographic data of the participants is shown in table 2.
Based on our findings, relating to whole-body Mizaj, FBS, Hgb, Hct, MCHC, WBC, and monocytes, were higher in people with hot Mizaj than cold Mizaj. Blood group and Rh were not significantly different between groups.
Besides, relating to the Mizaj of heart, Chol was high-

Table1. The Mizaj of the heart, brain, and liver of participants

Mizaj (n=72)	Hot	Moderate	Cold	Disagreement
Whole-body (n (%))	40(55.6%)	excluded	32(44.4%)	excluded
Heart (n (%))	33(45.8%)	13(15%)	19(26.3%)	7(9.7%)
Brain (n (%))	22(30.5%)	6(8.3%)	37(51.3%)	7(9.7%)
Liver (n (%))	20(27.8%)	21(29.7%)	25(34.7%)	6(8.3%)

Table2. The demographic data of participants

Mizaj (N=72)	Whole-body			Heart				Brain				Liver			
	Hot	Cold	p-value	Hot	Moder-ate	Cold	p-val-ue	Hot	Moderate	Cold	p-val-ue	Hot	Moder-ate	Cold	p-val-ue
Age (year) Mean±SD	37.3±8.1	35.5±9.2	0.381	37.1± 7.3	39.5±8.4	36.1± 7.7	0.461	36.9±7.8	36.5± 9.4	37.2±8.7	0.978	36.2± 9.3	37.1± 8.3	36.5±8.1	0.942
Sex (female) n (%)	27 (67.5%)	20 (62.5%)	0.685	21 (63.6%)	9 (69.2%)	13 (68.42%)	0.908	14 (63.6%)	5 (83.3%)	23 (62.2%)	0.599	14 (70%)	13 (61.9%)	16 (64%)	0.852
BMI	22.1±6.8	24.6±5.2	0.091	22.5± 3.8	23.4±3.5	23.8± 4.2	0.479	23.1±2.6	24.1± 3.5	24.±3.1	0.114	21.9± 4.6	22.5± 3.2	24.1± 2.9	0.108

er in people with moderate Mizaj than individuals with cold Mizaj. Also, MCV and MCH were significantly higher in hot individuals than in cold ones.

In the study of hotness/coldness of brain FBS, Hgb, Hct, MCV, MCH, lymphocytes were significantly higher in hot people than in colds.

In the study of hotness/coldness of the liver, FBS and TG in hot Mizaj were higher than cold Mizaj. Also, cortisol was higher in people with hot Mizaj than cold and higher in moderates than cold. Hgb, Hct, MCH, MCHC were significantly higher in the hotness of the liver than colds and moderates. MCV was higher in hot-Mizaj

Table3. Biochemical and hormonal indices in whole body and main organ mizajes

		FBS	TG	CHOL	LDL	HDL	BUN	Alb	T3	T4	TSH	Cortisol
		mg/dL	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL	g/dL	ng/mL	µg/dL	mIU/L	nmol/L
Whole Body Mizaj	Hot (n=40)	89±16	165±108	210±46	130±40	37.5±10.1	11.5±2.2	4.6±0.3	1.2±0.5	7.3±1.1	2±1.2	175.3±62
	Cold (n=32)	79±13	112±59	192±54	125±47.2	42.1±10.12	11.6±3.21	4.6±.036	1.30±0.8	7.2±1.10	2±1.2	158.2±64.3
	P-value	0.004	0.029	0.091	0.347	0.065	0.935	0.602	0.800	0.563	0.874	0.482
Heart Mizaj	Hot(n=33)	88.30± 13.69	148.53± 92.16	211.58± 46.67	139.05±40.56	37.29±10.04	12.16±2.08	4.57±0.29	1.12±0.54	7.41±1.03	2.02± 1.27	165.50±65.50
	Moderate (n=13)	87.77± 17.01	155.90± 137.91	226.23± 64.25	132.83±61.17	44.53±12.59	10.49±1.99	4.74±0.35	1.11±0.43	7.24±1.26	2.26± 1.06	151.13±87.23
	Cold (n=19)	78.88± 16.10	141.88± 70.34	182.47± 39.24	111.28±29.25	38.30±12.06	11.73±3.55	4.50±0.41	1.31±0.59	7.03±1.05	1.68± 1.12	178.78±46.20
	P-value	0.111	0.933	0.034	0.083	0.142	0.153	0.161	0.448	0.483	0.394	0.480
Brain Mizaj	Hot (n=22)	90.18± 15.14	159.45± 104.77	217.24± 45.97	137.37±39.85	37.07±9.77	12.01±2.02	4.58±0.32	1.13±0.53	7.34±1.05	2.06± 1.24	169.84±64.81
	Moderate (n=6)	79.40± 13.20	184.40± 117.24	204.00± 52.46	114.12±50.20	43.20±12.22	10.21±3.68	4.50±0.53	1.32±0.50	7.48±1.48	1.77± 1.03	221.55±71.13
	Cold (n=37)	79.27± 14.06	121.09± 65.14	189.55± 54.41	124.30±48.20	41.02±13.04	11.85±3.16	4.61±0.33	1.19±0.56	7.22±1.21	1.83± 1.20	159.19±63.58
	P-value	0.025	0.157	0.113	0.145	0.244	0.054	0.687	0.534	0.737	0.786	0.253
Liver Mizaj	Hot (n=20)	90.95± 16.36	177.90± 109.04	208.68± 49.88	125.06±41.88	39.06±10.65	11.62±2.49	4.59±0.32	1.26±0.57	7.17±1.16	1.83± 1.04	174.03±51.91
	Moderate (n=21)	87.10± 17.12	159.11± 100.77	195.10± 50.92	119.12±37.08	36.31±12.82	11.59±2.73	4.63±0.29	0.99±0.39	7.02±0.89	2.08± 1.48	191.49±64.03
	Cold (n=25)	77.83± 7.90	97.67±28.64	213.60± 50.52	146.28±48.37	42.62±10.43	11.70±2.89	4.59±042	1.26±0.59	7.62±1.08	2.03± 1.03	129.77±59.56
	P-value	0.022	0.020	0.475	0.107	0.211	0.991	0.614	0.173	0.187	0.751	0.004

Table 4. Cell Blood Counts in whole body and main organ mizajes

		WBS	RBC	HGB	HCT	MCV	MCH	MCHC	PLT	RDW	NEUT	LYMPH	MONO	EO	BASO
		10^3/UL	10^6/UL	g/dl	%	fl	Pg	g/dl	10^3/UL	10^3/UL	10^3/UL	10^3/UL	10^3/UL	10^3/ UL	10^3/UL
Whole Body Mizaj	Hot (n=40)	7.6±1.7	4.7±0.5	13.3± 1.6	39.2±3.8	83.1±5.6	28.1±2.2	33.8±1.1	242±59	15.5±8	3.9±1.3	2.7±0.7	0.6±0.2	0.3±0.3	0.03± 0.02
	Cold (n=32)	6.8±1.8	4.7±0.6	12.1± 1.3	36.5±3.1	73.2±20	26±4	33.2±1.1	237±80	13.80± 1.97	3.27±0.92	2.35±0.54	0.6±0.1	1.4±3.6	0.03± 0.02
	P-value	0.041	0.72	0.002	0.004	0.054	0.055	0.020	0.756	0.635	0.108	0.079	0.038	0.603	0.748
Heart Mizaj	Hot (n=33)	7.50± 1.83	4.70± 0.52	13.20± 1.70	38.89±3.91	83.02±6.02	28.09± 2.40	33.83±1.16	235.13± 63.06	15.07± 7.32	3.92±1.37	2.61±0.72	0.65± 0.19	0.27± 0.26	0.02± .014
	Moderate (n=13)	7.06± 1.56	4.77± 0.43	12.43± 1.90	37.46±4.73	78.53±7.16	26.04± 3.04	33.06±1.20	262.92± 90.40	13.97± 1.07	3.08±0.76	2.78±0.61	0.65± 0.15	0.50± 0.46	0.03± 0.01
	Cold (n=19)	7.15± 1.96	4.85± 0.71	12.29± 1.118	37.04±2.80	69.39±24.69	25.82± 4.35	33.14±1.01	225.16± 69.05	15.51± 6.54	3.62±0.85	2.24±0.63	0.61± 0.15	0.63± 1.01	0.03± 0.01
	P-value	0.698	0.661	0.114	0.207	0.007	0.031	0.045	0.327	0.793	0.087	0.068	0.740	0.123	0.133
Brain Mizaj	Hot (n=22)	7.60± 1.78	4.71± 0.51	13.26± 1.63	39.10±3.78	83.20±5.69	28.14± 2.27	33.82±1.11	239.47± 62.10		3.82±1.34	2.72±0.72	0.66± 0.19	0.35± 0.37	0.02± 0.01
	Moderate (n=6)	6.44± 0.66	5.11± 0.31	12.90± 1.54	38.81±3.66	76.20±9.32	25.34± 3.64	33.18±1.11	236.00± 27.31		3.22±0.49	2.39±0.35	0.59± 0.15	0.19± 0.09	0.03± 0.01
	Cold (n=37)	7.05± 1.98	4.74± 0.67	12.00± 1.41	36.24±3.42	70.37±22.89	25.69± 4.04	33.02±1.12	236.00± 92.33		3.50±0.93	2.28±0.63	0.62± 0.13	0.61± 0.94	0.03± 0.01
	P-value	0.265	0.291	0.015	0.020	0.006	0.006	0.021	0.978		0.434	0.049	0.568	0.184	0.870
Liver Mizaj	Hot (n=20)	7.37± 1.62	4.77± 0.48	13.73± 1.52	40.20±3.60	84.32±3.87	28.74± 1.54	34.09±1.04	23668± 62.85	16.48± 9.65	3.60±0.91	2.71±0.64	0.66± 0.19	0.36± 0.31	0.030±0.017
	Moderate (n=21)	7.26± 1.95	4.87± 0.73	12.40± 1.29	37.34±2.91	70.11±23.91	25.92± 4.05	33.15±1.08	220.45± 69.69	14.22± 2.34	3.83±1.34	2.48±0.81	0.65± 0.19	0.26± 0.20	0.024±0.008
	Cold (n=25)	7.28± 1.92	4.62± 0.46	11.98± 1.52	36.13±3.69	78.62±8.95	26.07± 3.48	33.08±1.13	258.50± 78.93	13.75± 1.23	3.60±1.27	2.36±0.58	0.61± 0.13	0.67± 1.02	0.03± 0.01
	P-value	0.976	0.681	≤0.001	0.001	0.006	0.004	0.003	0.235	0.285	0.764	0.218	0.594	0.089	0.088

than moderates. The details are shown in tables 3 and 4.

Discussion

In this study, FBS and Hgb as two important factors involved in metabolism had the main relationship with whole-body Mizaj. Also, the relationship between cortisol and the hotness of the liver may be suggested as one of the theories of hotness by increasing sympathetic activity.

Mizaj is one of the principles of PM that tries to categorize all individuals based on some criteria in three fields of physical, physiological, and psychological factors. Although 10 qualitative criteria have been presented in the references of PM to assess Mizaj, it is important to find quantitative values for this concept. Previous studies in this field did not have a reliable method to assess Mizaj [17,19,21,22,24]. As there is not

any valid and reliable questionnaire to determine Mizaj, the expert panel can be the best accessible and reliable way. Increasing the reliability of the diagnosis, the Mizaj assessment was performed in two separate sessions with at least two weeks intervals, not to affect the first session on secondary session for each individual.
As the theory of Mizaj, Mizaj of three main organs (heart, brain, liver) are important in shaping the whole-body Mizaj [15], their Mizaj were assessed in expert panels.
Helping to find the best significant difference, persons with moderate whole-body Mizaj were excluded from the study; nevertheless, the Mizaj of their main organs may be moderate. However, in cases where there was disagreement, in each of these diagnoses, they were excluded from the analysis.
Since in this study, we examined some of the factors affecting the body's metabolism and its products, it seems that a more precise relationship should be sought between the organs involved in metabolism and these factors. So, it was predictable that Mizaj of heart and brain may have had less relationship with these factors. On the other hand, the relationship between liver Mizaj, which is somehow involved in the metabolism of foods, should have been more important. It seems that since whole-body Mizaj is the result of the main organs Mizaj, rather than seeking the relationship between paraclinical factors with whole-body Mizaj, this relationship with organs Mizaj should be studied.
Another point is that the correlation between each value with Mizaj separately could be misleading. From a holistic viewpoint, the sum of parameters may be different in various Mizaj, without any significant difference in all of them.
Based on the results, in the area of hotness/coldness of whole-body Mizaj, FBS was significantly higher in the hot Mizaj. Since glucose is one of the most important energy sources of cellular metabolism and heat in the body, this finding can support the theory of PM about Mizaj. In addition, blood factors such as Hgb, HCT, MCV, MCHC, and MCH in the hot Mizaj group were significantly higher than the cold group that is consistent with the theory of PM that links blood production (as a symbol of hotness and wetness) to heat. It can also be said that as RBCs are responsible for delivering nutrients and oxygen to the cells and delivering waste products to the body's excretory tracts, this increase could be a sign of higher levels of cellular metabolism.
In case of WBC, the average count in people with a hot Mizaj was higher than that of people with a cold Mizaj. Also, this difference in the neutrophil count was significant.
As a new aspect in this study, in addition to the whole-body Mizaj, the Mizaj of the three main organs of the body was also determined.
The important finding is that all these factors in the hot Mizaj of liver were significantly higher. Also, the amount of cortisol was significantly higher than cold and moderate liver Mizaj. Besides, TG levels were higher in the hot Mizaj of the liver than in the colds.
These findings are important since the metabolism of the whole body is affected by the liver. It seems that it is necessary to pay more attention to the Mizaj of the liver in future studies.
In the field of brain Mizaj, as well as whole-body Mizaj, FBS, Hgb, Hct, MCV, MCH, MCHC, and lymphocytes were higher in the hot Mizaj.
However, there was no significant difference between the groups in indices in hot and cold Mizaj of the heart, except in MCV and MCH.
In this study, as a priori theory, we expected to find a correlation between thyroid factors with the hotness/coldness of Mizaj, but the results were not significant between the two groups. It may be proposed that metabolism in hot Mizaj individuals may be increased for reasons other than the involvement of thyroid hormones. For example, stimulation of the sympathetic and parasympathetic systems may be effective in this finding. This theory is consistent with elevated cortisol in hot-Mizaj groups of the liver as well as elevated blood sugar, which may be a consequence of elevated glucocorticoids. It is also consistent with the Shahabi et al. study that suggested an increase in sympathetic activity in people with a hot Mizaj [24].
Our study had some limitations. Lack of valid and reliable tools for Mizaj assessment, lead to design an expert panel as a gold standard. We tried to improve its validity with the repeat of the expert panel with two weeks interval and a novel process of agreement achievement. It means that despite the lack of complete reliable validity, probably is the best available way and has better validity than the Mizaj assessment in other studies. So, the results may be more reliable.
One of the limitations of the study is the small sample size due to the restricted protocol of the Mizaj assessment. But on the other hand, they had the most agreement in diagnosis between experts.
Because of the number of enrolled participants in this study, the Mizaj assessments were performed in a period of four months. Since in this period, the hotness of climate changed naturally, it can also have some effect on people's Mizaj, according to the theory of PM. Therefore, it would be better if it was possible to determine the Mizaj in a shorter period. However, blood samplings were performed on a fixed day, after all Mizaj assessments, on two consecutive days in a moderate season (spring) to avoid the effect of hotness or coldness of climate on the body Mizaj.

Conclusion

Assessing the Mizaj of the main organs of the body

is the most important strength of this study that could help develop new theories in the field of Mizaj. The findings of this study can help researchers design larger and more precise studies in this field. It is suggested that in addition to specific biochemical factors related to metabolism, more general indicators be matched with Mizaj in future assessments.

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Conflict of Interests
The authors have no conflict to declare.

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References
[1] Organization WH. WHO global report on traditional and complementary medicine 2019, World Health Organization, 2019.

[2] Mahmoudpour Z, Shirafkan H, Mojahedi M, Gorji N, Mozaffarpur SA. Digesters in traditional Persian medicine. Caspian J Intern Med 2018;9:1-6.

[3] Behmanesh E, Delavar MA, Kamalinejad M, Khafri S, Shirafkan H, et al. Effect of eryngo (Eryngium caucasicum Trautv) on primary dysmenorrhea: A randomized, double-blind, placebo-controlled study. Taiwan J Obstet Gynecol 2019;58(2): 227-33.

[4] Kolangi F, Shafi H, Memariani Z, Kamalinejad M, Bioos S, et al. Effect of Alpinia officinarum Hance rhizome extract on spermatogram factors in men with idiopathic infertility: A prospective double-blinded randomised clinical trial. Andrologia. 2019;51:e13172.

[5] Farahi O, Mozaffarpur S, Saghebi R, Mojahedi M. The significance of tongue in traditional Persian medicine. J Babol Univ Med Sci 2016;18:73-79.

[6] Zhang GG, Lee W, Bausell B, Lao L, Handwerger B, et al. Variability in the traditional Chinese medicine (TCM) diagnoses and herbal prescriptions provided by three TCM practitioners for 40 patients with rheumatoid arthritis. J Altern Complement Med 2005;11:415-421.

[7] Zhang GG, Lao L, Bausell B, Berman B, Handwrger B, et al. The variability of TCM pattern diagnosis and herbal prescription on rheumatoid arthritis patients. Altern Ther Health Med 2004;10:58-63.

[8] Ryu H, Lee H, Kim H, Kim J. Reliability and validity of a cold–heat pattern questionnaire for traditional Chinese medicine. J Altern Complement Med 2010;16:663-667.

[9] Okitsu R, Iwasaki K, Monma Y, Takayama S, Kaneko S, et al. Development of a questionnaire for the diagnosis of Qi stagnation. Complement Ther Med 2012;20:207-217.

[10] Mozaffarpur SA, Naseri M, Kamalinejad M, Shams MA, Memariani Z, et al. Nine steps to discover new medicines from traditional sources: the example of Persian medicine. J Altern Complement Med 2020;26:365-368.

[11] Mojahedi M, Naseri M, Majdzadeh R, Keshavarz M, Ebadiani M, et al. A review on identification mizaj (temperament) indices in Iranian traditional medicine (ITM). Med Hist 2012;4.

[12] Ansari AH, Zulkifle M, Ali M. An analytical study of concordance between Mizaj and diseases in adult patients of NIUM hospital, Bangalore. Anc Sci Life 2010;30:7-11.

[13] Vahedi A, Zamani M, Mojahedi M, Mozaffarpur S, Saghebi R, et al. Role of anthropometric dimensions of human body in identifying temperament in traditional Persian medicine. J Babol Univ Med Sci 2016;18:24-33.

[14] Mojahedi M, Alipour A, Saghebi R, Mozaffarpur SA. The relationship between mizaj and Its indices in persian medicine. Iranian Red Crescent Med J 2018;20:e57820.

[15] Salmannejad H, Mojahedi M, Mozaffarpur S, Saghebi R. The review of indices of Mizaj-e-Damagh (temperament of brain) identification in persian medicine. J Babol Univ Med Sci 2016;18:71-79.

[16] Salmannezhad H, Mojahedi M, Ebadi A, Mozaffarpur SA, Alipoor A, et al. Design and validation of mizaj identification questionnaire in persian medicine. Iran Red Crescent Med J 2018;20:e66709.

[17] Dar FA, Zaidi IH, Sherani F. Physiological variation of serum alkaline phosphatase level in damawi and balghami males in a sample population. Indian J Tradit Knowl 2011;10:741-744.

[18] Rothbart MK. Biological processes in temperament. In: Temperament in childhood. G. A. Kohnstamm, Ed, JE Bates, MK Rothbart. John Wiley & Sons, 1989; pp 77-110

[19] Carden CP, Judson IR. Humors of tumors: potential for interaction between cancer type and treating oncologist's personality? J Clin Oncol 2008;26:1566-1567.

[20] Bhikha R. Tibb and the concept of temperament. J Nat Med 2005;22:1-4.

[21] Ali SM, Alam M. A scientific correlation between blood groups and temperaments in Unani medicine. 2007.

[22] Furukawa T. A study of temperament and blood-groups. J Soc Psychol 1930;1:494-509.

[23] Salmannezhad H, Mojahedi M, Ebadi A, Montazeri A, Mozaffarpur SA, et al. An assessment of the correlation between happiness and Mizaj (temperament) of university students in Persian medicine. Iran Red Crescent Med J 2017;19:e55627.

[24] Shahabi S, Zuhair MH, Mahdavi M, Dezfouli M, Torabi Rahvar M, et al. Evaluation of the Neuroendocrine System and the cytokine pattern in warm and cold nature persons. Physiol Pharmacol 2007;11:51-59.