

The Review of Indices of Mizaj-e-Damagh (Temperament of Brain) Identification in Persian Medicine

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J Babol Univ Med Sci; 18(11); Nov 2016; PP: 71-9

Received: Jun 14th 2016, Revised: Jul 27th 2016, Accepted: Sep 28th 2016.

ABSTRACT

BACKGROUND AND OBJECTIVE: In Persian medicine (PM), total physical, physiological and psychological properties of individuals are known as “general Mizaj (temperament)”, whereas particular signs of each organ is known as “organ Mizaj”. 9 categories of Mizaj (temperament) have been introduced and each individual belongs to one category according to dominance of a specific quality. To present proper health care directives for each individual, it is necessary to identify his general Mizaj or the Mizaj of important organs of body such as brain, liver and heart. The purpose of this study is to survey and summarize the views of PM references regarding diagnostic signs of Mizaj-e-Damagh (temperament of brain).

METHODS: In this review article, we searched online databases such as ScienceDirect, PubMed, Scopus, Magiran as well as PM references for some key words such as “brain”, “temperament”, “Mizaj” and their Persian equivalent. Articles related to Mizaj-e-Damagh were surveyed and summarized.

FINDINGS: Out of total 181 articles, 170 articles were related to psychological views on personality types and only a few articles were dedicated to Mizaj in PM. No article was found to be directly related to indices of Mizaj-e-Damagh. Various signs were noted in these books regarding Mizaj-e-Damagh identification, but no reference has been made to the accurate methods of evaluation and their role in Mizaj identification. Condition of touch and hair, condition of sleep and wakefulness, nasal secretions level and psychic functions were the common points in these references regarding intended indices.

CONCLUSION: Considering the results of this research, conducting clinical studies to identify the extent and intensity of relationship between diagnostic signs of Mizaj-e-Damagh and Mizaj from the viewpoint of PM scholars is advised.

KEY WORDS: *Persian medicine (PM), Mizaj-e-Damagh (Temperament of Brain), Su-e-Mizaj (Abnormal Temperament).*

Please cite this article as follows:

Salmannegad H, Mojahedi M, Mozaffarpur SA, Saghebi R.. The Review of Indices of Mizaj-e-Damagh (Temperament of Brain) Identification in Persian Medicine. J Babol Univ Med Sci. 2016;18(11):71-9.

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Introduction

With respect to patient dissatisfaction regarding some types of treatment by modern medicine and many people's lack of access to modern health in some regions of the world, world health organization (WHO) in recent decades has adopted new strategies to develop traditional medicine worldwide while emphasizing on fewer side effects of traditional medicine (1-3).

Persian medicine (PM) with its particular diagnostic principles including a set of certain health care directives and treatment orders was used for diagnosis, prevention and treatment of diseases from the age of Ancient Persia to recent centuries. The basis of diagnosis in PM is paying attention to interpersonal differences as Mizaj (temperament) differences and dominance of humor in body and its treatment method is based on excretion of pathogenic humor and modification of patient's Mizaj (4, 5).

The concept of Mizaj in PM is a key to define health and disease. In this approach, each person has particular physical, physiological and mental properties and Mizaj is defined as a combination of these signs (6, 7). Accordingly, as long as each person's Mizaj is temperate, his biological functions are normal. However, if a person's Mizaj is out of balance, he will suffer from Su-e-Mizaj (Abnormal Temperament) (8).

PM references have categorized diseases associated with deviation from healthy Mizaj into a big category of diseases named "Su-e-Mizaj diseases" and noted specific treatments for each Su-e-Mizaj (6, 9). According to this view, a significant part of health care directives in PM is modified based on peoples Mizaj and the treatment method will also be modified according to the type of Su-e-Mizaj (10). In PM, close attention has been paid to patient's Mizaj in each stage of treatment, even in regard with diseases that are not included in the categorization of Su-e-Mizaj (11). In addition to general Mizaj of body, each organ has its own Mizaj and a considerable portion of healthcare measures are presented according to the properties of that organ's Mizaj.

For instance, dietary measures for a person with warm stomach or liver is different from or sometimes opposite to a person with cold stomach or liver (12). Among body organs, brain, heart and liver are known as chief organs of body (13). Brain Mizaj refers to qualities of warmness-coldness and wetness-dryness of brain, which is identified according to a set of signs

mentioned in the discussions of general Mizaj and organ Mizaj (8). PM references have proposed various indices to identify Mizaj. Regarding general Mizaj, these indices are widespread and include several biological properties of human. However, regarding organ Mizaj, these indices are more limited and act according to the functions of that particular organ. Some PM references have mentioned the target indices in exclusive chapter or among other chapters. Collecting and summarizing the views of PM references regarding indices of Mizaj identification are the priorities of fundamental PM researches. The views of PM references regarding indices of general Mizaj identification were collected and summarized in previous studies, but none of them were dedicated to chief organ mizaj (13, 14). The aim of this study is to summarize the views of PM references regarding indices of brain Mizaj identification as one of the chief organs.

Methods

To conduct this review article, authentic PM references were studied including: "The Canon of Medicine", "Zakhireh kharazmshahi", *Kāmil al-Sinaā al Tibbiya*", "Almansuri-fi-Teb", "Eksir Azam", "*Kholasat al-Hikmah*" and "*Mofarah al- Gholoob*" and their views regarding indices of Mizaj identification were extracted and summarized. In addition, we searched online databases such as Iran medex, Majiran, SID, Google scholar, Pub Med, Scopus, SienceDirect for some key words such as "Mezaj", "Mizaj", "Temperament", "Brain Mizaj", "Brain Temperament" and their Persian equivalent.

Results

Of total 181 articles, 170 articles were related to views on personality types in the field of psychology. Authors of these articles have done researches about the relationship between personality types and brain function or para-clinical indices without making a reference to brain Mizaj. Tuominen et al. examined the relationship between serotonin in the brain and personality types (15). Laricchiuta et al. evaluated the difference between micro and macro indices of basal ganglia in two personality types based on Cloninger's personality types (16). Hakamata et al. studied the relationship between personality types and glucose metabolism in various parts of brain (17). Searching

for the phrase "Brain temperament", no reference was found to be directly associated with brain Mizaj based on psychological views.

Among published articles, no article was found to be exclusively dedicated to Mizaj-e-Demagh (Brain Mizaj, Brain temperament) and the limited number of relevant articles made references to Mizaj or Su-e-Mizaj while discussing Demagh (Brain) diseases in PM but did not address different aspects of brain Mizaj and its diagnostic indices separately (18).

Brain Mizaj (Mizaj-e-Demagh) in PM references

1. Definition of Mizaj: Mizaj is an infinitive meaning fusing and blending (19, 20). In PM references, Mizaj is a technical term that refers to one of the principles of this medicine and is defined as a quality that is a combination of components of creatures, including human body (19).

In PM, the resultant of various mental and physical properties of human and even other creatures is assessed based on two spectrums of qualities including warmness-coldness and wetness-dryness. PM scholars have introduced Mizaj as a median quality that is a result of interaction between components of creatures including human body (19). The primary components of human body, known as the four elements, include fire (with warm and dry quality), air (with warm and wet quality), water (with cold and wet quality) and earth (with cold and dry quality).

Each of these elements induces the four qualities of warmness, coldness, wetness and dryness. The median quality of a compound material, as the resultant of the aforementioned qualities, is known as Mizaj (19, 21). Several conditions are possible for Mizaj including four singular conditions (warm, cold, wet and dry), four compound conditions (warm and wet, warm and dry, cold and wet, cold and dry) and one moderate (temperate) condition. Each person is placed in one of these categories according to the dominance of various qualities in his body and manifestation of these qualities in his mental and physical properties. This is called general Mizaj (12). In this approach, each organ benefits from its peculiar Mizaj and it involves the same categorization of general Mizaj. Since brain, heart and liver are the chief organs of body, their Mizaj is of great importance (21, 22).

2. Indices of brain Mizaj identification: PM references have mentioned various indices for identification of general Mizaj and organ Mizaj including brain; some of them can be mutually used for general and organ Mizaj, whereas some of them are

exclusive to organ Mizaj. In identification of Mizaj and Su-e-Mizaj of brain, in addition to general properties of warmness, coldness, dryness and wetness, more specific indices such as hair condition, physical and spiritual interactions, eye properties and nasal discharge are also considered (8, 12, 22-24). Following PM references and to simplify the proposed indices, symptoms of healthy brain Mizaj are stated independently of symptoms of Su-e-Mizaj in this study. First, results associated with symptoms of healthy brain Mizaj are noted here:

A. Hair: Hair condition is one of the main indices of brain Mizaj identification (8, 11). In this case, five main properties of hair including color, amount, diameter, growth rate and hairstyle has been considered. Premature baldness as well as black thick (messy) hair that tends to red and has rapid growth and high density indicate warmness of brain Mizaj (12). People with cold brain Mizaj have less hair with slower growth rate and the color of their hair is lighter than others and tends to white and yellow. In addition to thickness, rapid growth, premature hair loss and high density of hair in warmness of brain Mizaj, curly hair is also a sign of dryness of brain Mizaj. If someone's brain Mizaj is wet, he will have softer and thinner hair in addition to slow growth and loss of hair (delayed baldness or lack of baldness) (22, 24). In compound Mizaj, hair condition can be predicted according to the dominance of each quality. For example, in people with warm and dry brain Mizaj, the hair is more black, curly and bushy (24), whereas in people with warm and wet brain Mizaj, growth rate and premature hair loss decrease as the dominance of humidity increases. Overall, hair growth rate and baldness have a direct relationship with warmness and wetness of brain and an inverse relationship with coldness and wetness of brain (12). In case of temperate brain Mizaj, all these conditions reveal themselves somewhere between warmness-coldness and wetness-dryness (8, 11, 12).

B. Physical and psychic actions: People with warm brain Mizaj have more physical and spiritual movements. Therefore, these people have faster physical movements, hasty in giving comments and have less consistency (11), while benefitting from more intelligence and stronger senses. There is also a type of light-mindedness or levity in these individuals, called "Tish" in PM references (20). If warmness is accompanied with dryness, this condition will be intensified. A person with warm brain Mizaj is lively,

has strong manhood and is less affected by his environment (8).

In people with cold brain Mizaj, an opposite condition is expected. Therefore, slow movements and fatigue, idleness and stupidity as well as consistency are among some of their characteristic and they are more easily affected by the environment (22). People with dry brain Mizaj have faster movements, their senses are more manifest and therefore they have stronger and sharper senses (8, 11).

Accordingly, slow movements (fatigue), slow psychic actions (Beladat), slow thinking and amnesia in wet brain Mizaj are more intense than dry brain Mizaj.). In compound Mizaj, various physical and psychic actions may be observed according to the dominance of each of the four qualities. For example, fatigue, slow psychic actions and slow thinking in people with cold and wet brain Mizaj are more than people with just wet or cold brain Mizaj. People with warm Mizaj or Su-e-Mizaj are harassed by warmth and warm conditions and benefit from coldness. The situation is opposite for cold Mizaj. The mentioned principle is also true for wetness and dryness (11, 22).

C. Secretions and waste matter: In people with warm brain Mizaj, ductal secretions in head, particularly brain, are little with moderate consistency. However, level of secretion changes depending on the intensity of warmth and its association with wetness or dryness. In dry brain Mizaj, the mentioned secretions decrease and even dryness or emptiness of ducts are observed (8, 11, 12).

In people with cold or wet brain Mizaj, there are more secretions and they are diluted and their amount and dilution increase based on the intensity of coldness, wetness and their association. In warm and dry brain Mizaj, the secretions are little and concentrated and their amount and concentration increase based on the intensity of warmth, dryness and their association (22, 24).

D. Eye: Protuberant and red blood vessels in the eye, warm touch and fast blinking indicate warm brain Mizaj and opposite conditions such as thinness and lack of red blood vessels, cold touch of eye and slow blinking are signs of cold brain Mizaj. Regarding dry brain Mizaj, thinness of vessels, decreased secretions, dry touch of eye and clear senses are mentioned. Ahvazi has confirmed lack of red blood vessels in dry brain Mizaj (8,11,12).

Wetness of brain Mizaj increases secretions, wetness of touch and opacity of eye senses. As Ahvazi

indicated, people with wet brain Mizaj have blue (Azraq) eyes (12). If the mentioned qualities are combined, common conditions of eye will be observed. For example, swollen and red vessels, warm touch and opacity of eye senses are observed in warm and wet brain Mizaj. Other types of compound Mizajes also intensify common signs and modify non-common signs (12, 22, 24).

E. Sleep: People with warm brain Mizaj have light and short sleep, while people with cold brain Mizaj sleep too much and always have sleepy face. Severe insomnia is a sign of dry Mizaj, while excessive sleepiness and deep sleep indicate wet brain Mizaj. In compound qualities, common properties are intensified and non-common properties are modified (8, 11, 12). For example, insomnia and light sleep are intensified in warm and dry brain Mizaj and modified in warm and wet brain Mizaj (22, 24). The precise details and the terminology used for indices of various brain Mizaj are presented in table 1 and the sources are cited.

3. Indices of Su-e-Mizaj of brain

A. Simple Su-e-Mizaj of brain: In PM references, distinct properties are mentioned as symptoms and signs of dominance of qualities and pathogenic humor known as signs and symptoms of Su-e-Mizaj of brain. These indices are actually the intensified conditions of healthy Mizaj plus some exclusive indices of Su-e-Mizaj. In all Su-e-Mizaj chapters, there are two main categories including simple Su-e-Mizaj, in which there is only dominance of quality without dominance of humor as well as material Su-e-Mizaj, which is the result of dominance of humor.

In warm Su-e-Mizaj of brain, there are various degrees of anxiety and impatience (25), anger, insomnia and seeing nightmares depending on the severity of heat. When dryness is added to warmth, the symptoms will be intensified, which may ultimately lead to confusion (8, 11).

In cold Su-e-Mizaj of brain, fatigue and disorder occurs in brain-related functions such as memory and imagination and if dryness is added to coldness, a condition of solidity (strict moral code, depression) will be created (8, 11, 25).

In dry Su-e-Mizaj of brain, intensified dryness symptoms, sharpness of brain-related senses such as memory, decreased secretions, dryness of nose and insomnia is observed (11), while in wet Su-e-Mizaj of brain, various symptoms similar to coldness of brain including fatigue, too much sleep and disorder in brain-related functions is observed. Amnesia is also

one of the manifestations of cold and wet brain Mizaj that is intensified as wetness increases (8, 11). The precise details for indices of various Su-e-Mizaj of brain are presented in table 2 and the sources are cited.

B. Material Su-e-Mizaj of brain: In material Su-e-Mizaj, there are symptoms exclusive to material (humor) in addition to symptoms of simple Su-e-Mizaj. For example, heaviness, rapid pulses, swelling of eye and face and too much sleep are observed in sanguine Su-e-Mizaj of brain. In melancholic brain Su-e-Mizaj, there isn't much heaviness, yet thirst, dryness

and stinging feeling in the nose and insomnia are more pronounced. In phlegmatic brain Su-e-Mizaj, heaviness is more pronounced than other types of brain Su-e-Mizaj and other symptoms such as too much sleep, fatigue and frailty are present here, too. In choleric brain Su-e-Mizaj, there is less heaviness, but brain-related functions are more affected and the symptoms include obsession and corrupt mind (not thinking naturally). In addition, insomnia is more pronounced than other types of material brain Su-e-Mizaj (table 3) (8, 11).

Table 1. Signs of simple and compound brain Mizaj

Index	Warmness	Coldness	Dryness	Wetness
Hair	- Black (8, 12, 24), blond (11, 22) - Dark black (warm and dry) (8, 11, 12, 22, 24) - Reddish yellow (11, 12, 22, 24) - Fast growth (8, 11, 12, 22, 24) - Neither fast nor slow growth (warm and wet) (12) - Bushiness (24) - Curliness (8, 11, 12, 22, 24) - Intense curliness (warm and dry) (8, 11, 12, 22, 24) - Soft (warm and wet) (8, 11, 24) - Strength or thickness (8, 11, 24) - Speed of baldness (8, 11, 22, 24) - extreme speed of baldness (warm and dry) (8, 11, 12, 22, 24) - Delayed baldness (warm and wet) (8, 11, 12, 24)	- Light black (8, 12, 24) - Rate of greying hair (8, 11, 12) - Black (12) or yellowish (cold and dry) (8, 22), red and white or yellow (cold and wet) (22) - Slow growth (8, 11, 12) - Fast growth (cold and dry) (12) - Soft (8, 11, 12), soft (cold and wet) (12) - Straight (22, 24) - Thinness with coldness and dryness (22) - Slow baldness (8, 11) or normal baldness (cold and dry) (12) - Lack of baldness (12), delayed baldness (cold and wet) (8, 11)	- Fast growth (8, 11, 12, 22, 24) - Curliness (8, 11, 22) - Strength (8, 11) - Thickness (22) - Premature baldness (8, 11, 12, 22, 24)	- Slow growth (8, 11, 24) - Soft (8), very soft (11, 24) - Thin (24) - Delayed baldness (8, 11) - Lack of baldness (12, 24)
Actions and symptoms	- Fast movements (12) - Hasty (warm, warm and dry) (8, 12) - Intense psychological actions (warm and dry) (8, 12) - Hasty in giving comments (8, 12) - Inconsistency (8, 11, 12, 24) - Tish (8), Tayyash (warm and dry) (12) - Mental disorder and delusion (8) - Cheerful (12) - Clear senses (8, 11, 12), strong understanding and memory (8), opaque senses and delusional (12) (warm and dry) - Neither hasty nor slow (12, 24), slow senses (24) (warm and wet)	- Slow movements (8, 24) - Slow mind (8, 12) - Fatigue and idleness (8, 12) - Consistent will (8, 11, 12) - Speed of being influenced by disorders (8, 11) - Clear senses in youth and weak senses in old age (11, 22), good senses in old age (8) (cold and dry) - Dumb, forgetful (12), corrupted senses (8, 11) (cold and wet)	- Fast movements (12) - Clever and clear senses (8, 11, 12, 24)	- Slow movements (12) - Opacity of senses (8, 11) - Slow senses (24) - Forgetfulness (12)
Secretions and waste matter	- Little (12) - Ripe (8, 11) - Little excreta (12), lack of excreta (8, 11) (warm and dry) - Much excreta (8, 11) (warmer) - Fast rheums (12, 24)	- Much excreta (8, 11, 12) - Much catarrh and rheum (8, 11, 12, 24) - Moderate or unripe excreta (12) (cold and dry) - Much and unripe excreta (8, 11, 12), gets sick quickly (12), Fast rheums (8, 11) (cold and wet)	- Purity of excreta tract (8, 11) - Concentrated excreta (12)	- Diluted and much excreta (8, 11, 12)
Eye	- Red (12, 22, 24) - Red (8, 11), warmth of eyelid (8) (warm and dry) - Redness of face (8, 11) - Warmness of eye and face (24) - Warmness of touch (8, 11) - Lucidity of vessels (8, 11, 12, 22, 24) - Opacity of eyesight (12) (warm and wet)	- Thinness of vessels (8, 11, 12, 22) - Slow movement of eyelid (8, 11)	- Clear senses (12) - Thinness of vessels (12) - Coldness of touch (12)	- Dark blue (12) (indigo or blue) (20) - Wet (12) - Opacity of senses (12)
Sleep	- Lack of sleep (8, 11, 12, 24), Insomnia (12, 24), sleeplessness (8, 11) (warm and dry) - Too much sleep (12, 24), too much dreaming (12) (warm and wet) - Light (8, 11, 22)	- Too much sleep (11) - Lack of sleep (cold and dry) (12) - Too much sleep and deep sleep (cold and wet) (8, 11, 12, 22, 24) - Sleepy face (8)	- Lack of sleep (8, 11, 12, 22, 24) - Sleeplessness (8, 11)	- Too much sleep (12) - Sleepy (24) - Deep sleep (8, 22)
Impressibility of qualities	Quickly affected by warmth and warm natural foods (11, 22)	Quickly affected by coldness and cold natural foods (11, 22)	Quickly affected by dryness (11, 22)	Quickly affected by wetness (11, 22)

Table 2. Signs of simple and compound brain Su-e-Mizaj

Index	Warmness	Coldness	Dryness	Wetness
Actions and symptoms	- Priority of warm things ^(8, 11)	-Fatigued ^(8, 11)	-Priority of Excretions ⁽⁸⁾	-Fatigued ^(8, 11)
	- Troubled movements ^(8, 11)	-Weakness in brain functions ⁽¹¹⁾	-Clear senses ⁽¹¹⁾	-Much amnesia ⁽¹¹⁾
	- Anxiety in imaginations ^(8, 11)	- lack of imagination ^(8, 11)	-Intensity of memorize ⁽¹¹⁾	-Opacity of senses ⁽¹¹⁾
	- Impatient ^(8, 11)	- Fearful ⁽⁸⁾		
	- confusion ^(8, 11) , light head ⁽¹¹⁾ (warm and dry)	solidity ^(8, 11) (cold and dry) - Amnesia ⁽¹¹⁾ (cold and wet)		
Secretions and excreta	- Little secretions ⁽¹¹⁾ (warm and dry)	- Much secretions ⁽⁸⁾ - Easily catch catarrh ⁽⁸⁾	-Dryness of nose ^(8, 11) -Purity of excreta tract ^(8, 11)	-Lack of or moderate secretions ⁽⁸⁾ , much secretions ⁽¹¹⁾
Eye	- Red ^(8, 11)	- Whiteness ^(8, 11)		
Skin color	- Red ^(8, 11)	- Whiteness of face ⁽⁸⁾		
Sleep	- Lack of sleep ^(8, 11)			
	- Dominance of sleep, yet not stupor ^(8, 11) (warm and wet)	- Sopor ^(8, 11) (cold and wet)	- Sleeplessness ^(8, 11)	-Dominance of sleep ^(8, 11)
	- Nightmares ⁽¹¹⁾ (warm and wet)	- Much sleep (cold and wet) ⁽⁸⁾		
	- Impatient for sleep ⁽⁸⁾ (warm and wet)			
Impressibility of qualities	Annoyed by warmness and warm natural things ^(11, 22)	Annoyed by coldness and cold natural things ^(11, 22)	Annoyed by dryness ^(11, 22)	Annoyed by wetness ^(11, 22)
	Benefit from coldness and cold natural things ^(11, 22)	Benefit from warmness and warm natural things ^(11, 22)	Benefit from wetness ^(11, 22)	Benefit from dryness ^(11, 22)

Table 3. Exclusive signs of material brain Su-e-Mizaj

Index	Sanguine	Melancholic	Phlegmatic	Choleric
Head	- Much heaviness ^(8, 11) - Pulse ⁽⁸⁾ - Swelling of face and eye ⁽⁸⁾ - Prominence of vessels ⁽⁸⁾	-Dryness, irritation, burning and inflammation of nose ^(8, 11) - Heaviness ⁽¹¹⁾ - Thirst ⁽¹¹⁾	- Fatigue and idleness ^(8, 11) - Amnesia ^(8, 11) - Perceptible coldness ⁽⁸⁾ - Length of annoyance ^(8, 11) - Perceptible heaviness most of the times ^(8, 11)	- Length of illness ⁽¹¹⁾ - Obsession ^(8, 11) - Corrupted mind ^(8, 11) - Less heaviness than all others ^(8, 11)
Eye	- Warm and red ⁽¹¹⁾ - Red color ⁽⁸⁾	- Yellowness of eye and face ⁽¹¹⁾	- Whiteness ⁽¹¹⁾ - redness of eye and face ⁽⁸⁾ - Leadens color of eye, face and tongue ⁽⁸⁾	- Light black eye ⁽⁸⁾ - Light black face ⁽⁸⁾ - Light black color of all organs ⁽⁸⁾
Sleep	- Too much sleep ⁽¹¹⁾	- Severe insomnia ⁽¹¹⁾ - Sleeplessness ⁽⁸⁾	- Sopor and Dominance of sleep ^(8, 11)	- Sleeplessness more than all others ^(8, 11)
Secretions		- Irritant, bitter, yellow and warm secretion from nose ⁽¹¹⁾ - Dryness of nose ⁽⁸⁾		
Impressibility of qualities	Annoyed by warmness and warm natural things ^(11, 22) Benefit from coldness and cold natural things ^(11, 22)	Annoyed by coldness and cold natural things ^(11, 22) Benefit from warmness and warm natural things ^(11, 22)	Annoyed by dryness ^(11, 22) Benefit from wetness ^(11, 22)	Annoyed by wetness ^(11, 22) Benefit from dryness ^(11, 22)

Discussion

Of the 50 isolated *staphylococcus aureus* strains in this study, 34 strains (68%) were resistant to methicillin (MRSA) and 12 strains (24%) were

resistant to ciprofloxacin. The antibiotic resistance caused by efflux pump is one the major mechanisms in *staphylococcus aureus* and has become a very

important topic for researchers in recent years (18, 19). In this study, MRSA of strains was studied using disk diffusion method and PCR. The study of Moradi et al. using 104 *staphylococcus aureus* samples demonstrated that highest levels of susceptibility of strains was to vancomycin (96.2%), chloramphenicol (88.2) and rifampin (81.7) and resistance of strains to cefoxitin was 40.4% (MRSA) (20). To assess the resistance to ciprofloxacin and its relationship with efflux pump in this study, screening of strains was done to examine the existence and function of *norA* gene using PCR method and minimum inhibitory concentration of ethidium bromide in proximity to efflux pump inhibitor (CCCP). *norA* gene was present in all ciprofloxacin-resistant strains. *norA* gene is located inside chromosome and is highly protected among strains. Similar results have been reported in other studies.

Results of a study by Pourmand et al. revealed that *norA* gene is present in all ciprofloxacin-resistant strains and its gene expression increases in proximity to biocide hexahydroquinolone (21). According to a study by Saiful et al., of 19 isolated MRSA strains, 16 strains contained *norA* gene and all strains had active efflux pump (22). A comparison between our study and previous studies shows similar results, confirming the presence of efflux pump genes in ciprofloxacin-resistant strains. To examine the activity of efflux pump, CCCP decreased by 2-4 times the level of MIC in strains compared with ethidium bromide and

ciprofloxacin, which was noted in most of the similar studies. Ethidium bromide is a typical substrate of efflux pump that is used as a positive control in most studies to measure the activity of efflux pump.

Costa et al. demonstrated that efflux pump plays a key role in decreasing resistance to antibiotics and biocides (23). As mentioned in "Results" section, level of MIC in ethidium bromide decreases in the presence of CCCP, which is in line with other studies and confirms that efflux pump induces resistance to antibiotic. In this study, gene expression of *norA* in ciprofloxacin-resistant strains was assessed.

Our results demonstrated that ciprofloxacin-resistant strains have different expression of *norA* gene and the more resistant strains revealed higher relative gene expression of *norA*, which was in line with similar studies. Results of a study by Huet et al. demonstrated that *norA* efflux pumps were present in all strains and their expression increased in proximity to biocides (24). Results of the present study showed that *norA* efflux pump is directly related to ciprofloxacin-resistance and therefore, developing efflux pump inhibitors can help to control the risk of efflux pump-resistant strains.

Acknowledgments

Hereby, we express our deepest sense of gratitude to our colleagues in Islamic Azad University of East Tehran, Mr. Arian Rahimi and all those who participated in this study.

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