[TAM METİN SÖZLÜ BİLDİRİLER]

[FULL TEXT ORAL PRESENTATIONS]

FT-1

[INVESTIGATION OF THE RELATIONSHIP BETWEEN MOVEMENT SYSTEM AND CLINICAL DATAS IN DIABETIC NEUROPATHY] [DİYABETİK NÖROPATİDE HAREKET SİSTEMİ VE KLİNİK VERİLER ARASINDAKİ İLİŞKİLERİN İNCELENMESİ]

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Abstract
Objective: Hyperglycemia complications have negative effect on the movement system like all other systems in patients with diabetic neuropathy. In these patients, it is necessary to get a real clinical diagnosis and it is important to understand the relationship between movement system and hyperglycemia. Our purpose was to investigate the relationship between hyperglycemia values and changes in the movement system in patients with diabetic peripheral neuropathy.
Materials and Method: We included 33 patients aged between 45-76 with Type 2 diabetic neuropathy. Patient's demographic characteristics and duration of disease were recorded. Measurements related with the movement system are performed with biosensoric Biodex Balance SD balance systems. Adaptation time to gravity change and postural sway parameters were examined in the movement system.
Results: The mean age of the patients was 60.72± 7.05(48-76) years. There was a positive correlation between adaptation time to gravity change and age (r:0.445). In addition, there was a positive relationship between adaptation time to gravity change and body mass index measurements (r:0.429). There wasn't found any relationship between hyperglycaemia parameters (HbA1c and fasting blood glucose) and movement measurements.
Conclusion: There wasn't found direct relationship between hyperglycemia and movement system.
Keywords: Diabetic neuropathy, movement, biosensors
Conflict of interest: Authors do not have a conflict of interest
Öz
Amaç: Diyabetik nöropatisi olan olgularda hiperglisemi kaynaklı komplikasyonlar tüm diğer sistemler gibi hareket sistemini de olumsuz etkilemektedir. Bu hastalarda gerçek klinik tanıyı almak ve hareket sistemi ile hiperglisemi ilişkisini anlamak gerekmektedir. Çalışmanın amacı; diyabetik nöropatili hastalarda hiperglisemi değerleri ile hareket sistemindeki değişiklikler arasındaki ilişkiyi incelemektir.
Gereç ve Yöntem: Araştırmamıza 45-76 yaş aralığında olan Tip 2 diyabetik nöropatisi olan 33 hasta dahil edilmiştir. Hastaların diyabet süreleri ve demografik özellikleri kaydedilmiştir. Hareket sistemi ile ilgili olan ölçümleri biyosensörlü Biodex Balance SD denge sistemleri ile yapılmıştır. Hareket sisteminde gravite değişikliğine uyum süresi ve postüral salınım indeksi değerlendirilmiştir.
Tartışma: Hastaların yaş ortalaması 60.72± 7.05 (48-76) yıldı. Olguların gravite değişikliğine uyum süresi ile yaşları arasında pozitif ilişki saptanmıştır. (r:0,445) Ayrıca gravite değişikliği uyum süresi ile vücut kitle indeksi ölçümleri arasında anlamlı pozitif ilişkinin olduğu görülmüştür (r:0,429). Hiperglisemi parametreleri (HbA1c ve açlık kan glikozu) ile hareket ölçümleri arasında ilişki saptanmamıştır.
Sonuçlar: Hipergliseminin hareket sistemi ile direkt ilişkisi gözlenmemiştir.
Anahtar Kelimeler:Diyabetik nöropati, hareket, biyosensörler
Çıkar çatışması: Yazarların çıkar çatışması bulunmamaktadır.

Introduction
Diabetes is one of the most common health problems worldwide. According to the estimates of 2035, it is expected that 592 million people worldwide and 12 million people in Turkey will be affected by diabetes [1]. Diabetic peripheral neuropathy is the most common complication of diabetes. Peripheral nerve neuropathy has a wide range of neuropathic complications affecting the peripheral nerves at all levels in both acute and chronic forms [2].
The effects of metabolic and vascular factors are displayed responsible although the pathophysiology of peripheral nerve neuropathy is not fully explained [3]. High levels of blood glucose play an important role in metabolic and vascular dysfunction. High glucose remaining after cell use leads to increased sorbitol levels. Sorbitol transforms the protein kinase C activity and activates the anaerobic polyol-sorbitol pathway [2,4-5]. Sorbitol also causes osmotic stress. Advanced glycation end products (AGE) metabolic products are formed by binding of glucose to amino groups of proteins or lipids by non-enzymatic reaction. All these processes due to hyperglycemia leads to damage in the Shwann cells. Nerve hypoxia occurs with decreased endoneurial blood flow and diabetic neuropathy has emerged [2,4-6]. Sensory, motor and autonomic symptoms can be observed in diabetic neuropathy. Because the distal nerve fibers are away from the trunk, the involvement of neuropathy is seen often from the distal to the proximal segments. The nerves become more subtle in diameter as they branch out into the distal segments and this is why the early involvement the distal part of the peripheral nerves. As a result, distal nerves are more vulnerable to damage [7]. The symptoms of peripheral neuropathy are often characterized as prickling, deep aching, like electric shock, sharp pain and burning accompanying hyperalgesia and allodynia on the occasion of inspection [8]. Decreasing in vibration and pressure senses, losing the temperature perception and diminish/absence in ankle reflex are seen in sensorimotor types of neuropathy [9]. Hyperglycemia plays a key role in neuropathy caused by axonal and microvascular complications [2]. Symptoms induced diabetic neuropathy complications have negative effects on the movement system. These can be listed as decrease in activity level, loss in power, worsening of the motor control, diminishing in walking stability, gravitational insecurity and increased risk of falling [10-16]. However, the direct relation between the movements over the biosensoric platform and the glycemic data is not revealed. In our study, we aimed to investigate whether these values are directly related with each other or not. The aim of this study was to investigate the relationship between hyperglycemia values (HbA1c-fasting blood glucose level) and changes in the movement system in patients with diabetic peripheral neuropathy

Materials and Methods
Our study was carried out at the Neurology Department and the Physical Therapy and Rehabilitation School of the Dokuz Eylul University between September 2018 and December 2018. All patients signed the informed written consent. We included 33 patients with Type 2 diabetic neuropathy who were diagnosed with laboratory tests, neurological examination and electromyography (EMG). Diabetes type was determined according to the American Diabetes Association Criteria [17]. Patients aged between 45-76 and walking 500 meters independently were our inclusion criteria.Vascular, endocrinological and other causes leading to polyneuropathy, serious orthopedic problems, vascular, neurological and visual problems which were affecting mobility, active malignancy, alcohol-drug dependence, autoimmune diseases were the criteria of exclusion. HgA1C and fasting blood glucose revealing hyperglycemia and disease duration were recorded. Besides; demographic characteristics such as age, gender, and body mass index were also recorded. Measurements related with the movement system were performed by biosensoricBiodex Balance SD balance systems. Within this system; we evaluated the compliance time to the gravity replacement and postural sway index. We evaluated the time to adapt of the gravity change with the ''Limits of Stability (LOS)'' test and postural sway index assesment with the ''Fall Risk'' test. Biodex Balance SD: Biodex balance is a computer-based biosensoric measuring system with the visual feedback. Patients are asked to maintain balance with the ankle movements during functional skills. It has a circular platform which is used in both statically and dynamically . Dynamic platform mobility can be adjusted from level 12 (easy) to level 1 (most difficult) [18-20]. Participants stood barefoot bilateral stance over the platform. Measurements were performed by using visual feedback and patients were not permitted to take hand support. The first test to recognize the device was planned as a trial test. a) Limits of Stability Test (LOS): The LOS test measures the time and accuracy of the participants transfer their estimated ground reaction force to the target. There are 8 targets located in 45° intervals around the central target on the display screen. The ground reaction force corresponding to the gravity center

of the patients is transmitted to the display screen via a cursor. Patients were asked to direct the cursor to the target with gravity changes by using their ankles. Each target is randomly lighted up, and the patients is directed to the target by leaning and returning to the middle position before the next target is shown on the screen. According to the standard software configuration system, the test ends when all 8 targets have been reached and test is applied as 3 replicates. The average time of 3 measurements was calculated by the system [21]. Static platform was used for the test. b) Fall risk test: Postural sway index is calculated from the oscillation from the center point of the patients on the dynamic platform. As reported by standard software configuration system 3 evaluation of 20 seconds are calculated.at each unsteady grade There is 10 seconds break between assesments. Platform stability switches from the 12th stability level to the 8th level during 20 seconds. During this time the patients were asked to keep the cursor at the midpoint on the screen. The average score of the ossilations from the midpoint is calculated by the system [22].In our study; we compared the results of the HgA1C and fasting blood glucose measurements which are important in the clinical diagnosis of peripheral diabetic neuropathy and the movement system parameters by using biosensoric balance system.

Statistical Analysis

Statistical analysis was performed with IBM SPSS Statistics version 22. Continious data were analyzed by means±standart deviation (SD) and qualitative data expressed as percentages. The relationship between the data was performed by pearson correlation analysis.

Results

Table 1 shows the gender, age, body mass index and diabetes duration of the patients. Stability test performed with biosensoric balance device showed that there was a positive correlation between age and adaptation time to the gravity change of the patients [Table 2]. There was also found correlation between patient's body mass index and adaptation time to the gravity change [Table 2]. There wasn't found any correlation between the movement system parameters and hyperglicaemia parameters.

Table 1. Demographic characteristics and diabetes duration of the patients

%	Mean SD (min-max)	
Female (n):18	Male (n):15	%54.54
Age	60.72± 7.05(48-76)	
Body mass index	29.77± 4.86 (20.34-40.09)	
Diabetes duration	13.89±7.84 (1-30)	

Table 2. Correlation of the demographic characteristics and hyperglycemia parameters with the movement system parameters

***: positive correlation**

Clinical values		Limits of stability
	Fall Risk	
Age		r:0.445*
	r:0.103	
Diabetes duration		r:0.267
	r:0.200	
Body mass index		r:0.429*
	r:0.138	
HbA1c		r:0.225
	r:0.015	
Fasting blood glucose		r:0.062
	r:0.191	

Discussion

We examined if there was relationship between the movement system's change and age, body mass index, diabetes duration, HbA1c, fasting blood glucose level in patients with diabetic peripheral neuropathy. The correlation between the movement system and HbA1c is quite limited in the literature. HbA1c has been found to be associated with ankle immobility however it has been demonstrated that the presence of neuropathy is not sufficient for explaining movement problems [23]. We didn't encounter any studies investigating their relationship between fasting blood glucose level and movement system. According to Newton's 3rd rule, ground reaction force is defined as the inverse force of the same magnitude given by the ground as the gravity force vector of the human standing. Therefore, the change in gravity force defines the change of ground reaction force parallel in opposite direction. In a meta-analysis of studies evaluating the ground reaction force during baseline contact and take-off, there wasn't any significant difference found between the patients with diabetic neuropathy, patients without neuropathy and non-diabetic patients [13]. As distinct from the change of the gravity force we assesed the adaptation time of the gravity change. Adaptation time to gravity change was not associated with HbA1c level, fasting blood glucose level and a diabetes duration [Table 2]. It was observed that the adaptation time to gravitational change increased in direct proportion to the age and body mass index of the patients. It has been shown in the literature that the mobility of the patients with type 2 diabetes with high body mass index is much less than those with lower body mass index. [24]. It has also been shown that the effort required to correct the postural balance in obese individuals also increases [25]. In a study comparing geriatric patients with young individuals, geriatric individuals were reported to have lost their flexibility. Accordingly, postural compensatory strategies have been found to be quite inadequate when they were trying to adapt to the dynamic ground [26]. The results of our study showed that the patients have difficulties in terms of duration while shifting and regain of their gravity line with increasing age and body mass index. Somatosensory system is affected negatively in patients with diabetic neuropathy. Inappropriate motor responses and improper postural oscillations appear. Patients with diabetic neuropathy are weak in providing postural control [27-28]. These unsuitable responses lead to an increased risk of falling [15,29]. In our study, we evaluated the amount of postural sway by the fall risk test in the biosensoric biodex balance system. Postural sway index on the dynamic platform was independent from age, body mass index, diabetes duration and hyperglycemia criteria [Table 2]. Our results are not compatible with these studies in terms of postural sway in diabetic neuropathy. However, another study have found that when sensory feedback was used in patients with diabetic neuropathy they developed rigid posture adaptation by using excessive force to protect their postural oscillations [30]. The absence of a relationship between postural sway and clinical data may be explained due to the possibility of the subjects in the study had developed this adaptation. In another study evaluating risk factors for neuropathy, the duration of diabetes and body mass index has not been associated with neuropathy [31]. In our study, postural sway was not associated with diabetes duration and body mass index. However it is not known whether the postural oscillation originates from neuropathy. Therefore, the main causes of changes in the movement system should be investigated. We investigated the relationship between changes in movement system and hyperglycemia and we also questioned the affiliation between movement system and demographic characteristics. We observed that HbA1c and fasting blood glucose values were not associated with adaptation time to the gravity change and postural sway. Compliance to the body's gravitational change becomes more difficult as increasing age and body mass index. The most important limitation of our study was unsatisfactory number of patients. We thought that the number of subjects should be increased in order to support the results of the study. Motion system is a system with too many variables. For this reason, hyperglycemia-induced neuropathic symptoms that are thought to adversely affect the movement system should be planned and with comprehensive regression studies should be determined to what extent these parameters affect the system. The underlying mechanisms should be examined by looking for the relationship between the symptoms and the movement system. Results of our study shows that, the changes of the movement system have not been found in relation to HbA1c and fasting blood glucose values in patients with diabetic neuropathy.

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FT-2

[DNA-CHIP FOR DETERMINATION OF POINT MUTATIONS] [NOKTA MUTASYONLARININ TAYİNİNE YÖNELİK DNA ÇİPİ]

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Abstract
Objective: Molecular diagnostics lies at the milestones of modern-day biochemistry. The identification of genetic and proteomic targets and patterns relies on the proper choice of detection methods, a combination of biochemical recognition architecture and signal processing. DNA-chip technology, by placing multiple probes on a single flats surface, allows for the storage of a tremendous amount of information and massively parallel detection of target species. The aim of this study, developing a new fast DNA-chip procedure for the detection of point mutations.
Materials-Method: For this study, quartz crystal (qcm) electrodes surface modified with Poly Hema-Mac nanopolymer than, DNA-probes performed schiff base with the nanopolymer. Samples of genomic DNA were extracted from venous blood leukocytes and than, Arms (Amplification-Refractory Mutation System) method was used for creating amplicons. The amplicons were detected by using qcm DNA-chip. We compare the results with gel electrophoresis.
Results: The samples of wild type, α-thalassemia heterozygote, and homozygote samples PCR products were applied on the genosensor. When hybridization occurs on the electrode surface, quartz crystals frequency changes depend on the length of the probes. In this way, we can recognize the mutation type.
Conclusıon: DNA-chip was evaluated for multiplex recognization of α-thalassemia mutations. This nanopolymer based piezoelectric DNA-chip can be using an alternative technique for the determination of α-thalassemia mutations because it has more advantageous. For example, when this genosensor compared with conventional methods, it is faster, cheaper, more specific and less hazardous exposure.
Keywords: Point mutation, Genosensor, DNA-chip
Conflict of interest: The authors have declared that no competing interests exist
Öz
Amaç: Moleküler tanı, modern biyokimyanın mihenk taşlarındandır. Genetik ve proteomik hedeflerin ve modellerin tanımlanması, uygun tespit yöntemlerinin seçilmesine, biyokimyasal tanıma mimarisinin ve sinyal işlemenin bir kombinasyonuna dayanır. DNA çip teknolojisi, elektrot yüzeyine birden fazla prob yerleştirerek, yüksek miktarda bilginin elde edilmesine ve tüm analitlerin sonuçlarının eş zamanlı olarak alınmasına olanak tanır.
Bu çalışmanın amacı, nokta mutasyonlarının tespiti için yeni bir hızlı DNA çip prosedürü geliştirmektir.
Gereç Ve Yöntem: Bu çalışma için, kuvars kristal (qcm) elektrot yüzeyi, Poly Hema-Mac nanopolimeri ile modifiye edildikten sonra, DNA probları nanopolimer ile disülfit bağı kurarak biyoaktif tabaka hazırlanmıştır. Genomik DNA örnekleri venöz kan lökositlerinden elde edildi ve amplikon oluşturmak için Arms (Amplification-Refractory Mutation System) yöntemi kullanıldı. Amplikonlar, qcm DNA-çipi kullanılarak tespit edildi. Sonuçları jel elektroforezi ile karşılaştırıldı.
Tartışma: Genosensöre α-talasemi bakımından normal, heterozigot ve homozigot örneklerden elde edilen amplikonları örnek olarak eklendi. Elektrot yüzeyinde hibridizasyon oluştuğunda, kuvars kristallerinin frekans değişiklikleri probların uzunluğuna bağlı olarak değişiklik

gösterdi. Elde edilen frekans değişiklerinden yararlanarak, mutasyon tiplendirilmeleri yapıldı.
Sonuçlar: DNA çipi, α-talasemi mutasyonlarının multipleks analizi yönünden değerlendirildiğinde. Nanopolimer tabanlılı piezoelektrik DNA-çipinin, a-talasemi mutasyonlarının belirlenmesi için klasik yöntemlere alternatif bir teknik olarak kullanılabileceği düşünülmektedir. Örneğin, genosensör geleneksel yöntemlerle karşılaştırıldığında, daha hızlı sonuç vermekte, daha ucuz, daha spesifik ve daha az tehlikeli bir yöntem olduğu gözlenmektedir.
Anahtar kelimeler: Nokta mutasyonu, Genosensör, DNA-çipi.
Çıkar çatışması: Çıkar çatışması bulunmamaktadır.

Introduction
Alpha-thalassemia is one of the most common hemoglobin genetic abnormalities and is caused by the reduced or absent production of the alpha globin chains. [1, 2] Alpha-thalassemia is prevalent in tropical and subtropical world regions where malaria was and still is epidemic, but as a consequence of the recent massive population migrations, alpha-thalassemia has become a relatively common clinical problem. [3, 4] Therefore rapid mutation identification systems like DNA Chip technologies gain importance for this health problem. [5, 6]. The DNA hybridization events has become the main principle in the construction of DNA biosensor devices which consisting of single-stranded DNA (ssDNA) probes layer immobilized on a transducer surface in order to recognize its complementary DNA target to form a DNA double helix formation. This hybridization event is converted into a quantified signal by the transducer in the form of electrochemical and piezoelectric resonance for detection. [7] The electrochemical transducer has gained an interest for DNA hybridization detection because of its simplicity and direct convert to the hybridization events into the electrical signal. [8, 9] The major advantages of electrochemical DNA biosensor chip compared to other DNA sensor are it is amenable to miniaturization, compatible with microfabrication technique, required simple instrumentation, provide a remarkable sensitivity and selectivity, poses rapid response, easy to operate and has high portability, minimum power requirements and low-cost production. [6, 10]
Materials and Methods
Chemicals
All chemicals used in biosensor establishement were purchased from Sigma Chemical Co., USA. All solutions were prepared freshly just before experiment.
Apparatus
PalmSenspotentiostat, and corundum ceramic based screen printed gold quartz cyristal electrode combined with the reference Ag/AgCl electrode, and the auxiliary AuPd (98/2%) electrode were usedto perform the electrochmeical measurements. In the experiments, automatic pipets, a yellow line magnetic stirrer, and a thermostat were used. Ultra-pure water in the preparation of solutions was obtained water purification system.
Preparation of the DNA Chip biosensor
Prior to coating with nanopolymer, the surface of au quartz cyristal electrode was polished with alumina slurries on microfiber cloth to obtain a mirror surface. The polished electrode was rinsed with double distilled water. In order to remove undisered absorbable particules, the electrode was sonicated first in pure ethanol and later in double distilled water for 10 minutes. In the next step, the electrochemical cleaning of electrode was accomplished by five successive cyclic voltammetric sweeps between −1.0 and +1.0 V in 0.1 M HNO3 solution [11]. The bioactive layer was prepared by immobilizing DNA probes on the gold electrode with self assembled mono layer of Poly-Hema-Mac on the gold surface. All the measurements were executed in a thermostatic reaction cells [12, 13], contained varying amounts of DNA samples concentration on the bioactive surface. The principle is based on the specific DNA hybridisation between DNA probes and ssDNA samples. Finally, the arrisedplasmone resonance difference during these reactions was measured by QCM.
Results
Electrochemical characterisation of the DNA chip's plasmon resonance
When using different concentrations normal, alpha thalassemia heterozygote and alpha thalassemia homozigote of DNA samples on the chip surface the resonance classied by hybrisidation affinity. (Fig.1.)

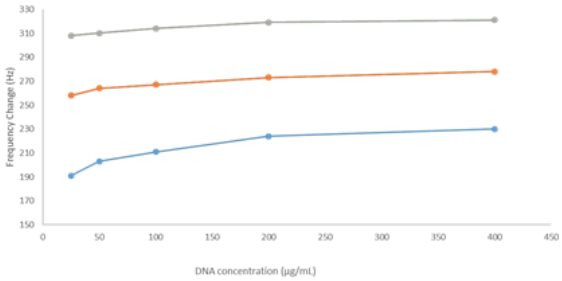

Figure 1. The plasmon resonance of normal, heterozygote and homozigote samples frequance (Grey: heterozygote, Red: normal, Blue: homozygote)
Effect of pH on the biosensor response
Biosensors based on an hybrizidation depends on a suitable buffer system and pH medium for obtaining the best responses[14]. To detect the effect of the pH value on the biosensor response, different buffer systems were investigated. For this aim, acetate (50 mM, pH 4.0-5.0-6.0), phosphate (50 mM, pH 7.0), and Glycine/NaOH (50 mM, 8.0) buffers were used in the experiments. The optimum pH value was 7.5. Below and above pH 7.5 causes a decreases in the biosensor response. (Fig. 2.)

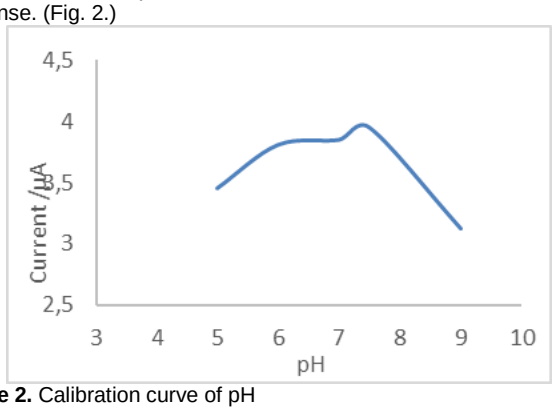

Figure 2. Calibration curve of pH
Measurement of different concentrations of DNA sample
25- 100 µg/ml of DNA samples concentration were prepared and measurements were taken under optimized conditions. When the DNA concentration increases, the response current rises at the mutation levels interval. (Fig. 3.)

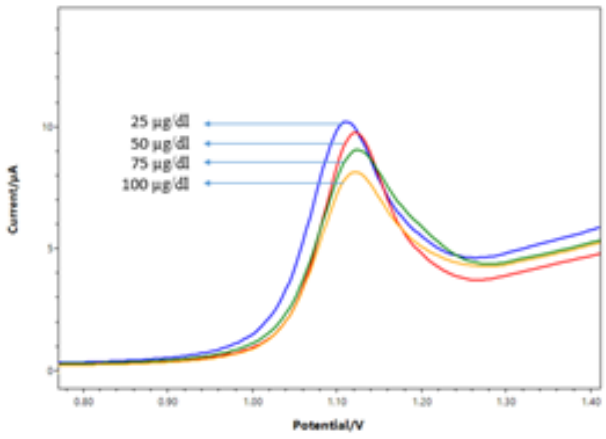

Figure 3. Measurement of different DNA amounton the chip surface
Conclusions
As a result of this work, determination of alpha thalassemia DNA samples by using biosensor DNA chip method is a new approach. [15, 16].Determination of point mutations with this method is also possible at low concentrations. According to literature the DNA biosensor chip studies have known to be very sensitive, specific, simple and less time-consuming methods. Consequently, we can be suggested that development of the method would be an original and useful procedure

for DNA samples determination. Therefore next step towards making the sensor for in vivo studies and more portable involves further miniaturization allowing in situ monitoring of signals.

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FT-3

[FABRICATION OF METAL OXIDE-BASED GAS SENSORS FOR BREATH ANALYSIS]
[NEFES ANALİZİ İÇİN METAL OKSİT TABANLI GAZ SENSÖRLERİNİN ÜRETİLMESİ]

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Abstract

Objective: Recently, breath analysis has attracted much attention for disease monitoring and clinical diagnostics due to its non-invasive nature. Several substances in human breath are considered as biomarkers or tracer compounds which are correlated to different diseases such as ammonia (kidney disease), HCN (bacterial infection) or acetone (diabetes). To achieve accurate diagnosis, minimum detection limit for a sensor should be in the range of sub-ppm (parts per million) or ppb (parts per billion) level. Detection of these low concentrations is major challenge in development of a breath analyzer sensor. Moreover, selective detection is required for precise diagnosis. For point-of-care diagnosis, portable, low cost and user-friendly sensors are also desirable. To meet these requirements, emphasis is placed on metal-oxide (MOX) based chemiresistive gas sensors. Chemiresistive sensors, which consist of various semiconducting MOXs such as SnO_2, ZnO and NiO have been studied for use as exhaled breath sensors due to their adequate reaction with biomarkers.

Materials and Method: Nanostructured MOXs; TiO_2 and CuO were fabricated as sensing materials by hydrothermal method. Fabricated sensor devices were tested toward some biomarkers such as acetone, ammonia and HCN in the range of ppb and sub-ppm levels.

Results: TiO_2 and CuO based sensors exhibited sensitive behavior for acetone and HCN, respectively in the range of disease concentration. Therefore, these sensors can be good candidates for diagnosis of diabetes and bacterial infection.

Conclusion: According to this study, sensor arrays (electronic nose) can be developed with MOX based sensors for breath component analysis to detect diabetes and bacterial infections.

Keywords: Breath analyses, gas sensor, metal oxide, electronic nose.
Conflict of Interest: The authors have no conflict of interest.

Öz

Amaç: Son yıllarda nefes analizi, müdahale gerektirmeyen doğası nedeniyle hastalıkların izlenmesi ve klinik tanı açısından oldukça dikkat çekmektedir. İnsan nefesinde bulunan amonyak (böbrek rahatsızlıkları), HCN (bakteriyel enfeksiyon) ya da aseton (diyabet) gibi bazı nefes belirteçleri veya izleyici bileşikler çeşitli hastalıklarla ilişkilendirilmektedir. Nefes analizinde kullanılacak sensörün doğru hastalık teşhisi için minimum algılama limiti sub-ppm (milyonda bir parçacık) ya da ppb (milyarda bir parçacık) olmalıdır. Bu düşük konsantrasyonları algılayabilecek sensörlerin geliştirilmesi nefes analizi için en büyük zorluktur. Ayrıca doğru tanı konulabilmesi için seçici bir algılama da gereklidir. Hasta başı teşhisi için, portatif, düşük maliyetli ve kullanıcı dostu sensörlerin üretilmesi de arzu edilen bir durumdur. Bu gereklilikleri karşılamak için metal oksit (MOX) tabanlı kemirezistif gaz sensörleri ön plana çıkmaktadır. Bu tip sensörlerde kullanılan SnO_2, ZnO, NiO gibi çeşitli yarıiletken metal oksitler, nefes belirteçlerine karşı yeterli reaksiyonları göstermeleri nedeniyle nefes analizi için çalışılmıştır.

Gereç ve Yöntem: Nano yapılı TiO_2 ve CuO tabanlı sensörler hidrotermal yöntem kullanılarak üretilmişlerdir. Üretilen sensörler, aseton, amonyak ve HCN gibi bazı nefes belirteci gazlara karşı sub-ppm ile ppb aralığında test edilmişlerdir.

Tartışma: TiO_2 ve CuO tabanlı sensörler hastalık seviyelerinde HCN ve asetona karşı algılama göstermişlerdir. Bu sonuçlarla beraber üretilen sensörler diyabet ve bakteriyel enfeksiyon teşhisinde kullanıma aday birer sensör olarak ön plana çıkmaktadır.

Sonuçlar: Yapılan bu çalışmaya göre metal oksit tabanlı gaz sensörleri kullanılarak oluşturulacak bir sensör dizisi (elektronik burun), nefes analizi uygulamalarında diyabet ve bakteriyel enfeksiyon teşhisi için kullanılabilir.

Anahtar Kelimeler: Nefes analizi, gaz sensörü, metal oksit, elektronik burun.
Çıkar Çatışması: Yazarların çıkar çatışması yoktur.

Introduction

In recent years, human exhaled breath analysis has attracted much attention for disease monitoring due to its non-invasive, effortless, painless and fast diagnostic ability [1,2]. Human exhaled breath consists of various gases, such as nitrogen, oxygen, some of the inert gases and volatile organic compounds (VOCs) at different concentration range from ppt (parts per trillion) to ppm (parts per million). Up to now, 1756 VOCs are found in healthy human exhaled breath [3]. In case of increment in concentration of some gases to the higher levels than that of healthy human exhaled breath levels, this increment in concentration might be related to some diseases. For instance, exhaled acetone concentration is in the range of 0.2-1.8 ppm for healthy people, while in the range of 1.25-2.5 ppm for people with diabetes disease [4,5]. On the other hand, higher concentration level than healthy exhaled breath of hydrogen cyanide (HCN) is attributed to cystic fibrosis [6]. Detection of such a low concentration is the major step for breath analysis sensor. There are several detection techniques for breath analysis such as gas chromatography, ion mobility spectroscopy, chemical sensors including electronic nose and

sensor arrays. Gas chromatography is the most sensitive and reliable method for breath analysis. However, it is not an ideal system due to its high cost, complex system requirements and size [7]. Also, it requires technically trained personnel. On the other hand, ion mobility spectrometry is fast, portable and sensitive. However, it requires radioactive source and offers a poor selectivity to interferent gases [7]. For point-of-care diagnosis, a portable, low-cost, environmentally and user-friendly sensors are desirable to assure patient comfort [8]. Therefore, semiconductor metal-oxide (MOX) based chemical gas sensors are one of the most promising candidates. MOX based chemical sensors have been proposed for use in exhaled breath analysis due to their superior properties such as their easy production process, and reactivity against wide range of VOCs [2, 9-13]. MOXs based chemical gas sensors have been reported to detect VOCs; however, their sensor performances should be still developed especially in terms of sensitivity and selectivity. Gas sensing mechanism of a MOX based sensor is related to chemical reactions between target gas species and surface atoms and molecules of the MOX [14-19]. Therefore, some surface modifications such as nanostructural morphology can improve sensor properties. Nanostructured MOXs have attracted considerable interest due to their high surface-to-volume ratio and unique electrical properties. These specific properties make them very good candidates for highly sensitive and selective breath analysis sensors [20-23]. In this study, nanostructured TiO_2 and CuO based chemical gas sensors were fabricated via hydrothermal method that is a very simple and productive technique [24,25]. Then these sensor devices were tested toward some breath biomarkers such as acetone, HCN, H_2S, NO and ammonia in the range of ppb and sub-ppm levels.

Materials and Methods

Fabrication of nanostructured MOXs based sensors

All the chemical reagents used in the experiments were commercially purchased and used without further purification. Tetrabutyltitanate (99+% purity) and hexamethylene tetraamine (99+% purity) were purchased from Alfa Aeser. Diethanolamine (≥99% purity), copper acetate monohydrate (≥99% purity) and copper nitrate trihydrate (≥99% purity) were purchased from Merck. HCl (36.5-38% purity) was purchased from Sigma-Aldrich. Deionized water (DI) was used for all experiments.

Fabrication of TiO_2 nanorods

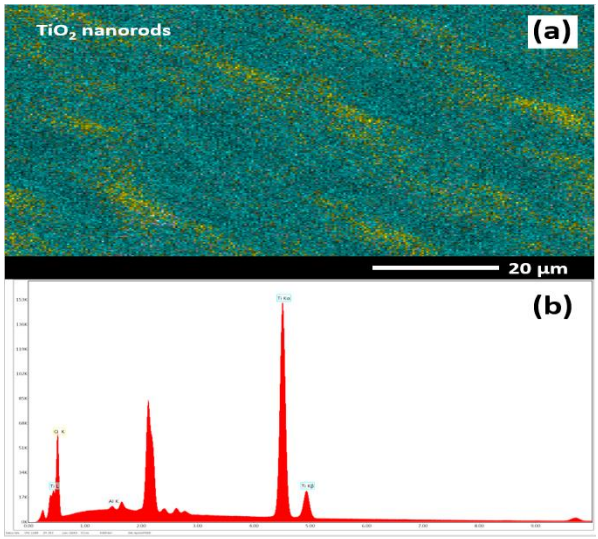
Tetrabutyltitanate, ethanol and diethanolamine were mixed under vigorous stirring at room temperature for 2 hours. Then, the mixture of ethanol and DI water solution was added dropwise above solution under vigorous stirring. After continuously stirring for 2 hours, the solution was aged at room temperature for approximately one day and a homogenous sol was obtained to be used in a seed layer formation for the next step. At the second step, this sol was coated on an alumina (Al_2O_3) substrate by spin coating. Afterwards, the seed layer-coated substrates were annealed at 500°C for 2 hours in the ambient air. At the last step, substrates were placed in a steel autoclave which contains a solution of tetrabutyltitanate, HCl and DI water (1:30:30) and kept at 150°C for 6 hours. After hydrothermal process, all samples were washed with DI water and dried in air. Finally, TiO_2 nanorods were obtained on the Al_2O_3 substrate.

Fabrication of CuO nanorods

Before the hydrothermal route, an ethanolic solution with 10 mM copper acetate monohydrate was prepared for seed layer. Then, this sol was coated on Al_2O_3 substrate by spin coating. This spin coating procedure was performed 5 times. At first 4 steps, substrates were annealed at 130°C for 5 minutes. After the last coating step, annealing time and temperature were increased to 1 hour and 300°C, respectively. Afterwards, substrates were placed in a steel autoclave which contains an aqueous hydrothermal solution of equimolar copper nitrate trihydrate and hexamethylene tetraamine and kept at 85°C for 4 hours. After the hydrothermal process, all samples were washed with DI water and dried in air. Finally, CuO nanorods were obtained on the Al_2O_3 substrate.

Characterization of MOX nanostructures

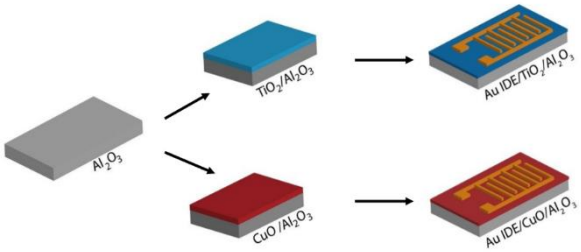
To investigate the phase and crystal structure of fabricated samples, X-ray diffraction (XRD, Rikagu Smartlab) with Cu Kα radiation (λ = 1.54059 Å) was used. The nanostructural morphologies and elemental distribution on the surface was observed by energy-dispersive X-ray



spectroscopy equipped scanning electron microscope (SEM and EDX, Philips XL 30 SFEG).

Sensor fabrication and gas sensor tests

For the gas sensor measurements, Au interdigital electrodes (Au-IDEs) were thermally evaporated on the samples surface with a shadow



mask. Keithley electrometer (model 6517A) was used for time dependent current measurements under the gas flow, and temperature was controlled with Lakeshore 340 temperature controller.

Figure 1. Device structure schemes of nanostructured TiO_2 and CuO based gas sensors.

TiO_2 and CuO based chemical gas sensors were investigated toward some breath biomarkers and interference gases such as acetone, ethanol, HCN, H_2S (hydrogen sulphide) and NO (nitric oxide). Sensors were mounted in a stainless-steel test chamber of 1 L volume capacity. All measured gases obtained from an Owlstone V-OVG gas generator equipped with permeation tubes. Concentration of gases were adjusted by mixing the carrier and analyte gases at different flow rates. Baseline signal of the sensor was determined under dry air flow condition. After the exposure of target gases, recovery of the sensor from saturated conditions back to the baseline was achieved under dry air flow.

All gas measurements were given as sensor response, defined as $S_R = {}^{\Delta I}/_{I_0}$,

here ΔI is the change in the current value when sensors were exposed to target gas molecules, I_0 is the baseline current value of the sensors was determined under dry air flow condition.

Results And Discussion

Structural Characterization

TiO_2 nanorods

XRD spectrum of TiO_2 nanorods is given in Figure 2. It can be seen from XRD pattern (JCPDS Card Number 88-1 175) that TiO_2 nanorods are in rutile phase. The strongest peak related with rutile phase was observed at 35.28° which indicates (101) crystal plane of TiO_2. The diffraction peaks at 27.55°, 52.67°, 61.43° and 68.30° are corresponds to (110), (211), (002) and (112) crystal planes, respectively.

Figure 2. XRD pattern of the TiO2 nanorods

To identify the morphological structure and elemental distribution of the TiO_2 nanorods, SEM scan and EDX mapping were performed. SEM images of TiO_2 nanorods are given in Figure 3.

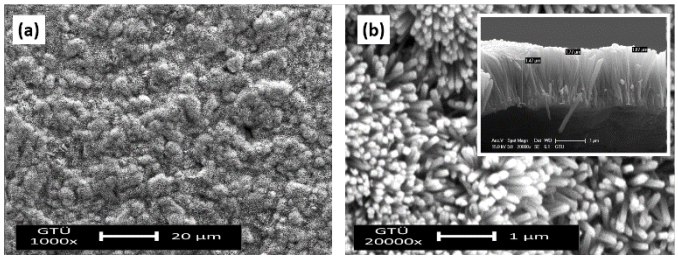


Figure 3. SEM images of TiO_2 nanorods; magnification of (a) 1000x and (b) 20000x.

SEM images show that nanorods homogenously cover the surface of the Al_2O_3 substrate (Figure 3a) and are vertically aligned (inset of Figure 3b). TiO_2 nanorods have tetragonal like shape (Figure 3b). The average length of the TiO_2 nanorods is around 1.5 µm as seen Figure 3b inset. EDX mapping and spectrum of TiO_2 nanorods are given in Figure 4. The presence of Ti, O and Al was observed from EDX spectrum.

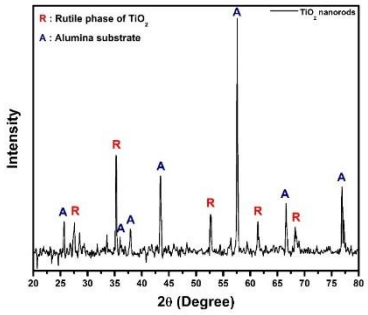


Figure 4. EDX (a) mapping image and (b) spectrum of TiO_2 nanorods. In the mapping results, yellow represents O, grey represent Al and green represent Ti.

CuO nanorods

XRD pattern of CuO nanorods is given in Figure 5. Monoclinic CuO phase formation was observed from XRD spectrum [25].

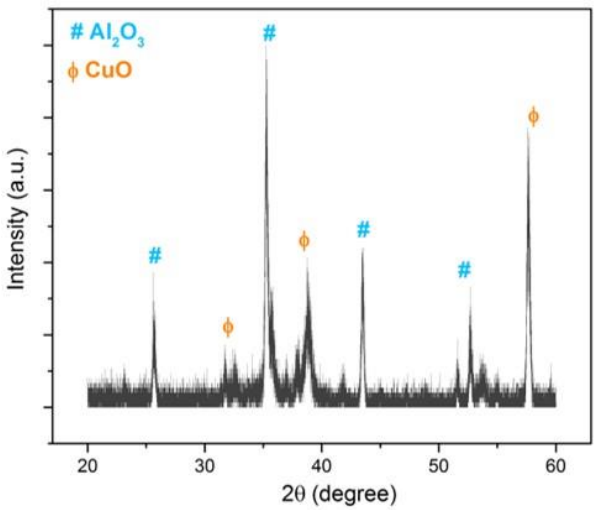


Figure 5. XRD pattern of CuO nanorods.

To identify the morphological structure of CuO nanorods, SEM scan was performed as seen in Figure 6. According to SEM images, CuO nanorods homogenously cover the surface of the Al_2O_3 substrate. Nanorods have spherical shape and approximately 230 nm length as seen Figure 6a inset.

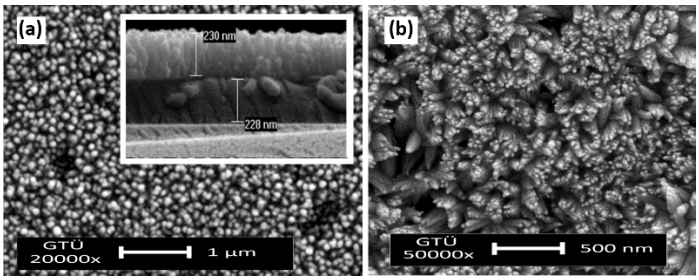


Figure 6. SEM images of CuO nanorods; magnification of (a) 20000x and (b) 50000x.

3.2. Gas Sensing Measurements

3.2.1. TiO_2 nanorods

Sensor measurements toward 3.8 ppm acetone, 0.6 ppm ethanol, 4.4 ppm HCN, 7 ppm H_2S and 3.4 ppm NO were performed under a constant voltage in a temperature range between 50-300°C. Gas sensor response versus operation temperature curves for TiO_2 nanorods are shown in Figure 7.

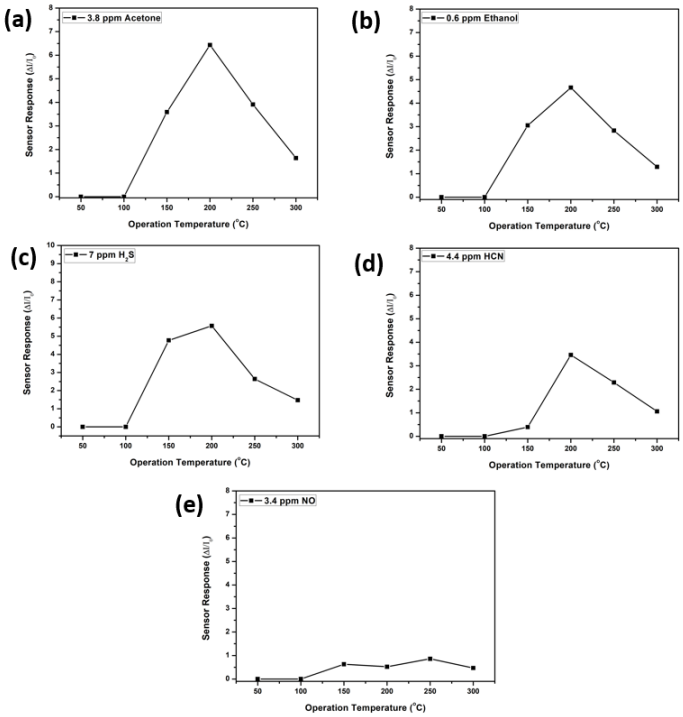


Figure 7. Sensor responses of TiO_2 nanorods between 50-300°C toward (a) 3.8 ppm acetone, (b) 0.6 ppm ethanol, (c) 4.4 ppm HCN, (d) 7 ppm H_2S and (e) 3.4 ppm NO.

The optimal operation temperature of TiO_2 nanorods for acetone, ethanol, HCN and H_2S detection was determined as 200°C while it is found to be 250°C for NO as can be seen in Figure 7. At optimal operation temperatures, sensor responses for ethanol, HCN, H_2S and NO are 4.66, 3.46, 5.57 and 0.86, respectively. Among the tested gases, best sensor response of TiO_2 nanorods observed toward acetone with a sensor response of 6.48 at 200°C. Sensor response of TiO_2 nanorods toward all gases at 200°C and dynamic sensor response at different acetone concentrations can be seen in Figure 8a and 8b. A summary of acetone sensing performance of TiO_2 nanorods is shown in Table 1.

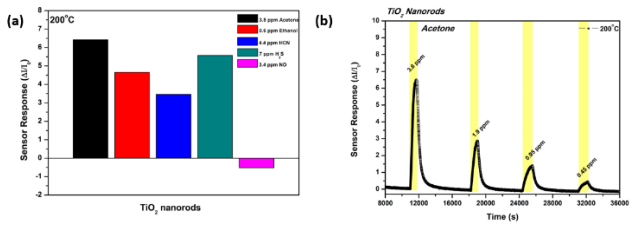


Figure 8. Sensor response of TiO_2 nanorods (a) toward all gases and (b) dynamic sensor response peaks toward different acetone concentration at 200°C.

Table 1. Sensor properties of TiO_2 nanorods toward acetone at optimal operation temperature.

Acetone Concentration (ppm)	Operation Temperature (°C)	Sensor Response ($\Delta I/I_0$)	Response Time (minute)	Recovery Time (minute)
3.8	200	6.58	14.46	32.08
1.9		3.12	15.76	29.56
0.95		1.65	21.68	27.71
0.45		0.42	19.36	26.11

As a typical n-type MOX semiconductor, gas sensing performance of TiO_2 depends on the phenomena of the exchange of charges between adsorbed gas species and MOX surface. In atmosphere ambient, oxygen species adsorb on TiO_2 surface, and electron transfer occurs from surface to oxygen species. Therefore, space-charge layer is enlarged in semiconducting TiO_2, and electrical resistance of the surface is increased. This process might be formulated as [26-28];

$$O_{2gas} \leftrightarrow O_{2ads}$$
$$O_{2ads} + e^- \leftrightarrow O_{2ads}^-$$
$$O_{2ads}^- + e^- \leftrightarrow 2O_{ads}^-$$
$$O_{ads}^- + e^- \leftrightarrow O_{ads}^{2-}$$

When the sensor is exposed to acetone gas, this reductive gas reacts with the adsorbed oxygen species on the surface. Then electrons are released back to the conduction band again and it causes the thinner space-charge layer and lower potential barrier. Therefore, electrical resistance of the surface is decreased, and this process can be described as [28-30];

CH_3COCH_3 (gas) ↔ CH_3COCH_3 (ads)
CH_3COCH_3 (gas) + $8O^- \rightarrow 3CO_2$ (gas) + $3H_2O$ (gas) + $8e^-$
CH_3COCH_3 (ads) + $4O_{2ads}^- \rightarrow 3CO_2$ (gas) + $3H_2O$ (gas) + $4e^-$

The high surface-to-volume ratio of the TiO_2 nanorods surface brings a larger contact area between target gas molecules and the surface. Therefore, nanostructural morphology increase the yield of all chemical reactions on the surface and gives a great contribution to the sensor performance.

CuO nanorods

Sensor response of CuO nanorods for different concentration of acetone, ethanol, HCN and H_2S in a temperature range of 50-300°C is given in Figure 9.

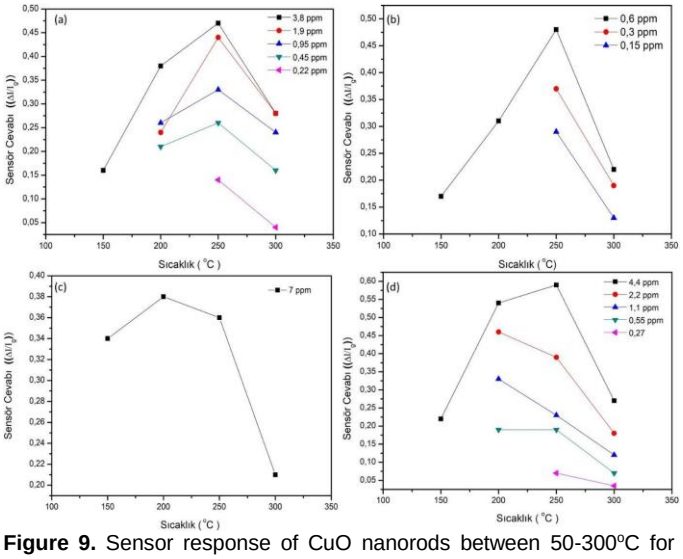


Figure 9. Sensor response of CuO nanorods between 50-300°C for different concentration of (a) acetone, (b) ethanol, (c) H_2S and (d) HCN.

The optimal operation temperature of CuO nanorods for acetone and ethanol is 250°C. However, it decreased to 200°C for H_2S. According to Figure 9d, the best sensor performance of CuO nanorods was observed at 250°C for HCN as sensor response of 0.59 for 4.4 ppm. Moreover, at this operation temperature, CuO nanorods show a sensitive behavior toward HCN in a concentration range between 4.4 ppm and 0.27 ppm. A summary of gas sensor performance of CuO nanorods for all gases is shown in Table 2. Dynamic sensor response of different HCN concentration at 250°C can be seen in Figure 10.

Table 2. Sensor properties of CuO nanorods.

Gas	Concentration (ppm)	Operation Temperature (°C)	Sensor Response ($\Delta I/I_0$)	Response Time (minute)	Recovery Time (minute)
Aceton e	3.8	250	0.47	14.4	47.7
Ethanol	0.6	250	0.48	15.3	51.3
HCN	4.4	200	0.59	9	29.7
H_2S	7	250	0.44	29.7	76.5

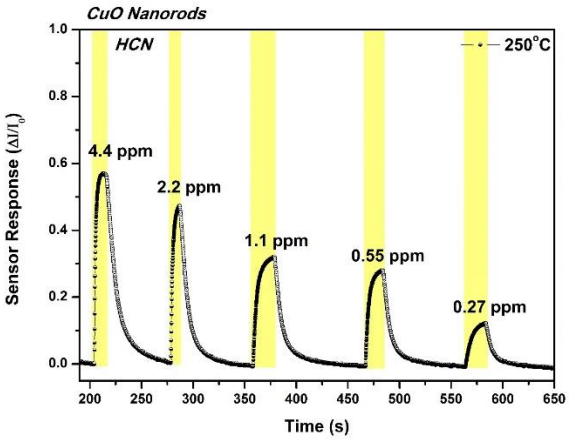


Figure 10. Dynamic sensor response peaks of CuO nanorods for different HCN concentration at 250°C.

Conclusion

TiO_2 and CuO nanorods were fabricated via hydrothermal process. The structural and morphological characterizations confirmed rutile phase TiO_2 nanorods formation with the length of approximately 1.5 µm and monoclinic CuO nanorods formation with the length of approximately 230 nm. The sensing characteristics of TiO_2 and CuO

nanorods were studied at different temperatures and concentrations. It has been shown that TiO_2 nanorods can be used to sense diabetes biomarker, acetone, with a concentration level of 0.45 ppm at 200°C. Also, CuO nanorods sense 0.27 ppm HCN at 250°C, which is a biomarker for cystic fibrosis. Both TiO_2 and CuO based gas sensors are very good candidates for further sensor array (electronic nose) applications to diagnose diabetes and cystic fibrosis diseases.

Acknowledgements

The authors would like to thank Ahmet Nazım for SEM work. This research was performed within the 116M201 project financed by Scientific and Technological Research Council of Turkey (TÜBİTAK).

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FT-4

[AN ELECTROMYOGRAPHY BASED ERGONOMİC INTERVENTION: IMPLEMANTATION OF REST BREAKS DURING GRASPING]

[ELEKTROMİYOGRAFİ TABANLI BİR ERGONOMİK MÜDAHALE: KAVRAMA SIRASINDA DİNLENME ARASI UYGULAMASI]

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Abstract

Objective: Grasping varies depending on some criteria such as age, gender, general health status and anthropometric characteristics. Grip strength is an important measure of dexterity and functionality. Working with heavy loads, applying excessive force without rest breaks has been mentioned as an important ergonomic risk factor in the literature. The aim of this study is to investigate the effect of rest breaks during grasping activity.

Materials and Methods: 70 individuals were included in the study. Individuals were randomly divided into 3 groups as without break working cycle group, 1 min. Break working cycle group, 2 min. break working cycle group. The electromyography measurements were taken from the flexor and extensor wrist muscle group with BIOPAC (Biopac Systems, Santa Barbara, CA, USA) body signal devices during grasping 10 min and 10 kg grasping force. The dynamometer of same devices was used for measurement of grasping force.

Results: 70 voluntary participants were participated to the study. 24 (17 male, 7 female) of them were in the without break group, 24 (11 male, 13 female) of them were in the 1 minute break group, 22 (10 male, 12 female) of them were 2 minute break group. It was observed that muscle activation of the working group increased significantly with 2 minutes break cycle ($p < 0.001$).

Conclusion: According to our study, muscle activation increased by 2 minutes break. According to this result, we think that giving appropriate rest breaks is important for preventing of work related musculoskeletal disorders.

Keywords: Grasping; Rest Break; Muscle Activation; Electromyography

Öz

Amaç: Kavrama yaş, cinsiyet, genel sağlık durumu ve antropometrik özellikler gibi bazı kriterlere bağlı olarak değişir. Kavrama gücü, el becerisi ve işlevsellik için önemli bir ölçüdür. Ağır yüklerle çalışmak, hiç durmaksızın aşırı kuvvet uygulamak literatürde önemli bir ergonomik risk faktörü olarak belirtilmiştir. Bu çalışmanın amacı, dinlenme molalarının kavrama aktivitesi sırasındaki etkisini araştırmaktır.

Gereç ve Yöntem: Çalışmaya 70 kişi dahil edildi. Bireyler, rastgele yöntemle aralıksız çalışma grubu, 1 dakika ara grubu ve 2 dakika ara grubu olarak 3'e ayrıldı. Elektromiyografi ölçümleri 10 dakika kavrama ve 10 kg kavrama kuvveti uygulaması sırasında BIOPAC (Biopac Systems, Santa Barbara, CA, ABD) vücut sinyal cihazı ile fleksör ve ekstansör bilek kas grubundan alındı. Kavrama kuvveti ölçümü için aynı cihazın dinamometresi kullanılmıştır.

Bulgular: Çalışmaya 70 gönüllü katılımcı katıldı. Aralıksız çalışma grubunda 24 birey (17 erkek, 7 kadın), 1 dakikalık ara grubunda 24 birey (11 erkek, 13 kadın), 2 dakikalık ara grubunda 22 birey (10 erkek, 12 kadın) vardı. Çalışma grubunun kas aktivasyonunun 2 dakikalık mola döngüsü ile anlamlı olarak arttığı gözlendi (p <0,001).

Sonuç: Çalışmamıza göre kas aktivasyonu 2 dakikalık mola ile arttı. Bu sonuca göre, işle ilgili kas-iskelet hastalıklarının önlenmesinde uygun dinlenme molaları vermenin önemli olduğunu düşünüyoruz.

Anahtar Kelimeler: Kavrama; Dinlenme Molası; Kas Aktivasyonu; Elektromiyografi

Introduction

Today, the world population is estimated to be about 6.9 billion and it is estimated that there are 3.1 billion workers in more than 55 main sectors. This situation provides job opportunities for people but also causes many occupational injuries in industrial sectors due to lack of awareness about occupational health and safety. Musculoskeletal Disorders(MSD) in Turkey with a rate of %9.9 ranks 3rd among the causes of disability. These disorders are legaly considered as work related disorders [1]. Production workers are often exposed to MSD in sectors such as automotive and poultry [2-3]. Muscle and skeletal system disorders may also develop due to insufficient environmental accessibility conditions [4]. Health and education workers are also in this risk group, especially nurses and teachers (5). Despite this situation, employees and employers do not have enough information about the incidence of MSD, risk factors, insurance payments for lost workdays, preventive trainings and the effectiveness of ergonomic approaches [6]. Ergonomic risk factors often called the workplace risk factors and individual factors [6-7]. An important question in the operational environment is to give the workers how much time they need to work to recover after a duty cycle, so that they will perform best during the next duty cycle [8]. According to the studies, one of the work accident factors is stress and fatigue [9]. In addition, awkward postures also trigger musculoskeletal disorders [2]. However, the employer may implement a work program that does not take into account the workload as long as the company fulfills its objective. Working without break can lead to fatigue or discomfort and this situation can lead to absenteeism, low productivity and medical costs. The rest break program is one of the implementations that can be applied to reduce fatigue for long working time [10].The upper extremity is the most important part of our body with its complex and functional structure. The upper extremity region, which is also important for the workers, is a region with important functions such as grip and touch. The upper extremity region, which we use a lot during the work, production and self-care.Therefore, the region where work-related musculoskeletal diseases are the most common. Carpal tunnel syndrome and shoulder injuries are among the most common muscles and skeletal problems in the upper extremities [11-12].Therefore, in our study, we decided to examine the effect of rest breaks on muscle activation during grip activity which is one of the most important activities of upper extremity.

Material and Methods

Study Group: 70 individuals were selected on the basis of the inclusion criteria, and the subjects were randomly included in the without break, 1-minute break and 2-minutes break. Criteria for inclusion in the study: Being 18-30 years old, having not worked for a long time (6 months or more) in a job involving the use of the hand before, agreeing to sign an informed consent form. Exclusion criterias: to be over 30 years old, have known and diagnosed diabetes or insulin resistance, have any history of surgery previously involving the neck and upper extremities, in the last 1 month, the dominant side has pain in one or more joints in the upper extremity, the presence of numbness, the presence of musculoskeletal disorders involving the upper extremity, the presence of a history of trauma involving the upper extremity and not agreeing to sign the ınformed voluntary consent form.

Electromyography Measurement

Electromyography measurement was performed with BIOPAC (Biopac Systems, Santa Barbara, CA, USA) MP36 model body signal device (Picture 1) during grasping with 10 kg force for 10 minutes [4]. The signal was constructed to have two channels from the flexor and extensor wrist muscle groups. For grasping force, dynamometer of BIOPAC body signal device (SS25LB Model) was used.

Figure 1. BIOPAC MP 36 Body signal devices

In our study, BIOPAC EL503 model silver / silver chloride (Ag /AgCl) superficial electrodes with contact surface 11 mm were used. SS25LB dynamometer used in the study is approximately 0.75 kg sensitivity. This dynamometer, measuring a maximum of 90 kg, gives the measured force in kilograms or pounds. Before the measurements, training have been given to the participant to grasp with 10 kg. The individuals who could not complete the test measurement successfully were excluded from the study (Picture 1).After the electrodes were placed, physiotherapist asked to keep elbow flexion angle 90° and wrist neutral position according to hand dynamometer measurement rule (Picture 2) [2-4]. Hand dynamometer measurement was taken with kg measurement unit. Calibration has been performed in this position before start to measurement. For other channels, the frequency range of 5-1000 Hz is selected.

Figure 2. Measurement position

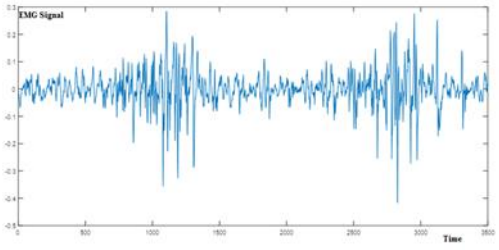

Figure 3. EMG signal processing

Biosensors

The EMG signals were measured by BIOPAC Body Signal Device (Biopac Systems, Santa Barbara, CA, USA) 2 kHz with frequency. In signal processing) calculations were performed with the MATLAB software [2-4].

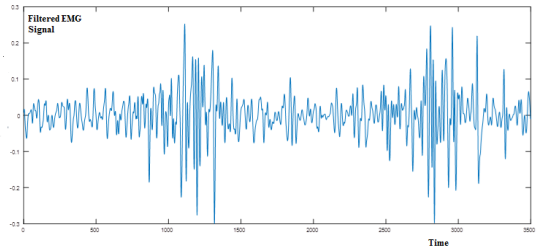

Figure 4. Filtered EMG signal

After the signals were filtered, the analysis was performed with MATLAB rogram (Picture 3-4). The calculated values of the signal are respectively Root Mean Square (RMS), Average values, minimum and maximum values (Table 1). For statistical analysis, the first 30 and last 30 seconds of EMG signals of each individual were considered (Table 1).

Table 1: Value formulas derived from signals.

		N	Mean	Std. Deviation			Mean	Std. Deviation
RMS_2_first	without break	24	0,0585	0,0211	RMS_3_initial	without break	0,0856	0,0415
	1 min. break	24	0,0623	0,0282		1 min. break	0,0959	0,0406
	2 min. break	22	0,0684	0,0347		2 min. break	0,0938	0,0334
	Total	70	0,0629	0,0282		Total	0,0917	0,0385
RMS_2_last	without break	24	0,0633	0,0237	RMS_3_end	without break	0,1065	0,0735
	1 min. break	24	0,0921	0,0586		1 min. break	0,1283	0,0775
	2 min. break	22	0,0982	0,0455		2 min. break	0,1332	0,0690
	Total	70	0,0842	0,0469		Total	0,1224	0,0734

Results

70 voluntary participant were participated to the study.70.8% (n = 17) of the without break group were male and 29.2% (n = 7) were female. 1 minute break group 45.8% (n = 11) were male and 54.2% (n = 13) were female. 2 minutes break group 45.5% (n = 10) male, 54.5% (n = 12) female. The mean age of the subjects was 22.30 ± 2.65 in females and 21.31 ± 1.31 in males. BMI averages of females were 24.37 ± 3.49; calculated as 25,67 ± 4,49 in males. The RMS values of the flexor group muscles with Channel 2 of the without break group the average of the first 30 sec (RMS_2_first) was 0.0585 ± 0.0211; The RMS2 value is the average of the last 30 sec (RMS_2_last), 0.0633 ± 0,0237. The mean value of the first 30 sec of RMS 2 with 1 min break was 0.0623 ± 0.0282; The average value of the last 30 sec of RMS 2 is 0.0921 ± 0.0586. The mean value of the first 30s of RMS 2 who worked with 2 min break was 0.0684 ± 0.0347; The average of RMS 2 last 30 sec values is 0.0982 ± 0.0455. The first and last values of RMS 2 belonging to individuals are given in table 2.The RMS values of the extensor group muscles with Channel 3 of without break group the average of the first 30 sec (RMS_3_first) was 0,0856±0,0415; The RMS2 value is the average of the last 30 sec (RMS_3_last) 0,1065±0,0735. The mean value of the first 30 sec of RMS 3 with 1 min break group was 0,0959±0,0406; The average value of the last 30 sec of RMS 3 is 0,1283±0,0775. The mean value of the first 30 sn of RMS 3 who worked with 2 min break group was 0,0938±0,0334; The average of RMS 3 last 30 sec values is 0,1332 ±0,0690. The first and last values of RMS 3 belonging to individuals are given in Table 2.

Features	Equations
RMS	$\sqrt{\frac{1}{N}\sum_{i=1}^{N} S_i^2}$
AVG	$\sqrt{\frac{1}{N}\sum_{i=1}^{N} S_i}$
MAX	$max \sum_{i=1}^{N} S_i$
MIN	$min \sum_{i=1}^{N} S_i$
VAR	$\frac{\sum_{i=1}^{N}(S_i-\mu)^2}{N}$
$\Delta RMS = \sqrt{\frac{1}{N_{ilk}}\sum_{i=1}^{N_{ilk}} S_i^2} - \sqrt{\frac{1}{N_{son}}\sum_{j=N}^{N_{son}} S_j^2}$	[1]
$\Delta VAR = \frac{\sum_{i=1}^{N_{ilk}}(S_i-\mu)^2}{N_{ilk}} - \frac{\sum_{j=N}^{N_{son}}(S_j-\mu)^2}{N_{son}}$	[2]

Table 2: RMS values for first 30 and last 30 seconds for channels 2

According to RMS 2 homogeneity test results, the data were not homogenous in the 10 kg group (Table 3) (p <0.05). As the data were not homogeneous, the Welch Brown-Forsythe test was used to determine the differences between the RMS 2 values between the first and the last. According to the results of the test, there was no difference between RMS 2 first 30 sec (p> 0.05) (Table 4). A statistically significant difference was found between RMS 2 last 30 sec (p <0.05) (Table 4). According to the results of the Tuckey-Scheffe test, a statistically significant difference was observed between the groups that had a uninterrupted and 2-minute break (p <0.05) (Table 5).

Table 3. RMS 2 homogeneity test of 10 kg working group

Test of Homogeneity of Variances				
	Levene Statistic	df1	df2	Sig.
RMS_2_first RMS_2_last	3,260	2	67	,045
	3,222	2	67	,046

Table 4. Welch Brown-Forsythe test made to measure whether RMS 2 differs between first and last.

Robust Tests of Equality of Means

		Statistics	df2	Sig.
RMS_2_first	Welch	,678	42,020	,513
	Brown-Forsythe	,697	56,022	,502
RMS_2_last	Welch	6,530	38,121	,004
	Brown-Forsythe	4,021	50,634	,024

Table 5. Tuckey-Scheffe Test for determining which group is the difference between the RMS 2 last values

Multiple Comparisons
Dependent Variable: RMS_2_last

	(I) grup	(J) grup	Mean Difference (I-J)	Std. Error	Sig.
Tukey HSD	without break	1 min break	-0,0288	0,0129	,075
		2 min. break	-0,0349*	0,0132	,028*
	1 min. break	without break	0,0288	0,0129	,075
		2 min break	-0,0060	0,0132	,891
	2 min. break	without break	0,0349*	0,0132	,028
		1 min break	0,0060	0,0132	,891
Scheffe	without break	1 min break	-0,0288	0,0129	,093
		2 min break	-0,0349*	0,0132	,037*
	1 min break	without break	0,0288	0,0129	,093
		2 min break	-0,0060	0,0132	,900
	2 min break	without break	0,0349*	0,0132	,037*
		1 min break	0,0060	0,0132	,900

Discussion

MSD are a heavy burden for all employees and employers. It is important to carry out appropriate and effective ergonomic intervention programs for the prevention of these disorders. Appropriate workplace design and reduction of individual risk factors are the most well-known ergonomic interventions, but there are few studies on resting yet [2]. In our study, we examined the effect of rest breaks on upper extremity muscle activation. According to the results of our study, the muscle activation was changed when a 2-minute break was continued for 10 minutes with a 10 kg hand grip force. According to the results of our study, a 2-minutes break hand grasping resulted in a bigger muscle activation than the without break hand grasping. At the beginning of our study, we thought that repetitive and long-term grasping activity would cause muscle fatigue in the upper extremity and this would lead to coordination problem and in the long term we thought that it would cause upper extremity injuries by creating cumulative trauma effect. As a result of our study, 2-minutes break should be in 10-minute work cycle. Zakaria et al. Reported that stress and fatigue were one of the important causes of occupational accidents [9]. In this respect, our study show that repetitive work would lead to MSD and cause work accidents. Rocha et al. have done an ergonomics study on the problems of uninterrupted work in call center employees and they decided that there should be a 10-minute break after 50 min of work [13]. They have a well-designed design for a healthy work environment and provide the appropriate rest breaks. In our study, individuals were operated with a grip force of 10 kg. As a result of our study, we decided that there should be a break of 2 min after 10 min. When we compared this situation with the call center employees, it was concluded that our study should be interrupted in a shorter period of time. Balchi and Aghazadeh stated that workers should take 10 minutes break for 60 minutes and 5 minutes break for 30 minutes. In this respect, our study is consistent with other studies in the literature [14]. Van Dongen et al. have studied how to adjust the timing of rest during the working cycle. According to their studies, they found that resting is necessary for both circadian rhythm and mental recovery. This study supports the idea and results of our study [8]. Rahman et al. investigated the importance of having a rest period for long-term standing and emphasized that 10-minute rest breaks were required in long-term standing work [10]. In our study, we evaluated the dynamic working time of the upper extremity region with continuous grip. For this reason, we suggest that it is appropriate 2 minutes break during grasping activity, but this should be repeated more frequently than the lower extremity. Kinali et al. Used superficial EMG electromyography in a study on the causes of cumulative trauma disorders in the upper extremity [2-4]. In our study, we used surface Electromyography and signal processing method to observe muscle activation during dynamic study. According to the results of our study, rest breaks during the work cycle is necessary for protection from MSD. According to this result, we conclude that giving appropriate rest breaks is important implementation of preventing the MSD in production lines.

Acknowledgements

We would like to thank the Istanbul Gelisim University Rectorate, the Board of Trustees, the Directorate of Health Sciences and the students for their support during the completion of this study. We would like to thank to Engineer Mehmet Göl for his contribution to signal processing.

Conflict of İnterests Statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Çıkar Çatışması Beyanı

Yazarlar bu makalenin yayınlanmasıyla ilgili olarak herhangi bir çıkar çatışması olmadığını beyan etmektedir.

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FT-5

[EVALUATION OF BIOTIN INTERFERENCE ON TSH AND FREE THYROID HORMONE MEASUREMENT]
[TSH ve SERBEST TİROİT HORMON ÖLÇÜMÜNDE BİYOTİN İNTERFERANSININ DEĞERLENDİRİLMESİ]

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Abstract
Objective: The interaction between biotin and streptavidin is the most powerful non-covalent biological interaction known, and some test manufacturers design tests using this strong interaction. High dose biotin-containing preparations used by patients may cause interference in these principled immunoassay systems. In our study, we aimed to evaluate biotin interference for thyrotropin (TSH), free thyroxine (fT4) and free triiodothyronine (fT3) which are exist in thyroid function test panel.
Materials and Method: Biotin (Sigma-Aldrich, USA) was added at 7 different levels (5-12.5-25-50-100-200-500 ng/mL) to serum pools containing 3 different levels (low, normal, high) of TSH (0.072-1.107-8.663 mIU/L), fT4 (0.46-1.073-2.86 ng/dL) and fT3 (1.56-2.71-5.8 ng/L). All analyzes were performed using the chemiluminescent immunoassay in Beckman Coulter DXI 800 (Beckman Coulter Inc., Fullerton, USA) analyzer. Following the addition of biotin, the change of the recovery value that > 10% accepted as significant interference.
Results: In low, normal and high-level serum pools, the biotin levels at which the interference started (positive interference) were 12.5-25-25 ng/mL for fT3 and 5-12.5-5 ng/mL for fT4, respectively. No interference was observed after addition of biotin in the TSH assay.
Conclusion: In the presence of a test result which does not correlate with the clinic, laboratory specialists should be aware that biotin may interfere with immunoassays, causing erroneous high or low results.
Keywords: biotin interference, immunoassay, thyroid function tests
Conflict of interest: No potential conflict of interest was reported by the authors.
Öz
Amaç: Biyotin ve streptavidin arasındaki etkileşim bilinen en güçlü non-kovalent biyolojik etkileşimdir ve bazı test üreticileri bu güçlü etkileşimi kullanan testler tasarlamaktadırlar. Hastalar tarafından kullanılan yüksek doz biyotin içeren preparatlar bu prensiple çalışan immunoassay sistemlerinde interferansa neden olabilmektedir. Çalışmamızda, tiroit fonksiyon test panelinde bulunan tirotropin (TSH), serbest tiroksin (sT4) ve serbest triiyodotironin (sT3) için biyotin interferansını değerlendirmeyi amaçladık.
Gereç ve Yöntem: 3 farklı düzeyde (düşük, normal, yüksek) TSH (0.072-1.107-8.663 mIU/L), sT4 (0.46-1.073-2.86 ng/dL) ve sT3 (1.56-2.71-5.8 ng/L) içeren serum havuzlarına 7 farklı düzeyde (5-12.5-25-50-100-200-500 ng/mL) biyotin (Sigma-Aldrich, ABD) eklendi. Tüm analizler Beckman Coulter DXI 800 (Beckman Coulter Inc., Fullerton, ABD) analizöründe kemiluminesan immunoassay kullanılarak gerçekleştirildi. Biyotin eklenmesini takiben geri elde değerindeki > %10 değişiklik anlamlı interferans olarak kabul edildi.
Tartışma: Düşük, normal ve yüksek düzey serum havuzlarında, interferansın (pozitif interferans) başladığı biyotin düzeyleri sırasıyla sT3 için 12.5-25-25 ng/mL, sT4 için 5-12.5-5 ng/mL idi. TSH testinde biyotin ilavesini takiben interferans gözlenmedi.
Sonuç: Klinik ile ilişkili olmayan bir test sonucu varlığında, laboratuvar uzmanları biyotinin immunoassaylerde interferansa yol açarak hatalı yüksek veya düşük sonuçlar oluşabileceğinin farkında olmalıdır.
Anahtar kelimeler: biyotin interferansı, immunoassay, tiroit fonksiyon testleri
Çıkar çatışması: Yazarlar tarafından potansiyel çıkar çatışması bildirilmemiştir.

Introduction
Biotin (vitamin B7 or vitamin H) is a water-soluble vitamin and an essential cofactor for carboxylases in mammals. Biotin-dependent carboxylases are involved in various cellular metabolic pathways such as fatty acid metabolism, leucine degradation, and gluconeogenesis. Biotin is widely available in foods and the recommended daily intake of biotin is 30 µg per day [1]. Foods relatively rich in biotin include egg yolk, liver, whole cereals, or some vegetables. In Western societies, the daily dietary intake of biotin is 35-70 µg per day, which meets the daily requirement [2]. Oral biotin is completely absorbed from the digestive system and is 100% bioavailable. Although the daily requirement is easily met from foods, supra-physiologic biotin supplementation is increasingly marketed as an over the counter remedy for common hair, nail, and skin problems as well as beneficial for weight loss. Relatively small doses of biotin for pharmacologic use includes inherited metabolic diseases such as genetic biotin deficiency and biotin-thiamine responsive basal ganglia disease. High doses (up to 300 mg per day in some cases) have been using in seconder progressive multiple sclerosis, in hemodialysis patients, and in patients with malabsorption syndromes or in total parenteral nutrition [3]. To survive in competitive and unfriendly environments, living organisms usually develop highly specific defense mechanisms. Scientists try to unravel such defense mechanisms and in the next step they use it in vitro, in a completely different context. For example, following the molecular biologists recognized bacterial restriction endonuclease enzymes as a cheap, easy, and highly specific and versatile way of cutting nucleic acids, a revolution in the field of molecular cloning happened. Streptavidin, a similar protein found in Streptomyces avidinii, binds with a very high affinity to biotin. The binding with streptavidin of biotinylated enzymes that participate in CO2 transfer inactivates the enzymes and thus inhibits the growth of bacteria that depend on biotinylated enzymes. This finding suggests the researchers that the interaction of streptavidin with biotin represents a natural defense [4]. The biotin-streptavidin interaction has some unique characteristics and the affinity between biotin and streptavidin is the most powerful non-covalent interaction known, provides the manufacturers to use this as a general bridge system in many diverse applications. Various immunoassays are readily available in different brands that use the strong interaction between biotin and streptavidin. However, biotin supplements in varied doses may cause interference in these principled immunoassay systems. In our study, we aimed to evaluate biotin interference for thyrotropin (TSH), free thyroxine (fT4), and free triiodothyronine (fT3).

Materials and Method
Aliquots of serum were prepared from pooled clinical specimens with 3 different levels (low, normal, high) for TSH (0.072-1.107-8.663 mIU/L), fT4 (0.46-1.073-2.86 ng/dL) and fT3 (1.56-2.71-5.8 ng/L). These aliquots were spiked with a 0.9% NaCl solution containing 10000 ng/mL biotin (Sigma-Aldrich; St. Louis, MO, USA) to achieve final biotin concentrations of 5, 12.5, 25, 50, 100, 200 and 500 ng/mL. All analyzes were performed using the chemiluminescent immunoassay in Beckman Coulter DXI 800 (Beckman Coulter Inc., Fullerton, USA) analyzer. Following the addition of biotin, the change of the recovery value that > ±10% accepted as a "significant interference" [5]. The samples that the laboratory analysis completed selected for the study and no additional blood samples were taken from the inpatients or outpatients.

Results
In low, normal and high-level serum pools, the biotin levels at which the significant interference started (positive interference) were 12.5-25-25 ng/mL for fT3 and 5-12.5-5 ng/mL for fT4, respectively. No interference was observed after addition of biotin in the TSH assay. It was determined that when the concentration of added-biotin increased, both the recovery values (%) of ft3 and ft4 raised even up to 636 and 731, respectively (Table 1).

Conclusions

that we use were based on biotin-streptavidin interaction and we

Untreated Serum Pools		Biotin-Spiked Serum Pools													
		5 ng/mL**		12.5 ng/mL**		25 ng/mL**		50 ng/mL**		100 ng/mL**		200 ng/mL**		500 ng/mL**	
		Result	Recovery (%)	Result	Recovery (%)	Result	Recovery (%)	Result	Recovery (%)	Result	Recovery (%)	Result	Recovery (%)	Result	Recovery (%)
fT3 (ng/L)	1.56	1.50	96.2	1.84*	117.9*	2.09*	134.0*	2.48*	159.0*	4.00*	256.4*	6.46*	414.1*	9.93*	636.5*
	2.71	2.77	102.2	2.71	100.2	3.26*	120.3*	3.34*	123.2*	6.68*	246.5*	9.53*	351.7*	14.5*	535.1*
	5.80	5.95	102.6	5.82	100.3	6.70*	115.5*	7.25*	125.0*	11.20*	193.1*	18.07*	311.6*	24.68*	425.5*
fT4 (ng/dL)	0.460	0.507	110.2*	0.527*	114.6*	0.550*	119.6*	0.752*	163.5*	1.037*	225.4*	1.802*	391.7*	3.363*	731.1*
	1.073	1.165	108.6	1.202*	112.0*	1.199*	111.7*	1.485*	138.4*	2.007*	187.0*	3.130*	291.7*	4.910*	457.6*
	2.860	3.410	119.2*	3.400*	118.9*	3.480*	121.7*	3.610*	126.2*	4.280*	149.7*	5.250*	183.6*	7.410*	259.1*
TSH (mIU/L)	0.072	0.074	102.8	0.073	101.4	0.076	105.6	0.072	100.0	0.071	98.6	0.072	100.0	0.071	98.6
	1.107	1.130	102.1	1.177	106.3	1.119	101.1	1.142	103.2	1.149	103.8	1.163	105.1	1.117	100.9
	8.663	8.806	101.7	9.138	105.5	8.937	103.2	8.927	103.0	9.050	104.5	9.024	104.2	8.429	97.3

Immunoassays are universally used to analyze a wide variety of biochemical markers including protein and steroid hormones, tumor markers, micronutrients, and therapeutic drugs. In general, two-site “sandwich” or noncompetitive and competitive assay formats used in immunoassays. Both assays can be designed to incorporate biotin–streptavidin linkage, and therefore, use of biotin supplement may interfere this interaction resulting in a decreased signal, that cause a falsely increased or decreased result depending on the assay type. In the noncompetitive assay format, the concentration of the analyte of interest is directly proportional to the signal intensity. However, the presence of excess biotin in patient samples can decrease the signal and lead to a falsely decreased test result. In the competitive assay format, the concentration of an analyte is inversely proportional to the signal intensity. In contrast to the noncompetitive assay, the signal reduction caused by excess biotin can lead to a falsely increased test result [6]. In addition, the thresholds at which interference occurs may vary from system to system depending on assay design. If the therapeutic use of biotin was the only issue, the frequency of immunoassay interferences would be rare. Biotin is marketed to improve the health of hair, skin, and nails as well as therapeutic use. For this purpose, biotin containing multi-vitamins and with a wide range of different formulations are available over-the-counter. Hair, skin, and nail supplements usually contain 5-10 mg biotin but multi-vitamins and supplements with higher biotin concentrations, up to 100 mg, are also available. Biotin supplement sales in the US were 49.6 million units between July 2016 and July 2017 and the most requested tablets were ranging from ≤ 2.5 mg up to 10 mg [7]. Biotin supplements have become increasingly popular, especially in higher concentrations. Unfortunately, the rise in the use of biotin leads to an increase in the risk of clinically significant analytical error especially in people using high-dose of biotin supplements. In recent years more paper published related to the test results which are not clinically compatible due to the biotin interference. As the thyroid function tests are one of the most frequently requested tests in immunoassays, the reported number of interferences related to these tests are more remarkable. Elston and et al. reported a patient with biotin-associated abnormal thyroid function test that mimicking Graves’ disease [1]. Batista et al. also reported a similar case that mimicking Graves’s disease [8]. As a result of increased awareness on this topic, on November 28, 2017, the Food and Drug Administration (FDA) issued a safety communication warning that biotin may interfere with laboratory tests [9]. The safety communication noted that biotin in patient samples may cause erroneous high or low results, depending on the test. The announcement also noted that several supplements on the market contain biotin levels up to 650 times the recommended daily intake of the vitamin. As we expected, following the addition of biotin, fT3 and fT4 test results were increased in our study, because fT3 and fT4 tests analyzed these tests in a competitive immunoassay. On the other hand, TSH test results did not show a significant change following the addition of biotin as we expected, because this test did not design by the manufacturer to incorporate biotin–streptavidin linkage. This study has certain limitations. We were not equipped in our center to verify the final biotin concentrations that were spiked, biotin concentrations were estimated by calculation. In conclusion, awareness of potential biotin interference has spread of last years owing to the rising popularity of biotin usage. Many people may not consider biotin as a medication and not report to their physician. This situation may result in misdiagnosis and potentially inappropriate treatment. Thus, in the presence of a test result which is not clinically compatible, laboratory professionals should be aware of that biotin may interfere with immunoassays, causing erroneous high or low results.

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Biosensors

FT-6

[DEVELOPMENT OF NEW, COLORIMETRIC AND AUTOMATIZED CERULOPLASMIN FERROXIDASE ACTIVITY MEASUREMENT METHOD]

[YENİ KOLORİMETRİK VE OTOMATİK SERULOPLAZMİN FERROKSİDAZ AKTİVİTESİ ÖLÇÜM YÖNTEMİ GELİŞTİRİLMESİ]

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Abstract

Objective: Ceruloplasmin also known as ferroxidase shows oxidase activity against ferric iron (Fe^{+2}), polyphenols and polyamines. In the study we aimed to develop a method of kinetic measurement to determine the activity of ferroxidase in serum with a unique, cheaper, user friendly method than its counterparts in the world.

Material and Methods: Ferrous ions are oxidized to ferric iron ions by ferroxidase activity, the color intensity of the product formed is directly proportional to enzyme activity. In our method, chromogen is not used during measurement and the product is directly measured. The analytical performance characteristics of the new method such as linearity, discrimination ability, inhibition and correlation were investigated.

Results: There was a strong correlation between our new method and the conventional method of ferroxidase measurement. Our method appeared to have an effective discriminating ability and linear absorbance increase. Also, No activity was observed in the sample inhibited with sodium azide during inhibition study.

Conclusion: This kinetic method developed by us for the first time, by performing preliminary work is a direct measurement method of the resulting product (Fe^{+3}) without using any chromogen. Also, It significantly cost low when compared to counterparts with a short reaction time.

Keywords: Ceruloplasmin, ferric iron, ferrous iron, ferroxidase

Öz

Amaç: Ferroksidaz olarak da anılan seruloplazminferrik demir, polifenoller ve poliaminlere karşı oksidaz aktivitesi gösterir. Çalışmada, serumdaki ferroksidaz aktivitesini dünyadaki benzerlerinden daha ucuz, kullanıcı dostu ve eşsiz bir yöntemle belirlemek için kinetik ölçüm yöntemi geliştirmeyi amaçladık.

Yöntem: Ferröz demir iyonları, ferroksidaz aktivitesi ile ferrik demir iyonlarına oksitlenir, oluşan ürünün renk yoğunluğu enzim aktivitesi ile doğru orantılıdır. Bizim yöntemimizde, ölçüm sırasında kromojen kullanılmaz, oluşan ürün doğrudan ölçülür. Ayrıca, Yeni yöntemin; doğrusallık, diskriminasyon yeteneği, inhibisyon ve korelasyon gibi analitik performans özellikleri de araştırılmıştır.

Bulgular: Yeni yöntemimiz ve geleneksel ferroksidaz ölçüm yöntemi arasında güçlü bir korelasyon bulundu. Yöntemimizin etkili bir diskriminasyon yeteneğine ve doğrusal absorbans artışına sahip olduğu gözlenmiştir. Ayrıca, inhibisyon çalışması sırasında sodyum azid ile inhibe edilmiş numunede hiçbir aktivite gözlenmemiştir.

Sonuç: Ön çalışmaları gerçekleştirilerek, ilk kez tarafımızca geliştirilen bu kinetik yöntem, herhangi bir kromojen kullanmadan, oluşan ürünün (Fe^{+3}) doğrudan saptandığı bir ölçüm yöntemdir. Ayrıca, kısa reaksiyon süresine sahip olan bu yöntem, muadilleriyle karşılaştırıldığında oldukça düşük maliyetlidir.

Anahtar kelimeler: Ferrik demir, ferroksidaz, ferröz demir, seruloplazmin

Introduction

Ceruloplasmin (Cp) is an α2-globulin with a molecular weight of 151 kDa, containing a significant proportion of total serum copper. Ceruloplasmin also known as ferroxidase shows oxidase activity against ferric iron (Fe^{+2}), polyphenols and polyamines [1]. However, ferrous iron ($Fe^{+}2$) is considered as biological substrate of ceruloplasmin and its affinity to ferrous iron is higher than other substrates. Ferroxidase is vital in regulating the ionic state of iron. It oxidizes ferrous iron ions (Fe^{+2}) to ferric iron ions (Fe + 3) to bind to the transferrin and thus prevent the formation of toxic iron products [2]. Plasma ferroxidase levels are significantly lower in protein synthesis disorders (such as liver failure and malnutrition), protein-losing enteropathy, nephrotic syndrome, hereditary hypoceruloplasminemia, Wilson's disease, Menkes disease and nutritional copper deficiency [2]. Nowadays, Cp measurement methods can be basically divided into two groups. 1) Quantitative measurement of ceruloplasmin amount 2) Measurement of oxidase activity of ceruloplasmin enzyme. The most commonly used measurement method for ferroxidase in routine analyzes is the immunometric method in which the mass is measured. In the methods used to determine the activity of the enzyme, ferrous iron (biological substrate), O-dianizidine, p-phenylenediamine and N, N-dimethyl-p-phenylenediamine (non-biological substrates) are used as substrates [3,4]. The aim of this course is to develop a method of kinetic measurement which will determine the activity of ferroxidase in serum with a unique method, cheaper than its counterparts in the world, ready to use, user friendly and has the potential of widespread use.

Materials and Methods

Ferrous ions (Fe^{+2}) are oxidized to ferric iron ions (Fe^{+3}) by ferroxidase activity as defined in the following reaction. The color intensity of the product formed is directly proportional to enzyme activity of ferroxidase and is measured kinetically in 412 nm wavelength. In our method, chromogen is not used during measurement and the product is directly measured. Two reagents are used for our newly developed method. Reagent 1 contains 450mM acetate buffer. Reagent 2 contains 20mM ammonium ferrous (II) sulfate hexahydrate. For this method, 100 µL of sample, 750 µL of reagent 1 and 200 µL of reagent 2 are needed. When ferrous iron ions used as substrates were exposed to oxidation by ferroxidase, an activity increase associated with the activity of the enzyme was observed at 415 nm. This increase in absorbance is due to the yellow Fe^{+3} ions formed by oxidation of colorless Fe^{+2} ions, which are used as substrates by ferroxidase enzyme.

Results

The analytical performance characteristics of our method such as linearity, discrimination ability, inhibition and correlation were investigated. Linearity and discrimination ability: In the reaction graph where the kinetic activities of the samples having low (1. sample), medium, (2.sample), high (3. sample) with ferroxidase activity were monitored over a period of time, our method appears to have an effective discriminating ability and linear absorbance increase (Figure 1).

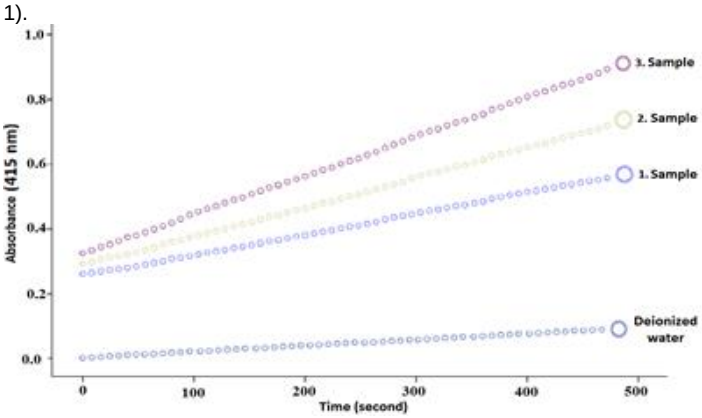

Figure 1. Samples having low (1. sample), medium (2.sample), high (3. sample) ferrooxidase activity and reaction graph in which the deionized water sample is superposed.

Inhibition: For ferroxidase enzyme inhibition; serum pool is divided into two equal parts. The inhibitory (2 mM sodium azide) was added to first part, deionized water was added to second part and the ferroxidase activity was measured according to the our newly devoloped method. As seen in Figure 2, while no activity is observed in the sample inhibited with sodium azide, the non-inhibitory serum sample continues its usual activity.

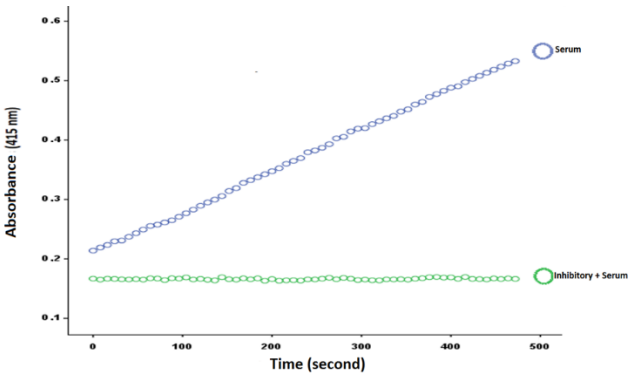

Figure 2. Reaction graphs of ferroxidase activities of serum samples with and without inhibitory.

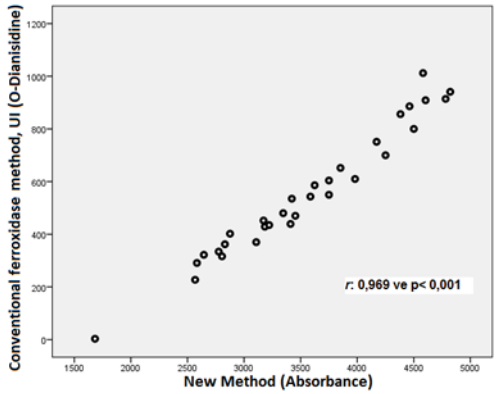

Figure 3. The correlation graph of conventional ferroxidase measurement method and newly developed measurement method.

Correlation: As seen in Figure 3, between the method we developed and the conventional method of ferroxidase measurement correlation results were statistically significant (r: 0.969 and p <0.001).

Conclusion

All of the current ferroxidase activity measurement methods use chromogen. This situation brings along some disadvantages.Conventional methods can measure indirectly by means of a chromogen. For this indirect measurement method; a buffer system with the appropriate pH and concentration to the quality of the chromogen is required. Also for long-term stability of chromogen stabilizers and / or protective chemicals are required. Thus, the increased number of reagents also significantly increases the interference and the cost of the method.

This kinetic method developed by us for the first time, by performing preliminary work is a direct measurement method of the resulting product (Fe^{+3}) without using any chromogen. It significantly cost low when compared to counterparts. It has short reaction time and gives quick results. Thus the method is constructed and can be measured easily, it can be automated in a simple way.

Conflict of interest: None declared.

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FT-7

[Investigation of the Effects of Exercise Training with Biosensor Device in Multiple Sclerosis]

[Multipl Skleroz'da Biyosensörlü Cihazla Verilen Egzersiz Eğitiminin Etkilerinin İncelenmesi]

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Abstract

Objectives: Balance disorders are common in patients with multiple sclerosis (MS). In balance disorders, exercise therapy is effective and different approaches such as group or individual training are applied. In our study, Biodex Balance System was applied to patients with multiple sclerosis with the biosensor device developed for balance measurement and training. The effects on disability, balance, fatigue, vestibular system and quality of life were investigated.

Materials-Methods: A total of 31 MS patients diagnosed were included in our study. When evaluating patients; Neuro-otologic tests for EDSS, Berg Balance Scale for Balance, International MS Quality of Life Scale for Quality of Life (Musiqol) were used and the patients were evaluated twice before and after treatment. The biosensor device developed for the balance measurement and training of Biodex Balance System SD was applied to the participants twice a week for 8 weeks.

Results: The mean age of the patients was 49.32 ± 8.20 (35-65) years. Significant differences were found in EDSS scores before and after exercise in MS patients (p <0.05). When the scores of the tests in the MS group were compared with the Biodex Balance Systems before and after the exercise; There was a significant difference between the stance test of the balance parameters, the use of gravity change in different directions (mediolateral and anteroposterior) and the Fall Risk Test scores (p <0.05). MS patients before and after exercise; Significant changes were observed in their balance and neurotologic evaluations (p <0.05). There was no difference in quality of life scores of MS patients before and after exercise (p> 0.05).

Conclusion: Biosensor biodex balance device in patients with multiple sclerosis, balance and vestibular system were found to be effective on the training.

Keywords: Multiple Sclerosis, Biosensor, Exercise

Conflict of Interest: No conflict of interest was declared by the authors.

Öz

Amaç: Multipl Sklerozlu (MS) hastalarda denge bozuklukları oldukça sık görülmektedir. Denge bozukluklarında egzersiz tedavisi etkilidir ve grup yada bireysel eğitim gibi farklı şekillerde egzersiz yaklaşımları uygulanmaktadır. Çalışmamızda Multipl Skleroz hastalarına Biodex Balance Sistem 'SD' isimli denge ölçümü ve eğitimi için geliştirilmiş olan biyosensörlü cihaz ile verilen egzersizlerin; özürlülük, denge, yorgunluk, vestibuler sistem ve yaşam kalitesi üzerine olan etkileri incelenmiştir.

Gereç-Yöntem: Çalışmamıza; tanısı konmuş 31 MS hastası alındı. Hastaları değerlendirirken; Özürlülük için EDSS, Denge için, Berg Denge Ölçeği, Vestibüler sistem için Nöro-otolojik testler, Yaşam kalitesi için Uluslararası MS Yaşam Kalitesi Skalası (Musiqol) kullanıldı ve hastalar tedavi öncesi ve sonrası olmak üzere iki kez değerlendirildi. Katılımcılara Biodex Balance System SD isimli denge ölçümü ve eğitimi için geliştirilmiş olan biyosensörlü cihazla haftada 2 kez olmak üzere 8 haftalık egzersizler uygulandı.

Tartışma: Hastaların yaş ortalaması 49.32 ± 8.20 (35-65) yıldı. MS hastalarının egzersiz öncesi ve sonrası EDSS skorlarında anlamlı farklılıklar bulundu (p<0.05). MS grubundaki olguların egzersiz öncesi ve sonrasında Biodex Denge Sistemleri ile yapılan testlerin skorları karşılaştırıldığında; denge parametrelerinden olan dik duruş testi, gravite değişikliğini farklı yönlerde kullanma testi (mediolateral and anteroposterior), düşme riski test skorlarında anlamlı fark bulundu (p<0.05). MS hastalarının egzersiz öncesi ve sonrası; denge ve nörootolojik değerlendirmelerinde anlamlı değişiklikler ortaya çıktı (p<0,05). MS hastalarının egzersiz öncesi ve sonrasındaki yaşam kalitesi skorlarında farklılık saptanmadı (p>0.05).

Sonuç: Multipl Skleroz hastalarında biyosensörlü biodex denge cihazı ile verilen eğitimin, denge ve vestibüler sistem üzerine etkili olduğu bulundu.
Anahtar Kelimler: Multipl Skleroz, Biyosensör, Egzersiz
Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Introduction

MS is a chronic, autoimmune, demyelinating, degenerative disease of the central nervous system (CNS) [1]. The problems that related with balance are observed at the rate of 59% in MS patients. Balance problems and in coordination are among the most complex and resistant symptoms, and usually together with other symptoms [2]. Besides body ataxia which can be observed during sitting and standing lead to loss of postural control, balance problems can reveal depending upon muscle weakness, somatosensory, visual and vestibular systems [3]. Balance problems are one of the most frequent problems in MS patients and it is highly important. Because, difficulties are experienced in functional activities such as transition from one position to another, maintain the upright position and walking and turning and all causes the loss of balance and falls [4,5].

Balance and postural control (PC) disorders are among the most frequent motor disorders symptoms are present in 20% of patients with MS at onset and chronic in 80% of cases [6]. Most patients report that balance and gait difficulties are the leading causes of disability [7]. Balance and PC are closely concept that require the CNS integration of visual, vestibular and somatosensory (proprioceptive a cutaneous) information as well as the proper activation of neuromuscular control mechanisms [8]. Another subject as important as exercises which is given to develop postural control is to right measure of lack of postural control. Different assessment strategies have been offered for impairments in MS. Clinicians have available clinical tests to quantify postural control and sensory interaction such as force plates and postural stabilometries [7,9]. In addition, Neuro-otologic tests are important tests which evaluate the postural control and vestibular system symptoms. In MS patients that are findings of vestibular system, modalities such as aerobics, yoga, and endurance exercise are mentioned in literature. Although the benefits of regular exercise for patients with MS are known, wide variety of exercise types to reduce deficits in mobility, fatigue and balance focus on strengthen, stretching, aerobic, endurance, core exercises and yoga [10,11]. Besides these sort of exercises, exercises which are given with computer-based systems have gained importance in recent years. However, computerized stabilometry has become an important objective tool for assessing sensory integration for balance and postural control, there is no study that evaluates the sensory interaction and postural control in patients with MS by Biodex Balance System before and after exercise training.

The aim of our study; Biodex Balance System was applied to patients with multiple sclerosis with the biosensor device developed for balance measurement and training; the effects on disability, balance, fatigue, vestibular system and quality of life were investigated.

Materials and Methods

This was a single blind, experimental before-after cohort, controlled study. Our study was approved by Dokuz Eylül University Ethics Committee of Non-Invasive Researches with the approval number 615 dated 05.09.2014. Informed consent was signed for all patients who participated in our study.

Participants:31 MS patients was included in the study. A neurologist confirmed diagnosis, eligibility criteria and Expanded Disability Status Scale (EDSS) scores. EDSS and neuro-otologic tests are performed by the other neurologist.

Inclusion criteria were;
18 years and older
Ability to walk 500 meters independently (EDSS 5 and under)
Voluntary participate the study

Exclusion criteria were;
Having the cardiovascular disease, thyroid disorders, gout or limitation of orthopedic
Having the psychiatric disorder
Not having the visual acuity
Having the severe cognitive impairment
Regular non-participation will be excluded from the study.

Exercise Procedure:Training was provided for postural control with BiosensorsBiodex Balance System to the patients a total of 8 week and 2 days per week. Home exercises for balance were given to the patients in order to make at home for the remaining days of the week. While postural control training at BiosensorsBiodex Balance Systems is made, it were asked to patients go on the platform as barefoot. In the first 4 weeks of the treatment, 30 minutes postural control training was given and the platform was motionless, but in the last 4 weeks of the treatment, 45 minutes postural control training was given and the platform was motile. Exercises progressed from easy level to difficult level. The platform was stable in BiosensorsBiodex Balance System for the first 4 weeks and the exercises became harder in the final 4 weeks by mobilizing the platform. Test Procedure:The demographics of participants were noted before test trials. Each participant was assessed by the blinded examiner. Disability:A neurologist evaluated physical disabilities of MS patients by using **'**Krutzke's Expanded Disability State Score' (EDSS) [12]. MS patients, with their EDSS score ranging between 0 and 5 (including 0 and 5), participated in the study. 8 different functional systems were assessed in this scale. Please refer to Page 13 for explanations on disability. The values range between 0 and 10 in this scale (0: normal neurological examination, 10: MS-caused death) [13,14]. Biosensors Biodex Balance Systems:BiosensorsBiodex balance systems were used to evaluate postural control. Postural Stability Test, Stability Limit Test and Fall Risk Test, included in biodex menu, were applied for postural control evaluation. The patients repeated each test 3 times. Average of the 3 tests was found and recorded as the result measurement. The patients were asked to stand on the platform barefoot and hold the platform rails when performing tests in BiosensorsBiodex Balance System The patients were provided detailed information about test performance before doing the test. Postural Stability Test (PST): The platform was stabilized when performing the test. The patients were asked to take normal postural position on the platform and the angles of their feet were recorded. The patients were asked to catch the target on the monitor for 20 seconds. Stability Limit Test(SLT): The patients were asked to stand on the platform barefoot and hold the platform rails when performing this test. The platform was stabilized when performing the test. The patients were asked to take normal postural position on the platform and the angles of their feet were recorded. The patients were asked to turn off the flickering lights on the monitor with their body motions and their test completion times were recorded. Fall Risk Test (FRT): The platform was mobile when performing the test. The patients were asked to take normal position on the platform and the angles of their feet were recorded. The patients were asked to focus on the target on the monitor for 20 seconds and they were allowed to rest for 10 seconds at the end of the 20 seconds. Balance Berg Balance Scale was used for balance measurement:Berg Balance Scale (BBS) is used for functional balance evaluation. BBS functional balance evaluation is characterized as a "golden standard" test. BBS is used for balance evaluation of Parkinson patients, patients with stroke episode, MS patients, and elderly. BBS consists of 14 general balance activities evaluating the skill for maintaining static position during decrease in support surface, which causes changes in the orientation of body weight center according to the support surface. The observer assesses the patient during activities and grades each activity in a 5-point scale with scores ranging between 0 and 4. The highest score is given as to correspond to rapid and easy completion of the activity. The highest total score is 56, reflecting a good balance function. The fall risk increases as the score goes from 36 to 56. A high score represents a good balance. BBS is a reliable and valid test for MS patients.

41-56 = low fall risk
21-40 = intermediate fall risk
0 –20 = high fall risk
<67% = fall risk in elderly; falls in the future are anticipated [15].

Neurootologic Tests:MS patients before and after exercise training as a neurotologic tests;;Romberg Test, Utenberger Test , Walking Test, Eye Movement Examination, Eye Examination,Caloric Test, Positional Test Hearing Examination, Vibration Evaluation done[16].

Life Quality MUSIQOL'Scale of Multiple Sclerosis International Quality of Life' (MUSIQOL)', a life quality scale specific for the disease, was used for assessing life quality of MS patients. This scale consists of a total of 31 items under 3 sub headings, namely psychological, physical and psychosocial. A low score indicates high level life quality [17].

Statistical analysis:The data were analyzed by using SPSS (version 23.0) software. The results were given as average ± standard deviation (SD) and in percentages. The categorical variables for the demographic and clinical data of the MS patients were compared by

using the chi-square test. The variables with normal distribution were compared by using a parametric statistical test (student's t test) and the variables with no normal distribution were compared by using a non-parametric (Mann-Whitney U test) statistical test. The statistical data were interpreted at $p<0.05$ significance level.

Results

Table 1 summarizes demographics of MS patients.

Table1. Demographics and clinical characteristics of MS patients

31 MS patients were included in the study. Whereas demographic characteristics including age, gender, BMI and dominancy were compared. MS patients included in the study were homogeneous. Neuro-otologic test results of MS patients are shown in Table 2.

	MS PATIENTS (n=31)	
	n	**%**
Sex		
Female	21	67.7
Male	10	32.3
State of education		
Primary and secondary education	5	16,1
High school	9	29.0
University	17	54.8
Dominant side		
Right	28	90.3
Left	3	9.7
Age (year)		
X±SD (min-max)	49.32 ± 8.20 (35-65)	

Table 2. Neuro-otologic Test Results of MS Patients

NEURO-OTOLOGİC TESTS	**MS PATIENTS (B.E, n:31)**		**MS PATIENTS (A.E, n:31)**		IMPROVEMENT ON	
					n	**%**
Romberg	Positive	26/31	Positive	24/31	2	6.45
	Negative	5/31	Negative	7/31		
Unterberger	Normal	11/31	Normal	17/31	6	19.35
	Rotation On	20/31	RotatinOn	14/31		
Walking	Normal	1/31	Normal	4/31	3	12.90
	Ataxic	30/31	Ataxic	27/31		
Spontaneous Nystagmus	On	3/31	On	3/31		
	Off	28/31	Off	28/31	-	
İno Nystagmus	On	10/31	On	9/31	1	3.22
	Off	21/31	Off	22/31		
Positional Test	Normal	24/31	Normal	25/31	1	3.22
	Vertigo	7/31	Vertigo	6/31		
Caloric Test	Normal	8/31	Normal	16/31	8	25.80
	Abnormal	23/31	Abnormal	15/31		
Hearing	Normal	28/31	Normal	29/31	1	3.22
	Influence On	3/31	Influence On	2/31		
Eye Movement	Normal	17/31	Normal	17/31		
	Influence On	14/31	Influence On	14/31	-	
Eye	Normal	25/31	Normal	24/31		
	Influence On	6/31	Influence On	7/31	-	
Vibration top Extremity	Normal	6/31	Normal	6/31		
	Off	25/31	Off	25/31	-	
Vibration lower Extremity	Normal	1/31	Normal	2/31	1	3.22
	Off	30/31	Off	29/31		
Central Influence	On	21/31	On	13/31	8	25.80
	Off	10/31	Off	18/31		
Peripheral Influence	On	10/31	On	7/31	3	12.90
	Off	21/31	Off	24/31		
Two Side Influence	On	9/31	On	4/31	5	16.12
	Off	22/31	Off	27/31		
Four Side Influence	On	10/31	On	9/31	1	3.22
	Off	21/31	Off	22/31		
Improvement describing	IMPROVEMENT ON				28	90.30
	IMPROVEMENT OFF				3	9.70
n: Number %: Percentage B.E= before exercise, A.E= after exercise						

When compared the neuro-otologic test results made before and after exercise of case in MS group, differences have been observed in Romberg (% 6,45) , Unterberger (% 27.70), walking (% 12.90), caloric tests (% 25.80), inonistagmus (% 3.22), positional test (% 3.22), hearing (% 3.22), vibration lower extremity (% 3.22). Compared to before, after the exercise, an improvement has been observed in 8 of 21 patients that have central vestibular influence and in 3of 10 patients that have peripheral vestibular influence. After exercise improvements have been observed in 5 of 9 patients who have two sides back cordon influence before exercise. After exercise, improvement has been observed in 1 of 10 patients who have four sides back cordon influence before exercise. When they were asked whether they benefited from the treatment or not, the 28 of 31 patients claimed they did. Postural control assessment results of the patients are shown in Table 3.

Table 3. Postural control assessment of the patients by using Biosensors Biodex Balance Systems

BIODEX	MS PATIENTS (B.E, n=31) I X ± SD (min-max)	MS PATIENTS (A.E, n=31) II X ± SD (min-max)	P (I-II)
PST 1	0.58±0.67 (2.0-0.0)	0.22±0.49 (2.0-0.0)	0.001*
PST 2	0.22±0.49 (2.0-0.0)	0.096±0.30 (1.0-0.0)	0.043*
PST 3	0.12±0.34 (1.0-0.0)	0.096±0.30 (1.0-0.0)	0.325*
SLT 1	53.38±18.90 (111.0-34.0)	45.93±22.66 (117.0-28.0)	0.038*
SLT 2	44.16±14.21 (65.0-10.0)	53.77±17.27 (70.0-12.0)	0.001*
DRT	0.96±0.91 (4.0-0.0)	0.58±0.80 (4.0-0.0)	0.001*
BBS	45.61 ±9.89 (55.0-10.0)	51.09 ±4.19 (56.0-42.0)	0.001*

In the comparison of the postural control test scores in Biosensors Biodex Balance Systems of the patients in MS group before and after the exercises, there were significant differences found in the scores of postural stability test (all scores, mediolateral and anteroposterior), stability limit test (completion period and all scores), and fall risk test (all scores) ($p<0.05$).

In the comparison of the life quality scores of the patients in MS group before and after the exercise, there was no significant difference found ($p>0.05$).

Discussion

The aim of this study was to investigate the effects of postural control exercises of MS patients on disability, balance, vestibular system and life quality. It was found that postural control exercises performed by MS patients had positive effects on disability, balance, vestibular system. MS is a disease that can create lesion in the connection of vestibular nuclei in the brain stem, cerebellum and dorsal column and therefore it can cause the plaints about all types and changeable stage of balance. For example, it causes blindness, blurred vision or seeing double because of the influence of optic tractus and a lesion exist in sensory pathways of back cordon cause deterioration in sensation of proprioception and vibration. Being intense of fibers related vestibular system around 4th ventricular and showing tendency of demyelination occurred in MS to especially keep the periventricular region are among the reasons why vestibular symptom and signs often occur [18,19]. MS is a disorder seen in young adults most frequently [20]. In this study, the average age of the individuals was found as 49.32 ± 8.20 years old (the youngest was 35 and the eldest was 65). In literature, it has been reported that MS develops at the ages of 15-50 years old in general [21]. In this context, the profiles of the patients included in the study are in line with the literature. After this all damage caused by disease as also in the other vestibular system damages, a process which is called as vestibular compensation begins. It is declared that compensation will be developed progressively with stimulating by showing movements which cause symptoms in the result of experiments in human and animal, to the organism as active and fresh, vestibular system function with 'error' mechanism. This information is the character of vestibular rehabilitation. Vestibular rehabilitation is a treatment based on specific exercise and aims to trigger the compensation mechanism[19]. In the review of the studies conducted on MS patients, it is seen that group studies are prevalent in general. In our study, a personal exercise program was applied differing substantially from the other studies. The patients were evaluated before the exercise, and the exercise program was adjusted to the individual and progressed. As differing from the literature, somatosensory and visual stimuli were given to the patients with Biosensors Biodex Balance Systems and balance training was provided in our study. Proprioception and stabilization training can be offered by using Biosensors Biodex Balance Systems. This system focuses on body muscles particularly. Control of cerebellum over body muscles is prevalent to control over extremity muscles. Balance disorders in MS patients must be assessed with the amount of postural responses when the set is changed and when visual adjustments are made. In line with the advancements made in technology today, exercise and evaluation methods used in the rehabilitation field have started to change. Recently, computer-supported systems have become remarkable for static and dynamic assessment of balance function. Biosensors Biodex Balance Systems are superior to other computer-supported balance systems because it offers both the assessment and treatment opportunities in the same device. This situation facilitates clinicians' works. In our study, we assessed postural stability and stability limits of the MS patients and healthy group and fall risk of MS patients by using Biosensors Biodex Balance Systems [22,23,24,25]. In the research of Soysal et al conducted on the effect of pilates exercises on emotional interaction, postural control and fatigue, 12 MS patients and the control group were included. Biosensors Biodex Balance Systems were used to assess postural control. There were significant differences found in the fatigue and emotional interaction (eyes open, eyes closed) test as a result of the study [26]. In the one of the study which includes vestibular rehabilitation program, Jackson and his friends [27] gave six-week standardized balance home exercise that is prepared according to individual after six-week control process to the MS patients who have balance problem the EDSS scores varying between 1.5 -6.5. According to the evaluations, significant improvement in the 'maximum deviation (farthest distance of the center of gravity during the goal orientation)' and 'outcome deviation (uninterrupted first distance from the goal orientation)' of the Limits of Stability Test in the situation of 'open eyes, motile area' and 'open eyes, visual environment, motile area' of Sensory Organization Test which is measured with Smart Balance Master has been observed statically for BBS with balance exercise. In our study, 8-week postural control exercises with computer-based systems were given to the MS patients. Significant improvement was observed in vestibular test that is made after evaluation. The most improvement was obtained from caloric test with 25.80%. It was observed that vestibular symptoms of 28 MS patients who their vestibular influence is determined by these tests have improvement. In our study, Berg Balance Scale, an objective method, and neurological tests were used to evaluate the balance influences of the patients. Neurological tests are clinical tests used for examination of vestibular system. Functional disorder of vestibular system is characterized by the combination of perceptional, oculomotor, postural and autonomic indicators prevalently. Vertigo, ataxia, nystagmus and nausea are seen in persons with the dysfunction of vestibular system. Findings emerging in the dysfunction of vestibular system show

different relationships of vestibular system with the central nervous system. Tedious neurologic examination aids usto determine whether the vestibular influence is peripheral or central. Neurootologic tests focus on eye examination generally. Eyes are one of the organs affected first in MS. In literature, there is only scarce number of researches applying neurootologic tests in MS patients. In our study, neurologic tests were performed before and after exercises in MS patients. Considering the results, there was significant improvement in vestibular test results of the patients after the exercises. Learnmonth et al compared 4 of the functional balance evaluation tests, including BDÖ scale, used for assessment of mobility and balance in multiple sclerosis. These were 25-step walking, 6-minute walking, timed standing and walking tests and BDÖ balance scale in turn. 24 MS patients, with their EDSS scores ranging between 5 and 6.5, participated in this study. Based on the collected data, reliabilities of the tests were found to be very close to each other and as similar. In conclusion, it has been reported that these four tests could be used for evaluation of balance functionally in patients diagnosed with MS [28]. In our study, BDÖ was applied to MS patients before and after exercising and there was significant difference found in their BDÖ scores ($p<0.01$). Balance dysfunctions affect locomotor system in MS patients substantially. Based on the influence of walking along with locomotor system, life quality of persons affects the level of fatigue. In our study, there was no change found in fatigue level of MS patients before and after exercising. There was significant improvement seen in the physical, psychosocial, cognitive sections of Fatigue Impact Scale and the total score, but it was not significant statistically. Since there was no intervention possible for the living places of the patients and the exercises lasted for eight weeks, it is thought that these results were obtained according to the evaluations made immediately after they were completed. The effects of postural control exercises on fatigue can be determined better by long-term exercise applications and the assessment of the results. In the study of Ertekin et al [29] , 12-week standardized balance and home stretching exercise program was applied for 40 patients, with their EDSS scores ranging between 2.0 and 6.0, and Scale of Multiple Sclerosis International Life Quality was applied to evaluate their life quality. In addition to observing significant improvement in objective (BBS) and subjective (ABC) balance parameters of the patients, who were evaluated before and after the exercise program, it was seen that their life quality improved significantly. In our study, there was no significant difference ($p>0.05$) in the assessment of life quality of MS patients before and after exercises. There was no significant difference found in life quality evaluations before and after exercises in MS patients and between life quality scores of the patient-healthy groups ($p>0.05$). Majority of the healthy individuals was patients' next of kin. It was interpreted that the differences seen in the results were due to high fatigue scores received because of patient care responsibility of healthy participants. As for our study's limitations; it can be thought that the treatment process is short but when looked into the literature, it will be observed that balance parameter has a significant improvement between 3-12 weeks with exercise program in the study of searching the effectiveness of specific balance exercises on MS patients. In also our study, significant improvement has provided in objective and subjective balance parameter of the patients that individualized postural control exercises are performed during 8 weeks. It has been observed the progress in the balance together by improvement of vestibular system with doing the balance exercises enough time on a regular basis. Other limitations of our study were failure to access to 39 MS patients, who were subject to strength analysis, since the patients usually experienced attacks, and the long-term effects of the exercises could not be followed. Our study carries the specialty of being the first in the literature as a study on MS patients which measure the vestibular system influence and training influence with neuro-otologic tests. As a result, postural control exercise given with Biodex Balance SD is an effective method for treatment of vestibular system on MS patients. In the study, the effects of postural control exercises are evaluated with neuro-otologic tests. Neuro-otologic tests are very important and diagnostic about emerging the vestibular influences.

Conclusion

When we evaluate the effect of 8 weeks postural control, it is determined that postural control exercises have a positive impact on vestibular system. Evaluation and treatment program with biosensor balance systems in MS patients were found to be effective in balance disorders.

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FT-8

[OUR LABORATORY EXPERIENCE IN SUBSTANCE ABUSE ANALYSIS WITH CARD TESTS]

[KART TESTLER İLE MADDE BAĞİMLİLİĞİ ANALİZLERİNDE LABORATUVARİMİZİN DENEYİMİ]

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Abstract
Objective: Different methods can be used in clinical laboratories for the evaluate of drug and stimulant tests. Card tests are used in some hospitals in order to perform these tests quickly. However, relatively different results can be reported with this method. The aim of this study is to report our false positive rate with card tests and to raise awareness about the use of the method.
Materials-Methods: At the weekend, drug addiction requests of our emergency department are being worked with card tests. In this method, the patient's urine is applied to the immersion zone on the card and moves along the test strip by capillary action. In this way free, gold conjugated antibodies, located near the immersion area, are transported along the test strip together with the urine sample. If there is no analyte in urine, the antibody reacts with the test analyte found in the reaction line. Consists of a red line. The test is negative. If the drug in the urine is present in an amount greater than the threshold, the sample after administration, the analytes in the urine are bound to the all antibody and since all the binding sites are filled, there is no antibody left to bind to the antigen present in the pad at the test site, with no red lines. In this case, test is positive. The results that requested from the emergency department and reported as positive with card tests are repeated in the following working days with an immunochemical method (Syva Emit II plus, Siemens), by an automatic analyzer (DimensionExL). Samples which positive results were determined by card test but found to be below the threshold by automated immunochemical method, although positively reported by card test were evaluated retrospectively. Thus, false positive results given by card test were determined.
Results: In the 548 card test analysis study, the actual positive and false positives of the tests were as follows: For amphetamine; 66/1, for ecstasy; 60/13, for cocaine; 19/1, In cannabis analysis; 100/10, benzodiazepine analysis; 63/16, for opiate; 15/5.
Conclusions: Even if the red line is too light in the test zone, the result should be reported as negative. The assessment of the existence of the line is relative to the personal knowledge and skill. A false positiv or false negative result may lead to different treatment. False positives can lead to criminal penalties in criminal cases. In our study, the repetition of the test was carried out by immunochemical method. However, there may be errors due to cross reactions with this method. The true gold standard method is chromatographic separation and mass analysis (LC-MSMS, GC-MSMS).
Keywords: Substance abuse tests, rapid drug test, false positivity
Declaration of Conflicting Interests: Author declares no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Öz:
Amaç: Uyuşturucu ve uyarıcı madde testlerinin çalışılması için klinik laboratuvarlarda farklı yöntemler kullanılabilir. Bu testlerin hızlı yapılabilmesi için bazı hastanelerde kart testler kullanılmaktadır. Ancak bu yöntemle verilen sonuçlar göreceli olarak raporlanabilmektedir. Bu çalışma ile amacımız kart testlerle yanlış pozitiflik oranımızı bildirmek ve yöntemin kullanımı ile ilgili farkındalık oluşturmaktır.
Gereç ve yöntem: Hafta sonu, acil servisimizin madde bağımlılığı test istemleri kart testlerle çalışılmaktadır. Bu yöntemde hastanın idrarı kartta bulunan immersiyon bölgesine ekilir, idrar kart üzerinde kapiller hareketle yürür. Stripin İmmersiyon bölgesinde hazır bulunan serbest gold konjuge antikorlar, idrarla birlikte strip boyunca sürüklenir. İdrarda aranan analit yoksa, sürüklenerek gelen serbest antikorlar reaksiyon çizgisinde pette bulunan test analiti ile reaksiyona girer, kırmızı çizgi oluşur. Test negatiftir. İdrarda aranan ilaç eşik değerin üzerinde miktarda mevcutsa, örneğin uygulamasından sonra idrardaki analitler tüm antikorlara bağlanır ve bütün bağlanma sahaları dolmuş olacağından test bölgesindeki pedde hazır bulunan antijene bağlanacak antikor kalmamıştır, kırmızı çizgi oluşmaz. Test pozitifdir.
Acil servisten istenilen ve kart testler ile çalışma sonrasında pozitif olarak raporladığımız sonuçlar takip eden iş gününde immun kimyasal yöntemle (Syva Emit II plus, Siemens), otomatik analizör (DimensionExL) ile tekrarlanmaktadır. Laboratuvarımızda bir yıl boyunca kart test yöntemi ile çalışılan testler retrospektif olarak incelenmiştir. Kart test ile pozitif raporlandığı halde, otomatize immunkimyasal yöntemle eşik değerin altında alınan sonuçlar kaydedilmiştir. Böylece kart testle verilen yanlış pozitif sonuçlar belirlenmiştir.
Bulgular: 548 kart test madde analizi çalışmasında, gerçek pozitif ve yanlış pozitif test sayıları sırasıyla, amfetamin için; 66/1, ekstazi için; 60/13, kokain için; 19/1, esrar analizinde; 100/10, benzodiazepin analizinde; 63/16, opiat için; 15/5' dir.
Sonuçlar: Kartlarda test bölgesinde kırmızı çizginin çok hafif olması durumunda bile sonuç negatif olarak raporlanmalıdır. Çizginin varlığının değerlendirilmesi kişisel bilgi, beceriye bağlı olarak görecelidir. Yanlış pozitif, yanlış negatif sonuç tedaviyi farklı yönlendirebilir. Yanlış pozitif verilen bir sonuç, adli vakalarda suçsuz cezalandırmalara sebep olabilir. Çalışmamızda test tekrarı immunkimyasal yöntemle yapılmıştır. Ancak bu yöntem ile de çapraz reaksiyonlara bağlı hatalar olabilir. Gerçek altın standart yöntem kromatografik ayırım ve kütle analizidir(LC-MSMS, GC-MSMS).
Anahtar Kelimeler: Madde bağımlılığı testleri, kart testler, yanlış pozitiflik
Çıkar çatışması: Yazarlar arasında çıkar çatışması beyan edilmemektedir.

Introduction

In our country, substance dependence tests are carried out in some clinical laboratories of hospitals. Substance abuse test requests from the laboratory are come from Alcohol and drug addiction (Amatem) units of hospitals, emergency services and intensive care units.
It takes time to send samples to central laboratories [1-3]. Rapid detection of a positive urine sample may be critical in some cases. Drug abuse is an increasing and very important cause of emergency room visits. Drug addiction test requests from emergency departments may be due to a forensic event or due to emergency health problems related to substance use. And rapid completion of the testing process is required [4]. There are various biochemical methods for substance dependence tests to recognize the abuse of drugs and stimulants. Among these methods, analysis of urine by LC MSMS or GC MSMS, which includes separating the substance in urine or blood sample by chromatographic method and then performing mass analysis, was considered as the most accurate analysis method. This is the first preferred method. [5,6]. The mass of the analyte is measured and there is no risk of measuring cross-reactants in this method. However, the installation or purchase costs of the devices are high with this. Another disadvantage of this method is that it requires experience for using the device and interpreting the tests. [5-7]. The other method that can be used is to recognize the drug components is immunochemical method. This method is easier than mass analysis. In this method, commercial kits are used. The kit contains antibodies that will react with the analyte in the patient sample. Sometimes antibodies may be bound to different chemical substances with structural similarity [4]. In this case the patient result may be interpreted as false positive. The third method in substance abuse tests is the card test method. This method is the fastest method. Drug screen rapid tests

are competetive immunoassays for the qualitative determination of various drugs and drugs metabolites in human urine [8]. After the patient's urine sample is collected and delivered to the laboratory, the matter can be completed and analyzed in minutes with the card test method. The rapid tests provide visual, qualitative preliminary analytical test result. Because of the results of the studies performed with this method are evaluated with the color formed on the card, there is a risk that the results can be reported due to the evaluator. Especially in cases of positive result with card test, a secondary analytical method should be performed. These devices enable qualitative screening for commonly abused drugs or drug classes, including amphetamine, barbiturates, benzodiazepines, buprenorphine, cocaine, cannabinoids, methadone, methamphetamine, methylenedioxymethamphetamine (MDMA), opiates and tricyclic antidepressants (TCA). Threshold values, recommended by Substance Abuse and Mental Health Services Administration (SAMHSA)-recommended cut-off concentrations for these drugs or selected threshold values by the laboratory may be used on the cards [9]. In these assays, if a given drug is present in a urine sample, it competitively binds to a specifically colored antibody, which prevents the antibody from binding to antibody-drug conjugates that are present in the detection region of the test. Consequently, the test line disappears, indicating a positive result for drug [9].

Urine samples are usually used for substance dependence tests. Urinary threshold levels were defined for these tests. The results over the threshold value are interpreted as positive. The goal of the this study is, show the result of the our laboratory analyzes carried out with card tests, to report our false positivity rate. To create awareness about the importance of true evaluation of substance analysis with card tests.

Materials and Methods

In our routine practice, in the course of normal working hours during weekdays, the drug addiction test requests made from our emergency department are studied by automatic analyzer (Dimension EXL, Siemens) with the immun analysis method (Syva Emit II Plus, Siemens). Test requests made during the weekend are concluded by working with the card test method (The nal von Minden drug screen test, Germany) in our emergency biochemistry laboratory. Each test which is evaluated as positive by card test is repeated with the immunoassay procedure during the following day time working hours and corrective action is taken if false positive result is given before. In this study, the results of the card test analysis which were conducted in our laboratory between January 2018 and December 2018 and which were followed by the immunochemical method were evaluated retrospectively. We recorded for the same sample, the results obtained under the threshold value with automatic immun analyzer but given positive with the card test before. The rate of giving false positive results was determined in one year period. Our drug rapid test principle is as follows; all drug rapid tests are based on the same test principle. The patient urine sample is applied to the immersion area of the tests and moves along the test strip by capillary action. Free conjugated antibodies, located near the immersion area, are transported along the test strip together with the urine sample. In the negative sample, these reach the test line region, where the target substance of the test is immobilised. These immobilised drugs are bound by the conjugated antibodies. Hence, particles accumulate at the test line region causing a red line to become visible. This situation indicates a negative test result. If a drug is present in the sample, it is bound by the conjugated antibodies after application of the sample. If patient sample's drug concentration is higher than the cut off level of the test strip, all binding sites of the conjugated antibodies are saturated and conjugated antibodies. And it cannot be bound at the test line. In the case of positive sample above the cut off level, the test line does not seem. In our laboratory, card tests are tried with calibrator for each new card test rapid screen box is opened. Thus, the accuracy of the threshold values given on the card is checked. The results of these tests were used to assess test performance around the cut-off levels of the devices. All calibrators for the preparation of trial study are obtained from Siemens Syva Emit.

Statistical analysis:

True positive and negative results were interpreted based on the drug concentrations of the same urine sample that were verified by immun chemistry Syva Emitt method. The results generated by the devices were compared with the recorded results to calculate positive and negative agreement fractions. The results were given as statistical ratios.

Results

Scanned substances in our laboratory and their threshold values are given in table 1. All parameters in table 1 are screening in each urine with one rapid card. 548 urine samples worked with rapid screen card tests in one year period.

Table1. Drug or metabolites and cut off levels used in our laboratory rapid drug screen card test.

Drug/metabolite	Available cut off levels (ng/ml)
Amphetamine	500
3-4 methylendioxyMetamphetamine (MDMA)	500
Benzoylecgonine (Cocaine metabolite)	150
Oxazepam (Benzodiazepine)	300
Opiate	2000
11-nor-Δ9-THC-9-COOH (cannabinoids ,THC)	50

Table 2 shows the our rate of positivity in all urine for each parameter. Last column shows the number of results which below the threshold value when the urine samples were screened again for the second time by the immunochemical method (False positive number and false positivity rate with card test).

Table 2: The rate of positivity in all urine for each parameter

Substance in card tests	Total number of parameters reported with card tests	Number of positive reported results	Rate of false positive results with card test
3-4 MethylendioxyMetamphetamine (MDMA)	548	73	13/73 (17,8%)
Oxazepam (BZD)	548	79	16/79 (20,3 %)
Opiate	548	20	5/20 (25,0 %)
11-nor-Δ9-THC-9-COOH (THC)	548	110	10/110 (9,1 %)

Discussion

Because of working with card tests are easy, they are still used as the only method in many hospital laboratories for the identification of substance use. In some hospitals, due to the small number of patients, no method other than card tests may be used. This device allows the person performing the analysis with his own eyes to read changes in the color of the control and test lines and to interpret the results in the same manner. Card test strips were also visually inspected by automatic test readers, and test positivity was determined based on the intensity of the test line. According to the all manufacturer's instructions, if a red or pink test line appears, the test should be considered negative. Even if the red line is too light in the test zone, the result should be reported as negative. Unfortunately the assessment of the existence of the line is relative based on personal interpretation. It should be noted that, false positive and false negative result will be able to differentiate the treatment and the patient's follow-up. False positivities can lead to criminal penalties in criminal cases. As a chance in our laboratory, the repetition of the test was carried out by immunechemical method. But it is not possible to analyse again with second method in many laboratories. In this case laboratory workers attention and sensitivity is very important. On the other hand, the probability of giving false negative results with card tests is low.

Other point to remember, threshold values defined by the authorities in drug analysis. The threshold values stated in the package insert of the card tests can be more than one. It is important for the laboratory specialist to choose a proper card based on the threshold value to be used. For example, if the used card test morphine threshold is 300 ng/ml, in this case all patients results which morphine analyte is over 300 ng/ml in urine will be reported as positive results. However, the legal threshold for the use of opiates is 2000 ng/ml. When the card test with a threshold of 300 is used for opiate screening, all patients reports who will have a result between 300 and 2000 will be reported as false

positive. Several studies have been conducted to investigate the accuracy and precision of card tests [1,9]. In these studies, the importance of threshold values and visual evaluation of the test are emphasized as similarly of our study. According to Kim and his friends, devices with automated readers are designed to reduce errors by eliminating the potential subjectivity of the visual inspection of test results. Also automatically generated test results provide traceability of the individual who operated the reader [9]. But we have also tested the card tests in our laboratory with automatic reader system. However, sometimes we have seen incorrect readings in their systems. It is better not to use this method and other quantitative or semi quantitative methods should be preferred. Antibody-based immunchemical semi quantitative screening methods working with automatic analyzer should be used both day and night hours especially in hospitals where a lot of patients are admitted to the emergency department [10]. In addition it should be noted that all screening tests provide only a preliminary analytical test result. The true gold standard method is chromatographic separation and mass analysis with LC-MSMS or GC-MSMS.

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FT-9

[DEVELOPMENT OF A NOVEL QUARTZ CRYSTAL MICROBALANCE IMMUNO-ASSAY FOR THE MEASUREMENTS OF SEROLOGICAL AUTOLOGOUS ANTI-TUMOR ANTIBODIES IN SMALL-CELL LUNG CANCER]

[KÜÇÜK HÜCRELİ AKCİĞER KANSERİNDE SEROLOJİK OTOLOGO ANTI-TÜMÖR ANTİKORLARININ ÖLÇÜLMESİ İÇİN YENİ BİR KUVARS KRİSTAL MİKROBALANSI IMMUNO-DENEYİNİN GELİŞTİRİLMESİ]

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Introduction

Estimates point out to above 18 million cancer diagnosis and almost 10 million cancer related deaths in 2018 [1]. This high cancer incidences were expected to continuously increase till 2030 in all cancers but colon and prostate, and the rate of increase is expected to be more dramatic in developing countries like Turkey, when compared to developed countries [2, 3]. Lung Cancer is the leading cause of death among all cancers and it is expected to keep that status in 2030 predictions [2]. Small-Cell Lung Cancer (SCLC), representing 15% of all Lung Cancers, is the most aggressive type of this cancer with its 5 year survival rate under 7% [4]. Although, median survival times and prognosis are better in Limited Disease (LD), compared to Extended Disease (ED) and untreated patients, quick progression and early distant metastasizing nature of the disease makes the early diagnosis extremely difficult [5, 6]. Currently, chest radiography, PET scan and Low Dose Computed Tomography (LDCT) are available in screening of the disease [7, 8]. Screening results are validated by histological evaluation of the samples taken with invasive methods like bronchoscopy [9]. However, all of the methods in use are prone to high false detection and sampling methods are invasive for the patients [10, 11]. Therefore, development of new methods for the diagnosis of the disease at early stages before the disease spread to adherent and distant tissues is important for both patient survival and prognosis. Biomarkers are valuable tools to be utilized for diagnosis. Especially autologous anti-tumor antibodies are abundantly and stably elicited in serum [12]. Many reported cases show that autologous anti-tumor antibodies are highly elicited against tumor antigens and are predictive of the disease, years before cancer patients display symptoms [13, 14]. Therefore, they have the potential to be used alone in early diagnosis or in conjunction with other screening methods to improve false detection rates. At this time, the only kit in the market utilizing autologous anti-tumor antibodies in SCLC diagnosis is OncimmuneEarlyCDT-Lung [15-17]. Its results are insufficient to be extensively used in clinics. However, with improved discrimination power, kits based on serum autologous anti-tumor antibodies have the potential to be used widely in the clinics. Previously in our lab, through the work of Dr. Atakan, several panels of autologous anti-tumor antibodies were discovered via Fetal Brain Protein Array (PA), Testis PA and custom PA screenings carried in sera of 50 SCLC patients and 50 healthy controls (unpublished data) [18]. To this date, one of the highest discrimination power achieved in our lab between SCLC patients and healthy controls is 60% sensitivity at 100% specificity using a panel of 4 autologous anti-tumor antibody biomarkers (POLB, SOX2, FKBP8, P53) (unpublished data) (Table 1). Enzyme-linked immunosorbent assay (ELISA), the traditionally employed method in measurements of protein based biomarkers, is handicapped by its technological limitations, such as time consuming detection, excessive use of expensive reagents, manual measurements, highly qualified technician and expensive instrument requirements [19]. Biosensors, on the other hand, has reusable expendables, allows fast & consecutive measurements, open to automation and does not require expensive instruments or qualified technicians [20-21]. Moreover, different types of biosensors based on optical, electrochemical, colorimetric and mass measurements has been successfully used in detection of several cancer protein biomarkers [21]. However, only a limited number of examples exist for biosensor guided detection of antibodies in complex matrix [22], let alone autologous anti-tumor antibody biomarkers. Such autologous antibody biomarkers have lower affinity to their antigens in the early stages of the disease, due to their yet incomplete affinity maturation process at that stage [22]. This complicates auto-antibody biomarker detection with immunoassays, especially those employing reagents with high ionic strength and detergent content, such as ELISA. In this paper we describe the development of a simple and rapid detection method for anti-tumor antibody biomarker analysis using a quartz crystal microbalance (QCM) biosensor. A QCM consists of a thin quartz crystal placed in between a pair of electrodes. The crystal, with its piezoelectric properties, oscillates under an electric voltage. This resonance frequency of the quartz is affected by the mass addition to or loss from the sensor surface and hence can be used to measure the molecular interactions occurring on the sensor surface without any label requirements [23]. This study is hitherto the first demonstration of the use of QCM in the context of autologous anti-tumor antibody measurements. Proof of concept for applicability of QCM immunosensor in autologous anti-tumor antibodies is displayed

using the example of anti-SOX2 monoclonal antibody (mAb) measurements spiked-in in PBS buffer. SOX2 protein, the antigen of the target antibody, is employed as the recognition element in QCM immunosensor chip surface. ELISA measurements of anti-SOX2 mAb were taken for cross-validation. As a result, QCM anti-SOX2 mAb immunoassay were able to detect the spike-in anti-SOX2 mAb in PBS at concentrations as low as 34 ng/mL.

Materials and Methods

Materials: All chemical reagents used in QCM experiments were from Sigma Aldrich (Taufkirchen, Germany). Lyophilized recombinant SOX2 protein (cat no: SRP3157) and lyophilized bovine serum albumin (BSA) (cat no:A2153) were purchased from Sigma Aldrich (Taufkirchen, Germany). Lyophilized anti-SOX2 mAb (cat no: MAB2018) was purchased from R&D Systems (Wiesbaden, Germany). QCM sensor chip (5 MHz, AT-cut) (cat no: QSX301) was purchased from Q-Sense (AB, Sweden). Sensor Surface Modification: Gold layer on AT cut 5 MHz Quartz Chip surface was cleaned by pulse sonicating in degassed double distilled water. Procedure was repeated 3 times for 3 minutes (replacing water each time). Sonication process was repeated again, this time in degassed analytical grade ethanol (Sigma-Aldrich). Procedure was repeated 3 times for 3 minutes (replacing ethanol each time). After sonication, chips were dried under nitrogen gas and dipped in piranha solution (3:1 mixture of sulfuric acid and 30% hydrogen peroxide) for 10 seconds to etch the organic residues on the surface. As the last step of cleaning, chips were rinsed three times with degassed double distilled water and three times with degassed ethanol. Initially gold coated cleaned chip surface was coated with self-assembled monolayer (SAM) by immersing the chip in 2mM ethanol solution of 11-Mercaptoundecanoic Acid (11-MUA). Immersed chips were incubated overnight in the dark at room temperature. After the 11-MUA incubation, chips were rinsed with ethanol and dried under Nitrogen gas. Dried chips were primed with PBS buffer in QSense Explorer QCM-D device (Biolin Scientific) at a flow rate of 27 uL/sec. Surface is treated with a freshly prepared 1:1 mixture of 400 mM EDC and 100 mM NHS for a duration of 8 min at 27 uL/sec flow rate (total volume 216 uL). EDC-NHS treatment immediately followed by 50 ug/mL SOX2 protein injection (in filter sterilized PBS, pH: 5.5) for 8 min (total volume 216 uL). After 10 min PBS injection, surface was blocked with 50 ug/mL BSA (in PBS, pH: 7.4) for 8 min (total volume 216 uL). Unreacted NHS esters were capped with 1M ethanolamine solution (pH: 8.5) treatment for a duration of 8 min. Chips were primed with PBS (pH: 7.4) for a short while to stabilize the frequency before the measurements were carried. Illustration of chip modification can be seen in Figure 1. Anti-SOX2 mAb Detection via QCM: Samples for QCM measurements were prepared by spiking-in anti-SOX2 mAb in filter sterilized PBS (pH: 7.4). 11 concentrations in a concentration range between 4096 ng/mL and 4 ng/mL were prepared by half step serial dilutions (4096 ng/mL, 2048 ng/mL, 1024 ng/mL, 512 ng/mL, 256 ng/mL, 128 ng/mL, 64 ng/mL, 32 ng/mL, 16 ng/mL, 8 ng/mL, 4 ng/mL). Measurements were taken consecutively from the lowest concentration to highest. For each sample, an 8 minute sample injection was followed by a 15 minute PBS injection, both at 27 uL/min flow rate. Signal changes after anti-SOX2 injection on SOX2 immobilized surface were recorded. Chamber temperature was 18 oC at all steps. The data collected throughout the QCM binding experiments were utilized to calculate KD (binding affinity constant) of the interaction between anti-SOX2 mAb and surface bound SOX2 protein. Simple Langmuir Isothermal 1:1 interaction model was used to describe the behavior of molecular adsorption. Here, the frequency change is related to the adsorbed anti-SOX2 mAb, that is, $\Delta f = (f\max \times C)/(KD + C)$, where Δf corresponds to the frequency change of the QCM at any given anti-SOX2 concentration whereas C is the corresponding anti-SOX2 concentration. Pearson R correlation analysis were also carried to determine correlation levels between the model curve and the experimental results. ELISA Measurements: Protocol for our in house anti-SOX2 ELISA is described elsewhere [18]. Samples were prepared by spiking-in anti-SOX2 mAb in either different serum (anti-SOX2 mAb negative serum) concentrations or PBS. 6 ELISA standard curves were generated by spiking-in anti-SOX2 mAb in 1:400, 1:1600, 1:3200, 1:6400, 1:12800 serum dilutions and PBS buffer. Standard curve dilutions were covering between 200 ng/mL and 0.2 ng/mL. 5 dilution steps were prepared by serially diluting 200 ng/mL with 1/4 dilution steps (200 ng/mL, 50 ng/mL, 12.5 ng/mL, 3.1 ng/mL, 0.8 ng/mL, 0.2 ng/mL). Diluent was blocking buffer in serum dilutions. Standard curves were compared to each other using Pearson R correlation analysis to see the effects of non-specific binding in each serum dilution within the given concentration range. Lastly, ELISA standard curve in PBS is compared to the QCM measurements in PBS. Data Analysis: All of the data analysis were carried in GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA, USA).

Results and Discussion

Chip Modification, Although, EDC-NHS coupling is frequently used as a covalent immobilization method due to its high efficiency, the method is not error free and can result in side reactions that hinder the intended coupling [24]. Therefore, unreacted NHS-esters were capped with BSA containing blocking buffer and 1M ethanolamine solution. SOX2 immobilization resulted in a 21 Hz frequency drop and capping of unreacted esters resulted in a further 2.5 Hz frequency drop in the system, indicating a successful SOX2 immobilization on the surface.

Anti-SOX2 mAb Detection via QCM Anti-SOX2 mAb binding (8 min at 27 uL/min) in a concentration range from 4 ng/mL to 4096 ng/mL was carried on to SOX2 protein immobilized QCM chip surface and frequency changes on the AT cut 5 MHz crystal were recorded. Anti-SOX2 mAb QCM immune assay were able to measure anti-SOX2 at concentrations as low as 32 ng/mL and was linear between the range 32 ng/mL and 512 ng/mL (Figure 2). Recorded changes between 32 ng/mL and 512 ng/mL were plotted to generate a calibration curve. Anti-SOX2 QCM measurements were then fitted to Langmuir isothermal 1:1 binding model to determine the KD value. KD value between the anti-SOX2 mAb and the surface immobilized SOX2 protein was calculated as 11.2 nM in PBS buffer. Pearson R correlation analysis between the raw frequency changes and Langmuir isothermal 1:1 binding model curve resulted in an R square value of 0.94 with a significant two-tailed p value of 0.0064, indicating a great fit between the data and the model. QCM raw frequency changes for sample concentrations between 32 ng/mL (0.94 nM) and 512 ng/mL (30.12 nM), 1:1 Langmuir isothermal binding model curve, and Pearson correlation analysis results can be seen in Figure 2. ELISA Standard Curves, Protein Array validations were carried by measurements at 1:400 and 1:1600 serum dilutions via ELISA. In contrast, proof of concept experiments for anti-SOX2 QCM immunoassay were carried using samples prepared in PBS buffer (pH 7.4). Therefore, to evaluate the effect of non-specific binding [23,24] between serum ELISAs and PBS ELISA, 6 ELISA standard curves were generated by spiking-in anti-SOX2 mAb in 1:400, 1:1600, 1:3200, 1:6400, 1:12800 serum dilutions and PBS buffer (Figure 3). All standard curves highly correlated with each other, based on Pearson R analysis (Data not shown). Standard curves generated with ELISA in different assay matrix conditions indicate that there is no matrix (serum) interference in any of the ELISA standard curves (Figure 3). Hence, anti-SOX2 mAb ELISA standard curve measurements in PBS buffer can be used interchangeably with standard curves generated in 1:400 and 1:1600 serum dilutions, which were used in cPA validations.

A comparison was made between anti-SOX2 mAb standard curves of ELISA (0.20 ng/mL - 200 ng/mL concentration range in PBS) and QCM (32 ng/mL – 512 ng/mL concentration range in PBS). Results show a similar detection range between QCM and ELISA measurements (Figure 4). Interaction between anti-SOX2 mAb and SOX2 protein was investigated for the first time using QCM and Langmuir Interaction model revealing a strong interaction between the antibody-antigen couple. Herein we report a novel QCM based immunosensor capable to measure spike-in anti-SOX2 mAbs in PBS at concentrations as low as 32 ng/mL. This proof of concept is a great step towards quick, cheap and reliable measurements of serological autologous anti-tumor antibodies. Anti-SOX2 mAb QCM assay already has a comparable range to ELISA. It is possible to employ secondary Ab modified gold or magnetic nanoparticles to further amplify mass change, improve signal-noise ratio and enhance assay sensitivity [23]. Therefore, with further optimizations and change in assay matrix from PBS to serum, our auto-antibody panel and QCM assay hold a promising future in the SCLC diagnosis.

Acknowledgements

This work was supported by TUBITAK 1512 project (2180273).

Ethical Issues

Ethics committee approval for the sera used in this study was obtained from Atatürk Chest Disease and Chest Surgery Education and Research Hospital, Ankara, Turkey. (146-20.08.2008)

Ethics committee approval by Atatürk Chest Disease and Chest Surgery Education and Research Hospital is re-issued and re-

approved by Bilkent University Human Research Ethics Committee, Ankara, Turkey. (2018_04_13_03)

Conflict of Interest

The authors have no conflict of interest.

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