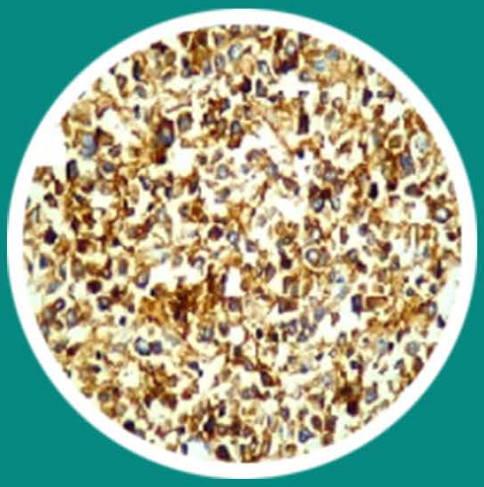
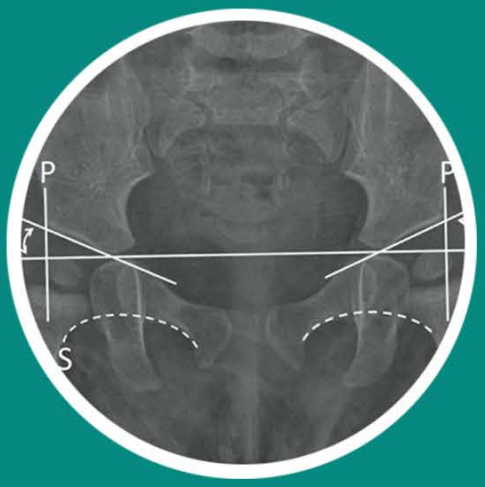
Volume 5 Issue 6 November 2019

e-ISSN: 2149-3189

The European Research Journal





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Abstracting and Indexing

The journal is abstracted and indexed with the following: TR Index (ULAKBİM TR Dizin), Google Scholar, Index Copernicus (ICV 2018: 100), EMBASE, ProQuest Central, ROAD, SciLit, MIAR, J-Gate, SHERPA/RoMEO, BASE, EZB, CrossRef, JournalTOCs, WorldCat, TURK MEDLINE, Turkish Citation Index.

Publisher

The European Research Journal (EuRJ)
The Association of Health Research & Strategy
Kırcaali Mah. Fevziçakmak Cd. Göktaş İş Mrk. Kat:3 No:62/12
Osmangazi/BURSA-TURKEY
www.dergipark.org.tr/eurj/

e-ISSN: 2149-3189

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Case Reports

The European Research Journal 2019;5(6):928-938 ORIGINAL ARTICLE

Diagnostic utility of clinical and epidemiologic features in fever of unknown origin

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DOI: 10.18621/eurj.441463

ABSTRACT

Objectives: To assess the diagnostic utility of clinical features in the major diagnostic categories of Fever of unknown origin (FUO).

Methods: One hundred and thirty-three patients meeting the classic criteria of FUO were included in the study. A structured diagnostic protocol was used in all cases. Sensitivity, specificity, positive and negative predictive values (PPV and NPVs), and likelihood ratios of positive and negative tests (LR+ and LR-) were estimated with 95% confidence intervals (95% CIs) for all clinical findings.

Results: Clinical and epidemiologic features with best diagnostic utility indexes for the three major diagnostic categories were: weight loss of 15 pounds or more (sensitivity, 68.4%, 95% CI: 52.33-84.52), pallor of the skin and mucous membranes (sensitivity, 65.7%, 95% CI: 49.39-82.19), prior medical history of cancer (PPV, 63.6%, 95% CI: 30.66-96.61; LR+, 4.38, 95% CI: 1.36-14.09), lymphadenopathy (LR+, 2.2, 95% CI: 1.11-4.74), for neoplasms; arthritis (PPV, 72%, 95% CI: 51.84-93.61), prior family history of collagen diseases (PPV, 100%, 95% CI: 91.67-100.00), neurologic disorder (LR+, 5.1, 95% CI: 1.37-19.68), myalgia (LR+, 4.1, 95% CI: 1.45-11.88) and skin lesions (LR+, 3.0, 95% CI: 1.51-6.22) for noninfectious inflammatory diseases; weight loss of 15 pounds or more (sensitivity, 50%, 95% CI: 27.91-72.09), epidemiological history of previous tuberculosis or tuberculosis exposure (LR+, 9.0, 95% CI: 1.76-46.77), and jaundice (LR+, 2.73, 95% CI: 0.7-10.63) for infections.

Conclusions: We identified clinical data emerging from the anamnesis and physical examination that may help to guide the diagnostic process in FUO.

Keywords: Fever of unknown origin, clinical features, diagnostic utility

Received: July 7, 2018; Accepted: July 4, 2019; Published Online: July 21, 2019

Fever of unknown origin (FUO) is a syndrome described more than 50 years ago. In 1961 Petersdorf and Beeson [1] defined FUO as an illness with temperature exceeding 38.3°C on at least three occasions, evolving during at least three weeks, with no diagnosis reached after one week of inpatient investigation.

Despite advances in diagnostic techniques, the diagnostic process in patients with FUO remains a major clinical challenge. An enormous number of diseases and unusual presentations of common diseases have been reported as causes of FUO [2-4]. Clinicians are often unaware of the diagnostic significance of symptoms or physical findings in evaluating those patients. Besides, many symptoms are vague or seemingly insignificant [2].

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e-ISSN: 2149-3189

The value of finding clinical clues in the diagnostic process was accepted since the first FUO case series [5]. In 1997 De Kleijn *et al*. [6] gave much weight to the presence of potentially diagnostic clues (PDCs) in history and physical examination even when many of them were misleading in their report. Gaeta, Vanderschueren and Knockaert also highlighted the importance of PDC to reach a diagnosis in FUO patients [7, 8].

On the other hand, the body of FUO literature consists of case series and cohort studies and the yield from a complete history review and meticulous physical examination is not well known [9-11]. This has resulted in excessive diagnostic testing because there is not an efficient and effective diagnostic approach [3, 9].

Authors like Cunha [3,12] and Tolia [2] have emphasized the value of history and physical examination to identify the cause of FUO. The knowledge of clinical aspects in FUO patients will lead physicians to be focused on what to look for and its diagnostic significance [10]. Diagnostic approach in FUO requires considering clinical presentation that can group patients in specific categories: infectious, noninfectious inflammatory diseases (NIID), neoplasms, and miscellaneous disorders, as has been suggested by Cunha [10, 12].

Herein we present a prospective 10-year study on 133 patients presenting with FUO to a tertiary care center. Our objective was to estimate the diagnostic utility of clinical and epidemiological features to recognize whether the disease is infectious, noninfectious inflammatory or neoplastic.

METHODS

Between January 2000 and December 2009, we recruited consecutive patients with FUO at the "Hermanos Ameijeiras" Hospital in Havana, Cuba. This is a 644-bed hospital facility for adults with 14 clinical and 12 surgical wards. All the patients included in this study were admitted in one of the four Internal Medicine wards existing now in this tertiary care center. The study was approved by the hospital Research Ethics Committee.

Eligibility Criteria

Patients had to meet the classic criteria of FUO for being included in the study: febrile illness persisting for three weeks or more, fever of more than 38.3°C on at least three occasions and an unclear diagnosis after one week of hospital investigation. Patients were excluded if they were known to have HIV infection or if they did not present fever during their hospitalization. All patients were older than 16 years.

Diagnostic Work-up

Since 1998 our hospital has a structured diagnostic FUO protocol. Data registered include history, physical examination and investigations required in the diagnostic process. All this information has been prospectively registered in a structured data collection form. This protocol was not applied in a rigid manner in all patients.

The protocol consists in one week of hospitalization for cases with fever lasting three weeks or more. During the first week of hospitalization patients undergo a standardized history and standardized thorough physical examination. Fever existence is confirmed during this week and all medications are withdrawn if possible.

Investigations are categorized as obligatory tests, first-level tests and second-level tests. Obligatory tests are part of the initial diagnostic evaluation of every patient, they must be performed in all patients in the first week of admission and are required to qualify as FUO. These tests include a battery of nonspecific laboratory tests, basic imaging tests and blood cultures. First-level tests and second-level tests are done in cases who still meet FUO criteria after this first week. First-level tests include investigations with more complexity without biopsy procedures, such as: immunological tests, serological tests, microbiological tests, imaging techniques, endoscopic procedures, and endocrinological tests. Second-level tests comprise invasive procedures (Table 1).

Clinical presentation and results of obligatory investigations were considered to determine if first or second-level tests should be indicated. If a patient remains without definite diagnosis after first-level tests results, second-level tests are to be considered. Liver biopsy was considered only in patients with

Table 1. Summary of tests in the diagnostic protocol

Obligatory tests performed in all patients
Hemoglobin; leukocyte count and differential count; platelet count; sedimentation rate; creatinine; aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, blood cultures (n = 3), urinalysis (microscopic examination), chest X-ray, abdominal ultrasonography of abdomen.
First-level tests: immunological tests, microbiological tests, serological tests, imaging techniques, endoscopic procedures, endocrinological tests
Lactate dehydrogenase; protein; protein fractions; ferritin; creatine phosphokinase; bilirubin; ANA; anti-DNA; rheumatoid factors; ANCA; anticardiolipin antibodies; serum protein electrophoresis; cryoglobulin; C3 and C4 levels; blood cultures; urine cultures; HBsAg; anti-HCV; HIV serology; Western blot test; serology for citomegalovirus, *Epstein-Barr* virus, *Brucella* serologic tests; tuberculin skin test; AFB smear and culture of sputum and effusions (pleural, peritoneum, pericardium); ultrasound searching lymphadenopathy; complete lower extremity ultrasound; abdominal CT; chest CT; transthoracic echocardiography; transesophageal echocardiography, colonoscopy, gastroscopy, bronchoscopy, thyroid function tests (TSH, T4).
Second-level tests: invasive procedures
Bone marrow aspiration and biopsy; liver biopsy; lymph node dissection; kidney biopsy; skin and muscle biopsies; solid tumors biopsy; temporal artery biopsy, aspiration of pleural fluid or ascites, pleural biopsy, peritoneum biopsy, exploratory laparotomy.

ANA= antinuclear antibodies, anti-DNA = anti-native DNA antibody, ANCA= antineutrophil cytoplasmic antibody, HBsAg = hepatitis B surface antigen, anti-HCV=hepatitis C virus antibodies, HIV=human immunodeficiency virus, AFB = acid-fast bacilli, CT = computed tomography

abnormal liver function test.

In selected cases without definite diagnosis empirical therapy was required according to their clinical condition and to their presumptive diagnosis. Such empiric therapy included antimicrobial agents, anti-tuberculous drugs or corticosteroids.

For our study, during hospitalization all patients were assessed weekly by the first author besides the attending physician. The final diagnosis was established by the attending physician and two members of the FUO Study Group of the hospital (including the first author). The conclusive factors in the establishment of the final diagnosis included diagnostic test results or classification criteria. In some patients, final diagnosis was made based on their response to specific therapy or based on their clinical course with exclusion of other diseases. In each patient the final diagnosis was grouped in four separate diagnostic categories: infections, noninfectious inflammatory diseases, neoplasms or miscellaneous category. When the final diagnosis did not fit into one of the three major FUO groups it was considered a miscellaneous category.

The patients discharged without final diagnosis were followed until a diagnosis could be made or for one and a half year. This follow-up was performed by the attending physician and the first author at our institution.

Diagnostic tests and clinical criteria followed to establish definitive diagnosis and to place the patients in one of the major diagnostic categories of FUO are detailed in Table 2. The miscellaneous group and patients without final diagnosis are not included.

The presence or absence of potentially relevant findings in the clinical history and physical examination for approaching the diagnosis of FUO, were registered as:

Prior medical history: neoplastic disorders, collagen diseases, history of heart murmur, history of surgical-invasive procedures during the last six months before the onset of fever, history of dental work or periodontal disease during the last three months before the onset of fever, history of blood transfusion, history of glucocorticoids or immunosuppressive treatment during the last three months before the onset of fever.

Prior family illnesses (parents, brothers, children, grandparents, uncle): neoplastic disorders, collagen

Table 2. Tests for definitive diagnosis in major diagnostic categories of Fever of unknown origin

Diagnostic categories	Definitive diagnostic tests					
	Histology	Inmunology	Imaging	Microbiology	Laboratory	Clinical
Neoplasms	bone marrow biopsy, lymphadenopathy biopsy, lymphadenopathy cytology, solid tumors biopsy, post-mortem examination					
Noninfectious inflammatory diseases	muscle biopsy, skin biopsy, renal biopsy	ANA, anti-DNA, RF, ANCA, anticardiolipin antibodies	Axial contrast enhanced CT of supra-aortic trunks		*†ESR >50 mm/h, ‡leukocytosis >10.000 (>80% granulocytes), ‡liver function tests, serum ‡ferritin	*Age (≥50 years), headache, visual symptoms, jaw claudication. †Aching and stiffness of shoulders and hips. ‡Arthralgia, arthritis, fever >39^{0}C, transient erythema, sore throat, generalized lymphadenopathy, splenomegaly. *†‡Response to corticosteroid therapy. *†‡Exclusion of infections, malignancies and rheumatic diseases
Infections	lymphadenopathy biopsy, liver biopsy, bone marrow biopsy, post-mortem examination		abdominal ultrasonography, abdominal CT, transthoracic echocardiography, transesophageal echocardiography	urine culture, pleural fluid cultures AFB positive, HBsAg, Brucellosis serologic tests, HIV serology, Western blot test		clinical course, response to antibiotic therapy, response to antituberculous therapy

HIV = human immunodeficiency virus, ANA = antinuclear antibodies, anti-DNA = anti-native DNA antibody, ANCA = antineutrophil cytoplasmic antibody, RF = rheumatoid factor, CT = computed tomography, AFB = acid-fast bacilli, HBsAg = hepatitis B surface antigen, ESR = erythrocyte sedimentation rate.

Noninfectious inflammatory diseases: Clinical and laboratory criteria were followed to establish the diagnosis of *giant cell arteritis, †polymyalgia rheumatic and ‡adult-onset Still disease.

diseases.

Prior epidemiological history: previous tuberculosis or tuberculosis exposure, previous cattle or bird exposure, travel history.

History: recurrent fever (a fluctuating fever pattern with fever-free intervals of at least two weeks), duration of fever longer than six months, night sweats, chills, myalgia, arthralgia, visual complaints (amaurosis fugax, decreased visual acuity, blurred vision), headaches, abdominal pain, new onset of back pain, chest pain, diarrhea, shortness of breath, dry cough, sputum, dysuria, dysphagia, lower gastrointestinal bleeding.

Physical findings: pallor of the skin or mucous membranes, weight loss of 15 pounds or more, mouth ulcers, heart murmur, hepatomegaly, splenomegaly, localized or generalized lymphadenopathy, abdominal tumor, arthritis, temporal artery tenderness, skin lesions (erythema, macule, papule, ulcers, purpura, necrosis), tachycardia, pulmonary auscultation abnormalities (crackles, rhonchi, wheezes, absent breath sounds), jaundice, diminished or absent pulse, neurologic disorder (seizures, mental status changes, cranial nerve palsies, peripheral neuropathy, sensory loss, motor deficit, neuropsychiatric manifestations with cognitive dysfunction).

Statistical Analysis

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratio of a positive test and likelihood ratio of a negative test (LR+ and LR-) were estimated (with 95% confidence intervals [95% CI]) for each of the clinical and epidemiological features to assess their contribution to the diagnosis of the major etiologic categories of FUO: infectious, noninfectious inflammatory diseases and neoplastic disorders. The gold standard was the definitive diagnosis at the end of the clinical workup. We performed Statistical analysis with SPSS v 13.0, and Epidat 3.1 (Xunta de Galicia, Conselleria de Sanidade, Direccion Xeral de Saúde Pública, https://dxsp.sergas.es).

We considered a clinical or an epidemiological feature useful for diagnosis if at least one of the following criteria were met: sensitivity higher or equal to 50%, likelihood ratio of a positive test higher or equal to 2, positive predictive value superior to 60%, in one or more FUO groups.

RESULTS

Between January 2000 and December 2009, 150 adult patients were admitted to our internal medicine wards with prior diagnosis of FUO. Of these patients, 133 were eligible for inclusion and were retained for analysis; 17 cases were excluded (in nine patients, diagnosis was achieved during the first week of hospital investigation, and in eight patients, temperature over 38.30C could not be documented during hospitalization).

The mean age of our cases was 50.4 ± 18 years (range, 16 to 91 years). Fifty-two patients (39.1%) were elderly (over 60 years old). Sixty-five patients (48.9%) were male. The mean duration of hospitalization to establish definitive diagnosis was 28.1 ± 20.9 days (range, 5 to 149 days). Before admission to our institution, sixty-five patients (49.6%) were hospitalized at least once in general Internal Medicine wards and fifty patients (37.6%) were referred by internists of other university hospitals.

The most prevalent diagnostic categories of FUO were: 38 neoplasms (28.6%) and 37 noninfectious inflammatory diseases (27.8%) followed by 24 infections (18%), 18 miscellaneous diseases (13.5%), and 16 undiagnosed patients (12%). Malignant lymphoma, adult-onset Still disease and abdominal abscess were the most common causative diseases in each FUO group respectively (Table 3).

Patients in whom no final diagnosis was made were followed-up for 18 months, 14 patients recovered spontaneously, and in two patients fever subsided after empirical treatment with corticosteroids.

In six patients, diagnosis could be achieved by autopsy: two non-Hodgkin lymphomas, one adrenocortical carcinoma, one bone marrow toxicity, one miliary tuberculosis, and one bacterial liver abscess.

Diagnostic utility for clinical history and physical examination

In the neoplasms category, the sensitivity of

Table 3. Final diagnosis in 133 patients with Fever of unknown origin

Diagnostic category	No. of patients (%)
Infections	**24 (18)**
Bacterial	
Abdominal abscess	5
Endocarditis	4
Tuberculosis	4
Other *	
Viral	
HIV infection	4
Chronic hepatitis B or C	3
Neoplasms	**38 (28.6)**
Haematological	24
Lymphoma	19
Chronic myeloproliferative disorder	3
Other †	
Solid tumours	14
Metastasis of unknown origin	4
Other ‡	
Non-infectious inflammatory diseases	**37 (27.8)**
Connective tissue diseases	21
Adult Still's disease	10
Systemic lupus erythematosus	6
Rheumatoid arthritis	3
Other §	
Vasculitis syndromes	15
Temporal arteritis/Polymyalgia rheumatic	7
Polyarteritis nodosa	4
Wegener's granulomatosis	2
Other ‖	
Miscellaneous	**18 (13.5)**
Factitious fever	3
Deep vein thrombosis	2
Other ¶	
No diagnosis	**16 (12)**

* includes Brucellosis 2 cases, urinary tract infection 1 case, biliary tract infection 1 case.
† includes multiple myeloma 1 case, acute myelogenous leukemia 1 case.
‡ includes solid malignant tumors: lung 2 cases, colon 1 case, prostatic 1 case, bladder 1 case, adrenal gland 1 case, thyroid 1 case, uterus 1 case, mediastinal angiosarcoma 1 case, gastric GIST 1 case.
§ includes polymyositis 1 case, dermatomyositis 1 case.
‖ includes Takayasu's arteritis 1 case, microscopic polyangiitis 1 case, anti-phospholipid syndrome 1 case.
¶ includes Castleman's disease 1 case, Kikuchi's disease 1 case, sarcoidosis 1 case, inflammatory pseudotumor 1 case, nonhematologic bone marrow fibrosis 1 case, autoimmune hepatitis 1 case, chronic meningitis 1 case, idiopathic granulomatosis 1 case, aortic dissection 1 case, iliac artery aneurysm 1 case, common variable immunodeficiency 1 case, drug-induced liver injury 1 case, interstitial pneumonia 1 case.

weight loss of 15 pounds or more (68.4%, 95% CI: 52.33 - 84.52) and pallor of skin or mucous membranes (65.7%, 95% CI: 49.39-82.19) is noticeable. Almost 64% (95% CI: 30.66-96.61) of patients with prior medical history of neoplastic disorder had cancer as the cause of FUO (positive predictive value). Other clinical features showed low sensitivity, but specificity was over 90% for medical history of neoplastic disorder, medical history of immunosuppressive treatment and jaundice. Only one clinical aspect exhibited a fair likelihood ratio of a positive test: medical history of neoplastic disorder (LR+=4.38, 95% CI: 1.36-14.09).

In noninfectious inflammatory diseases category: pallor of skin or mucous membranes showed the higher sensitivity (56.76%, 95% CI: 39.44-74.07). Noticeable positive predictive values were present for visual complaints (75%, 95% CI: 20.07-100.00), arthritis (72.73%, 95% CI: 51.84-93.61), mouth ulcers (70%, 95% CI: 36.60-100) and prior family illnesses of collagen diseases (100%, 95% CI: 91.67-100.00). Clinical features as visual complaints, mouth ulcers, arthritis, neurologic disorder, diminished or absent pulse, myalgia, arthralgia, skin lesions and headaches were found between eight and three times more often in patients of the EINI group than in patients of other FUO groups (LR+) but only arthritis showed an important confidence interval (LR+ = 6.92, 95% CI: 2.93-16.32). For this group of patients, specificity was very high for all features listed in Table 4 except pallor of the skin or mucous membranes.

In infectious diseases category, likelihood ratio of a positive result was fairly high for previous tuberculosis or tuberculosis exposure (LR+ = 9.08, 95% CI: 1.76-46.77). Sixty-six percent of patients with epidemiological history of previous tuberculosis or tuberculosis exposure had an infectious disease as cause of FUO (PPV, 95% CI: 20.61-100%). Specificity was high for previous tuberculosis or tuberculosis exposure (98.17, 95% CI: 95.19-100) and jaundice (95.41, 95% CI: 91.03-99.80).

In Table 4 we display the diagnostic utility indexes of clinical history and physical exam features considered useful (see the methods section) for each of the three main diagnostic categories.

We found some other interesting and more specific clinical features for which no diagnostic utility indexes were estimated since they were present in very few cases, but we still consider them worthy of exposing. In the neoplasms group, the period between prior history of malignancy and FUO ranged from two to six years. Twenty-one percent of patients with malignant lymphoma had recurrent fever. Four patients with jaundice had a hematologic malignancy, two had a malignant lymphoma, one had acute myeloid leukemia, and one had a chronic myeloproliferative disorder. Lymphadenopathy was localized in eight patients and generalized in three patients in this group of FUO: five were cervical, three were inguinal, two were, supraclavicular, and one was axillary.

Regarding the noninfectious inflammatory diseases group, arthritis occurred mainly in adult-onset Still disease and in systemic lupus erythematosus (five patients each). Adult-onset Still disease was the main cause of skin lesions, sometimes rash persisted for a few days. Recurrent fever pattern was not present in patients with this inflammatory disorder.

Visual complaints in systemic inflammatory diseases were amaurosis fugax (Takayasu's arteritis) and blurred vision or decreased visual acuity (giant cell arteritis, adult-onset Still disease).

Neurologic disorders present in systemic inflammatory diseases were: peripheral mononeuropathy (polyarteritis nodosa) in two patients, diplopia due to the sixth cranial nerve palsy (one giant cell arteritis, and one adult-onset Still disease), hemiparesis (one patient with secondary antiphospholipid syndrome), and neuropsychiatric manifestations in one patient with lupus erythematosus.

In the infectious diseases group, chronic hepatitis B (three patients) and AIDS (three patients) were the most common etiology for weight loss of 15 pounds or more. The most common causes of jaundice in this group were pyogenic liver abscess, chronic hepatitis B, and AIDS (one patient each).

DISCUSSION

In this prospectively collected series of FUO, we attempted to assess the utility of the clinical aspects in the categories of neoplasms, noninfectious

Table 4. Epidemiologic and clinical features with diagnostic utility* in major diagnostic categories

Neoplasms	**Sens** (95% CI)	**Spec** (95% CI)	**PPV** (95% CI)	**NPV** (95% CI)	**LR+** (95% CI)	**LR-** (95% CI)
medical history of neoplastic disorder (n = 11)	18.42 (4.78-32.06)	95.79 (91.22-100.00)	63.64 (30.66-96.61)	74.59 (66.46-82.73)	4.38 (1.36-14.09)	0.85 (0.73-1.00)
medical history of immunosuppressive treatment (last 3 months) (n = 7)	10.53 (0.00-21.60)	96.84 (92.80-100.00)	57.14 (13.34-100.00)	73.02 (64.87-81.16)	3.33 (0.78-14.19)	0.92 (0.82-1.04)
weight loss ≥ 15 pounds (n = 66)	68.42 (52.33-84.52)	57.89 (47.44-68.35)	39.39 (26.85-51.94)	82.09 (72.16-92.02)	1.63 (1.18-2.24)	0.55 (0.33-0.90)
pallor of the skin or mucous membranes (n = 67)	65.79 (49.39-82.19)	55.79 (45.28-66.30)	37.31 (24.99-49.64)	80.30 (69.95-90.66)	1.49 (1.08-2.05)	0.61 (0.38-0.99)
lymphadenopathy (n = 23)	28.95 (13.21-44.68)	87.37 (80.16-94.57)	47.83 (25.24-70.41)	75.45 (66.96-83.95)	2.29 (1.11-4.74)	0.81 (0.65-1.01)
jaundice (n = 8)	10.53 (0.00-21.60)	95.79 (91.22-100.00)	50.00 (9.10-90.90)	72.80 (64.60-81.00)	2.50 (0.66-9.49)	0.93 (0.83-1.05)
Noninfectious inflammatory diseases	**Sens** (95% CI)	**Spec** (95% CI)	**PPV** (95% CI)	**NPV** (95% CI)	**LR+** (95% CI)	**LR-** (95% CI)
family illnesses of collagen diseases (n = 6)	16.67 (3.10-30.23)	100.00 (99.40-100.00)	100.00 (91.67-100.00)	73.68 (65.16-82.21)	0.00	0.83 (0.72-0.96)
myalgia (n = 13)	21.62 (7.01-36.24)	94.79 (89.83-99.76)	61.54 (31.25-91.83)	75.83 (67.76-83.91)	4.15 (1.45-11.88)	0.83 (0.69-0.99)
arthralgia (n = 27)	43.24 (25.93-60.56)	88.54 (81.65-95.43)	59.26 (38.87-79.64)	80.19 (72.13-88.25)	3.77 (1.94-7.36)	0.64 (0.48-0.86)
headaches (n = 11)	18.92 (4.95-32.89)	95.83 (91.32-100.00)	63.64 (30.66-96.61)	75.41 (67.36-83.46)	4.54 (1.41-14.61)	0.72 (0.72-0.99)
pallor of the skin or mucous membranes (n = 67)	56.76 (39.44-74.07)	41.57 (41.57-62.60)	31.34 (19.49-43.20)	75.76 (64.66-86.85)	1.18 (0.83-1.68)	0.83 (0.55-1.26)
arthritis (n = 22)	43.24 (25.93-60.56)	93.75 (88.39-99.11)	72.73 (51.84-93.61)	81.08 (73.34-88.82)	6.92 (2.93-16.32)	0.61 (0.45-0.81)
skin lesions† (n = 24)	35.14 (18.40-51.87)	88.54 (81.65-95.43)	54.17 (32.15-76.18)	77.98 (69.74-86.22)	3.07 (1.51-6.22)	0.73 (0.57-0.94)
mouth ulcers (n = 11)	18.92 (4.95-32.89)	96.88 (92.87-100)	70.00 (36.60-100)	75.61 (67.61-83.61)	6.05 (1.65-22.18)	0.84 (0.71-0.98)
visual complaints‡ (n = 4)	8.11 (0.0- 18.25)	98.96 (96.41-100.00)	75.00 (20.07-100.00)	73.64 (65.65-81.63)	7.78 (0.84-72.47)	0.93 (0.84-1.02)
neurologic disorder§ (n = 9)	16.22 (2.99-29.44)	96.88 (92.87-100.00)	66.67 (30.31-100.00)	75.00 (66.98-83.02)	5.19 (1.37-19.68)	0.86 (0.75-1.00)
diminished or absent pulse (n=3)	5.41 (0.0- 14.04)	98.96 (96.41-100.00)	66.67 (0.0- 100.00)	73.08 (65.07-81.09)	5.19 (0.48-55.53)	0.96 (0.88-1.04)
Infections	**Sens** (95% CI)	**Spec** (95% CI)	**PPV** (95% CI)	**NPV** (95% CI)	**LR+** (95% CI)	**LR-** (95% CI)
previous tuberculosis or tuberculosis exposure (n = 6)	16.67 (0.00-33.66)	98.17 (95.19-100)	66.67 (20.61-100)	84.25 (77.52-90.98)	9.08 (1.76-46.77)	0.85 (0.71-1.02)
weight loss ≥ 15 pounds (n = 66)	50.00 (27.91-72.09)	50.46 (40.61-60.30)	18.18 (8.12-28.24)	82.09 (72.16-92.02)	1.01 (0.65-1.57)	0.99 (0.64-1.54)
pallor of the skin or mucous membranes (n = 67)	54.17 (32.15-76.18)	50.46 (40.61-60.30)	19.40 (9.19-29.62)	83.33 (73.58-93.08)	1.09 (0.72-1.65)	0.91 (0.57-1.46)
jaundice (n = 8)	12.50 (0.00-27.81)	95.41 (91.03-99.80)	37.50 (0.00-77.30)	83.20 (76.25-90.15)	2.73 (0.70-10.63)	0.92 (0.78-1.07)

Sens = sensitivity, Spec = specificity, PPV = positive predictive value, NPV = negative predictive value, LR+ = likelihood ratio of a positive test, LR- = likelihood ratio of a negative test.

* Diagnostic utility = All clinical or epidemiological aspects meeting at least one of the following criteria: sensitivity higher or equal to 50%, likelihood ratio of a positive test higher or equal to 2, positive predictive value superior to 60%,in one or more FUO groups.

† includes erythema, macule, papule, ulcers, purpura, and necrosis.

‡ includes amaurosis fugax, decreased visual acuity, blurred vision.

§includes seizures, mental status changes, cranial nerve palsies, peripheral neuropathy, sensory loss, motor deficit, neuropsychiatric manifestations with cognitive dysfunction.

|| Diagnostic value indexes for all features are available from the author upon request.

inflammatory diseases and infections. We used the common diagnostic utility indexes for their summarizing capacity and acceptance. We also described some features present in a reduced number of patients, worthy of a mention. FUO is a complex syndrome always difficult to etiologically diagnose and thus a challenge for clinicians worldwide [3, 10]. It is also an infrequent syndrome, our series is not a small one, considering other published series, and perhaps one of the few case series paying attention to the epidemiological and clinical history of the patient [5, 11, 13-15].

According to a previous report, the prevalence of FUO groups in our center has changed during the past two decades [16]. Neoplasms (40%) and infections (29.5%) frequency has declined but neoplasms still represent the main diagnostic category [16]. The prevalence of NIID in the present series was higher than in the previous report (16.1%) [16]. This trend could be the result of: 1) improvements in diagnostic methods for certain entities (endocarditis, abdominal abscesses, tumors), 2) referral patterns to our tertiary care center, 3) diagnosis of some NIID (Still disease, vasculitis) is rarely obtained at an early stage, 4) hematologic malignant diseases and small metastasis remain difficult to diagnose.

The large mean duration of hospitalization in this series revealed the major difficulty in establishing the cause of FUO. Other authors report similar values for this variable with periods ranging from 21 to 27 days [13, 14, 17].

De Kleijn *et al*. [13] and Iikuni *et al*. [15] found common clinical findings in FUO patients such as: relevant diseases in the past (78.4%), weight loss (55.7%), lymphadenopathy (41.2%), arthralgia (28.7%), skin rash (16.3%), hepatosplenomegaly (14.4%), and heart murmur (12.4%). Some of these clinical findings were also frequently observed in this report.

These report findings (high positive predictive value and high likelihood ratio of a positive test) led to consider the neoplastic etiology in patients with prior history of cancer, no matter how remote. In such cases we found spread of the tumor or its transformation in cases with prior hematological malignant diseases. Similarly, other authors [2] recognize the value of this diagnostic clue and De Kleijn *et al*. [13] suggest to always search for recurrence of the tumor in these cases.

The patients who receive immunosuppressive treatment may become neutropenic and the fever will be due to infections in most of such cases [18, 19]. However, in this series the patients with prior history of immunosuppressive treatment were not neutropenic, and an infection was not the cause of their fever.

Based on the current study results a weight loss of 15 pounds or more pointed in first place to the neoplastic category and in second place to the infectious group. Hirschmann *et al*. [20] and Cunha *et al*. [10, 12] reported that weight loss accompanied by dramatic loss of appetite suggests a neoplastic etiology. Bleeker-Rovers *et al*. [14] considered weight loss as a clue with diagnostic value in FUO patients. It is well known that weight loss can be a remarkable clinical finding in common neoplastic or infectious causes of FUO such as malignant lymphomas, tuberculosis, subacute bacterial endocarditis and HIV infection [10, 21-27].

In this study, palpable lymphadenopathy mainly pointed to the neoplastic group. Clinical lymphadenopathy can be found in non-neoplastic diseases but since 1982 and thereafter some authors have considered this physical finding as a helpful clue to diagnose neoplasms, particularly malignant lymphomas [5, 10, 11, 28, 29]. Unlike other reports [3, 6, 30], in this series lymphadenopathy confined to the inguinal region was also useful in establishing a definitive diagnosis.

The high positive predictive value and the high likelihood ratio of a positive test of some clinical findings in the NIID category confirmed them as clues with diagnostic utility in this group. Similar observations have been indicated by Cunha *et al*. [3, 10, 12] who stated the family medical history of rheumatic disorders, eye symptoms, joint swelling and effusion, myalgia, arthralgia, mouth ulcers and headache as helpful clues in this FUO category. In addition, Iikuni *et al*. [15] reported arthralgia (42.2%), skin rash (31.1%), and myalgia (13.3%) as relevant findings in this category.

Our observations also confirm that the presence of certain visual complaints (amaurosis fugax, decreased visual acuity, blurred vision) or neurologic disorders

(cranial nerve palsies, peripheral mononeuropathy, motor deficit, neuropsychiatric manifestations) pointed strongly to the diagnosis of vasculitis syndromes. In agreement with this result, some authors [3, 10, 11, 31] have described these clinical findings mainly in temporal arteritis, Takayasu's arteritis, periarteritis nodosa and systemic lupus erythematosus.

Tuberculosis presenting as FUO can be difficult to diagnose due to commonly nonspecific signs and symptoms [25, 32, 33]. This study found high positive predictive value and high likelihood ratio of a positive test for previous tuberculosis or tuberculosis exposure in the infectious category. The epidemiological value of these antecedents has been recognized in other reviews [10].

We found jaundice associated with involvement of the liver by non-TB infections or neoplasms. Prior studies do not describe jaundice in FUO patients [2,3,13-15]. Its presence has been related with biliary obstruction by tuberculous lymphadenitis [32].
Pallor of the skin or mucous membranes showed a high sensitivity in all of the FUO groups evaluated. This result is in accordance with the statement that low hemoglobin increases the chance of reaching a diagnosis in FUO patients [6, 34].

Our results confirmed that recurrent fever and fever lasting longer than six months were unhelpful clues. De Kleijn *et al*. [13] and Knockaert *et al*. [35] have associated these clinical findings with low probability to make a diagnosis.

Limitations

Finally, we point out some limitations of our study. The first and foremost is the relatively low number of cases that prevented accurate estimates of the diagnostic indicators. The fact that the study is based on information from real patients in whom the indications of diagnostic procedures depend on the patient and his treating physician, is the second important limitation of the study since it might bias the diagnostic value indicators. Although we have a diagnostic protocol for FUO, due to ethical considerations total compliance is not possible to achieve in all patients.

The study was conducted in a tertiary care center, so extrapolations to primary or secondary care facilities should be done with caution.

CONCLUSION

In conclusion, the present study reveals clinical and epidemiological features with diagnostic utility in the major diagnostic categories of FUO and quantifies this utility.

Authors Contribution

VRC = Study concept and design, data collection and interpretation, drafted and revised the article; RSJP = Design of the study and statistical planning, data analysis and interpretation; and HMRS = Study concept and design, data interpretation. All authors participated in the critical revision of the article and approved the final version to be published.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The author disclosed that they did not receive any grant during conduction or writing of this study.

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The relationship between exercise dependence, cognitive style and personality characteristics in candidates participating in physical education and sports school special talent examination

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DOI: 10.18621/eurj.445554

ABSTRACT

Objectives: It has been suggested that there is a relationship between exercise dependence (ED), perfectionism, self-esteem and some personality characteristics.In the present study, the relations between ED and dysfunctional attitudes, self esteem and personality characteristics were evaluated.

Methods: Subjectswere 438 canditates entering special talent examination of Bülent Ecevit University School of Physical Education and Sports, Zonguldak, Turkey. Participants were evaluated with demographic data form prepared by investigators, Exercise Dependence Scale-21 (EDS-21), Dysfunctional Attitude Scale Turkish short form (DAS-R), Eysenk personality quetionnary revised form (EPQR-A) and Rosenberg Self Esteem Scale (RSES).

Results: Of the subjects participating in the study, 88 (20.1%) were in dependent (D), 303 (69.2%) in non-dependent-symptomatic (NDS) and 47 (10.7%) in non-dependent-asymptomatic (NDA) groups. There was significant difference in weekly duration of exercise hours between groups ($p = 0.003$). There was significant difference between groups in terms of DAS-R P/A (Dysfunctional Attitude Scale Turkish short formPerfectionism/achievement) scores. ($p = 0.013$) In post-hoc Dunn test carried out to determine the significance of the difference in DAS-R P/A scores between groups, no significant difference was found between D and NDS. However, there was significant difference between D and NDA and between NDAand NDS ($p < 0.05$). In multinomial regression model, it was found that when weekly duration exercise increases 1 unit in the D, 1.082 unit increase occurs compared to NDA ($\beta=1.082$, $p = 0.012$).

Conclusions: It was established that in D, perfectionist attitude was seen at a significantly higher rate than non dependent groups and duration of exercise was predictive of ED.

Keywords: Exercise dependence, perfectionism, self-esteem, personality, dysfunctional attitude

Received: July 19, 2018; Accepted: August 21, 2018; Published Online: July 25, 2019

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e-ISSN: 2149-3189

Exercise, which may be defined as regular physical activity, has many physical benefits such as treatment of diabetes, and decreasing the risk of coronary diseases; and psychological ones such as increasing self-esteem, and decreasing anxiety and depression levels [1, 2]. It hasbeen reported that, in spite of the benefits of exercise, excessive exercise may lead to disruptive results. As in other addictions, exercise may become a compulsive behavior in time and tolerance may develop, which increases the amount of exercise, the need to exercise and withdrawal symptoms may occur in association with negative outcomes when exercise is not carried out [3]. Some investigators have termed excessive exercise as exercise dependence (ED) due to common characteristics it shares with other addicitions [4]. In the single study carried out on ED in the population, its prevalance rates were found to vary between 0.3% and 0.5% [5].

A relationship has beendemonstrated between exercise and perfectionism both in cross-sectional and longitudinal studies [6, 7]. Self-esteem is the self perception of the individual as valuable. People constantly try not to lose their self esteem and to increase its level [8]. Adaptive perfectionism is associated with high self-esteem while maladaptive perfectionism is associated with low level self esteem [9]. Subjects with maladaptive perfectionism doubt their own actions and capacity. They frequently criticise and despise themselves [10]. Perceived physical inadequacy is associated with lack of self-acceptence and self criticism with low self esteem [11]. Gotwals *et al*. [12] investigated the relation between perfectionism and self esteem in sportsmen. In their study, maladaptive perfectionism was found to be associated with low self-esteem whilst no relation was found between adaptive perfectionism and self esteem [12].

Although some studies cold not findany relationship [13], it has been suggested that there is apositive correlation between ED level and extroversion, which is one of the personality dimensions, and negative correlation with neuroticism [14, 15]. Hausenblas ve Giacobbi [16] suggested that whilst exercise is a coping strategy for concerns about being healthy and appearance,for some personality characteristics, excessive exercise may become dysfunctional in time [16]. In studies on internet addiction, which are behavioral addiction like ED, it has been reported that low self esteem and high neuroticism levels are associated with internet addiction [17]. It is known that there is a relation between perfectionism and personality characteristics such as narcissism [18]. Perfectionism is the predictor of neuroticism [19], and perfectionism and neuroticism are associated with psychiatric disorders [20].

Despite the literature, there is no consistent data on relationship between ED, perfectionism, personality characteristics, and self esteem. Contradictory results in the studies may be due to differences in the samples being studied. Risk groups need to be identified in order to prevent ED. ED may have relations with dysfunctional attitudes, self-esteem and personality characteristics. The aim of the present study was to investigate the relation between ED, and dysfunctional attitudes, self-esteem and personality characteristics in subjects attending special talent examination in physical education and sports school.

METHODS

Design and Subjects

The present cross sectional study was carried out with subjects taking special talent exam of Bülent Ecevit University School of Physical Education and Sports, Zonguldak, Turkey. All applicants from all regions of Turkey who are entering Physical Education and Sports School Special Talent Examination 2016 exam in Bülent Ecevit University were targeted for study. Because of the need for regular exercise habits to be able to pass the exam, and because the test is a specialized aptitude test on sports, the sample was chosen this way. During the six day exam period, a total of 489 male and 188 female subjects entered the exam. Five hundred thirty one candidates accepting to participate in the study and signed the consent forms. In order to prevent bias, which may stem from the use of data collection tools, scales were administered to subjects randomly without any selection. Participants filled the scales in mean 20 minutes. After scales with missing or mistaken coding were removed, statistical analysis was carried out with the data of the remaining 438 participants. The present study was approved by Ethics Committee of Bülent Ecevit University, School of Medicine Ethics Committee with approval no. 2016-90-27/07.

Scales

Demographical data form:It was developed by the investigators and includes sociodemographic information on the participants.

Exercise depencence scale-21 (EDS-21):It is Likert type (scored between 1-6) self report scale developed with the aim of determining ED [4].Test was developed to determine exercise dependence irrespective of the type of exercise. According to scoring of test, three categories are obtained. That is, dependent subjects (D), non-dependent symptomatics (NDS) and non-dependent asymptomatics (NDA). D criteria is obtaining 5 or 6 scores from an item. Those who obtain a score of 3-4 are classified as NDS while those who obtain a score of 1-2 are classified as NDA. Turkish validity and reliability study of the scale was developed by Yeltepe and İkizler [21]. In Turkish validity-reliability study carried out with exercise dependent sample, Cronbach alpha coefficient value was found to be 0.97 [21].

Dysfunctional attitude scale Turkish short form (DAS-R): Dysfunctional Attitude Scale is Likert type self reported scale and which includes 40 items scored between 1-7 developed to evaluate dysfunctional attitudes and beliefs [22]. Turkish validity and reliability study of the short form of the scale was performed by Batmaz ve Ozdel [23]. Its Turkish form includes 13 items scored between 1-7. Turkish version of the dysfunctional attitude scale are stratified into two groups. i.e. need for approval/dependency (NFA/D) and perfectionism/achievement (P/A). In Turkish form cronbach alpha value was found to be 0.84 for NFA/D subscale, 0.75 for perfectionism/achievement and 0.84 overall [23].

Eysenck personality questionnary revised short form (EPQR-A): It is a shortened from of Eysenck personality questionnary and includes 24 items answered as yes or no. Form, has four dimensions including extroversion, neuroticism, psychoticism and lie. It evaluates personality with the dimensions of extroversion, neuroticism and psychoticism. Lie dimension aims to prevent bias during administration of form and control its validity. There are 6 items for each dimension [24]. Cronbach alpha values found in Turkish validity study were as follows: 0.78 for extroversion, 0.65 for neuroticism, 0.42 for psychoticism and 0.64 for lie [25].

Rosenberg self esteem scale (RSES): Rosenberg Self esteem scale isa self report scale including 63 multiple choice questions. It has 12 subcategoriesin the context of the present investigation. Its first ten items wereused in order to measure self esteem. The higher the score obtained from the scale, the lower was self esteem score (0-1: was evaluated as high level of self esteem, 2-4: as moderate self esteem and 5-6: as low level of self esteem) [26]. In Turkish validity and reliability study, coefficient of validity was found to be 0.71 and reliability coefficient was found to be 0.75 using test-retest method [27].

Statistical Analysis

Statistical analyses were performed with SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). Distribution of data was determined by Shapiro-Wilk test. Continuous variables were expressed as mean±std. deviation or median (min-max), categorical variables as frequency and percent. The Chi-square test was used to determine for difference between for categorical variables. The Kruskal-Wallis test was used to determine for differences between three groups. The Dunn test was used for post-hoc test after Kruskal-Wallis test. Mutinominal logistic regression model was constructed to account for effects of prognostic factors. Significance levels were set at $p < 0.05$.

RESULTS

Of the participants in the study, 88 (20.1%) was in D, 303 (69.2%) in NDS, and 47 (10.7%)in NDA. Demographic and clinical characteristics of the groups are demonstrated in Table 1. There was significant difference in weekly duration of exercise hours between groups (p =0.003) (see Table 1).

Comparison of scale scores between groups is demonstrated in Table 2. There was significant difference between groups in terms of DAS-R P/A (Dysfunctional Attitude Scale Turkish short form Perfectionism/achievement) scores (p = 0.013) (see Table 2). In post-hoc Dunn test carried out to determine the significance of the difference in DAS-R P/A scores between groups, no significant difference was found between D and NDS. However, there was significant difference between D and NDA and between NDA and NDS.

Table 1. Comparison of demographic variables between the groups (n = 438).

	Dependent	Non-dependent symptomatic	Non-dependent asymptomatic	*p* value
[+]Age (yeras), Median (Min–Max)	19 (18-22)	19 (18-35)	19 (18-23)	0.123
[++]Sex				
Female, n (%)	31 (35.2%)	75 (24.8%)	16 (34.0%)	0.094
Male, n (%)	57 (64.8%)	228 (75.2%)	31 (66.0%)	
[+++]BMI (kg/m^2), Median (Min-Max)	20.9±2.3	21.5±2.2	21.2±2.3	0.702
[+]Overall duration of exercise (years), Mediam (Min-Max)	7 (1-15)	7 (1-25)	5.5 (1-14)	0.103
[+]Weekly duration of exercise (hours), Median (Min-Max)	12 (1-35)[a]	8 (1-60)[b]	8 (2-28)[b]	**0.003**
[++]Use of psychiatric drugs				
Yes, n (%)	1 (1.1%)	5 (1.7%)	0 (0.0%)	1.000
No, n (%)	87 (98.9%)	298 (98.3%)	47 (100.0%)	
[++]Previous history of psychiatric disorders				
Yes, n (%)	0 (0.0%)	10 (3.3%)	2 (4.3%)	0.109
No, n (%)	88 (100.0%)	293 (96.7%)	45 (95.7%)	
[++]Alcohol use				
Yes, n (%)	3 (3.4%)	25 (8.3%)	2 (4.3%)	0.280
No, n (%)	85 (96.6%)	278 (91.7%)	45 (95.7%)	
[++]Smoking				
Yes, n (%)	7 (8.0%)	51 (16.8%)	3 (6.4%)	**0.030**
No, n (%)	81 (92.0%)	252 (83.2%)	44 (93.6%)	
[++]Substance use				
Yes, n (%)	2 (2.3%)	5 (1.7%)	0 (0.0%)	0.700
No, n (%)	86 (97.7%)	298 (98.3%)	47 (100.0%)	

[+]Kruskal-Wallis, [++]Chi Square, [+++]ANOVA(Analysis of Variance), p<0.05, BMI=Body mass index

In order to test the predictive power of variables between groups, multinominal logistic regression analysis was carried out. Variables which meet the condition of $p < 0.250$, such as age, sex, overall duration of exercise, weekly duration of exercise, previous history of psychiatric disorders, smoking status, DAS-R P/A, EPQR-A lie and RSES were entered into regression model. According to model, when weekly duration of exercise increases 1 unit in D, there is 1.082 unit increase compared to NDA (β=1.082, $p = 0.012$) (Table 3).

DISCUSSION

Although the rate of ED is low in the general community, the rates found in studies carried out with college students at similar ages are comparable to our

Table 2. Comparison of scales between the groups (n = 438).

	Dependent	Non-dependent symptomatic	Non-dependent asymptomatic	*p* value
DAS-R				
P/A, Median (Min-Max)	20 (8-48)[a]	20 (8-51)[a]	15 (8-56)[b]	**0.013**
NFA/D, Median (Min-Max)	12 (5-31)	13.5 (5-33)	10 (5-35)	0.079
Total, Median (Min-Max)	32 (13-74)[ab]	33 (13-84)[a]	24 (13-91)[b]	**0.013**
EPQR-A				
Neuroticism, Median (Min-Max)	9 (6-12)	9 (6-12)	10 (6-12)	0.741
Extraversion, Median (Min-Max)	8 (4-12)	8 (4-12)	8 (4-12)	0.752
Psychoticism, Median (Min-Max)	7 (3-12)	7 (3-12)	8 (4-10)	0.999
Lie, Median (Min-Max)	10 (1-12)	10 (1-12)	9 (1-12)	0.164
RSES, Median (Min-Max)	1 (0.25-2.75)	1 (0.0-4.83)	1 (0.0-2.75)	0.193

Kruskal-Wallis, $p < 0.05$,
DAS-R = Dysfunctional attitude scale-revised, P/A = Perfectionism/achievement, NFA/D = Need for approval/dependency, EPQR-A = Eysenck Personality Questionnaire Revised/Abbreviated Form, RSES = Rosenberg Self-Esteem Scale

results [28, 29]. In the present study, the rate of ED was two fold higher in men than in women. Data on the relative prevalence of ED in genders are conflicting. In female sex, excessive exercise occurs more commonly as a symptom of eating disorders of with the aim of losing weight. Our study was carried out with subjects entering special talent examination of Physical education and Sports school. Therefore, our sample may include intrinsically motivated subjects [29, 30]. In the present study, weekly duration of exercise in D was significantly higher than that of NDS and NDA. Although it is not possible to give clear cutoff values for ED, Imm *et al*. [31] suggested that exercise at or over 5 hours a week can be considered as ED. Garman *et al*. [28] established, in another study carried out with college students, that the group defined as exercise dependent exercised 6 hours or more weekly. In the present study, weekly duration of exercise was found to be high in all groups. As our sample comprises subjects who wanted to enter physical education and sports department and performed exercise professionally, not as ahobby, longer exercise hours can be expected.

Perfectionism may be defined variably according to its personal and interpersonal multidimensional structure [32]. In the study by Hagan and Hausenblas [6] on university students, even when ED deriving from eating disorders was excluded, significant relation has been found between ED and perfectionism. In the study of Hall *et al*. [33] with a sample composed of sportsmen, it has been established that self-oriented and socially prescribed perfectionisms, which are among the dimensions of perfectionism, are associated with perfectionism. P/A which was one of the dimensions of DAS-R, used in the present study,is associated with high level of personal standarts, feeling anxious about negative evaluation of others, and evaluating faults and deficiencies as inadequacy [34]. In the present study, in D, DAS-R P/A dimension score was significantly higher than that in the groups without ED. This difference may be explained in different ways. Focusing on success involved with perfectionism is associated with excessively self critical and the idea of I am valuable if I am successful. Perfectionist people tend to be active physically. Anxiety about in adequacy and perception of failure may have led them to use exercise as a coping strategy [35]. Self theory

Table 3. Summary of multinomial logistic regression analysis

Group[a] and predictor	Wald x^2	df	B	Odds Ratio	*p* value	95% CI
Dependent						
Intercept	0.107	1	1.005		0.744	
Age	1.292	1	-0.75	0.839	0.256	0.621-1.135
Overall duration of exercise (years)	2.221	1	0.103	1.108	0.136	0.968-1.269
Weekly duration of exercise (hours)	6.249	1	0.079	1.082	**0.012**	1.017-1.151
DAS-R P/A	0.924	1	0.033	1.033	0.336	0.966-1.105
DAS-R NFA/D	0.023	1	0.007	1.007	0.880	0.919-1.104
EPQR-A Lie	1.738	1	0.109	1.116	0.187	0.948-1.313
RSES	0.77	1	-0.092	0.912	0.781	0.477-1.746
Sex						
Male	0.314	1	-0.296	0.744	0.575	0.264-2.096
Female		0	0[b]			
Smoking						
Yes	0.205	1	0.401	1.493	0.651	0.264-8.452
No		0	0[b]			
Non-dependent symptomatic						
Intercept	0.919	1	-2.460		0.338	
Age	0.625	1	0.098	1.103	0.429	0.866-1.404
Overall duration of exercise (years)	0.259	1	0.031	1.031	0.611	0.916-1.161
Weekly duration of exercise (hours)	0.277	1	0.015	1.016	0.599	0.959-1.076
DAS-R P/A	1.414	1	0.035	1.036	0.234	0.977-1.098
DAS-R NFA/D	0.052	1	0.009	1.009	0.820	0.932-1.092
EPQR-A Lie	3.704	1	0.136	1.146	0.054	0.998-1.316
RSES	0.028	1	-0.047	0.954	0.868	0.547-1.663
Sex						
Male	0.010	1	0.047	1.048	0.920	0.420-2.614
Female		0	0[b]			
Smoking						
Yes	2.007	1	1.087	2.965	0.157	0.659-13.337
No		0	0[b]			

[a]The reference category is: asemptomatik, [b]This parameter is set to zero because it is redundant.
DAS-R = Dysfunctional attitude scale-revised, P/A = Perfectionism/achievement, NFA/D = Need for approval/dependency, EPQR-A = Eysenck Personality Questionnaire, Revised/Abbreviated Form, RSES = Rosenberg Self-Esteem Scale

explains maladaptive perfectionism as the outcome of cognitive dissonance between real self and ideal self. In the context of theory of self, excessive exercise seems to be used as means towards the formation of perfect persona [36]. People with perfectionist attitude are known to have cognition triggering and maintaining stress and to be prone to psychiatric disorders. Increased stress levels may also have led to dependence [37]. Simply, perceived performance pressure from coaches and parents and the fear of failing to meet expectations of others may cause negative perfectionistic behavioral style. In today's fitness culture, it has been suggested that people with a perfectionist attitude can turn to exercise more for intrinsic social values [38].

In the study of Guidi *et al*. [39] with 79 Italian participants, it was determined that there is relation between low self esteem and ED. In the study of Bruno *et al*. [40] on 150 subjects from fitness clubs, low self esteem was found to be predictor of ED. In both of the above studies, scales other than self esteem scale we used were employed. In the study of Iannos and Tiggerman [41], when self-esteem, which was evaluated with a version of self esteem scale used in the present study, and some personality characteristics were compared between those who have ED and those who do not have such dependence, no significant difference was found. In the present study, there was no significant difference between groups interms of self-esteem, DAS-R NFA/D and personality dimensions. The relationship between ED, self-esteem and personality traits may vary according to the sample being studied. It has been found that people with ED associated with low self esteem and disordered eating pattern use excessive exercise to regulate their emotions and to cope with low self esteem [42]. Likewise, people with a risk of eating disorder and who exercise regularly have been found to have neurotic characteristics compared to those who are not at risk of having eating disorder [43]. These contradictory findings in the studies may be related to the motivation of people to exercise. While people motivate to exercise, to lose weight or have a better appearance, low self esteem and certain personality traits are associated with ED; this may not be related to situations when motivationis skill or career development [44]. Since the present study consisted of a sample of professionals who are interested in sports, no significant differences in self esteem and personality characteristics could be detected. Besides, Iannos veTiggerman [41] suggested that excessive exercisemay have influenced the measurement of self esteem and personality characteristics, removing the difference between groups. In order to remove the effect of excessive exercise on results, the analysis of scores obtained when the same participants quit sports or their exercise is prevented may be beneficial.

In the present study, it was found that according to regression model developed for identifying the predictors of ED, when weekly exercise duration increases 1 unit in D, compared to NDA, this increase becomes 1.082 unit. Though the length of exercise period increases risk for the development of dependence, heavy and long exercise carried out by professional sportsmen in order to be successful should be carefully distinquished from exercise [45].

Limitations

There are some limitations of the present study. DAS-R P/A dimension used in the present study does not measure perfectionism multi dimensionally. Scales based on self report were also used. Further studies including clinical interviews may be beneficial. Since the present study is a cross sectional one, it is not possible to establish cause and effect relations based upon our results. In the present study was conducted in a single center which restricts the generalization of results. It may be useful to carry out similar studies in multiple centers. It is thought that trait anxiety may be effective on ED. Trait anxiety levels were not evaluated, which may be considered another limitation of the study [46].

CONCLUSION

In the present study,in the ED group, perfectionism was significantly higher than non-dependent group and the duration of exercise is predictive of ED. In cases of exercise lasting for a long duration, the risk of dependence should be kept in mind and if necessary, perfectionism should be evaluated with structured scales.

Ethical standards

The study was performed according to the 1964

Helsinki declaration and its later amendments and was approved by the local ethics committee.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2019;5(6):948-956 ORIGINAL ARTICLE

Thiol/disulphide homeostasis in *H. pylori* infected patients

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DOI: 10.18621/eurj.443557

ABSTRACT

Objectives: The aim of the study was to evaluate the oxidative stress level in patients, diagnosed with *H. pylori* infection, using a novel marker (thiol/disulphide homeostasis) and to compare the level in infected individuals with that in healthy volunteers.

Methods: A total of 60 patients diagnosed with gastritis, erosive gastritis or ulcer by endoscopy were included and biopsied. The 30 patients diagnosed with *H. pylori* and 30 healthy individuals were enrolled. Medical histories, physical examination results, body mass index (BMI), hemogram, free triiodothyronine (FT3), free thyroxine (FT4), thyroid stimulating hormone (TSH), urea, creatinin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein) LDL and thiol/disulphide levels obtained in the study groups were compared.

Results: There was no significant difference between the total thiol, native thiol, disulphide/native thiol and dissulphide/total thiol ratios of the patient and control group. When the *H. pylori* patients were stratified by endoscopic evaluation as having mild (superficial gastritis or normal appearance) or severe (ulcer or erosive areas) symptoms, there were significant differences in disulphide, disulphide/native thiol, disulphide/total thiol and native thiol/total thiol levels. We also observed BMI and the total, native thiol levels of *H. pylori* patients were inversely related.(r: 0.562, $p = 0.001$; r: 0.0552, $p = 0.002$).

Conclusions: Thiol/disulphide homeostasis is likely to differ with both duration and severity of *H. pylori* infection. Further investigations are needed to investigate the effect of *H. pylori* on oxidative stress.

Keywords: *H. pylori*, thiol, oxidative stress

Received: July 13, 2018; Accepted: January 23, 2019; Published Online: July 22, 2019

Helicobacter pylori is a Gram-negative bacillus about 0. 6×3.5 micron in size, infecting more than 50% of the world's population and regarded as the most common infection worldwide [1].The disease is typically acquired in childhood and continues throughout life [2]. As in many chronic diseases, most patients are asymptomatic, but chronic inflammation may result in gastric ulcers and gastric cancer [3, 4]. Chronic inflammation due to *H. pylori*, also may play a role in the pathogenesis of extragastric diseases such

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e-ISSN: 2149-3189

as atherosclerosis, acute coronary syndrome, chronic immune thrombocytopenic purpura and insulin resistance [5-8].

Reactive oxygen species (ROS) are produced in the human body as a result of normal aerobic metabolism and a balance between production and inactivation is required. Excess production of ROS can lead to a situation of oxidative stress, which is responsible for many pathological processes [9].

Oxidative stress in the gastric mucosa as a result of *H. pylori* infection is a crucial contributing factor to gastric carcinogenesis. Reportedly, infection was shown to correlate with increased damage due to oxidative stress of the gastric mucosa [10]. Although reversible upon bacterial eradication [11], the consequences of oxidative stress are evident through observed changes in global lipid and protein expression [12] and an increase in damaged biomolecules, such as DNA (modification of bases, telomere shortening) [13]. Additionally, anti-oxidant capacity is also reduced due to decreased levels of antioxidant molecules, such as glutathione (GSH) [14] in the gastric mucosa of *H. pylori* infected patients. Chronic inflammation and bacterial virulence factors associated with microbial pathogens causes cellular damage through rupturing lysosome, damaging mitochondria, producing endoplasmic stress and dysregulation of cellular ions balance. These cellular dysfunctioning leads to oxidative stres cathepsin B production, nuclear and mitochondrial DNA damage and genetic instability [15].

Genetic instability resulting from *H. pylori* infection is not clear [16, 17]. Cells are exposed to reactive oxygen species during oxidative processes, and antioxidants like thiol groups, often referred as mercaptans, function to counteract the process that are induced [18]. Thiols are organic compounds that including a sulfhydryl (-SH) group bonded to a carbon atom. A very large proportion of the plasma thiol pool consists of albumin and other proteins, with smaller proportion of low molecular weight thiols such as cysteine, cysteinyl glycine, glutathione, homocysteine and γ-glutamylcystein [19]. The thiol proteins, thiol groups of low molecular weight compounds, cysteine residues and other control groups are reversible converted to disulphide bonds by oxidant molecules. Reduction of disulphide bonds regenerates thiol groups to maintain thiol/disulphide homeostasis [20, 21] (Figure 1). In this study, we compared the thiol/disulphide ratios and plasma thiol levels in *H. pylori* infected patients and healthy controls.

METHODS

This study was performed in accordance with the Helsinki Declaration and it was ethically approved by the institutional committee (ethics committee approval number: 37732058-53/671). The study was conducted between july 2016 and october 2016 at Erzurum Training and Research Hospital. Sixty patients admitted to gastroenterology clinic at various times were included, 30 with *H. pylori* infection and 30 uninfected controls.

Helicobacter infection was detected according to endoscopic biopsy. Patients without *H. pylori* infection as a result of endoscopic biopsy; evaluated as control group. Patients with *H. pylori* infection and control group were stratified by mild (superficial gastritis or normal endoscopic appearance) or severe (ulcer or

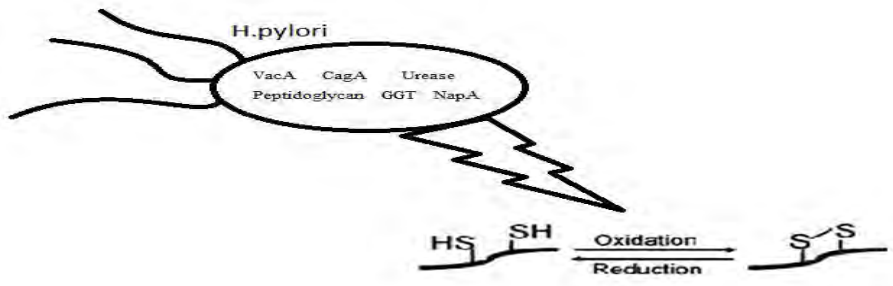

Figure 1. Oxidative stress leads to the formation of unwanted disulphide bonds in the cytoplasm, a process termed disulphide stress. Upon return to non-oxidative conditions, cellular reductases like thioredoxin reduce the cysteine modifications and restore the original protein activity.

erosive areas) symptoms. Patients with other infections, cardiovascular disease, diabetes mellitus, thyroid disease and histories of hypertension history or smoking were excluded. Patient medical histories were obtained, After the measurement of weight and height according to the standard protocol, body mass index (BMI) was calculated using the following formula: BMI (kg/m^2) = weight (kg) / height^2 (m^2).

Endoscopic biopsy specimens were evaluated in department of pathology at Erzurum Training and Research Hospital. Specimens were stained with hematoxylin eosin (H & E) and giemsa for detecting *H. pylori* infection. Results reported by the pathologist.

All blood samples were obtained after a fasting period of 12 hours. A complete blood count, free triiodothyronine (FT3), free thyroxine (FT4), thyroid stimulating hormone (TSH), urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein) LDL were measured. Biochemical analyses were studied by architectc16000 clinical chemistry analyzer (Abbot), complete blood count was studied by sysmex, and thyroid function tests were studied by immulite 2000×pi (Siemens).

The thiol/disulphide homeostasis assay has been previously described. Briefly, disulphide bonds were reduced to form free functional thiol groups. Unused sodium borohydride reductant was consumed and removed with formaldehyde, and both reduced and native thiol groups were determined after reaction with 5,5'-dithiobis-(2-nitrobenzoic) acid. Half the difference between total thiols and native thiols was recorded as the dynamic disulphide homeostasis value. After determination of native thiols (-SH), disulphide (-SS) and total thiols (-SH + -SS), disulphide/total thiol percent ratio (-SS/-SH+-SS), disulphide/native thiol percent ratio (-SS/-SH) and native thiol/total thiol percent ratio (-SH/-SH+-SS) were calculated. Blood samples drawn for determining the thiol/ disulphide level were centrifuges and the plasma was stored at −80 degrees until used. Assays were performed by an automated clinical chemistry analyser (Cobas 501, Roche), and the results were reported as µmol/L.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Qualitative variables were assessed by Chisquare test. Correlation analyses were performed using Pearson's correlation test or Spearman's correlation test. The differences between the different groups of controls and patients were analyzed by unpaired test or Mann-Whitney U test. A p value < 0.05 was considered significant. Data were analyzed with the SPSS® for Windows computing program (Version 17.0).

RESULTS

A total of 60 patients, 18 men and 42 women were included in the study; the demographic characteristics and laboratory results are shown in Table 1. The patients in the *H. pylori* group were significantly older (45 years of age) than participants in the control group (39 years of age, $p = 0.004$). There were no significant differences in BMI, biochemical test results, including lipid profiles and thyroid function tests in the two groups. There were no significant between-group differences in complete blood count results except haematocrit. The haemoglobin values were lower in the controls than in the *H. pylori* group, but the difference did not reach significance ($p > 0.05$).

Both total thiol (medians of 447 µmol/L and 442 µmol/L, p = 0.59) and native thiol (medians of 431 µmol/L and 410 µmol/L, $p = 0.55$) were lower in the *H. pylori* than in the control group, but the differences were not significant. Median disulphide levels were 14 µmol/L in the control group and 15 µmol/L in the *H. pylori* group, and no significant differences in disulphide/native thiol, disulphide/total thiol, native thiol/total thiol were found (3.6%-3.4%, 3.3%-3.2% and 9.32%-9.34%, respectively; p = 0.95).

When the *H. pylori* patients were stratified by mild (superficial gastritis or normal endoscopic appearance) or severe (ulcer or erosive areas) symptoms, significant differences were observed in mean disulphide, disulphide/native thiol, disulphide/total thiol and native thiol/total thiol levels (13.4 µmol/L and 17.4 µmol/L, $p = 0.008$; 3.27% and 4.21%, $p = 0.022$; 3.06% and 3.86%, $p = 0.022$ and 93.8 and 92.2%, $p = 0.022$, respectively)

In addition, native thiol level was found to decrease with both age ($p = 0.19$ and BMI ($p = 0.031$). Pearson correlation analysis confirmed significant

Table 1. Demographic characteristics and laboratory results of the study participants

Variables	Control group	*H. pylori* positive group	*p* value
Sex			
Male	9	9	0.78
Female	21	21	0.78
Age (years)	39	45	**0.004**
Native thiol (-SH) (µmol/L)	431 ± 47.31	410.8 ± 34.07	0.55
Total thiol (-SH + -SS) (µmol/L)	457 ± 42.34	442.2 ± 34.15	0.59
Disulphides (-SS) (µmol/L)	14.9 ± 5.49	15.02 ± 4.87	0.98
-SS/-SH (%)	3.602 ± 1.970	3.490 ± 1.272	0.95
-SS/Total -SH (%)	3.35 ± 1.67	3.26 ± 1.54	0.95
-SH/Total -SH (%)	93.2 ± 2.25	93.4 ± 1.19	0.95
BMI (kg/m²)	25.9	24.7	0.39
WBC (10^3/µL)	8.03 ± 2.49	8.16 ± 3.34	0.86
Hemoglobin (g/dL)	13.90 ± 1.72	14.65 ± 1.17	**0.05**
Hemotocrit (%)	42.70 ± 5.16	45.27 ± 3.39	**0.02**
Platelet (10^3/µL)	282.90 ± 69.24	277.10 ± 71.58	0.75
MCV (fL)	84.40 ± 6.80	87.03 ± 3.19	0.06
FT3 (ng/dL)	3.18 ± 0.57	3.40 ± 1.71	0.63
FT4 (ng/dL)	1.07 ± 0.13	1.79 ± 2.66	0.27
TSH (µIU/mL)	1.38 ± 1.10	1.07 ± 0.85	0.24
Glucose (mg/dL)	98.23 ± 16.28	98.13 ± 16.71	0.98
Creatinin (mg/dL)	0.71 ± 0.13	0.71 ± 0.26	0.91
Total cholesterol (mg/dL)	182.62 ± 60.81	183.73 ± 52.35	0.95
Triglyceride (mg/dL)	132.81 ± 66.67	131.83 ± 81.66	0.97
HDL (mg/dL)	47.68 ± 8.50	44.92 ± 9.34	0.41
LDL (mg/dL)	116.31 ± 50.05	123.92 ± 54.97	0.69
Total protein (g/dL)	7.35 ± 0.83	7.48 ± 0.58	0.60
Albumin (g/dL)	4.42 ± 0.30	4.44 ± 0.32	0.81
AST (U/L)	22.26 ± 8.23	23.30 ± 8.73	0.63
ALT (U/L)	21.60 ± 11.93	22.83 ± 12.35	0.69
GGT (U/L)	26.17 ± 26.36	24.23 ± 14.09	0.72
ALP (U/L)	138.13 ± 77.51	99.57 ± 50.51	0.31

BMI = Body mass index, WBC = White blood cell, MCV = Mean corpuscular volume, FT3 = Free triiodothyronine, FT4 = Free thyroxine, TSH = Throid stimulating hormone, HDL = High density lipoprotein, LDL = Low density lipoprotein, AST = Aspartate aminotransaminase, ALT = Alanine aminotransaminase, GGT = Gamma glutamyl transferase, ALP = Alkaline phosphatase

negative correlations of native thiol and total thiol levels with BMI (r = –0.278, $p = 0.031$ and $p = 0.0032$, respectively), blood urea nitrogen (r = −0.277, $p = 0.037$) and HDL level (r = –0.462 and r = –0.469, $p = 0.012$ and $p = 0.010$, respectively) (Table 2).

We found a significant correlation between BMI and total thiol and native thiol. No significant correlation was found in the control group. There was a significant negative correlation between the patient group and BMI. (Table 3).

Table 2. Pearson correlation analysis confirmed significant negative correlations of native thiol and total thiol levels with BMI, blood urea nitrogen and HDL level

		Native thiol	Total thiol
BMI	Pearson Correlation	-0.278	-0.278
	Sig. (2-tailed)	**0.031**	**0.032**
Glucose	Pearson Correlation	-0.065	-0.069
	Sig. (2-tailed)	0.625	0.602
Creatinine	Pearson Correlation	0.049	0.036
	Sig. (2-tailed)	0.711	0.788
Urea	Pearson Correlation	-0.277	-0.277
	Sig. (2-tailed)	**0.037**	**0.037**
GGT	Pearson Correlation	0.088	0.108
	Sig. (2-tailed)	0.506	0.416
Albumin	Pearson Correlation	0.143	0.107
	Sig. (2-tailed)	0.290	0.428
Total cholesterol	Pearson Correlation	0.217	-0.252
	Sig. (2-tailed)	0.240	0.172
Triglyceride	Pearson Correlation	-0.011	-0.020
	Sig. (2-tailed)	0.950	0.909
HDL	Pearson Correlation	-0.462	-0.469
	Sig. (2-tailed)	**0.012**	**0.010**
LDL	Pearson Correlation	-0.197	-0.239
	Sig. (2-tailed)	0.296	0.204
Hemoglobin	Pearson Correlation	0.159	0.186
	Sig. (2-tailed)	0.230	0.159
Platelet	Pearson Correlation	-0.022	-0.037
	Sig. (2-tailed)	0.871	0.779
FT3	Pearson Correlation	0.310	-0.307
	Sig. (2-tailed)	0.084	0.087
FT4	Pearson Correlation	-0.028	-0.003
	Sig. (2-tailed)	0.877	0.988
TSH	Pearson Correlation	-0.141	-0.192
	Sig. (2-tailed)	0.305	0.161

BMI = Body mass index, GGT = Gamma glutamyl transferase, HDL = High density lipoprotein, LDL = Low density lipoprotein, FT3 = Free triiodothyronine, FT4 = Free thyroxine, TSH = Throid stimulating hormone

Tablo 3. Pearson correlation analysis of BMI between patient and control group

	Total thiol		Native thiol	
Patient group	Pearson Correlation	-0.562	Pearson Correlation	-0.552
	Sig. (2-tailed)	**0.001**	Sig. (2-tailed)	**0.002**
Control group	Pearson Correlation	-0.072	Pearson Correlation	-0.061
	Sig. (2-tailed)	0.704	Sig. (2-tailed)	0.747

BMI = Body mass index

DISCUSSION

In this study; total thiol, native thiol and disulphide levels, which playing key roles in responding to oxidative stress in patients with *H. pylori* infection, were evaluated. No significant differences between the infected patients and control participants were observed even though total thiol and native thiol levels were lower in the *H. pylori* patients. Total and native thiol levels were correlated with BMI, HDL, BUN levels.

Thiols are organic compounds that include a sulfhydryl group with a critical role in reacting to oxidative stress in cells, and many previous studies investigated low molecular weight protein thiols. In this study, the method preciously described by Erel and Neselioglu [22] was used to determine plasma dynamic thiol disulphide level. In Naja *et al*.'s study [23], evaluated total thiol as oxidative stress level, has found no significant difference in the plasma thiol level in *H. pylori* infected patients compared with controls.

H. pylori has several virulence factors (CagA,VacA, urease, GGT, etc). These factors are strongly associated with carcinogenesis, severity of inflammation, duodenal ulcer, and probably different levels of oxidative stres [24]. There are some studies, emphasizing the importance of these virulence factors (vag A +, GGT +, NapA +) in relation to the severity of oxidative stress [25-27]. In our study when the endoscopic results were stratified as mild (normal or only superficial gastritis) and severe (ulcer or erosion formation), statistically significant differences in disulphide, disulphide/native thiol, disulphide/ total thiol and native thiol/ total thiol values obtained (Figure 2).

Thioredoxins are highly conserved throughout a wide range of organisms, and they are essential for the isurvival of oxygen-sensitive cells. The gastric pathogen *H. pylori* uses the thioredoxin system to maintain its thiol/disulfide balance. The glutathione-glutaredoxin (GSH) reduction system is often used in

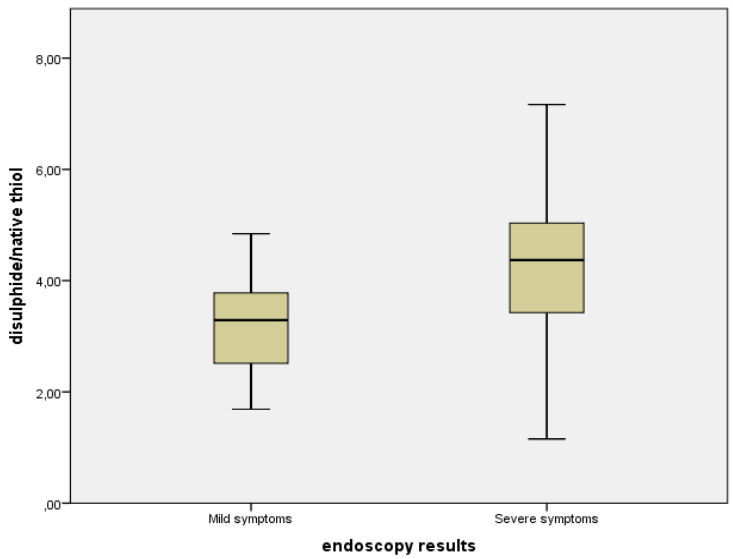

Figure 2. Disulphite/native thiol distribution in groups. Significant differences were observed in mean disulphite/native thiol levels when the *H. pylori* patients were stratified by mild or severe symptoms ($p = 0.02$)

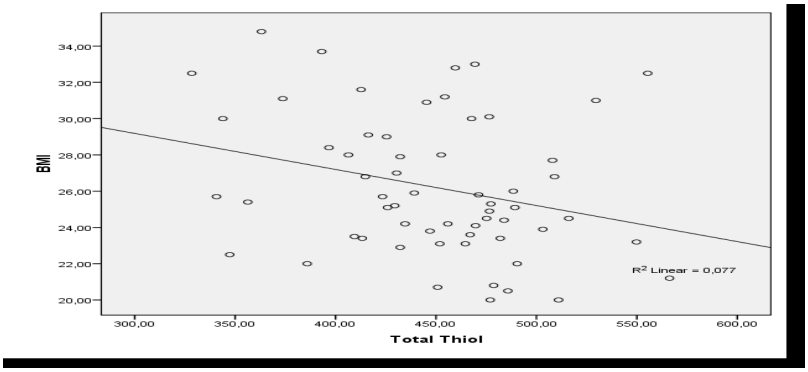

Figure 2. Pearson correlation analysis confirmed negative correlations of total thiol levels with BMI (*H. pylori* positive participants) (r = -0.562, p = 0.001).

addition to the Trx system in bacteria to maintain a reduced state inside the cell [22]. Organisms that lack GSH, such as Lactobacillus casei, Bacillus subtilis, Bacteroides fragilis, Staphylococcus aureus, and *H. pylori*, presumably must rely on the Trx system to maintain thiol/disulfide balance in the cell [21, 23-26]. The mechanisms for the maintenance of this balance in *H. pylori* have not been studied in great detail. Some studies suggest mutation of these genes are important for response to oxidative stress. Also infection with these mutant helicobacter strains may affect the oxidative stress level in host.

In addition to *H. pylori*, there are many other stressors affecting gastrointestinal tract such as nonsteroidal anti-inflammatory drugs, gastric acid, ischemia-reperfusion, and mental stresses. Theses stressors generate free radicals within gastrointestinal tissues. Generally, gastrointestinal tract can withstand such oxidative stresses to some extent by enhancing its antioxidant system [28]. Briefly, the difference results in oxidative stress levels might be due to both host-induced factors, h pylori and its different strains.

Many recent publications have evaluating the correlation between oxidative stress, and obesity [29-32]. Although this condition is thought to be caused by deterioration of oxidant-antioxidant equilibrium as a result of increased inflammation and body distribution along with obesity, there are also studies that have not been found to correlate with BMI and oxidative stress [33, 34]. In our study, We found a significant correlation between BMI, totol thiol and native thiol. However, when the patient and control group were evaluated separately, this negative correlation was evaluated only in the patient group in the pearson correlation analysis. No significant correlation was observed in the control group. We think that this situation is due to increased oxidative stress by addition of obesity in *H. pylori* patients with low total and ative thiol levels. (Figure 3).

Additionally, we also found a significant correlation between HDL, BUN levels and total, native thiol levels. This correlation was also found in other studies with similar method [29, 35, 36].

Limitations

One of the study limitations was that the number of participants in the severity-stratified groups was not large enough to allow for evaluation of subpopulations.

CONCLUSION

In conclusion, no significant correlations of total thiol, native thiol and disulphide levels with the presence of *H. Pylori* were observed, even though total thiol and native thiol levels were lower in the *H. pylori* group. However, total thiol, native thiol and disulphide levels were observed to be significantly decreased in patients with severe symptoms. And we also observed BMI and the total and native thiol levels of *H. pylori* patients were inversely related.

Ethics approval and consent to participate

The study was approved by the Erzurum Training and Research Hospital ethics committee (ethics committee approval number: 37732058-53/671), and was conducted following the ethical guidelines of the Declaration of Helsinki.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Funding

This work was supported by the Prof. Dr. Özcan EREL.

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The European Research Journal 2019;5(6):957-961 ORIGINAL ARTICLE

Family history in developmental dysplasia of the hip: should we follow-up?

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DOI: 10.18621/eurj.442402

ABSTRACT

Objectives: Developmental dysplasia of the hip (DDH) is an important problem. Ultrasonography (US) is a proper method before 6 months of age. For older children, plain radiographs can be useful. Six risk factors are emphasized: breech presentation, female sex, a positive family history, being first-born, left hip affected, and mode of delivery. In some centers, clinicians prefer to perform a control US examination or pelvic radiographs after 6 months of age for the children having a positive family history. We aimed to evaluate the necessity of control US/direct radiography examinations.

Methods: A total of 205 children with a positive family history for DDH are included. US examinations are performed according to Graf's method. We have evaluated direct radiographs by using Hilgenreiner, Perkin, and Shenton lines, acetabular angle.

Results: Initial US examinations are performed at a median age of 8.3 weeks. Seventy-four patients (36%) had a repeat ultrasound scan at a median age of 7 months; none of them demonstrated abnormal findings. One hundred and thirty-one patients (63.9%) had control radiographs at a median age of 8.2 months. Shenton line is considered as normal, and the upper femoral epiphysis is located in inferomedial quadrant according to Hilgenreiner and Perkin lines.

Conclusions: A positive family history for DDH may be a less important reason for performing control US or radiographic examination. Patients with a normal screening US result and having risk factors can be discharged from follow up safely, so that unnecessary examinations and family anxiety will be reduced.

Keywords: Developmental dysplasia of the hip, Graf method, ultrasound, family history, follow-up

Received: July 10, 2018; Accepted: November 11, 2018; Published Online: July 22, 2019

Developmental dysplasia of the hip (DDH) is a common and important problem, with a prevalence of 0.1 to 2/1000 children. Delayed diagnosis and treatment can cause premature degenerative joint disorder, functional impairments, chronic pain, permanent disability, etc. Screening with only physical examination provides a correct diagnosis approximately 50% of cases with dislocated hip by the first year of life. Screening with ultrasonography (US) reduces the rates of open reductions and complications by 46% [1-4].

Graf's US investigation technique is used widely as a screening tool for DDH diagnosis [5]. Screening of all children with US is not widely accepted. It is recommended to perform US to the cases that have a physical examination finding [6, 7]. US is a proper

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e-ISSN: 2149-3189

method for the children before 6 months of age, because at this time femoral head is largely cartilaginous. For the children older than this, plain anteroposterior pelvic radiographs can be useful [8, 9].

In the literature six common risk factors are widely emphasized for DDH: breech presentation, female sex, family history, first-born, left hip affected, and mode of delivery [10]. Before the usage of US screening, studies reported that the incidence of late DDH is higher in children with a positive family history [11]. In some centers, especially in the ones who are not specifically interested in DDH cases, clinicians prefer to perform a control US examination or pelvic radiographs after 6 months of age for the children having a positive family history for DDH [12].

In the current study, we aimed to evaluate the necessity of control US/direct radiography examinations for the children with a positive family history of DDH. So that, unnecessary examinations and exposure to ionizing radiation might be reduced.

METHODS

Approval for this prospective study was granted by the institutional ethics review board. The study was conducted retrospectively, in two different centers. We have retrospectively evaluated 683 children who were screened for DDH. We have excluded the ones with a US examination result other than Graf type 1 hip. We have included the ones with a positive family history and a follow up examination. US results were not normal (Graf type 1) in 81 cases, family history is negative in 77 cases, 320 cases are not reexamined. Finally, 205 children are included into the study.

We have evaluated screening US results, and control US/direct radiography results. US examinations are performed according to Graf's method (Figure 1). We have evaluated direct radiographs by using Hilgenreiner, Perkin, and Shenton lines, acetabular angle (Figure 2). Patient's ages and sex, accompanying risk factors, other than family history are also noted.

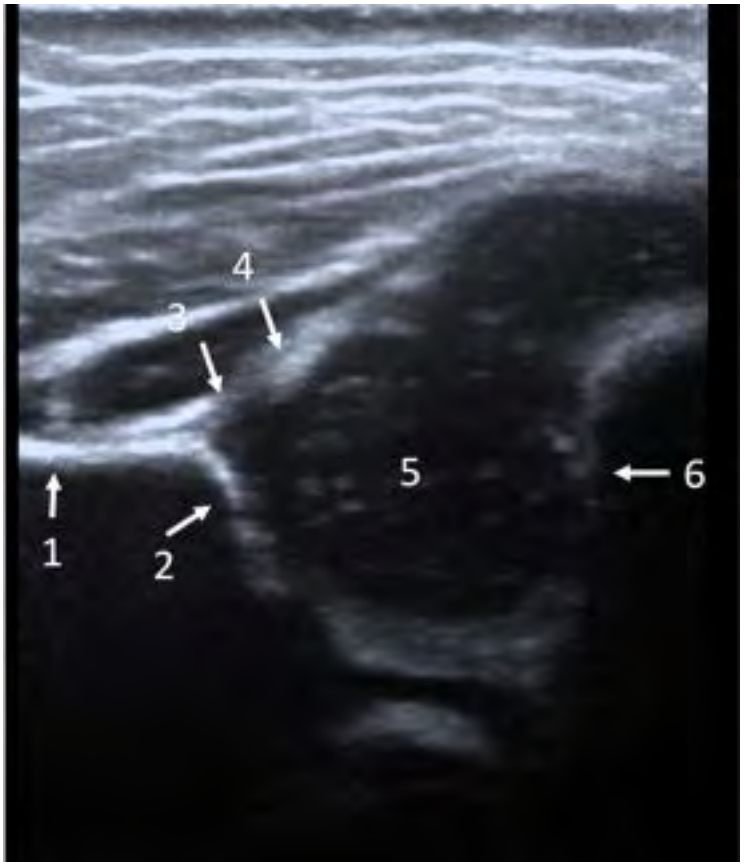

Figure 1. Coronal US view of a normal hip. 1, iliac bone; 2, lower limb of the ilium and bony acetabular roof; 3, cartilaginous acetabular roof; 4, acetabular labrum; 5, cartilaginous part of the femoral head (hyaline cartilage); 6, chondro-osseous junction between the bony part and the cartilaginous part of the femoral neck.

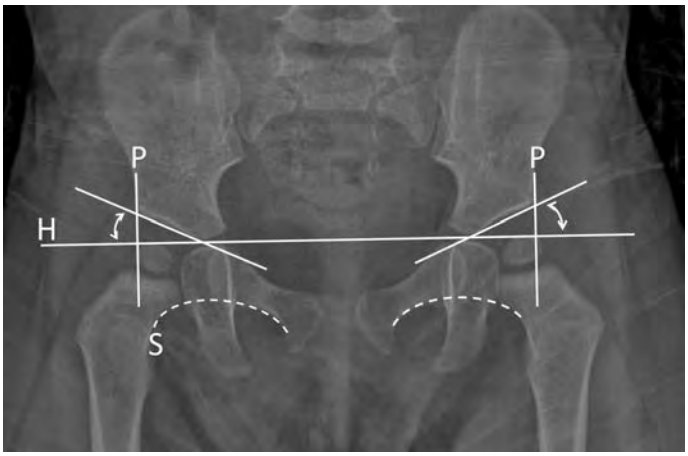

Figure 2. H: Hilgenreiner line, P: Perkin line, S: Shenton line, arrow: acetabular angle.

Statistical Analysis

All study information was recorded on patient data sheets, then entered into an Excel (2007, Microsoft Corp., Redmond, WA) spreadsheet for analysis. All data entries were double-checked by one of the investigators. Data were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows 20 (IBM SPSS Inc., Chicago, IL). Normal distribution of the data was evaluated with the Kolmogorov-Smirnov test. Numeric variables that had a normal distribution were shown as mean±standard deviation. The variables that did not have a normal distribution were shown as median (interquartile range). For comparison of the numeric variables between the two groups student's T test and Mann-Whitney U test were used.

Table 1. Patients having two control radiographs

Patient no	1st acetabular angle	2nd acetabular angle
1	26.5	21.8
2	27.3	21.8
3	26.7	20
4	27.1	21
5	26.8	20.8
6	27.2	21.3
7	27.1	21.7

RESULTS

Mean age of the population is 10 months ± 3 weeks. Population consists of 141 (68.8%) girls and 64 (31.2%) boys. The initial US examinations are performed at a median age of 8.3 weeks (range 6.3-12 weeks). 74 patients (36%) have a repeat ultrasound scan at a median age of 7 months (range 6-12 months); none of them demonstrate abnormal US or physical examination findings, and accepted as normal.

One hundred and thirty-one patients (63.9%) have control radiographs at a median age of 8.2 months (range 6-21.2 months). For all of the patients, Shenton line is considered as normal, and the upper femoral epiphysis is located in inferomedial quadrant according to Hilgenreiner and Perkin lines.

Mean acetabular angle is 24.3 ± 0.7. Seven patients (3.4%) have multiple direct radiography examination (2 direct radiographs). We have used the initial examination to calculate mean acetabular angle. Amongst these 7 patients, 4 have multiple risk factors for DDH. Initial acetabular angles of these 7 patients are all higher than 26.4, however their final acetabular angles are within normal limits, lower than 22 (Table 1).

Table 2. Patients having multiple risk factors

Patient no	Risk factors	Acetabular angle
1	BP, F	25
2	BP, F	26
3	BP, F, DB	27,3
4	F, BP	25,3
5	BP, F, DB	27,1

BP = Breech presentation, F = Female, DB = Difficult birth

5 patients have multiple risk factors (more than two) (Table 2). Amongst them, only 4 have multiple direct radiography examination (2 direct radiographs). Mean acetabular angle of these 5 patients is slightly higher than whole population, but it is not statistically significant (26.2 ± 0.1 vs. 24.3 ± 0.7, $p > 0.01$) (Figure 3).

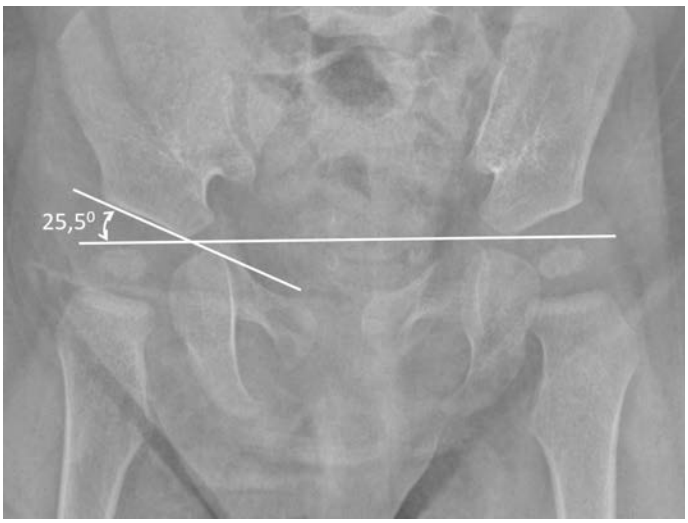

Figure 3. A patient with multiple risk factors. Acetabular angke is measured as 25.5 degrees.

DISCUSSION

DDH is still a common and important problem. An effective treatment, can prevent permanent disability [13]. The clinical evaluation for DDH is attributed to pediatrics professor Marino Ortolani. However, physical examination is not always enough to diagnose dysplastic, unstable or even dislocated hips. US, which were popularized by Graf in the 1980s, have been widely used to define and detect DDH [3, 14].

A positive family history is thought to be a strong risk factor for DDH, as it is stated in the literature that an abnormal US result is found three times more in children with a positive family history for DDH [15]. When the children is examined with only physical examination, it is found that late dysplasia occurs in 18 % of children [11]. This finding creates a clinical habit to perform a radiographic examination between 6-12 months.

Using both US examination and pelvic radiograph is still a method for some clinics. According to data obtained from British Society for Children's Orthopedic Surgery, 35 %of surgeons said that they request a control radiograph from the patients with a normal ultrasound scan [16]. Price *et al*. found that an abnormal Radiograph is found only 0.5 % of 11,000 patients with a normal initial US examination. They stated that a control radiograph is not necessary for patients with a normal US scan [17]. Specifically, some studies investigated the children with a positive family history for DDH to define the necessity of control radiographic examinations. It is stated that residual dysplasia in children with a family history of DDH and a normal hip ultrasound is not found significantly [16, 18]. Our results are consistent with the literature, we have not detected any cases of late DDH, with a normal US result and positive family history.

The acetabular angle measured by using Hilgenreiner line is generally less than 28º at birth. The angle will become smaller by age, and should measure less than 22º at and beyond 1 year of age [19]. All of the children, who were classified as normal at initial US examination, have acetabular angles within normal limits. All of the patients who have more than one control direct radiographs have higher acetabular angles in comparison with the population. It is showing that a relatively higher acetabular angle, even within normal limits, might cause an unnecessary control radiographic examination. In the literature, it is stated that relatively high acetabular indexes come to normal limits in control examinations [12], even in children with risk factors. Our results are consistent with the literature; we cannot detect any late DDH cases in children with a relatively high acetabular angle.

In the literature there is not a similar study examining the possible correlations between presence of the risk factors and acetabular angle values. According to our results, children with multiple risk factors had slightly higher acetabular angles, but this is not a statistically significant difference. This might be the result of our small population. Further studies with larger populations can enlighten a possible correlation.

Limitations

The study has some limitations. First, the retrospective nature of the study is a limitation. Second, we do not have enough cases with multiple risk factors. Control examinations are not the same for all patients (US for some cases, direct radiograph for others).

CONCLUSION

To conclude, having a positive family history for DDH is not a reason for performing control US or radiographic examination. Patients with normal screening US result and having risk factors can be discharged from follow up safely, so that unnecessary examinations and family anxiety will be reduced. If following up is still considered as necessary, US examinations can be performed instead of direct radiographs, as for avoiding ionizing radiation exposure.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2019;5(6):962-968 ORIGINAL ARTICLE

Autism spectrum disorders among adolescents and adults and comparison with schizophrenia

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DOI: 10.18621/eurj.441214

ABSTRACT

Objectives: Autism Spectrum Disorders (ASD) may be commonly misdiagnosed as schizophrenia due to common symptoms and accompanying psychotic manifestations in both adolescence and adulthood. The purpose of this study is to examine and compare the autistic symptoms and positive and negative symptoms of schizophrenia in cases diagnosed as Autism Spectrum Disorder.

Methods: Twenty-one patients between ages of 16-36 who have admitted to outpatient clinic have previously been diagnosed as autism spectrum disorders (autistic disorder, Asperger Syndrome, pervasive development disorder not otherwise specified) according to DSM-IV diagnosis criteria, have an IQ of 50 or above, have been included in the study. Control group have been composed of 21 patients between ages of 21-39 who have been diagnosed as schizophrenia according to DSM-IV diagnosis criteria and have an IQ of 50 or above. Psychiatric assessment has been made with Childhood Autism Rating Scale (CARS), Scale for the Assessment of Positive Symptoms (SAPS), Scale for the Assessment of Negative Symptoms (SANS), SCID-I and WAIS.

Results: The negative symptoms of ASD are found to be higher than schizophrenia cases where as the positive symptoms of schizophrenia cases are found to be higher than ASD cases. Twenty percent (n = 4) of OSB cases do not meet autism symptoms while none of the schizophrenia cases meet autism symptoms. In one case of the ASD group, additional schizophrenia diagnosis was present.

Conclusions: In this study, it has been found that negative symptoms of schizophrenia are widely observed in adolescent and adult patients followed with ASD diagnosis. Consequently, autism spectrum disorders are manifested common symptoms with schizophrenia in adolescence and adulthood.

Keywords: Autism spectrum disorders, shizophrenia, symptom

Received: July 6, 2018; Accepted: July 17, 2018; Published Online: January 18, 2019

Pervasive Development Disorders (PDD), is a group of neuropsychiatric disorder with a course of significant retardation an deviation in social, communication and cognitive development. In literature autistic disorder, Asperger Syndrome and pervasive developmental disorder not otherwise specified are referred to as "autism spectrum disorders (ASD)". Recent studies give ASD prevalence as 60-70/10,000 [1]. ASD symptomatology in adolescence and adulthood may not be as distinctive as in childhood [2]. For this reason, it is thought that it may be confused with some psychiatric disorders when in the diagnosis spectrum of Asperger Syndrome and Pervasive Developmental Disorder Not Otherwise Specified [3]. Before the stud-

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e-ISSN: 2149-3189

ies in adolescence and adulthood, its coexistence and common symptoms with childhood schizophrenia has attracted attention. While studies show high incidences of PDD symptoms in childhood schizophrenia [4, 5], a recent study asserts that 25% of childhood schizophrenia cases are diagnosed with additional PDD and considers two hypotheses. One of these is that autism-like symptoms may be the early stage symptoms of schizophrenia, and the other is that autism is a risk factor for the development of childhood schizophrenia [6]. In adulthood studies, some case statements support coexistence of ASD and psychotic disorders [7], while some studies assert that schizophrenia prevalence in ASD is not different from normal population [8]. Individuals with ASD accompanied by psychotic disorders show different autism symptoms than the cases without psychotic disorders [9]. Not only the cases with accompanying psychotic disorder but frequent accompanying of other psychiatric disorders may cause these individuals to apply to clinics and may result in the first diagnosis in adolescence or the main diagnosis to go unnoticed [10]. Considering all this confusion in diagnosis, DSM 5 released in 2013 has foreseen a single diagnosis category instead of different sub groups and diagnosis age has been annulled in order to improve the specificity of the autism diagnosis. But the new diagnosis system DSM 4-TR raised concerns that individuals diagnosed with pervasive developmental disorder may not be diagnosed. It has been shown that individuals diagnosed with ASD diagnosis according to DSM-IV TR diagnosis system may show distinctive autism symptoms, but they may not be diagnosed according to DSM-5 [11, 12]. With the symptom diversity, DSM 5 was abandoned to make categorical distinction and it was aimed to make it more homogenous by changes such as grading of symptom severity. But with the new diagnosis system, concerns about the diagnosis and comparison of the disorder with other disorders are on the agenda. In this study we tried to draw attention to difficulties at the diagnosis stage of ASD and to attributes which may be confused with schizophrenia in clinical practice.

METHODS

Forty-two patients (21 schizophrenia and 21 autism spectrum disorders) who were 16-36 years old, whose IQ is 50 and above were included in this study. Ethical board approval was obtained before the study, all patients were informed on interviews and scales and that their decision to join the study will not lead to a positive or negative change in their treatment and oral and written consent were taken from patients who accepted to contribute to the study. Structured Clinical Interview Scale for DSMIV Axis-I Diagnoses (SCID-I), Scale for the Assessment of Positive Symptoms (SAPS), Scale for the Assessment of Negative Symptoms (SANS), The Childhood Autism Rating Scale (CARS), Wechsler Adult Intelligence Scale (WAIS) and sociodeographic information form were applied to the patients.

Measures

Sociodemographic and Clinic Information Form: This form is applied by the interviewer to each patient to collect information on sociodemographical features and current and past clinical status Structured Clinical Interview Scale for DSMIV Axis-I Diagnoses (SCID-I): It is a structured clinical interview scale developed by First *et al.* [13] in 1997. Its adaptation to Turkish and reliability and validity study was carried out by Özkürkçügil *et al.* [14].

Scale for the Assessment of Positive Symptoms (SAPS): The SAPS was designed to assess positive symptoms, principally those that occur in schizophrenia. The instrument is intended to complement the SANS. The assessed positive symptoms include hallucinations, delusions, bizarre behavior, and positive formal thought disorder. The SAPS was developed by Andreasen [15]. The Turkish version was reported to be reliable by Erkoç *et al.* [16]. Scale for the Assessment of Negative Symptoms (SANS):The SANS assesses five symptom complexes to obtain clinical ratings of negative symptoms in patients with schizophrenia. These are affective blunting, alogia (impoverished thinking), avolition/apathy, anhedonia/asociality, and disturbance of attention. The final symptom complex seems to have less obvious relevance to negative symptoms than the other four complexes. Assessments are conducted on a 6 - point scale (0 = not at all to 5 = severe). The instrument was developed by Andreasen [17]. The Turkish version was reported to be reliable by Erkoç *et al.* [18].

The Childhood Autism Ratıng Scale (CARS): The

Table 1. Comparison of SAPS values

SAPS	ASD	Schizophrenia	*p value*
	Mean ± SD	Mean ± SD	
Hallucinations	0.6 ± 2.8	5.9 ± 4.8	**0.001**
Delusions	1.2 ± 2.7	11.7 ± 5.9	**0.001**
Bizarre Behavior	6.9 ± 3.8	2.62 ± 2.24	**0.001**
Positive Formal Thought Disorder	4.0 ± 3.6	4.1 ± 4.4	0.664
Inappropriate Affect	1.2 ± 1.1	0.1 ± 0.4	**0.001**
Total Score	14.2 ± 10.3	24.8 ± 13.4	**0.002**

ASD = Autism Spectrum Disorders, SAPS = Scale for the Assessment of Positive Symptoms, SD = standard deviation

scale assesses behavior in 14 domains that are generally affected by severe problems in autism, plus one general category of impressions of autism, with the aim of identifying children with autism, as differentiated from the other developmental disorders. The Turkish version was reported to be reliable a group from Dokuz Eylül Universıty Faculty of Medicine Child and Adolescent Health and Diseaeses Depertment. WAIS: The WAIS was developed by David Weschsler in 1955. It is translated to Turkish by Epir and Iskit [19].

Statistical Analysis

Data is analysed with SPSS 15.0 for Windows software. Besides descriptive statistical methods (mean, standard deviation), chi-square test is used to compare categorical variables and Independent-Samples T test is used to compare continuous variables .Mann Whithney U testis is used to compare non-normal distrubition variables. For all statistical analysis, $p < 0.05$ is reported as statistically significant.

RESULTS

Twenty-one ASD (11 autistic disorder, 4 development disorder not otherwise specified, 6 Asperger Syndrome) and 21 schizophrenia cases were included in the study. Both groups consisted of 2 women and 19 men. The average age of ASD cases was 20.9 ± 6.2 years (range; 16 to 36); and the average age of schizophrenia cases was 32.4 ± 4.5 years (range; 24-38). ASD group had average IQ of 78.6 ± 14.3 (range; 52-110), and schizophrenia group had average IQ of 86.4 ± 12.0 (range; 68-111).

Table 2. Comparison of SANS values

SANS	ASD	Schizophrenia	*p valu*
	Mean ± SD	Mean ± SD	
Affective Flattening or Blunting	16.0 ± 6.1	2.2 ± 4.4	**0.001**
Alogia	10.0 ± 4.7	6.7 ± 2.4	**0.024**
Avoliton-Apathy	7.1 ± 3.8	7.4 ± 2.8	**0.667**
Anhedonia-Asociality	14.5 ± 3.0	10.8 ± 3.4	**0.001**
Inattention.	4.2 ± 2.3	1.1 ± 1.3	**0.001**
Total Score	51.8 ± 17.5	35.5 ± 10.0	**0.002**
Total Score	51.8 ± 17.5	35.5 ± 10.0	**0.002**

ASD = Autism Spectrum Disorders, SANS = Scale for the Assessment of Negative Symptoms, SD = star deviation

The first application age of ASD cases was 7 ± 5.4 years, and those of schizophrenia cases were 24 ± 3.2 years. In the family histories of ASB group, 1 case had schizophrenia history, 1 case had pervasive developmental disorder history, 2 cases had mood disorder (depression) history, 3 cases had mental retardation history; in schizophrenia group, 6 cases had schizophrenia history, 2 cases had mood disorder history. ASB group had 3 cases with epilepsy history, while schizophrenia group had no cases with epilepsy or systemic disease history.

When we look at the SAPS values, general total score of schizophrenia group was significantly higher than ASD group (24.8 ± 13.4versus 14.2 ± 10.3) (Table 1); SANS values of ASD group was significantly higher than schizophrenia group (51.8 ± 17.5 versus 35.5 ± 10.0) (Table 2).

CARS values of ASD group was significantly higher than schizophrenia group (32.4 ± 4.4 versus 21.4 ± 1.3). In ASD group 5 cases (23.8%) did not meet the diagnostic criteria of autism according to CARS, 11 cases (52.4%) were mild to moderate autistic, 5 cases (23.8%) were highly autistic. In schizophrenia group none of the cases met the diagnostic criteria of autism (Table 3).

DISCUSSION

In this research study, adolescent and adult ASD and schizophrenia cases have been compared with respect to sociodemographic attributes, autism and schizophrenia symptoms. In this study, while the ASD group showed more indications of autism, none of the

Table 3. Comparison of CARS values

CARS	OSB	Schizophrenia	*p value*
	Mean ± SD	Mean ± SD	
Relating to People	2.9 ± 0.4	2.3 ± 0.3	**0.001**
Imitation	1.0 ± 0.4	1.0 ± 0	0.317
Emotional Response	2.6 ± 0.4	1.8 ± 0.3	**0.001**
Body Use	2.4 ± 0.6	1.2 ± 0.3	**0.001**
Object Use	2.0 ± 0.6	1.07 ± 0.2	**0.001**
Adaptation to Change	1.7 ± 0.8	1.1 ± 0	**0.001**
Visual Response	2.6 ± 0.5	1.7 ± 0.3	**0.001**
Listening Response	2.1 ± 0.5	1.3 ± 0.3	**0.002**
Taste, Smell, Touch	1.5 ± 0.8	1.0 ± 0	0.658
Fear or Nervous	1.8 ± 0.6	1.9 ± 0.3	**0.001**
Verbal Communication	2.2 ± 0.8	1.2 ± 0.2	**0.001**
Nonverbal Communication	2.3 ± 0.5	1.5 ± 0.3	0.083
Activity Level	2.2 ± 0.6	1.9 ± 0.2	**0001**
Level & Consistency of Intellectual Response	1.9 ± 0.4	1.1 ± 0.3	**0.001**
General Impression	2.6 ± 0.6	1.0 ± 0	**0.001**
Total Score	32.4 ± 4.4	21.4 ± 1.3	**0.001**
	n (%)	n (%)	
No Autism	5 (23.8)	21 (100)	
Mild-Moderate Autism	11 (52.4)	0	
Severe Autism	5 (23.8)	0	

ASD = Autism Spectrum Disorders, CARS = Childhood Autism Rating Scale, SD = standard deviation

patients in the schizophrenia group was diagnosed with autism. In the ASD group 5 patients who showed no symptoms of autism, had Asperger Syndrome. Although Asperger Syndrome is a variant of high functioning autism disorder by some clinicians, a definitive differentiation of these two disorders has not been clarified yet. While the discussion is ongoing, DSM 5 aims a scaling of the disorder based on severity rather than a categorical approach, and so a more sensitive definition. In our study, the first reason of having cases with no autism symptoms in the ASD group is that the CARS scale (although applicable in all pervasive development disorders) is a specific for autistic disorder, and the second reason is the mild course of symptoms of pervasive development disorders in adolescent and adult cases, especially with high IQ levels. It is known that in adolescence symptoms related to social interaction, and in adulthood symptoms related to rituals and limited area of interests tend to have a milder course [2]. In CARS scale, no meaningful difference was observed in the imitation, taste, smell and touch reactions subscale which are frequent symptoms of autism; this can be explained by the regression of autism symptoms and improved self-control of cases (especially with high IQ levels) in adulthood. The lack of difference in nonverbal subscales can be explained by the fact that this attribute is related to the negative symptoms of schizophrenia. According to CARS scale, the only subscale which is significantly higher in schizophrenia cases than ASD cases is fear and nervousness subscale. In schizophrenia, especially delusion of persecution results in high detections of this subscale.

The positive symptoms of schizophrenia patients included in our study are detected to be higher that those of the ASD group. But the ASD group was significantly higher than the schizophrenia group in bizarre behavior - and inappropriate affect subscale. The reason for this can be the core symptoms of ASD being recurrent, sometimes obsessive and sometimes bizarre behavior. However inappropriate affect does not allow diagnosis of paranoid schizophrenia. Surprisingly, there is no significant difference between the two groups in positive formal thought disorder subscale. While core symptoms of ASD as insufficient communication skills, undeveloped language skills, occasional difficulties in building sentences or use of pronouns, occasional inability of accentuation in speech, may all reflect as positive formal thought disorder; it can also be explained with the high IQ levels, and accordingly higher education and better language and communication skills of ASD cases. When we look at the negative symptoms; while ASD group is significantly higher in blunted affect, alogia, anhedonıa and social withdrawal, and attention subscales, there is no significant difference in avoliton-apathy subscale. Reduced gestures, emotional unresponsiveness, inability in accentuation, lack of eye contact in the blunted affect subscale are all frequent symptoms of ASD. Also, poor conversational content of the ASD group, which is due to inability of communication and social skills or limited areas of interest, may lead to significantly high alogia scores. Inability to make friends, inability of intimacy, lack of interest in entertainment or inability to express and share topics of interest, which are quite important for ASD diagnosis may lead to rise in anhedonia-asociality subscale. The fact that problems related to attention and ADHD is one of the most frequent comorbidities of ASD for especially children and adolescents; and that inability to socially recognize surroundings is a core symptom of ASD, may lead to rise in attention subscale. In ASD negative symptoms are on the forefront rather than positive symptoms [20, 21]. Nevertheless, although none of the schizophrenia cases in our study show autistic disorder diagnosis, there are studies showing autism symptoms in schizophrenia, which are frequently related to negative symptoms [22, 23]. Especially recent studies assert that schizophrenia accompanied with autistic symptoms is different in demographical, psychopathological and cognitive aspects; although positive symptoms are not on the forefront social withdrawal inability of executive functions are on the forefront; and that this is related to ASD symptoms [24].

In our study group 1 out of 21 cases (approximately 1%) is additionally diagnosed with schizophrenia. Various studies affirm coexistence of varying degrees of schizophrenia and psychotic disorder in adolescent and adult ASD groups [8, 25-28].

Limitations

There are limitations to our study. The cases assessed in this study, are cases followed up at the

child, adolescent and adult psychiatry clinics of a training research hospital. Individuals with ASD are frequently followed up at rehabilitation units and when they apply to clinics due to accompanying psychiatric complaints, more severe symptoms are expected and so it is difficult to generalize the collected data. Also, the fact that ASD group cases are adolescent and adults, may have affected the frequency and distribution of psychiatric problems which have not emerged yet. In consideration of ASD core symptoms, it might be more significant to use disorganized schizophrenia cases as control group. Low number of cases is also a limitation of the study. As the psychiatric assessment is cross sectional, we believe that a longitudinal study will be more useful.

CONCLUSION

Consequently, this study shows high incidence of negative symptoms of schizophrenia in adolescents and adults diagnosed with ASD. Accordingly, it is important to distinguish adolescent and adult ASD patients at the clinic and to take into consideration the confusion of diagnosis to common symptoms with other psychiatric disorders. Also, applicant to clinic with other psychiatric problems, having difficulties in social and communal interactions, NOS-PDD, AS, and HFA which are groups of autism with milder symptoms especially in adolescents, must be considered for diagnosis. Schizophrenia and ASD are to separate neurodevelopmental disorders with different courses, and it is clinically useful to understand the differences in accordance with the latest categorization of disorders. Although the border between the two disorders are not yet clear, there are studies showing neurobiological, epidemiological, cognitive and clinical relations [29, 30]. When we consider coincident symptoms and the probability of being seen together, clarity of diagnosis in clinical practice becomes even more important. For this, long term follow-ups, family studies, genetics and advanced neuroimaging are necessary.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The author disclosed that they did not receive any grant during conduction or writing of this study.

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Role of B-type natriuretic peptide in diagnosis of coronary artery disease

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DOI: 10.18621/eurj.447914

ABSTRACT

Objectives: B-type natriuretic peptide (BNP) has been extensively studied as a biomarker in heart failure. There is clear benefit of BNP in diagnosis and risk stratification of several cardiac diseases including acute coronary syndromes. Our aim was to evaluate diagnostic role of changes in BNP levels with exercise in coronary artery disease (CAD).

Methods: Fifty-one patients underwent exercise stress testing (EST) for suspected CAD and consequently underwent coronary angiography (CA) were prospectively enrolled. Patients with and without at least one significant diameter stenosis in major epicardial arteries (CA+ and CA-) versus patients with and without evidence of myocardial ischemia during exercise stress testing (EST+ and EST-) were classified into 4 groups, respectively (Group 1, CA+/EST+; group 2, CA+/EST-; group 3, CA-/EST+; and group 4, CA-/EST-). All patients underwent EST. Blood was drawn from patients for determination of BNP levels 10 minutes prior to, 10 minutes after and 4 hours after EST.

Results: EST parameters other than the parameters signifying myocardial ischemia didn't differ significantly among groups ($p > 0.05$). Pre-exercise, post-exercise and 4h-post exercise BNP values were significantly higher in group 1 and group 2 compared to group 3 and group 4 ($p < 0.05$ for all comparisons between the groups for pre-exercise, post-exercise and 4h-post exercise BNP). Exercise-induced increases in BNP were higher in group 1 and group 2. Patients with significant CAD involving LAD demonstrated higher basal and exercise-induced BNP as well as BNP increases, irrespective of the EST result.

Conclusions: Basal, maximal-exercise and post exercise BNP values predicted CAD, as well as CAD involving LAD irrespective of ischemic changes in EST. Our results point out potential role of BNP as an adjunct to EST in diagnosis and management of CAD.

Keywords: Natriuretic peptide, coronary artery disease, exercise stress test, myocardial ischemia

Received: July 25, 2018; Accepted: July 29, 2018; Published Online: July 30, 2018

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e-ISSN: 2149-3189

The idea that the heart is an endocrin organ was suggested by Kisch *et al*. [1] in 1956 with demonstration of secretory granules in atria of pigs. These secretory granules were later demonstrated to contain atrial natriuretic peptide (ANP). Subtypes and physiology of these peptides are studied extensively and have been defined since then. There are 3 main subtypes of natriuretic peptides: ANP, which is mainly secreted in atria; B-type natriuretic peptide (BNP) (known as brain natriuretic peptide), which is mainly secreted from ventricles [2]; Type-C natriuretic peptide which is mainly secreted from vascular endothelium, central nervous system and kidneys, and intestinal-type natriuretic peptide, secreted from gastrointestinal system mucosa [3].

Brain natriuretic peptide is synthesized in ventricle as a pro-BNP form consisting of 108 amino acids. This propeptide is later cleaved into active form, BNP, consisting of 32 amino acids and N-terminal pro-BNP by peptidases. Especially BNP has been extensively studied as a biomarker in mainly heart failure, and demonstrated prognostic significance in several different clinical settings. While there is clear benefit of BNP in diagnosis and risk stratification of several cardiac diseases including patients with acute coronary syndromes [4] and previous myocardial infarction [5], there is compelling evidence regarding role of this biomarker in management of transient myocardial ischemia in patients without concommitant structural heart disease [6].

Coronary artery diease (CAD) encompasses a wide range of clinical subsets from ST segment elevation myocardial infarction (STEMI) to stable angina pectoris. Common biomarkers used for diagnosis and management of CAD with acute presentation (unstable angina pectoris, non-ST segment elevation acute coronary syndrome, ST segment elevation acute coronary syndrome) such as creatin kinase-MB (CK-MB) and troponins are released into blood stream from irreversibly damaged myocardial tissue. In contrast, BNP is mainly secreted actively from reversibly damaged myocardial tissue. As a result, BNP was beneficial in assessing physiologic consequences of ischemia and infarct rather than the extent of damaged myocardial tissue. Our aim in this study was to evaluate diagnostic role of changes in BNP levels with exercise in CAD.

METHODS

Patients

This study was conducted in Bursa Yüksek İhtisas Training and Research Hospital. The patients were selected from patients who admit to outpatient clinic with signs and symptoms warranting exercise stress testing (EST). Twenty-six patients (18 males, 8 females, mean age 54.7 ± 1.7 years) with at least 50% narrowing in at least one of the 3 major epicardial coronary arteries, and 25 patients (15 males, 10 females; mean age 52.5 ± 1.8 years) with no narrowing or diameter stenosis < 50% in any of the major epicardial arteries were enrolled in this clinical study. Patients with severe valvular disease, New York Heart Association (NYHA) class III-IV heart failure, myocardial infarction in the last 2 weeks, with hepatic and/or renal dysfunction and patients with significant systemic disease other than diabetes mellitus (DM) were excluded from this study.

Study patients underwent detailed assessment for cardiovascular risk factors. Detailed past medical history, physical examination and laboratory parameters were evaluated for this purpose. Electrocardiography (ECG), chest X-ray, fasting plasma glucose, total cholesterol, LDL cholesterol, HDL cholesterol levels; hemoglobin, hematocrit, white blood cell counts were assessed.

All patients underwent standard transthoracic echocardiographic examination before EST. All patients underwent EST with standart Bruce protocol. Blood was drawn from patients for determination of BNP levels 10 minutes prior to, 10 minutes after and 4 hours after EST. The study protocol was approved by Bursa Yüksek İhtisas Training and Research Hospital ethical committee. Informed consent was obtained from all patients prior to enrollment in the study protocol.

Exercise Stress Testing

EST was performed in accordance with relevant guidelines on a treadmill. Beta blockers were witheld for at least 2 days, and digoxin was witheld for at least 2 weeks prior to EST. Electrocardiography, heart rate and blood pressure parameters and maximal exercise capacity in METs were recorder. At least 1-mm horizontal or downsloping ST-depression 60-80 msecs

after the end of the QRS complex, bundle branch blocks triggered by ischemia, atrial or ventricular dysrhythmias during EST was accepted as suggestive of ischemia.

Echocardiography

Echocardiography was performed on a 3.5 Mhz probe with a Vivid 7 Pro machine (General Electric, USA) in left lateral decubitus position. Parasternal short axis, long axis, apical 2 chamber, 3 chamber and 4 chamber views were acquired. Left ventricle diastolic functions were assessed with the use of mitral inflow patterns with pulsed-wave Doppler and tissue doppler imaging of basal septal and lateral walls of the left ventricle.

BNP Analysis

At least 5 cc of blood was drawn by phlebotomy into EDTA-tubes just before starting EST, within 10 minutes following completion of the EST and 4 hours after EST. Biosite Triage BNP test system (San Diego, California, Biosite incorporated) was utilized for determination of BNP levels. BNP levels are analysed and measured by immunoassay method and ersults are read by Triage MeterPlus (Biosite Co, San Diego, CA) in this system. Brain natriuretic peptide levels analyised by this method are affected by hypertension, diabetes, renal failure and chronic obstructive pulmonary disease [7]. Results are reported in picogram/milliliter (pg/mL). The minimum level of BNP that could be detected by this assay is 5 pg/mL.

Statistical Analysis

Statistical analysis was performed on statistical software SPSS version 17. Differences between groups with regard to continuous variables were examined by Mann-Whitney U test, differences with regard to categorical variables were examined by Pearson chi-square test and temporal changes in BNP levels were examined with the use of Wilcoxon signed-rank test. Findings were summarized as (percentage, mean, standard deviation). Threshold for statistical significance of the p value was accepted as ≤ 0.05.

RESULTS

Fifty-one patients who admitted to our cardiovascular diseases outpatient clinic, underwent EST for suspected CAD and consequently underwent coronary angiography were prospectively enrolled in this study. Patients with and without at least one significant diameter stenosis in major epicardial arteries (CA+ and CA-) versus patients with and without evidence of myocardial ischemia during

Table 1. Basal demographic characteristics of the study population

		Group 1 (CA+/EST+)	Group 2 (CA+/EST-)	Group 3 (CA-/EST+)	Group 4 (CA-/EST-)	Total
Age (years) (Mean ± SD)		54.0 ± 8.5	55.3 ± 9.0	50.4 ± 8.8	55.8 ± 9.4	53.6 ± 8.9
Gender, n (%)	Male	8 (66.7)	8 (57.1)	10 (66.7)	7 (70)	33 (64.7)
	Female	4 (33.3)	6 (42.9)	5 (33.3)	3 (30)	18 (35.3
Tobacco, n (%)	User	8 (66.7)	5 (35.7)	2 (13.3)	3 (30)	18 (35.3)*
	Not user	4 (33.3)	9 (64.3)	13 (86.7)	7 (70)	33 (64.7)*
DM, n (%)	Yes	1 (8.3)	3 (21.4)	2 (13,.)	3 (30)	9 (17.6)
	No	11 (91.7)	11 (78.6)	13 (86,.)	7 (70)	42 (82.4)
HT, n (%)	Yes	3 (25)	6 (42.9)	5 (33.3)	3 (30)	17 (33.3)
	No	9 (75)	8 (57.1)	10 (66.7)	7 (70)	34 (66.7)
Total cholesterol, n (%)	Yes	8 (66.7)	7 (50)	5 (33.3)	4 (40)	24 (47.1)
	No	4 (33.3)	7 (50)	10 (66.7)	6 (60)	27 (52.9)
Family history for CVD, n (%)	Yes	5 (41.7)	4 (28.6)	1 (6.7)	2 (20)	12 (23.5)
	No	7 (58.3)	10 (71.4)	14 (93.3)	8 (80)	39 (76.5)

DM = diabetes mellitus, HT = hipertension, CA = coronary angiography, EST = exercise stress test,*= $p < 0.05$

Table 2. Exercise stress test findings

	Group 1 (CA+/EST+)	Group 2 (CA+/EST-)	Group 3 (CA-/EST+)	Group 4 (CA-/EST-)	Total	*p* value
Achieved maximal MET	11.3 ± 2,6	10.6 ± 3.5	13.1 ± 3.2	11.8 ± 3.3	11.7 ± 3.1	0.77
Resting heart rate	86.6 ± 12.0	93.1 ± 17.6	88.4 ± 21.0	85.3 ± 14.9	88.6 ± 16.8	0.14
Maximal heart rate	142.1 ± 20.2	131.8 ± 18.3	176 ± 7.5	181.2 ± 16.4	157.7 ± 17.3	0.10
Heart rate recovery at 1st minute	25.5 ± 10.3	26.6 ± 8.4	31.4 ± 10.4	25.7 ± 14.2	27.5 ± 10.7	0.65

CA = coronary angiography, EST = exercise stress test, MET = metabolic equivalents

exercise stress testing (EST+ and EST-) were classified into 4 groups, respectively (Group-1, CA+/EST+; group 2, CA+/EST-; group 3, CA-/EST+; and group 4, CA-/EST-). Twenty-six patients (18 males, 8 females, mean age 54.7 ± 1.7 years) had at least 50% narrowing in at least one of the 3 major epicardial coronary arteries (Group 1 and group 2), and 25 patients (15 males, 10 females; mean age 52.5 ± 1.8 years) had no narrowing of more than 50% in any of the major epicardial arteries (Group 3 and group 4). All patients underwent EST with standart Bruce protocol. Blood was drawn from patients for determination of BNP levels 10 minutes prior to, 10 minutes after and 4 hours after EST.

Patients with and without significant stenoses in epicardial coronary arteries underwent EST and the patients were grouped in a factorial design into EST+ and EST-. Basal demographic properties of the study

Table 3. Transthoracic echocardiography findings

	Group 1 (CA+/EST+)	Group 2 (CA+/EST-)	Group 3 (CA-/EST+)	Group 4 (CA-/EST-)	Total	*p* value
Left ventricle ejection fraction (%)	54.8 ± 16.3	51.2 ± 11.4	65.6 ± 6.0	60.4 ± 6.8	58.0 ± 12.0	0.62
Left ventricle end-diastolic diameter (cm)	4.8 ± 0.5	4.6 ± 0.3	4.7 ± 0.5	4.5 ± 0.3	4.6 ± 0.4	0.91
Left ventricle end-systolic diameter (cm)	3.2 ± 0.5	3.2 ± 0.4	2.9 ± 0.3	3.1 ± 0.3	3.1 ± 0.4	0.95
E-wave amplitude (m/sn)	0.78 ± 0.26	0.65 ± 0.21	0.8 ± 0.21	0.79 ± 0.19	0.75 ± 0.22	0.88
A-wave amplitude (m/sn)	0.79 ± 0.19	0.79 ± 0.16	0.82 ± 0.3	0.78 ± 0.13	0.8 ± 0.21	0.46
E/A ratio	1.0 ± 0.39	0.83 ± 0.28	1.0 ± 0.4	1.0 ± 0.31	1.0 ± 0.35	0.67
Left atrium anteroposterior diameter (cm)	3.9 ± 0.4	3.7 ± 0.36	3.8 ± 0.43	3.6 ± 0.21	3.7 ± 0.36	0.23
Interventricular septum end-diastolic thickness(cm)	1.11 ± 0.12	1.12 ± 0.10	1.17 ± 0.22	1.0 ± 0.14	1.11 ± 0.16	0.09
Posterior wall end-diastolic thickness (cm)	0.99 ± 0.16	1.12 ± 0.10	1.17 ± 0.22	1.02 ± 0.14	1.11 ± 0.16	< 0.05

CA = coronary angiography, EST = exercise stress test

Table 4. Coronary angiography findings

Patient number	Group 1 (CA+EST+)	Group 2 (CA+EST-)	Group 3 (CA-EST+)	Group 4 (CA-EST-)
1-vessel disease	1 (8%)	0 (0%)		
2-vessel disease	2 (17%)	3 (21%)	0	0
3-vessel disease	9 (75%)	11 (78%)	0	0
LAD (%)	53 ± 39	47 ± 41.6	0	0
RCA (%)	33.3 ± 36.7	35.7 ± 40.3	0	0
Cx (%)	30.7 ± 35.3	44.6 ± 35.5	0	0

CA = coronary angiography, EST = exercise stress test, LAD = left anterior descending coronary artery, RCA = right coronary artery, CX = left circumflex coronary artery

patients are summarized in Table 1. When basal demographic characteristics of the study groups are compared, tobacco use was significantly more frequent in group 1 and group 2 compared to the remaining groups. Significant differences between groups with respect to DM, hypercholesterolemia were detected. Family history for cardiovascular disease was significantly more frequent in group 1 and 2 compared to the remaining groups, likewise.

EST findings of the patients are demonstrated in Table 2. No major complications such as ventricular arrhythmias or ST segment elevation were observed. There was no statistically significant difference between groups with regard to maximal achieved MET, resting heart rate, maximal heart rate and heart rate recovery at 1st minute of recovery.

Transthoracic echocardiography findings including left ventricle ejection fraction, end-diastolic and end-systolic diameters, E and A wave amplitudes of transmitral diastolic inflow waves, E/A ratios, left atrium anteroposterior diameter, and end-diastolic septal wall thickness didn't differ between groups. Posterior wall thickness was significantly higher in Group 1 compared to the remaining groups (Table 3). Coronary angiography revealed mainly 3-vessel disease in major epicardial coronary arteries in group 1 and group 2. Table 4 summarizes the severity of CAD in study groups. While left anterior descending coronary artery (LAD) lesions were apparently more dominant in group 1, left circumflex coronary artery (LCX) lesions in addition to LAD lesions were more severe compared to right coronary artery (RCA) lesions in group 2.

BNP levels before (BNP1), after (BNP2) and 4 hours after EST (BNP3) are summarized in Table 5. All values demonstrated significantly different values in group 1 and group 2 compared to group 3 and group 4. However, there were no significant differences when group 1 and group 2 and similarly group 3 and group 4 were compared with each other. Groups with

Table 5. Pre-exercise, post-exercise and 4-hours post-exercise BNP within study groups

	Group 1 (CA+/E+)	Group 2 (CA+/E-)	Group 3 (CA-/E+)	Group 4 (CA-/E-)	*p value*
BNP1 (pg/ml)	79.6 ± 46.8	42.5 ± 8.68	17.5 ± 5.8	11.8 ± 3.76	**< 0.05**
BNP2 (pg/ml)	88.8 ± 42.5	57.9 ± 12.1	18.8 ± 7.3	13.0 ± 3.66	**< 0.05**
BNP3 (pg/ml)	34.2 ± 31.6	41.9 ± 9.47	15.8 ± 4.9	10.0 ± 3.65	**< 0.05**
BNP2-BNP1 (pg/ml)	9.18 ± 6.02	15.37 ± 4.85	1.28 ± 1.66	1.19 ± 0.5	**< 0.05**
BNP3-BNP1 (pg/ml)	15.4 ± 4.9	26.8 ± 11.4	1.57 ± 0.4	1.1 ± 0.2	**< 0.05**

CA = coronary angiography, EST = exercise stress test

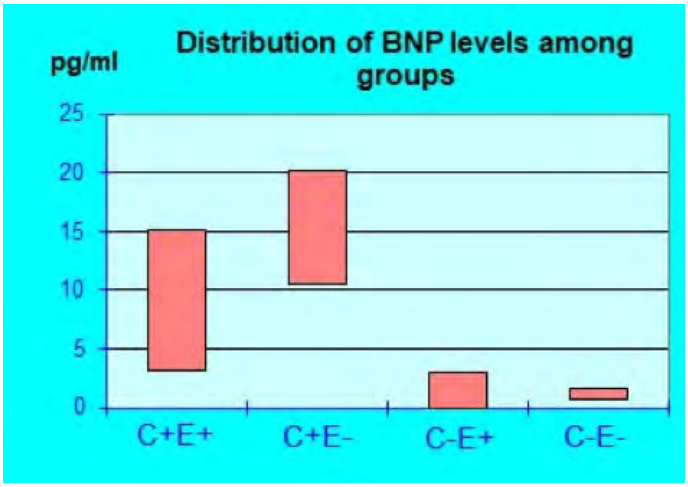

Figure 1. Distribution of BNP levels in study groups. C = coronary angiography, E = exercise stress test

significant CAD (group 1 and group 2) differed significantly from groups without significant CAD (group 3 and group 4) with regard to response of BNP levels to exercise (Figure 1).

DISCUSSION

There are 3 main findings of this study; (1) Pre-exercise BNP levels were significantly higher in patients with CAD compared to patients without significant CAD, (2) Exercise-induced increases in BNP levels are higher in patients with significant CAD, and (3) This difference between patients with and without CAD was independent of exercise induced ST segment depression and result of the EST.

EST retains its position as the first-line diagnostic test in non-invasive assessment of CAD. One-millimeter of ST segment depression during EST predicts CAD with a sensitivity of 75% and a specifity of 66%, while 2-mm ST depression predicts CAD with a 52% sensitivity and 86% specifity. EST demonstrated an overall diagnostic performance with a 46% sensitivity and 40% specifity in our study.

Diagnostic performance of temporal changes in BNP levels with exercise (BNP-2/BNP-1) was relatively higher compared to EST alone, with a sensitivity of 94% and a specifity of 70%. Diagnostic accuracy of EST was worse in our study compared to previous studies but diagnostic performance of temporal changes in BNP levels from pre-exercise levels to 10-minute levels was better compared to previous studies. Previous studies demonstrated a 80% sensitivity and 55% specifity of this assessment with a BNP cut-off value of 8 pg/mL [8]. As a result, addition of BNP assessments to EST might improve clinical decision making processes by potentially decreasing expenses, need for advanced invasive and noninvasive tests and complications due to invasive tests.

Several tests and parameters were suggested previously as an adjunct to EST to improve diagnostic accuracy of this test. Gender, age, chest pain, high blood total cholesterole levels, ST-segment depression were all shown to improve diagnostic accuracy of EST. Other predictors that increase EST sensitivity and specifity are exercise capacity, exercise induced angina pectoris, double product value, maximum systolic blood pressure, diabetes mellitus, tobacco use, abnormal resting ECG, high blood pressure, family history of CAD [9]. Similarly, adjunctive use of noninvasive tests such as cardiac tomography or myocardial perfusion scintigraphy were shown to increase accuracy of EST in detecting CAD, albeit with increased costs [10].

BNP is an important indicator of left ventricle dysfunction and myocardial ischemia. This biomarker increases in early phases following myocardial infarction [11]. Bassan *et al*. [12] previously showed that among patients presenting with ACS without persistent ST segment elevation, BNP levels were above 100 pg/mL in 70%, although only 50% of the study patients had elevated CK-MB or troponin I levels elevated at time of presentation. It was suggested in this study that BNP could be used in ruling out noncardiac cause of chest pain as well as in early diagnosis of myocardial ischemia [12].

BNP predicts LV remodelling and mortality following percutaneous coronary interventions in patients with acute coronary syndromes [13]. It similarly predicts heart failure and death independently in long-term follow-up in this patient group [14]. N-terminal BNP (NT-proBNP) is superior to troponin T in predicting prognosis in ACS patients and could be successfully used as an early indicator of myocardial ischemia. Typical temporal changes in BNP levels include a peak level reached within 24 hours following myocardial infarction and a following plateu. In large MI's a second peak occurs at 5th day

and this peak is suggested to be an indicator of remodeling [15].

Pre-exercise and peak exercise BNP levels were previously shown to be higher in patients with LAD and CX lesions compared with patients with RCA lesions independent of left ventricle end-diastolic pressures in patients presenting with unstable angina pectoris who had previous history of myocardial infarction [16]. Likewise, increases in BNP levels were higher in patients with LAD lesions compared to patients with CX and/or RCA lesions. This finding might be related with the size of myocardial territory supplied by the respective epicardial coronary artery.

Exercise-induced acute increases in BNP secretion is shown previously. Marumoto *et al* showed that increases in BNP levels with exercise as well as peak BNP values reached during maximal exercise were significantly higher in patients with exercise induced ischemia as shown by myocardial perfusion scintigraphy by Tl 201, compared to patients without inducible ischemia [17]. Brain natriuretic peptide was also shown to be an independent predictor of CAD in patients undergoing dobutamine stress echocardiography [18]. N-terminal pro-BNP was similarly found to have higher values and increase significantly more in patients with inducible ischemia by MPS compared to controls without ischemia. These values also correlated with severity of CAD in studies [18]. Another study yielded conflicting results with significantly increased pre-exercise, but not peak BNP values with CAD [16].

Accumulating evidence lead to rise of BNP as a potential candidate in diagnosis and risk stratification of CAD, so that ischemia-induced BNP levels were suggested as a biomarker for ruling-out ischemia, irrespective of left ventricle systolic dysfunction [5]. We excluded patients with left ventricular systolic and diastolic dysfunction. The fact that we observed consistent association between BNP levels as well as increases in BNP levels supports that BNP is an excellent biomarker for diagnosing and assessing the severity of CAD.

CONCLUSION

Pre-exercise and peak exercise values as well as increases in BNP levels are associated with presence and severity of CAD among patients undergoing EST independent of left ventricular systolic dysfunction. Our findings suggest that BNP should be used more actively in management of CAD in addition to its well-established role in diagnosis and management of heart failure.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2019;5(6):977-980 ORIGINAL ARTICLE

Neovascular age-related macular degeneration: 18-month outcomes of aflibercept treatment in patients resistant to ranibizumab

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DOI: 10.18621/eurj.446300

ABSTRACT

Objectives: Aim of this study is to investigate the effect of intravitreal aflibercept therapy in an 18-month period in patients with recurrent neovascular age-related macular degeneration resistant to intravitreal ranibizumab.

Methods: This is a prospective study of eyes with neovascular age-related macular degeneration switched to intravitreal aflibercept with at least 18 month of follow-up after the switch. All patients had had a minimum of 6 injections of ranibizumab before the switch. All patients received a loading dose of three intravitreal 2 mg aflibercept injections at 4-week intervals. Changes in best-corrected visual acuity, central macular thickness and the frequency of injections were compared.

Results: The study included 39 patients, each with one diseased eye. The studied eyes had received an average of 10.74 ± 4.38 previous intravitreal ranibizumab injections over a period of 28.31 ± 18.08 months. During the study, an average of 6.94 ± 2.58 intravitreal aflibercept injections were given in a period of 18 months. Mean central macular thickness at baseline, before switching to aflibercept, 6, 12, and 18 months after the aflibercept injection were 327.44 ± 120.57, 354.50 ± 127.79, 290.20 ± 112.25, 311.70 ± 119.47, and 299.29 ± 98.38 µm, respectively. A significant change was found in the macular thickness measured at intervals throughout the study. However, no significant improvement was found in visual acuity after 18 month after switching to aflibercept.

Conclusions: Switching from intravitreal ranibizumab, an inhibitor of vascular endothelial growth factor-A, to aflibercept, another inhibitor for such factors, has increased central macular thickness significantly without changes in visual acuity.

Keywords: age-related macular degeneration, aflibercept, ranibizumab, switch

Received: July 20, 2018; Accepted: August 28, 2018; Published Online: November 2, 2018

Age-related macular degeneration (AMD) is indicated as the leading cause of age-related severe vision loss in developed countries, especially in patients over 55 years [1]. Treatments involving the intravitreal administration of inhibitors of vascular endothelial growth factor-A (VEGF-A) have

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e-ISSN: 2149-3189

transformed the management of neovascular AMD (nAMD). Two such VEGF-A-inhibiting agents are ranibizumab (Lucentis®; Genentech, South San Francisco, CA, USA) and aflibercept (Eylea® ;Regeneron, Tarrytown, NY, USA). Both were approved by the United States Food and Drug Administration (FDA) for the treatment of various retinal diseases, the latter being the latest drug approved for the treatment of nAMD. Aflibercept is a recombinant protein produced by fusion of the VEGF-binding sequences of human VEGF receptors 1 and 2 along with the Fc backbone of human immunoglobulin G1 (IgG1) [2]. Aflibercept binds and inhibits the VEGF-A and VEGF-B isoforms as well as the placental growth factor, which is another member of the VEGF family, although binding of ranibizumab is restricted to VEGF-A isoform (2). In addition, aflibercept was reported to have a significantly higher binding affinity for VEGF compared to ranibizumab and bevacizumab, another such agent that had not been yet approved by the FDA but used off-label to treat retinal diseases [3]. Several studies have shown that anatomic response for ranibizumab and bevacizumab in n-AMD can be reduced over time, a phenomenon known as tolerance, tachyphylaxis, or resistance [4]. In this study, we aimed to investigate the efficacy of intravitreal aflibercept (IVA) in patients with nAMD who had been previously treated with intravitreal ranibizumab (IVR) but developed resistance to treatments. We have switched to and applied an 18-month aflibercept treatment, evaluating the changes in patients central macular thickness (CMT) along with their visual acuity in the course of treatment.

METHODS

The study adhered to the Declaration of Helsinki, and obtained exemption from full review by the local ethics committee. This is a prospective study of eyes with neovascular age-related macular degeneration switched to intravitreal aflibercept with at least 18 month of follow-up after the switch.All patients had had a minimum of 6 injections of ranibizumab before the switch. All patients received a loading dose of three intravitreal 2 mg aflibercept injections at 4-week intervals. Changes in best-corrected visual acuity (BCVA), central macular thickness (CMT) and the frequency of injections were compared.

Inclusion criteria were as follows: persistent intraretinal or subretinal fluid, at least six consecutive monthly injections with ranibizumab, and last injection of ranibizumab within one month of switching to aflibercept. We recorded demographic data, the total number of intravitreal ranibizumab injection, the time since initiation of anti-VEGF therapy, the interval between the last intravitreal ranibizumab injection and the first aflibercept injection.

All patients received a loading dose of three monthly aflibercept injections (2 mg/0.05 ml) and were followed up monthly. Retreatment with a single aflibercept injection was performed according to any of the following: visual acuity loss of at least five letters, with optical coherence tomography (OCT) evidence of fluid in the macula; persistent or recurrent intraretinal or subretinal fluid on OCT; new subretinal hemorrhage from choroidal neovascularization (CNV).

Fundus fluorescein angiography and indocyanine green angiography-ıf nessesary-were performed at the baseline visittoconfirmthe presence of AMD-related CNV and to exclude potential masquerade lesions such as polypoidal vasculopathy. At each visit, a full ophthalmic examination, including BCVA and IOP (intraocular pressure) assessment with Goldman applanation tonometry, spectral-domain optical coherence tomography (Heidelberg Spectralis; Heidelberg Engineering, Heidelberg, Germany) measurement of both eyes were conducted.

Statistical Analysis

All statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software (v22.0 for Windows; SPSS Inc., Chicago, IL, USA). Visual and anatomical outcomes comparing mean values was conducted by two-sample, two-sided t test. Statistical significance was defined as $p < 0.05$.

RESULTS

The study included 39 patients, each with one diseased eye. Throughout the study, the patients were given intravitreal injection of 2 mg/0.05 mL

Table 1. Desciriptive characteristics of the patient cohort at baseline

Characteristics	Data of the patients (n = 39)
Age, years (mean ± SD, range)	72.20 ± 7.14 (59-86)
Gender distribution, male / female n (%)	17 (44) / 22 (56)
Follow-up before switch, months (mean ± SD, range)	28.31 ± 18.08 (8-67)
Number of ranibizumab injections previous swicth (mean ± SD, range)	10.74 ± 4.38 (6-23)
BCVA, log MAR (mean ± SD)	0.98 ± 0.62
CMT (mean ± SD)	327.44 ± 120.57

BCVA = best-corrected visual acuity, CMT = central macular thickness, SD = standard deviation

aflibercept to the diseased eye to replace the ranibizumab injections. The mean age was 72.20 ± 7.14 years (range; 59-86 years). Of the patients, 22 were women and 17 were men. At enrollment, study eyes had an average of 10.74 ± 4.38 previous IVR injections over a period of 28.31 ± 18.08 months (range; 8-67 months).

The mean number of IVA injections during 18 months was 6.94 ± 2.58. At baseline, CMT was 327.44 ± 120.57 µm and best-corrected visual acuity (BCVA) was 0.98 ± 0.62 logMAR log of the Minimum Angle of Resolution) (Table 1). Before switching to aflibercept, CMT was 354.50 ± 127.79 µm and BCVA was 1.02 ± 0.49 logMAR. Mean CMT 6, 12, and 18 months after the aflibercept injection were 290.20 ± 112.25, 311.70 ± 119.47, and 299.29 ± 98.38 µm, respectively (Table 2). A significant difference in CMT was found between the visits (p = 0.02). The CMT was significantly lower at 6 months and 18 months after switching to aflibercept than that before switching ($p < 0.01$ and p = 0.04, respectively). Mean BCVA values 6, 12, and 18 months after the aflibercept injection were 0.87 ± 0.46, 0.97 ± 0.50, and 0.97 ± 0.50 logMAR, respectively (Table 2). No significant improvement in visual acuity was found in the 18-month period after switching to aflibercept (p = 0.59). No serious ocular and systemic side effects were reported during the 18-month period.

Table 2. Mean central macular thickness and best-corrected visual acuity

	CMT (mean ± SD)	BCVA (mean ± SD)
At baseline	327.44 ± 121.20	0.98 ± 0.62
Before switch	354.50 ± 127.79	1.02 ± 0.49
Six months after switch	290.2 0 ± 112.25	0.87 ± 0.46
Twelve months after switch	311.70 ± 119.47	0.97 ± 0.50
Eighteen months after swicth	299.29 ± 98.38	0.97 ± 0.50
p values	**0.02**	0.59

SD = standard deviation

DISCUSSION

In this prospective study, we investigated the anatomic and functional response to aflibercept treatment in patients with refractory nAMD after switching from intravitreal ranibizumab to aflibercept injection. Several studies have reported treatment outcomes after switching to aflibercept from other drugs [5-7].

Cho *et al*. [8] reported that the CMT was reduced from 295 µm to 274 µm through an average of 4.4 aflibercept injections over a period of 6 months. Grewal *et al*. [9] found that the initial CMT of 329 µm decreased to 292 µm through an average of 10.2 ± 1.2 aflibercept injections over a period of 12 months. Bakall *et al*. [10] reported that the CMT was reduced by 65 mm after 3 injections with no significant change in visual acuity. In our study, the average number of IVR injections before switching to aflibercept was 10.74. All of the patients were found to have persistent

subfoveal subretinal and/or intraretinal fluid in the diseased eye despite multiple injections.

In our study, the average CMT value of 349.97 ± 122 μm before switching to aflibercept was decreased to 299.29 ± 98.38 μm in the course of 18-month treatment. The results demonstrate that switching to aflibercept provided significant improvements in CMT, which were maintained during the longer follow-up periods up to 18 month.

Comparison of anatomical results showed that switching from ranibizumab to aflibercept delivered a significant decrease in CMT. Molecular and pharmacological characteristics of aflibercept might have contributed to improved outcomes [11]. Aflibercept was known to have a higher binding affinity for VEGF-A compared to ranibizumab or bevacizumab. In addition, it also binds and inhibits VEGF-B and placental growth factor, which were shown toinfluence neovascularization [12]. Our study provides evidence that there is a significant anatomical effect, resulting in decreased CMT after switching. Our study also indicated a modest improvement in BCVA after switching to aflibercept, albeit not significant. Significant foveal photoreceptor loss and development of subretinal scarring at an earlier stage of the disease might have contributed to the divergent results in terms of the anatomic improvement and functional outcome.

Limitations

Limitations of the current study include small sample size.

CONCLUSION

In conclusion, switching to aflibercept produced a significant decrease in central macular thickness after 6, 12, and 18 months. However, there was no significant change in visual acuity.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2019;5(6):981-989 ORIGINAL ARTICLE

The relationship between emotional appetite and bipolar features in obese and non-obese individuals

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DOI: 10.18621/eurj.433962

ABSTRACT

Objectives: It is known that many different positive and negative emotions can affect appetite and also, individuals who have bipolar features often have emotional fluctuations. In this study, it was aimed to investigate the relationship between emotional appetite and bipolar features in obese individuals.

Methods: One hundred and ninety obese individuals who applied for bariatric surgery and 136 non-obese individuals were evaluated with Emotional Appetite Questionnaire (EMAQ), Beck- Depression Inventory (BDI), Beck-Anxiety Inventory (BAI) and The Temperament Evaluation of Memphis, Pisa, Paris and San-Diego Auto-questionnaire (TEMPS-A) in the study.

Results: In obese individuals who applied for bariatric surgery, the frequency of bipolar disorder was found to be 2% and binge eating disorder (BED) frequency was 51.2%. It was found that scores of appetite in negative emotions were higher in obese individuals with BED compared to obese individuals and it was higher in obese individuals compared to normal weight individuals. Cyclothymic features explained 27.2%, 25.8% and 15.7% of scores of appetite in negative situations of obese individuals with BED, obese individuals without BED and normal weight individuals, respectively.

Conclusions: As a result of this study, it can be concluded that scores of appetite in negative situations may be affected by cyclothymic features in obese individuals with BED, in obese and normal weight individuals. Depression and anxiety symptoms are effective factors in explaining scores of appetite in negative situations of obese individuals with BED, obese and normal weight individuals.

Keywords: emotional appetite, bipolar features, obesity, cyclothymia

Received: June 14, 2018; Accepted: August 7, 2018; Published Online: January 21, 2019

Emotional appetite is defined as increase in the consumption of food in order to cope with unpleasant feelings and situations [1]. There are data indicating that emotional appetite may be related to individuals' body mass index (BMI) and emotional appetite may cause obesity [1, 2]. Furthermore, emotional appetite has been shown to be an effective factor in the relationship between eating disorders (binge eating disorder [BED] and bulimia nervosa) and obesity [3-5]. The presence of a relationship between obesity, eating disorders and emotional appetite has caused more research into emotional appetite. Consequently, Nolan *et al*. [2] defined appetite changes caused by psychological symptoms as a construct related to neg-

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e-ISSN: 2149-3189

ative and positive emotional states. In this study, it was found that compared to their usual eating habits, overweight individuals reported to consume more food in negative situations than underweight individuals, and underweight individuals reported to consume more food in positive emotions than overweight individuals.

BED is defined as an eating disorder characterized by recurrent periods of binge eating with the sense of loss of control over eating behavior [6]. It has been argued that problematic eating behaviors and emotional appetite are related in individuals diagnosed with BED [4]. In a study of obese women, emotional appetite was suggested to be one of the determinants of BED [7]. It has been indicated that emotional regulation problemshave significant effects on emotional overeating and eating pathologies in individuals with BED [8]. In studies conducted on individuals with a desire to lose weight, it was reported that individuals with BED could be experiencing serious problems in appetite control [9, 10]. Frequency of emotional appetite symptoms have also been found to be high in individuals with a desire to lose weight [11]. In a study comparing participants who had weight loss with bariatric surgery with participants who had diet-induced weight loss, it was shown that neurotic features were effective in weight loss in both groups and emotional appetite mediated this effect, and in both groups overeating behavior was decreased [12]. In another study conducted on post-bariatric surgery patients, obese patients were evaluated to have positive change in emotional appetite as their weight decreased [13].

Bipolar and related disorders are defined as mental disorders with manic, hypomanic and depressive symptoms. In DSM 5, bipolar diagnoses (bipolar I, bipolar II, cyclothymia, other specified, unspecified bipolar and related disorder) are classified according to the severity, frequency, duration and content of manic, hypomanic and depressive symptoms [14]. Studies have revealed that eating disorders are prevalent in individuals with bipolar disorder, at the same time bipolar disorder comorbidity is high among individuals with eating disorders [15]. In a study on young patients with bipolar disorder, patients with bipolar disorder were evaluated as more likely to exhibit overeating and emotional eating behaviors compared with individuals without bipolar disorder [16]. In a study investigating psychiatric diagnoses of individuals applying for bariatric surgery, current bipolar disorder rate was 13.7% and lifetime prevalence was 35.6% [17]. In another study evaluating 120 consecutive participants seeking bariatric surgery, bipolar disorder frequency was found to be 91.7% [18]. Despite these studies, in a study conducted on 935 obese patients, bipolar disorder rate was reported as 6%, still, this bipolar disorder ratio was higher than those of normal population [19].

Research on emotional appetite in obese individuals focus on eating disorders, depression, anxiety, anger and neurotic features [2, 20, 21]. Nevertheless, there are no studies in the literature investigating the relationship between bipolar features and emotional appetite in obese individuals. It is known that there are many different emotions effective in emotional appetite [2], including positive (confident, happy, relaxed, playful) and negative emotions (sad, bored, angry, anxious, etc) and individuals with bipolar features often have emotional fluctuations and rapid shifts in mood [14]. It is stated that especially in individuals with cyclothymic features, major emotional changes are seen during the day [22] and at the same time, appetite can be affected when individuals are in negative (when under pressure, after a heated argument, etc) and positive situations (when falling in love, after receiving good news, etc) [2]. Therefore, it was aimed to investigate the relationship between emotional appetite and bipolar features in obese individuals.

METHODS

Participants

Two hundred and eleven voluntary obese individuals (BMI > 34.9) who have applied for bariatric surgery between November 2013 and February 2016 were invited to participate in the study. Twelve individuals refused to participate in the study. Exclusion criteria of the study was having any mental or organic disease, psychotic disorder, bipolar disorder or bulimia nervosa. Before including the study, patients were evaluated if they had any psychiatric disorders that prevented them from participating in the study. Consequently, of 199 morbidly obese participants, 4 (2%) had diagnosis of bipolar disorder (3 of them bipolar I, 1 of bipolar II), 1 (0.5%) had psychotic disorder, 102 (51.2%) had BED and 4 (2%) had bulimia nervosa. Participants with a diagnosis of

bipolar disorder, bulimia nervosa, or of any psychiatric disorder that prevents from participating the study were excluded. So, in this study, nonpathological bipolar features were investigated in obese and normal weight individuals. Obese patients were divided into two groups according to having BED diagnosis. By this means, we attempted to control the confounding effect of this diagnosis on emotional appetite. The reason for this is that studies indicate that BED diagnosis and BED features can affect emotional appetite [4, 7, 8].

To compare data of obese individuals, we wanted to design a control group of non-obese individuals (18 < BMI < 30) with similar sociodemographic (age, education level, sex) characteristics with obese individuals. For this purpose, 149 individuals who applied to the family medicine department of our hospital were invited to participate in the study. The exclusion criteria in non-obese group was having a diagnosis of any organic or mental disorder, psychotic disorder, bipolar disorder or bulimia nervosa. In addition, a history of obesity (BMI > 30) or of having bariatric surgery were defined as other exclusion criteria. For this reason, 11 participants with a history of obesity and 2 participants with BED were excluded from the study. In both obese and control group, psychiatric diagnosis and exclusion criteria were evaluated by clinical interview. Consequently, psychometric data of 102 obese with BED, 88 obese without BED and 136 non-obese participants who were eligible according to exclusion criteria were examined.

Measures

Emotional Appetite Questionnaire (EMAQ) was used to assess emotional appetite of participants. Beck-Depression Inventory (BDI), Beck-Anxiety Inventory (BAI) and The Temperament Evaluation of Memphis, Pisa, Paris and San-Diego Auto questionnaire (TEMPS-A) were used to evaluate bipolar features. Besides these, all participants were given sociodemographic data form designed by the researchers including questions about age, sex, height, weight, past psychiatric admissions.

Emotional Appetite

EMAQ was developed by Nolan *et al*. [2] in 2010. The presence of emotional eating is evaluated in negative/positive emotions (14 items) and in negative/positive situations (8 items). The validity study of Turkish version of the Cronbach's alpha coefficient was 0.73 and the inter-item correlation coefficients of the scale were between 0.14-0.72 [23]. Every item in the scale is scored between -4 and +4 and there are four subscales in the scale as positive emotions (EMAQ-PE), negative emotions (EMAQ-NE), positive situations (EMAQ-PS) and negative situations (EMAQ-NS). High scores from the subscales of the scale indicate high level of emotional and situational appetite features.

Depression

BDI, which was developed by Beck *et al*. [24] to determine the severity of the depressive symptoms of an individual, was adapted to Turkish by Hisli [25]. This scale, consisting of 21 items, is evaluated based on a spectrum of 0 to 63 points, and its Cronbach's alpha coefficient is 0.80. It is used to assess the depression symptoms experienced during past week. The higher the score obtained on the scale indicates the more severe depressive symptoms the individual is experiencing.

Anxiety

BAI which was developed by Beck *et al*. [26] to determine the severity of the anxiety symptoms of an individual, was adapted to Turkish by Ulusoy *et al*. [27]. The correlation coefficients of the scale were between 0.45-0.72 and the Cronbach's alpha value was 0.93. This scale, consisting of 21 items, is evaluated based on a total score that ranges from 0 to 63. It is used to evaluate anxiety symptoms seen during past week. The higher the score obtained from the scale, the more severe anxiety the individual is experiencing.

Bipolar Features

Memphis, Pisa, Paris and San-Diego Auto-questionnaire (TEMPS-A) is a likert type of scale developed by Akiskal *et al*. [22]. It is a measure used to evaluate major and stable traits that are effective in emotional variations of individuals with and without bipolar disorder. Its Turkish version and validity and reliability study were done by Vahip *et al*. [28]. Five sub-temperament features are measured, which are cyclothymic, depressive, hyperthymic, irritable and

anxious. The test-re-test reliability of the Turkish version is between 0.73 and 0.93, and the Cronbach's alpha coefficient is between 0.75 and 0.84. Cyclothymic temperament involves emotional fluctuations (I get sudden shifts in mood and energy, my mood often changes for no reason) and polarity of emotions (My moods and energy are either high or low, rarely in between). Depressive temperament involves hopelessness, sadness (I am a sad, unhappy person) and lack of energy (I do not seem to have as much energy as other people). Hyperthymic temperament involves cheerful, optimistic, enthusiastic features (I am usually in an upbeat or cheerful mood). Irritable temperament is associated with pessimistic, grumpy, dissatisfied and irritable states (I am a grouchy person, I am by nature a dissatisfied person). Anxious temperament involves fear and anxiety (I am often fearful of someone in my family coming down with a serious disease), somaticcomplaints (When I am nervous, I often feel nauseous).

Procedure

Voluntary participants were recruited in the study. Prior to participation in the study, an informed consent form was signed by all participants. After evaluation of the psychiatric diagnoses, participants were individually assessed by sociodemographic data form and psychometric scales. DSM-5 was used in the evaluation of psychiatric diagnoses (mental disorders, bipolar and related disorders, BED, bulimia nervosa, psychosis). Approval from the ethics committee was obtained. In addition, the study was conducted according to the Declaration of Helsinki.

Statistical Analysis

Pearson correlation analysis was used to analyze the relationship between EMAQ, BDI, BAI and TEMPS-A in obese and non-obese individuals. Chi-square test was used to compare the ratios related to sociodemographic data of the individuals in the control group and obese patients. One Way ANOVA was used to compare the average scores of EMAQ, BDI, BAI and TEMPS-A between the obese with BED, obese without BED and control group. In addition the Tukey test was used to determine which groups differed from each other in the mean scores. Hierarchical regression analysis was used to measure

Table 1. The relationship between TEMPS-A, EMAQ, BDI, BAI and BMI

	EMAQ-NE			EMAQ-PE			EMAQ-NS			EMAQ-PS			BMI	
	O with BED	O without BED	NO	O with BED	O without BED	NO	O with BED	O without BED	NO	O with BED	O without BED	NO	O with BED	O without BED	NO
BMI	0.11	-0.12	0.17*	-0.06	-0.10	-0.03	-0.01	0.05	0.01	0.08	-0.07	-0.02			
Depressive	0.14	0.32**	0.04	-0.17	0.06	0.11	0.37***	0.47***	0.37***	-0.10	0.04	0.06	0.25*	0.14	-0.01
Cyclothymic	0.17	0.28**	0.02	0.07	0.04	-0.03	0.52***	0.51***	0.40***	0.08	0.01	-0.24**	0.05	0.15	0.05
Hyperthymic	0.01	-0.12	-0.08	0.05	-0.21*	0.02	-0.05	0.11	0.11	-0.07	-0.29**	0.01	-0.10	0.00	0.01
Irritable	0.12	0.22*	0.12	0.07	0.06	-0.03	0.43***	0.48***	0.38***	0.09	-0.01	-0.16*	-0.08	-0.01	0.00
Anxious	0.11	0.22*	-0.14	-0.07	0.06	0.05	0.47***	0.57***	0.60***	-0.01	-0.01	-0.19*	0.11	0.02	-0.12
BDI	0.22*	0.26**	-0.15	0.03	0.03	0.12	0.51***	0.50***	0.46***	0.01	-0.02	-0.07	0.02	0.09	0.01
BAI	0.13	0.12	-0.16	0.07	-0.05	-0.04	0.87***	0.88***	0.88***	-0.01	-0.08	-0.16	-0.11	0.03	0.06

O = Obese, NO= Non-Obese, BED = Binge Eating Disorder, TEMPS-A = The Temperament Evaluation of Memphis. Pisa. Paris and San-Diego Auto-questionnaire, BDI = Beck Depression Inventory, BAI = Beck Anxiety Inventory, BMI = body mass index, EMAQ = Emotional Appetite Questionnaire, PE = positive emotions, PS = positive situations, NE =negative emotions, NS =negative situations. *** $p < 0.001$. ** $p < 0.01$. * $p < 0.05$.

whether cyclothymic, depressive, hyperthymic, irritable and anxious temperaments scores were predicted by the abnormal EMAQ scores. Significance level for all the analyses was determined as $p < 0.05$. IBM SPSS Statistics 22.0 software was used in analyzing the data. Normal distribution assumptions were met for the Pearson correlation analysis, One Way ANOVA and Hierarchical Regression Analysis.

RESULTS

Obese patients between the ages of 18-68 were included in our study. The age range of the healthy control group was 20-62. Mean age of obese individuals with BED (34.44 ± 8.48 years), obese (36.77 ± 10.27 years) and normal weight (34.19 ± 8.6 years) individuals did not differ statistically (F = 2.41, $p = 0.091$). 21 (20.6%) of obese with BED, 17 (19.3%) of obese, 34 (25%) of normal weight individuals were male and sex ratios were similar for the three groups ($X^2 = 1.96$, $p = 0.550$). 40 (20.6%) of obese with BED, 20 (19.3%) of obese, 23 (25%) of normal weight individuals had a history of psychiatric treatment and psychiatric treatment history ratios were statistically differentfor the three groups (X2 = 15.75, $p < 0.001$).

A positive correlation was found between EMAQ-NE scores and BMI in non-obese participants (r = 0.17, $p < 0.05$). It was found that there was a positive relationship between EMAQ-NE scores and depressive (r = 0.32, $p < 0.01$), cyclothymic (r = 0.28, $p < 0.001$), irritable (r = 0.22, $p < 0.05$), anxious temperament scores (r = 0.22, $p < 0.05$) in obese participants. A positive correlation was found between EMAQ-NE scores and BDI scores in obese participants (r = 0.26, $p < 0.01$). There was a positive relationship only between EMAQ-NE and BDI in obese participants with BED (r = 0.22, $p < 0.05$). In the three groups, there was a negative relationship between EMAQ-PE scores and hyperthymic temperament in only obese individuals (r = -0.21, $p < 0.05$). There was a positive relationship ranging from 0.37-0.88 between EMAQ-NS scores and depressive, cyclothymic, irritable and anxious temperament, BDI, BAI scores in the three groups. There was a negative relationship between EMAQ-PS scores and cyclothymic temperament (r = -0.24, $p < 0.01$), irritable temperament (r = -0.16, $p < 0.05$), anxious temperament (r = -0.19, $p < 0.05$) in non-obese group. There was a negative relationship between EMAQ-PS

Table 2. Comparison of results of the TEMPS-A, EMAQ, BDI and BAI between the obese and non-obese group

	O with BED	O without BED	NO	F	*p* value
Depressive[a]	7.64 ± 3.29	6.61 ± 3.30	5.56 ± 3.30	11.66	< 0.001
Cyclothymic[b]	9.90 ± 4.33	8.30 ± 4.84	7.08 ± 4.42	11.31	< 0.001
Hyperthymic[b]	11.72 ± 4.00	11.55 ± 3.51	9.95 ± 3.87	7.80	< 0.001
Irritable[c]	3.97 ± 3.49	3.63 ± 3.80	3.13 ± 3.38	1.69	0.186
Anxious[a]	7.29 ± 4.56	6.22 ± 4.94	5.21 ± 4.18	6.20	0.002
BDI[b]	15.14 ± 8.08	12.87 ± 8.20	8.21 ± 7.61	23.75	< 0.001
BAI[b]	14.66 ± 10.24	13.37 ± 7.60	6.49 ± 7.23	25.79	< 0.001
EMAQ-NE[d]	2.02 ± 12.42	-7.35 ± 13.98	-12.86 ± 12.53	38.93	< 0.001
EMAQ-PE[c]	1.12 ± 6.43	-0.38 ± 6.23	1.47 ± 5.01	2.88	0.057
EMAQ-NS[b]	3.15 ± 2.77	2.69 ± 2.89	1.20 ± 1.94	19.95	< 0.001
EMAQ-PS[c]	-1.01 ± 5.19	-1.60 ± 4.87	-0.41 ± 4.18	1.73	0.178
BMI[e]	46.29 ± 5.47	46.90 ± 5.21	22.56 ± 2.60	1126.43	< 0.001

O = Obese, NO = Non-Obese, BED =Binge Eating Disorder, TEMPS-A = The Temperament Evaluation of Memphis. Pisa. Paris and San-Diego Auto-questionnaire, BDI = Beck Depression Inventory, BAI = Beck Anxiety Inventory, EMAQ = Emotional Appetite Questionnaire, PE = positive emotions, PS = positive situations, NE = negative emotions, NS = negative situations. [a]NO < O with BED, [b]NO, O without BED <O with BED, [c]NO, O without BED, O with BED, [d]NO < O without BED < O with BED, [e]NO < O without BED, O with BED.

Table 3. Hierarchical regression analysis results for EMAQ scores

	EMAQ-NE			EMAQ-PE			EMAQ-NS			EMAQ-PS		
	O with BED	O without BED	NO	O with BED	O without BED	NO	O with BED	O without BED	NO	O with BED	O without BED	NO
Step 1												
R^2	0.029	0.081**	0.001	0.005	0.002	0.001	0.279***	0.266***	0.163***	0.006	0.000	0.059**
Adjusted R^2	0.020	0.071**	-0.007	-0.005	-0.010	-0.006	0.272***	0.258***	0.157***	-0.004	-0.012	0.052**
Step 2												
R^2	0.034	0.121	0.106*	0.088	0.051	0.038	0.326***	0.365***	0.391***	0.074	0.089	0.142**
Adjusted R^2	-0.016	0.068	0.072*	0.041	-0.007	0.001	0.291***	0.326***	0.368***	0.026	0.033	0.109**
Step 3												
R^2	0.056	0.132	0.142**	0.113	0.066	0.060	0.776***	0.794***	0.801***	0.082	0.100	0.156**
Adjusted R^2	-0.014	0.057	0.096**	0.046	-0.015	0.009	0.759***	0.776***	0.790***	0.013	0.021	0.110**

Step 1 = Cyclothymic temperament scores, Step 2 = Depressive, hyperthymic, irritable and anxious temperament scores were added, Step 3= BDI and BAI scores were added. O = Obese, NO = Non-Obese, BDI = Beck Depression Inventory, BAI = Beck Anxiety Inventory, EMAQ = Emotional Appetite Questionnaire, PE = positive emotions, NE = negative emotions, NS = negative situations. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

scores and hyperthymic temperament scores in obese group (r = -0.29, $p < 0.01$). Among the three groups, there was a negative relationship between BMI and depressive temperament scores in only obese individuals with BED (r=0.25, $p < 0.05$) (Table 1).

Depressive temperament ($p < 0.001$), cyclothymic temperament ($p < 0.001$), hyperthymic temperament ($p < 0.001$), anxious temperament ($p = 0.002$), BDI ($p < 0.001$), BAI ($p < 0.001$), EMAQ-NE ($p < 0.001$) and EMAQ-NS ($p < 0.001$) scores differed between the three groups. Based on the Tukey test applied to the ANOVA analysis, only EMAQ-NS scores varied ($p < 0.001$) among the groups (Table 2).

According to hierarchical regression analysis (Table 3), it was found that the cyclothymic temperament scores accounted for 7.1% of the EMAQ-NE scores ($p < 0.01$) in obese participants (Step1). It was found that the cyclothymic, depressive, hyperthymic, irritable and anxious temperament scores accounted for 7% of the EMAQ-NE scores ($p < 0.05$) in non-obese participants (Step 2). By adding BDI and BAI scores into this step it accounted for 9.6% of the EMAQ-NE scores (Step 3). It was also found that cyclothymic temperament scores accounted for 27.2% ($p < 0.001$), 25.8% ($p < 0.001$), 15.7% ($p < 0.001$) of EMAQ-NS scores, in obese individuals with BED, obese and normal weight individuals respectively (Step 1). By adding depressive, hyperthymic, irritable and anxious temperament scores into this step, explanatory power increased to 29.1% ($p < 0.001$), 32.6% ($p < 0.001$), 36.8% ($p < 0.001$), respectively (Step 2). By adding BDI and BAI scores into this step, it was found that explanatory power increased to 75.9% ($p < 0.001$), 77.6% ($p < 0.001$), 79% ($p < 0.001$), respectively (Step 3). It was found that the cyclothymic temperament scores accounted for 5.9% of the EMAQ-PS scores ($p < 0.01$) in non-obese participants (Step1). By adding depressive, hyperthymic, irritable and anxious temperament scores into this step, it accounted for 10.9% of the EMAQ-PS scores (Step 2). By adding BDI and BAI scores into this step, it accounted for 11% of the EMAQ-PS scores (Step 3).

DISCUSSION

In our study, it was found that scores of appetite

in negative emotions were higher in obese individuals with BED compared to obese individuals and it was higher in obese individuals compared to normal weight individuals. It was also determined that cyclothymic and hyperthymic features were more frequent in obese individuals with BED compared with obese and normal weigh individuals. Also, scores of appetite in negative situations in obese individuals with BED, obese and normal weight individuals were explained statistically significantly by bipolar features, depression, anxiety scores and especially cyclothymic features. In literature, it has been shown that emotional appetite couldbe an effective factor in the link between obesity and eating disorders (BED and bulimia nervosa) [3-5]. In this study, higher scores of appetite in negative emotions in obese individuals diagnosed with BED, compared with obese and normal weight individuals, is congruent with the results of the previous studies in literature. Levels of impulsivity, depressive symptoms and anger were found to high in individuals diagnosed with BED [29, 30]. It is therefore thought that appetite control in individuals diagnosed with BED is negatively affected by impulsive features and mental disorders. In this study findings of higher rates of cyclothymic and hyperthymic features in individuals diagnosed with BED compared with obese and normal weight individuals, support this aspect.

In obese individuals, comorbidities and high psychopathology rates may be confounding factors in the relationship between emotional appetite and BMI. In other words, the low confounding impact of comorbidities and psychopathological features in normal weight individuals may increase the appearance of the relationship between BMI and emotional appetite. In this study, a significant positive correlation was found between depressive temperament features and BMI values of obese individuals with BED. It is argued that depressive temperament could facilitate the development of bipolar disorders and depressive disorders [31] and high negative emotionality and low persistence in childhood could cause alterations in appetite and nutrition in individuals [32]. For this reason, there may be a relationship between BMI of individuals with BED and depressive temperament features. So, in order to understand the relationship between obesity and depressive disorders, more research on stable depressive features would be conducted.

The correlation between temperament features other than hyperthymic features and scores of appetite in negative situations in obese individuals with BED, obese and normal weight individuals implies that appetite in negative situations can be affected by negative mood in obese and normal weight individuals. Relationship between scores of appetite in positive emotions, negative emotions, positive situations and bipolar features, anxiety and depression differed among the three groups. Scores of appetite in negative emotions increased as bipolar features (except hyperthymic features) increasedin only obese patients. This may suggest that appetite in negative emotions in obese individuals with BED is associated with more diverse variables.

In obese individuals with lively, energetic and active characteristics, the need to eat in relation to positive emotions and situations may be lesser. The need for reward-seeking behaviors in relation to positive situations and emotions may also be less in obese individuals and they may be controlling their appetites better. Since higher frequencies of impulsive features in obese individuals compared with normal weight individuals were reported and obese individuals with ADHD were evaluated to be affected more in psychopathological features [33]. Our findings show that as cyclothymic, irritable and anxious features increased, appetite in relation to positive situations is decreased in normal weight individuals, however remain unchanged in obese with BED and obese individuals. This indicates that appetite in relation to positive situations can be affected by cyclothymic, irritable and anxious features in normal weight individuals whereas not in obese with BED and obese individuals.

In this study, only 2% of participants applying for bariatric surgery were diagnosed with bipolar disorder. In other previous studies, in individuals applying for bariatric surgery, bipolar diagnosis was found at different rates of 6-91.7% [17-19]. It is stated that the incidence of bipolar disorder may change with age [34]. Therefore, despite the results of cross-sectional studies, the life time prevalence of bipolar disorder may increase in obese individuals. Therefore, in order to evaluate bipolar disorder diagnosis accurately in obese individuals, it may be essential to evaluate factors that affect the frequency such as age, sex and

culture. In addition to this, in studies reporting higher rates of bipolar disorder, establishing the diagnosis of bipolar disease and features by scales instead of interviews by clinicians suggests the methodological limitations in these studies. In our study, bipolar disorder diagnosis in obese individuals applying for bariatric surgery was evaluated by clinical interview. Our study findings of bipolar frequency was similar to that of normal population and bipolar features (cyclothymia and hyperthymia scores) that may increase the risk of bipolar disorder were more frequent in obese individuals.

Nolan *et al*. [2] suggested that appetite can be affected by certain negative (when under pressure, after a heated argumen etc.) and positive (when falling love, after receiving good news) situations. Besides that, the emotions of individuals with cyclothymic features were determined to change rapidly during the day without any obvious reason (I get sudden shifts in mood and energy, my mood often changes for no reason) [22]. The increasing power of cyclothymia in explaining scores of appetite in negative situations towards normal weight (15.7%), obese (25.8%) and obese (27.2%) individuals indicates that the cyclothymic features of obese individuals with BED make them more susceptible to negative situations. Another data supporting this finding is that after adding hyperthymic, anxious, irritable and depressive temperament scores to the model, the power to explain the negative situations score changed in normal weight (36.8%), obese (32.6%) and obese individuals with BED (29.1%). In other words, it can be suggested that cyclothymic features are primary in explaining appetite in negative situationsin obese individuals with BED, whereas other bipolar features are effective in obese and normal weight individuals. Furthermore, in the last model (Table 3), bipolar features, depression and anxiety can be considered to be effective at high and similar levels (79%, 77.6%, 75.9%, respectively) in explaining appetite in negative situations in normal weight, obese and obese individuals with BED, respectively.

Limitations

Limitation of this study is that the number of participants is relatively small and the number of male participants are lower than female. Another limitation is the assessment of depression, anxiety and bipolar features by self-administered scales. This could affect data of the study. Another limitation is that the data of the study are only generalizable to obese individuals (BMI > 34.9) applying for bariatric surgery.

CONCLUSION

As a result of this study, it can be concluded that scores of appetite in negative situations may be affected by cyclothymic features in obese individuals with BED, in obese and normal weight individuals. Depression and anxiety symptoms are effective factors in explaining scores of appetite in negative situations of obese individuals with BED, obese and normal weight individuals.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The author disclosed that they did not receive any grant during conduction or writing of this study.

Acknowledgements

The authors thank everyone who provided assistance for the study including medical secretaries.

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The European Research Journal 2019;5(6):990-995 ORIGINAL ARTICLE

The efficacy of bleb needling revision with 5-fluorouracil in encapsulated bleb after unsuccessful trabeculectomy

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DOI: 10.18621/eurj.425740

ABSTRACT

Objectives: To investigate the efficacy of the bleb needling revision (BNR) procedure with the adjunctive use of 5-Fluorouracil (5-FU) in encapsulated bleb after unsuccessful trabeculectomy.

Methods: We reviewed 16 eyes of 15 subjects who underwent BNR procedure due to encapsulated bleb after unsuccessful trabeculectomy. Demographic data, type of glaucoma, intraocular pressure (IOP) values of pre-BNR, IOP values of post-BNR at first day, first week and first month, follow up time and complications were recorded from patients' files, retrospectively.

Results: The mean follow up time was 53.1 ± 26.4 weeks. The average time between previous unsuccessful trabeculectomy and BNR was 11.12 ± 8.79 weeks. The mean IOP of pre-BNR was 26.0±4.4 mmHg and significantly decreased to 12.4 ± 5.0 mmHg post-BNR at the first day ($p < 0.001$). The mean IOP values of post-BNR at the first week was 13.3 ± 4.9 mmHg and at the first month was 14.8 ± 4.8 mmHg. According to the mean IOP of pre-BNR, the mean IOP values of post-BNR at the first week and at the first month were significantly lower ($p < 0.001$ and $p < 0.001$, respectively). Seven (44%) eyes were achieved success and eight (50%) eyes were achieved qualified success. One (6%) eye was classified as the failure.

Conclusion: The bleb needling revision procedure with the adjunctive use of 5-FU in encapsulated bleb after unsuccessful trabeculectomy is a simple, useful and repeatable method to restore the dysfunctional bleb.

Keywords: Bleb needling revision, 5-Fluorouracil, encapsulated bleb, trabeculectomy, filtration surgery

Received: May 21, 2018; Accepted: January 22, 2019; Published Online: June 30, 2019

Trabeculectomy remains the gold standard surgical procedure in cases that intraocular pressure (IOP) couldn't control with antiglaucomatous medication [1]. Trabeculectomy is a very effective procedure to reduce IOP at the early postoperative period. Nevertheless, its efficiency may decreases over time for some complications such as dysfunctional blebs. Localization of the bleb, episcleral fibrosis, and bleb encapsulation are the bleb-related reasons for the failure of primary filtration surgery [2].

Encapsulated bleb usually seen within 2-8 weeks after filtration surgery and the incidence is reported between 2.5% and 29% in previous studies [3, 4]. Encapsulated bleb was characterized as an elevated, smooth surfaced, tense, thickened, dome-shaped fibrotic membrane. The mechanism of development of encapsulated bleb is not well understood and many factors are responsible for its formation. The encapsulated bleb wall that limiting aqueous outflow is composed of a fibrous, collagenous connective tissue

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e-ISSN: 2149-3189

which is histopathologically produced as a result of fibroblastic proliferation [5]. Hence, inflammatory reaction and fibroblastic activity may increase the risk of developing encapsulated bleb [6].

The method of Bleb Needling Revision (BNR) which was first described by Ferrer in 1941, is now frequently preferred after unsuccessful filtration surgery [7]. The adjunctive use of antifibrotic agents such as 5-fluorouracil (5-FU) and mitomycin-C (MMC) in BNR is suggested to increase the rate of success of the procedure. 5-FU and MMC inhibit the scarring tissue formation and the proliferation of the fibroblasts which are the primary reasons for dysfunction of the blebs. Thus, BNR with antifibrotic agent is a simple and reliable method that can be an alternative treatment after dysfunctional blebs and reduce the IOP without causing additional healthy conjunctival surface loss and prevents patients from another glaucoma surgery. BNR has very good results but many factors such as age, the number of antiglaucomatous drug used before, history of previous ocular surgery, type of glaucoma, use of antimetabolite in primary filtration surgery and bleb morphology may affect the success of the procedure.

In addition, MMC has higher potency according to 5-FU but more complications have been reported due to use of MMC [1, 6]. Therefore, we prefer to perform the procedure with 5-FU. In this study, we aimed to evaluate the efficiency of the BNR procedure in patients who underwent BNR with adjunctive use of 5-FU after unsuccessful trabeculectomy.

METHODS

Sixteen eyes of 15 subjects who underwent BNR due to encapsulated bleb after unsuccessful trabeculectomy (IOP ≥ 21 mmHg) in Necmettin Erbakan University, Meram School of Medicine, Department of Ophthalmology between March 2015-March 2017 were evaluated retrospectively. The study was approved by the local ethics committee (No:2018/1156) and followed the tenets of the Declaration of Helsinki. Demographic data, type of glaucoma, IOP values of pre-BNR, IOP values of post-BNR at the first day, the first week and the first month which were measured by Goldmann applanation tonometry, follow up time and complications were recorded from patients files.

The BNR procedure was performed in the operation room using a 27-gauge needle with adjunctive use of 5-FU by two surgeons (MO and EM). After topical anesthetic (Proparacaine Hydrochloride 0.5%), 5% povidone-iodine solutions were instilled into the eye. A 27-gauge needle was inserted into the subconjunctival space to the tight Tenon's capsule around the bleb on the opposite site of the scleral flap, puncture the adherent tissue and lift the scleral flap. The flap was lifted with gentle side-to-side movement, breaking episcleral adhesions until the bleb is reformed. If simple flap dissection failed to form a nonsatisfactory bleb, the needle can be passed under the scleral flap into the anterior chamber. Then, the needle was removed and 5 mg (0.2 ml of 25 mg/ml solution) of 5-FU was subconjunctivally injected superior to the bleb. At the end of the procedure, antibiotic drops (moxifloxacin 0.5%) were instilled into the eye.

History of previous unsuccessful trabeculectomy surgery with or without antifibrotic agents, the presence of dysfunctional blebs due to encapsulated bleb, the presence of patent iridectomy were the inclusion criterion. History of previous multiple trabeculectomies or filtration surgery other than trabeculectomy, the presence of non-patent iridectomy, the presence of corneal epithelial complications, and leaky blebs were the exclusion criterion. The BNR procedure was considered as success IOP < 21 mmHg without antiglaucomatous medication; qualified success defined as IOP < 21 mmHg with antiglaucomatous medication and failure defined as IOP ≥ 21 mmHg with antiglaucomatous medication.

Statistical Analysis

SPSS for Windows (version 17; SPSS Inc, Chicago, Illinois, USA) were used for the analysis. The paired t-test was used to compare IOP. A P value < 0.05 was considered as significant.

RESULTS

Sixteen eyes of 15 subjects were included in the study. Three (20%) subjects were female, 12 (80%) subjects were male. The age of subjects ranged

Table 1. Demographic data and IOP values

Eye No.	Gender	Age	Type of Glaucoma	Pre-BNR IOP (mmHg)	Post-BNR IOP at first day (mmHg)	Post-BNR IOP at first week (mmHg)	Post-BNR IOP at first month (mmHg)
1	M	22	Traumatic	21.2	7	10	14
2	M	33	POAG	23.4	8	16	14,2
3	F	57	POAG	27	6	6	12
4	F	73	POAG	25.8	17	16.8	15.6
5	M	25	POAG	25,5	12	12.5	11.5
6	M	25	POAG	30.3	15.1	10.1	13.1
7	M	65	POAG	26.1	15.1	11.1	11.1
8	M	69	Pseudoexfoliation	23.7	17.7	10.7	14.7
9	M	54	Uveitic	25	5	7	8
10	M	45	Pseudoexfoliation	25.8	12.8	10.1	10.4
11	F	59	POAG	22.6	16.6	16.6	15.6
12	M	37	POAG	21.2	16.2	16.2	17.2
13	M	57	POAG	22.2	13.4	17.6	15.8
14	M	35	Traumatic	25.1	12.3	9.4	16.4
15	M	18	Traumatic	36.9	9.9	19.3	17.3
16	M	62	Neovascular	34.4	19.7	23.7	29.7

BNR = bleb needling revision, IOP = intraocular pressure, POAG = primary open-angle glaucoma, M = male, F = female

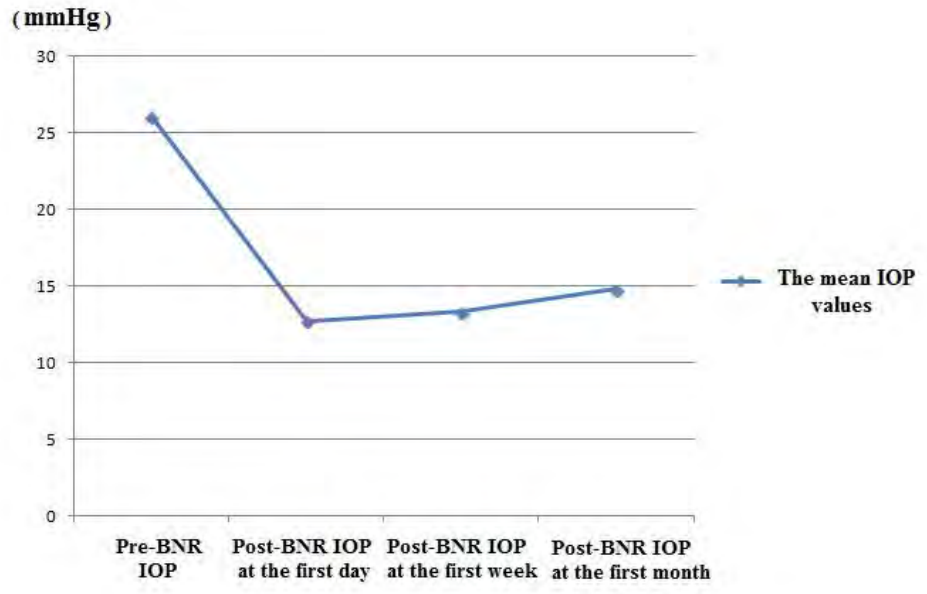

Figure 1. The mean IOP values of patients. BNR = bleb needling revision, IOP = intraocular pressur

Table 2. Pre-BNR and Post-BNR characteristics

Eye No.	Time between trabeculectomy and BNR (week)	Antimetabolite agent use in previous trabeculectomy	Follow up time (week)	The number of antiglaucomatous medication	Success	Complication
1	8	5-FU	26	None	Yes	None
2	4	5-FU	80	One	Qualified	None
3	4	None	124	None	Yes	None
4	10	None	44	One	Qualified	None
5	10	5-FU	48	None	Yes	None
6	7	5-FU	36	Two	Qualified	Late Leakage
7	9	None	25	None	Yes	None
8	16	None	76	One	Qualified	None
9	5	None	34	None	Yes	None
10	29	None	61	None	Yes	None
11	28	None	64	Three	Qualified	Bullous Keratopathy
12	8	None	76	Two	Qualified	Late Leakage
13	26	5-FU	53	One	Qualified	None
14	4	5-FU	34	None	Yes	None
15	6	None	40	One	Qualified	None
16	4	None	28	Three	Failure	None

BNR = bleb needling revision, IOP = intraocular pressure, 5-FU = 5-Fluorouracil

between 18-73 years old and the mean age of subjects was 46.0±18.2 years (Table 1). All eyes were underwent conventional trabeculectomy previously and six of them were with adjunctive use of 5-FU (Table 2).

There were nine (56%) eyes with primary open-angle glaucoma (POAG) and seven (44%) eyes with secondary glaucoma such as one eye had neovascular glaucoma, one eye had chronic uveitis, two eyes had pseudoexfoliation glaucoma, three eyes had glaucoma following serious trauma to the globe (Table 1). The average time between previous unsuccessful trabeculectomy and BNR ranged between 4-29 weeks and the mean time was 11.12 ± 8.79 weeks. The mean IOP of pre-BNR was 26.0 ± 4.4 mmHg and significantly decreased to 12.7 ± 4.4 mmHg post-BNR at the first day ($p < 0.001$). The mean IOP values of post-BNR at the first week were 13.3 ± 4.9 mmHg and at the first month was 14.8 ± 4.8 mmHg (Figure 1). The mean follow up time was 53.1 ± 26.4 weeks (range 25 to 124 weeks). According to the mean IOP of pre-BNR, the mean IOP values of post-BNR at the first week and at the first month were significantly lower ($p < 0.001$ and $p < 0.001$, respectively). Seven (44%) eyes were achieved success and eight (50%) eyes were achieved qualified success. One (6%) eye was classified as failure. Complications such as late-onset bleb leakage in two eyes and bullous keratopathy in one eye observed in three (19%) eyes as shown in Table 2.

DISCUSSION

One of the most important factors that determining the success of trabeculectomy and bleb function is the degree of healing process in the filtration zone [8, 9]. A variety of risk factors such as excessive surgical trauma and increased inflammatory process after an early period of filtration surgery which is related to the development of encapsulated bleb have been reported in the literature [5, 6]. This suggests that these risk factors are not a definite etiology for the presence of encapsulated bleb formation [6]. Even so, surgical trauma and increased postoperative inflammation may probably lead to increase the risk of encapsulated bleb

development [10]. Although there is currently no definitive treatment to prevent bleb failure and encapsulated bleb. Indeed, many drugs and different treatment methods are still being studied.

One of the drugs used to inhibit fibroblastic proliferation and enhance the success of the filtration surgery by allowing the bleb to remain functional is 5-FU. The use of 5-FU adjunctive with filtration surgery or BNR procedure and also injections after filtration surgery can be done. Many researchers have reported the efficacy of the BNR with 5-FU in unsuccessful bleb after filtration surgery [11, 12]. Notably, IOP decreased by an average of 10.5 mmHg (44.8%) immediately after the BNR procedure in the study of Kapasi and Birt [11]. Broadway *et al*. [13] showed 47% success rate in encapsulated bleb after BNR with adjunctive 5-FU and it was reported that the mean IOP being reduced from 26.5 mmHg to 15 mmHg after a median of 1 BNR procedure. Also, the mean IOP was lower in the BNR with 5-FU group (12.1 ± 2.8 mmHg) compared to the medical treatment group (15.1 ± 2.1 mmHg) in the study of Suzuki and Susanna [14]. In our study, it was found that the mean IOP values decreased by 51% (reduced from 26.0 ± 4.4 mmHg to 12.7 ± 4.4 mmHg) at the postoperative 1st month compared to the mean preoperative IOP values.

MMC is another antifibrotic agent in filtration surgery and in BNR procedure but more complications have been reported due to use of MMC [15, 16]. In a retrospective study comparing MMC and 5-FU, there was no difference in success between using MMC and 5-FU, and early postoperative IOP was reported to be the only predictor of success [17]. Complications such as hemorrhage, infection, late-onset bleb leakage, hypotonia, choroidal detachment and suprachoroidal hemorrhage can occur with rarely due to BNR procedure. Anand *et al*. [18] have reported that MMC is a more patent antifibrotic agent but also they reported that in the MMC group, blebitis and late bleb leakage were reported more frequently than in the 5-FU group. In this study, we observed late-onset bleb leakage in two eyes and bullous keratopathy in one eye.

Previous studies have reported that the short time between previous unsuccessful trabeculectomy and the BNR procedure has an adverse effect on bleb function [19, 20]. Shetty *et al*. [21] suggested that immediate BNR after trabeculectomy could be unsuccessful due to the ongoing episcleral inflammation caused by the previous surgery. On the other hand, Gutierrez-Ortiz *et al*. [20] proposed that the success of the BNR procedure was highly correlated with glaucoma filtration surgery performed less than 4 months previously. In this study, the time elapsed between previous trabeculectomy and BNR procedure was ranged 4 to 29 weeks and additionally in twelve eyes the time was ≤ 10 weeks. Indeed, we achieved success in seven of sixteen eyes and in eight eyes antiglaucomatous medication is needed after the BNR procedure (qualified success). The overall success rate of the BNR procedure in the study was 94%.

Moreover, there may be an eventual decrease in efficiency in the BNR procedure over time. IOP > 30 mmHg before the procedure, high IOP after the procedure, lack of use antimetabolite in previous filtration surgery, type of glaucoma are the risk factors that affect the efficiency of the BNR procedure [22]. Generally, repeated BNR with adjunctive 5-FU provide revive of filtration in dysfunctional bleb and prevent other surgery [23]. Indeed, additional surgery may be needed in some cases. Patients with neovascular glaucoma or secondary glaucoma (uveitic glaucoma, traumatic glaucoma, pseudoexfoliation glaucoma, etc.) constitute a high-risk group. In our study, in one risky eye which had neovascular glaucoma IOP couldn't control with antiglaucomatous medication after the BNR procedure and Ahmed glaucoma valve implantation had to be performed.

CONCLUSION

In conclusion, despite limitations of this study such as small sample size, retrospective design, and the absence of the control group, the BNR procedure with adjunctive use of 5-FU effectively reduces IOP or decreases the number of antiglaucomatous drugs in patients with encapsulated bleb and it is a simple, effective and repeatable method for the failure of bleb filtration after trabeculectomy.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2019;5(6):996-1000 ORIGINAL ARTICLE

Study of biofilm formation in *Salmonella* species isolated from food

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DOI: 10.18621/eurj.434298

ABSTRACT

Objectives: Biofilms are defined as communities of organisms attached to a surface and producing an extracellular matrix, in which the bacteria are imbedded. Infections with *Salmonella* species represent a major health problem and a significant burden on food industry. Biofilm formation is one of the causes of pathogenicity of *Salmonella* species, especially in the food industry, which allows bacteria to bind to different levels. Many outbreaks have been associated with biofilms, because they quickly resist anti-microbial and cleansing agents. The aim of this research was to study the capability of biofilm formation by *Salmonella* species isolated from food.

Methods: A total of 8 *Salmonella* species were isolated from 400 samples of red meat, chicken, eggs, and vegetables. Identification was carried out by conventional biochemical tests and serotyping. The capability of biofilm production was measured by titration in Crystal Violet microplate.

Results: In the phenotypic study of *Salmonella* isolates with turbidity method at 550 nm without acetic acid, only 2 (25%) of isolates were able to produce biofilm. both of isolates belonged to the group D of *Salmonella*.

Conclusions: The capability of the isolates to form biofilm reveals the potential ability to resist antimicrobial chemotherapy, therefore higher levels of hygiene in production, packaging, and supply are necessary.

Keywords: *Salmonella*, biofilms, foodborne disease

Received: June 17, 2018; Accepted: March 2, 2019; Published Online: July 21, 2019

The incidence of non-typhoidal salmonellosis in the United States is reported to be 1.4 million per year, with over 95% of these cases being foodborne diseases and 30% of these food infections results in death. Various studies have shown the high capability of *Salmonella* species to bind and form biofilm on different surfaces [1, 2]. A biofilm is any group of microorganisms in which cells stick to each other and

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e-ISSN: 2149-3189

often also to a surface. These adherent cells become embedded within a slimy extracellular matrix that is composed of extracellular polymeric substances [3]. The formation of biofilms reduces the susceptibility to antimicrobial treatment which will ultimately lead to high treatment costs for patients [4]. Food contamination in the production line through unsanitary surfaces is one of the most common problems in food processing plants. Improperly cleaned and residue levels are a good environment for binding and growth, of pathogenic bacteria and, consequently, biofilms formation. The passage of the processed product from contaminated surfaces causes its microbial contamination [5, 6]. The growth of bacteria in the biofilm on the surfaces makes it easier them to transport and difficult to eliminate them. Because biofilm cells exhibit greater resistance to biosolids and disinfectants compared with free cells [7, 8]. The growth of biofilms on food processing equipment causes microbial contamination in the process product, thus reducing the shelf life of the product and increasing the prevalence of food-borne diseases, in particular, those related to *Listeria monocytogenes* and *Salmonella* species. These biofilm contain pathogenic microorganisms [9, 10]. Since there was little information about the formation of biofilm from *Salmonella* species isolated from food in Iran the purpose of this study was to investigate thecapability of biofilm formation by *Salmonella* species isolated from food.

METHODS

A total of 8 *Salmonella* species were isolated from 400 samples of red meat, chicken, eggs, and vegetables. Identification was carried out by conventional biochemical tests and serotyping. Antibiotic sensitivity tests were carried out on the identified *Salmonella* species by using the Kirby-Bauer (Figure 1). Twelve antibiotic discs, namely amoxicillin, nalidixic acid, chloramphenicol, imipenem, tetracycline, ciprofloxacin, ceftriaxone, meropenem, streptomycin, cefepime, cefuroxim and cotrimoxazole. Results were analyzed according to Clinical and Laboratory Standards Institute (CLSI) [11].

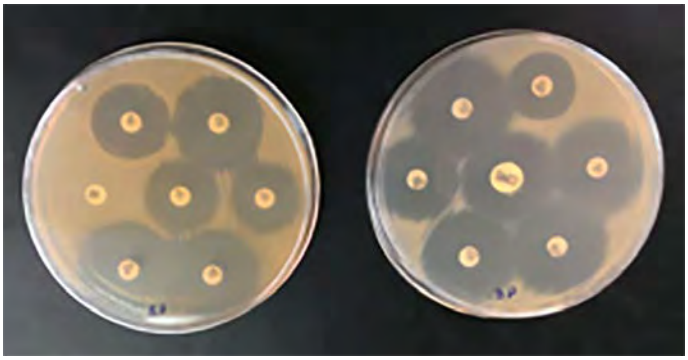

Figure 1. Antibiotic sensivity test.

Biofilm Production

The capability of biofilm production was measured by titration in Crystal Violet microplate according to the instructions used by Peeters *et al*. [12]. Samples was cultured in tryptic soy broth (TSB) and incubated at 37° C for 24 hours. After dilution in fresh TSB, 150 ml of cell suspension was poured into a 96 well flat-bottom polystyrene microplate and incubated at 37° C for 24 hours. The plate was washed three times with 200 µl of posphate-buffered saline (PBS) and air-dried. For fixation of biofilms, 100 µl of 99% methanol was used, after 15 minutes, alcohol was removed and plates were dried in air. 100 µl of 2% crystal violet was added to all of the wells and after 20 minutes the plates were washed with water to remove the color residues. The bonded colors were then released by adding 150 µL of 33% acetic acid. The light absorption (OD) of each well was measured at 570 nm using the ELISA reader. All measurements were repeated 4 times .This was repeated in three separate experiments. E. coli Top 10 and E.coli EAEC 042 strains were used as a negative and positive control respectively.

RESULTS

Of the eight isolated *Salmonella*, two isolates had the capability to produce biofilms, both of which belong to group D (Figure 2). The *Salmonella* isolates showed, the highest resistance 6 (75%) to nalidixic acid, 3 (37.5%) were intermediate to ciprofloxacin and cefuroxime amoxicillin. All isolates 8 (100%) were sensitive to chloramphenicol, imipenem, meropenem, ceftriaxone, cefepime, streptomycin, and cefotaxime. Serogroup D *Salmonella* has the highest resistance to nalidixic acid (75%). Serogroup A was susceptible to

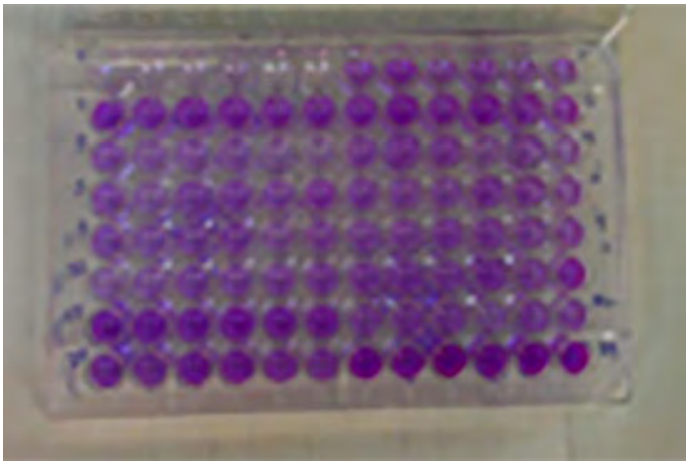

Figure 2. Biofilm production.

cefuroxime and nalidixic acid and intermediate to the rest of antibiotic. *Salmonella* serogroup B was resistant to nalidixic acid, tetracycline, cotrimoxazole, and amoxicillin and sensitive to the rest of the antibiotic. *Salmonella* serogroup C was resistant to nalidixic acid and tetracycline, intermediate to ciprofloxacin and sensitive to the rest of antibiotics. All non-typeable *Salmonella* showed 100% sensitivity to the entire tested antibiotic (Table 1).

DISCUSSION

Salmonella is an important foodborne pathogen and its prevalence in fresh food poses a threat to human. The increase in demand and consumption of raw vegetables has resulted in a rise in food-borne related illnesses and outbreaks. The biofilm formation is a mechanism of *Salmonella* to adapt to different environments. They have been of considerable interest in food hygiene since biofilms may contain spoilage and pathogenic bacteria which increases post-processing contamination and risk to public health. In addition, biofilm cells are more resistant to cleaning and disinfection processes in the food industry. A number of studies have shown that *Salmonella* spp. are capable of adhering and forming biofilms ondiverse surfaces including metal, glass and rubber surfaces [13-15]. The assessment of biofilm formation by *Salmonella* on microtitre plateshowed that all *Salmonella* isolates were able to form biofilms. Other research worker showed that the *Salmonella* were able to form biofilm on microtiter [16].

Pervious study also showed that *Salmonella* biofilms grown and established on stainless steel

Table 1. Antibiotic susceptibility profile of serogroup A, B, C & D and non-typeable *Salmonella*.

Antibiotics	Intermediate %	Sensitive %	Resistant%	Sensitive %	Intermediate	Sensitive %	Resistant%	Intermediate %	Sensitive %	Sensitive %
	serogroup A		serogroup B		serogroup C		serogroup D			non-typeable
Amoxicillin	100	0	100	0	0	0	100	100	0	100
Nalidixic acid	0	100	100	0	0	100	0	0	25	100
Chloramphenicol	100	0	0	100	0	0	100	0	100	100
Imipenem	100	0	0	100	0	0	100	0	100	100
Tetracycline	0	0	100	0	0	100	0	0	0	100
Ciprofloxacin	100	0	0	100	100	0	0	100	0	100
Ceftriaxone	100	0	0	100	0	0	100	0	100	100
Meropenem	100	0	0	100	0	0	100	0	100	100
Streptomycin	100	0	0	100	0	0	100	0	100	100
Cefepime	100	0	0	100	0	0	100	0	100	100
Cefuroxime	0	100	0	100	0	0	100	100	0	100
Cotrimoxazole	0	0	100	0	0	0	100	0	0	100

surfaces as well as meat thawing-loss broth (MTLB). This finding is a matter for concern, particularly for the poultry and meat processing industries using modern meat processing equipment. In these situations with mechanical and process automation, the surfaces are in repeated contact with raw meat, thusincreasing the opportunities for *Salmonella* transfer and attachment leading to biofilm formation [17].

The *Salmonella* isolates showed, the highest resistance 6 (75%) to nalidixic acid, 3 (37.5%) were intermediate to ciprofloxacin and cefuroxime amoxicillin. All isolates 8 (100%) were sensitive to chloramphenicol, imipenem, meropenem, ceftriaxone, cefepime, streptomycin, and cefotaxime. Serogroup D *Salmonella* has the highest resistance to nalidixic acid (75%). Serogroup A was susceptible to cefuroxime and nalidixic acid and intermediate to the rest of antibiotic. Several studies have documented high resistance of *salmonella* to the tetracyclines [18, 19], which is in agreement with the result obtained in this study. *Salmonella* serogroup B was resistant to nalidixic acid, tetracycline, cotrimoxazole, and amoxicillin and sensitive to the rest of the antibiotic. *Salmonella* serogroup C was resistant to nalidixic acid and tetracycline, intermediate to ciprofloxacin and sensitive to the rest of antibiotics. All non-typeable *Salmonella* showed 100% sensitivity to the entire tested antibiotic. A study carried in Canada showed the highest incidence of food-borne outbreaks, with the highest intake of vegetables and fresh fruits, with *Salmonella* with 50% had the highest incidence of this disease, while *Salmonella* isolates from food were 2% and vegetarians showed lower rates than chicken and meat [20]. A study a total of 48 strains of *Salmonella* enteritidis isolated from various sources in South America were investigated in terms of virulence factors including invasion, biofilm production, movement, presence of viral plasmid [21]. In this study, most strains were highly invasive and only three strains were low invasive. All the strains with low invasive did not produce biofilms, while 53% of high invasive produced biofilm [21]. In food industries, the binding of pathogenic bacteria and food corrosive to food contact levels in their production and packaging processes, and finally, the formation of microbial biofilms could be a potential source of contamination of food products and diseases and transmission of diseases. Biofilms on the surfaces of bacteria make it easier to transport and eliminate them. Because biofilm cells exhibit greater resistance to biosolids and disinfectants compared with free cells [7].

CONCLUSION

To consider the ability of producing biofilm by isolated *salmonella* from food samples and rising of *salmonella* gastroenteritis's especially group D, needing for more care and observance a higher level of health to preparation, producing, packing and supply of food seems.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Acknowledgment

This research was supported by Tehran University of Medical Sciences (TUMS) under contract number 32414. The authors thank the TUMS for their supportand Sara Pordel who help to implement this article.

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The European Research Journal 2019;5(6):1001-1006 ORIGINAL ARTICLE

Clinical significance of mean platelet volume/lymphocyte ratio and mean platelet volume/platelet ratio in the exacerbation of chronic obstructive pulmonary disease

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DOI: 10.18621/eurj.443660

ABSTRACT

Objectives: Studies showing the role of systemic inflammation in chronic obstructive pulmonary disease (COPD) are increasing. Particularly, importance of mean platelet volume (MPV) and neutrophil/lymphocyte ratio (NLR) for acute exacerbation of COPD has been reported. The use of MPV/lymphocyte ratio (MLR) and MPV/platelet ratio(MPR) in acute exacerbation of COPD patients was investigated in our study, considering that MPV alone may be a more valuable marker of inflammation.

Methods: Between March 2017 and March 2018, COPD patients who applied to Abant Izzet Baysal University School of Medicine, Chest Diseases outpatient clinic were examined. Results were retrospectively scanned from patient files after institutional approval. Sixty-four (60.4%) stable COPD and 42 (39.6%) acute exacerbation COPD patients were included in the study.

Results: Seventy-one (67%) of patients were male and 35 (33%) were female. NLR was 2.26 (0.93-6.48) in stable patients and 4 (1.18-36) in acute attack patients ($p < 0.001$); PLR was 137.44 (66.9-436.6) in patients with stable disease and 162.8 (85-1056.6) in patients with attack ($p = 0.068$). MLR was 5 (2.92-25) in acute attack patients and 4 (1.89-8.67) in stable patients; this difference was statistically significant ($p = 0.003$). MPV was found to be 7 (5.5-9.1) fL in patients with stable disease and 8 (5-13.4) fL in acute patients. This difference was statistically significant ($p < 0.001$). MPR was found to be statistically significantly higher in acute patients than in patients with stable disease ($p = 0.04$). WBC, neutrophil and CRP were found to be statistically significantly higher in acute patients than in stable patients ($p < 0.05$). There was a statistically significant correlation between WBC and NLR ($r = 0.269$, $p = 0.005$) and between CRP and NLR ($r = 0.379$, $p < 0.001$).

Conclusions: Hemogram parameters from routine laboratory tests in COPD patients are cheap and easily accessible. It is important to detect the presence of subclinical inflammation in the stable phase, as well as to identify patients at risk of exacerbation. Prospective studies are needed to demonstrate correlations with inflammatory markers.

Keywords: Mean platelet volume, neutrophil/lymphocyte ratio, lymphocyte, platelet, chronic obstructive pulmonary disease

Received: July 13, 2018; Accepted: January 29, 2019; Published Online: July 24, 2019

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e-ISSN: 2149-3189

Chronic obstructive pulmonary disease (COPD), reveals by developing a variety of particles as a result of exposure to persistent airflow limitation. It is thought that both the prevalence, the economic and social burden of the world will increase year by year [1]. One of the causes that affect the development of the disease is exacerbations. Early detection of this is a preventive measure in terms of major complications [2]. Recently, the potential role of hemogram parameters during follow-up of chronic diseases has been investigated. The mean platelet volume (MPV) and neutrophil/lymphocyte ratio (NLR) especially have been reported for acute exacerbation of COPD [3, 4]. It has been reported that thrombocytopenia is associated with poor outcomes in acute exacerbations [5], while thrombocytosis is associated with short and long term mortality after exacerbations [6]. The number of lymphocytes is known to decrease in inflammatory conditions [7]. In our study, the MPV/lymphocyte ratio (MLR) and MPV/platelet ratio (MPR) alone were considered to be a more valuable marker of inflammation than MPV. The purpose of hypothesis is; MLR and MPR could be used as biomarkers in acute exacerbation in COPD patients.

METHODS

COPD patients who applied to the Abant Izzet Baysal University School of Medicine Chest Diseases outpatient clinic between March 2017 and March 2018 were included in the study. Results were retrospectively scanned from patient files after institutional approval. Patients with stable COPD were evaluated according to the latest updated COPD combined staging [1]. Exacerbation of COPD was determined according to treatment options based on the detailed screening of symptoms such as dyspnea, cough, or sputum production and / or purulence, and whether acute deterioration resulted in symptoms requiring additional treatment [8] According to this; patients with exacerbation were grouped as mild, moderate and severe. Demographic characteristics, comorbidities, hemogram parameters and C-reactive protein (CRP) values were recorded. Blood was collected before the patients began taking systemic corticosteroids (intravenous), antibiotics (oral/intravenous), and theophylline. MLR was performed by lymphocytic cleavage of MPV; NLR, by neutrophilin lymphocyte cleavage; PLR was obtained by platelet lymphocytic cleavage and MPR by platelet cleavage of MPV. Patients who had another exacerbation during 3 months, who had obstructive sleep apnea, cor pulmonale, pneumonia, pulmonary thromboembolism, coagulopathy, malignancy, and drug use that could affect mean platelet volume were excluded from the study. The study was approved by the Chief Medical Officer of Abant İzzet Baysal University Medical Faculty Education and Research Hospital. The research protocol is in line with the 2000 Helsinki Declaration.

Statistical Analysis

In statistical analysis, statistical package program of IBM Statistics 16.0 (SPSS) was used. Normal distribution of continuous variables was evaluated by Kolmogorov Smirnov tests. Indepented t test was used for normal variables and Mann-Whitney U test was used for normal variables. For comparison of multiple groups, Kruskal-Wallis test was used for normal non-scattering data. The Spearman correlation test was used for the relationship between non-normally distributed continuous variables. Statistical significance was accepted as $p < 0.05$.

RESULTS

Sixty-four (60.4%) stable COPD and 42 (39.6%) acute exacerbation COPD patients were included in the study. Seventy-one (67%) of the patients were male and 35 (33%) were female. While 71 (67%) patients had additional disease, 35 (33%) had no additional disease. 12 (11.3%) of the patients never smoked, 65 (61.3%) were ex-smoker, 29 (27.4%) were active smokers. Patients with acute exacerbation had 4 (9.5%) heavy, 21 (50%) mild, 17 (40.5%) moderate activation. Twenty-two (34%) of the stable patients were stage A, 22 (34%) were grade B, 17 (27%) were stage C and 3 (5%) according to GOLD. The mean age of acute exacerbation patientspatients was 66.5 ± 9.3 years, while the mean age of stabilized patients was 63.9 ± 10.2 years ($p = 0.19$). There was no smoking history in 5, 23 were ex-smoker, 14 were active smokers. NLR was 2.26 (0.93-6.48) in stable patients and 4 (1.18-36) in acute exacerbation ($p <0.001$). PLR

Table 1. Laboratoryfindings of acuteexacerbationandstabilized COPD patients

	Stabil COPD	Active COPD	*p* value
Age (years)	63.9 ± 10.2	66.5 ± 9.3	0.19
Platelet (K/uL)	274.3 ± 69.8	277.05 ± 71.1	0.84
White blood cell (K/uL)	7.1 ± 1.38	11.6 ± 4.1	**< 0.001**
Neutrophil/lymphocyte ratiio	2.26 (0.93-6.48)	4 (1.18-36.6)	**< 0.001**
Platelet/lymphocyte ratio	137.4 (66.9-436.6)	162.8 (85-1056.6)	0.068
MPV/lymphocyte ratio	4 (2.89-8.67)	5 (2.92-25)	**0.003**
MPV/platelet ratio	0.026 (0.014-0.041)	0.028 (0.020-0.067)	**0.04**
Neutrophil (K/uL)	4 (2.1-9.7)	6(2-14.5)	**< 0.001**
MPV (fL)	7 (5.5-9.1)	8 (5.813.4)	**< 0.001**
C-reactive protein (mg/L)	3.3 (0.1-20)	26 (6.3-267.7)	**< 0.001**

Shown as mean ± standard deviation or median (minimum-maximum). COPD = Chronic obstructive pulmonary disease, MPV = Mean platelet volume

was 137.44 (66.9-436.6) in patients with stable disease and 162.8 (85-1056.6) in patients with exacerbation (p = 0.068). MLR was 5 (2.92-25) in patients with acute attack and 4 (1.89-8.67) in patients with stable disease, and this difference was statistically significant (p = 0.003). Similarly, MPR was statistically significantly higher in acute patients than in stable patients (p = 0.04) (Table 1). MPV was found to be 7 (5.5-9.1) fL in patients with stable disease and 8 (5-13.4) fL in acute patients. This difference was statistically significant (p < 0.001). Among the other inflammatory parameters, WBC, neutrophil and CRP were statistically significant (p < 0.05 for all) in acute patients compared to the stable patients (Table 1).

Correlation between WBC, CRP, NLR, MLR and MPR was statistically significant between WBC and NLR a correlation was found (r = 0.269, p = 0.005). There was a statistically significant correlation between CRP and NLR (r = 0.379, p < 0.001). Similarly, there was a statistically significant correlation between NLR and MLR (r = 0.805, p < 0.001). There was no correlation between MPR and inflammatory parameters. In patients with acute exacerbation, NLR, MLR and MPR were assessed

Table 2. Correlation of inflammatory parameters

		Number	Mean	Standard. deviation	*p* value
NLR	Mild	21	3.7538	2.43081	0.095
	Modarate	17	6.4442	8.00233	
	Severe	4	6.8714	3.84920	
MLR	Mild	21	5.7136	3.35507	0.178
	Modarate	17	7.5003	5.26600	
	Severe	4	4.7548	1.96772	
MPR	Mild	21	0.02991	0.008899	0.528
	Modarate	17	0.03443	0.012472	
	Severe	4	0.03181	0.008881	

NLR = Neutrophil/lymphocyte ratio, MPV = Mean platelet volume, MLR = MPV/lymphocyte ratio, MPR = MPV/platelet ratio

separately for disease severity. NLR levels were found to be higher in moderate and mild patients in severely ill patients but not statistically significant. MLR and MPR levels were lower in mild cases than in moderate to severe cases and moderate to severe cases. These values were not statistically significant ($p > 0.05$ for all) (Table 2).

DISCUSSION

In recent years, studies showing the role of systemic inflammation in COPD are increasing. Proinflammatory cytokines such as CRP, fibrinogen and IL-6 have been shown to increase in systemic circulation in patients with stable COPD [9, 10]. Thrombocytes are activated by the release of proinflammatory cytokines, especially C-reactive protein [CRP], TNF-α and IL-6 [11]. MPV is one of the important predictors of platelet function. Studies have reported that the MPV and platelet counts are inversely related to the normal population [12, 13], so MPV and platelet counts need to be interpreted as a significant proportion of independent variables [14,15]. When the literature is reviewed, no study has been found to demonstrate that MPV/lymphocyte ratio (MLR) and MPV/platelet ratio (MPR) can be assessed in acute COPD exacerbation.

The views on the possibility that MPV can be used as an inflammatory marker are contradictory [11,14-18]; Gasparyan *et al*. [11] reported that MPV decreased as the degree of inflammation increased. Conversely, another study comparing COPD patients with healthy controls [14] showed a significant increase in MPV. In another study, it was stated that MPV decreased during the attack period and increased during the stabilization period [15]. Agapakis *et al*. [16] also found that lower MPVs were found in the stabilization and exacerbation groups, while Wang *et al*. [17] have found that COPD patients have a lower MPV in the stable and acute exacerbation phases.

According to inflammation in COPD, platelets may be expected to increase in number and volume as a positive acute phase reactant, considering that COPD is present during both stabilization and exacerbation periods. However, there are contradictory mechanisms explaining this condition. Because of the inflammation secondary to hypoxia ,the increase in prothrombotic activity caused by endothelial injury and the stimulatory effect of proinflammatory cytokines such as fibrinogen on platelets, can be explained the increase in the production of larger reactive platelets, when cytokines are considered to stimulate thrombocytes. MPV is elevated in low-grade chronic inflammatory diseases predominantly , thrombosis such as atherosclerosis [18]. MPV is reduced in high-grade inflammatory diseases such as active rheumatoid arthritis, inflammatory bowel disease and familial mediterranean fever. Intensive consumption of larger platelets in inflammatory regions may account for reduced MPV in patients with COPD. This study found that MPV was significantly increased in patients with acut exacerbation, and this increase was significant ($p < 0.001$). PLT values were found to be insignificant although there was an increase in these patients ($p = 0.84$).

Recently, platelet/lymphocyte ratio (PLR) has been evaluated as a prognostic marker in various inflammatory and cardiovascular diseases. Kurtipek *et al*. [20] reported that PLR was elevated in patients with active COPD compared with patients with stable COPD. In a study by Karadeniz *et al*. [20], PLR was shown not only in acute exacerbation patients but also in patients with stable COPD. In their study, PLR values were negatively correlated with severity of airway obstruction [21]. This increase was not statistically significant in spite of the fact that PLR value was significantly higher in patients with acute exacerbation ($p = 0.068$).

It has been reported that lymphocyte counts can also be used to show inflammation in chronic diseases such as platelets and neutrophils [22]. It is predicted that MLR may be more valuable than these parameters in reflecting inflammation given the increased MPV and lymphocyte count alone. One study showed that MLR levels were a prognostic factor in diabetic and acute myocardial infarction (MI), patients with increased MLR had a greater thrombotic volume and worse angiographic characteristics; high MLR is reported to be a risk factor for early and late mortality after MI [13]. In our study, MLR levels in acute exacerbated COPD patients were significantly higher than those in stable COPD patients ($p = 0.003$). To our knowledge, it is the first study to show high MLR levels in acute exacerbation in COPD patients ($p = 0.178$). We showed that MLR and MPR were higher

in patients with acute exacerbation in COPD than in patients with stable disease, and these differences were statistically significant. ($p = 0.003$ for MLR, $p = 0.04$ for MPR). Our study showed high MPR levels in patients with acute exacerbatif COPD. As in MLR, MPR does not show the severity of the disease with exacerbation in acute COPD ($p = 0.528$).

Assessment of the neutrophil/lymphocyte ratio (NLR) assessment in patients presenting with COPD exacerbation may provide important keys [23]. Lymphocytopenia is observed with increased WBC and neutrophil, especially in inflammation caused by bacterial infections. NLR has been reported to show active inflammation in cardiovascular diseases, renal diseases, familial Mediterranean fever, active ulcerative colitis and acute coronary syndrome [24, 25]. In one study, a significant increase in NLR was shown in stable COPD patients compared to healthy controls [26]. It is showed that a lower number of eosinophils and a higher NLR (> 7) were independent risk factors for sepsis mortality in the study of Terradas *et al*. [27]. Göçmen *et al*.'s study [28] that investigating the relationship between COPD and NLR severity, they reported that NLR correlated positively with arterial PaCO2 and negative correlation with pH and FEV1/FVC. In the study of Gao *et al*. [29], It was shown that NLR is high in both exacerbation and stabilization period, and there is a positive correlation between exacerbation severity and NLR. In our study, NLR levels were higher in acute exacerbativ COPD patients compared to stable COPD, it was also meaningful. ($p < 0.001$) NLR did not show the severity of acute exacerbated COPD, such as MLR and MPR ($p = 0.095$).

CRP is the most well-known inflammatory biomarker and is increasing in most cases of infection, inflammation and tissue damage. Correlation between WBC, CRP NLR, MPR and MLR supports the hypothesis that these new markers (MPR and MLR) can be used as a marker of acute exacerbation in COPD.

Limitations

Retrospective design is the most important limitation of this study. However, since our study is performed in a limited chest disease clinic, our results can not be generalized for all patients. The limited number of patients, especially if the number of patients with severe exacerbation is low. Finally there are complex relationships between comorbid diseases (diabetes mellitus, hypertension, hypercholesterolemia, obesity, metabolic syndrome, atrial fibrillation), medications (statins, some antihypertensives, antiplatelet drugs) and smoking in COPD. There is a need for comparative studies with matched controls for age and previous exposure to cigarette smoke, as these factors can greatly affect MPV values.

CONCLUSION

Exacerbation is a serious problem in COPD, this exacerbation is detected early and mortality and morbidity decrease if treatment is started. Hemogram parameters obtained as part of a routine laboratory test in patients with COPD are simple, inexpensive and easily accessible tools. Before the adverse clinical outcomes occur, they can be used either individually or by measuring the proportions to each other. It is predicted that there may be subclinical inflammation in the stable period, as well as useful indications to evaluate the severity of acute attacks and the management of acute episodes. They should also be able to detect patients who are at risk of exacerbation. Prospective studies are needed to demonstrate correlations between other inflammatory markers.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2019;5(6):1007-1013 ORIGINAL ARTICLE

The relation between performance and oral health in male athletes

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DOI: 10.18621/eurj.432272

ABSTRACT

Objectives: .Oral health is as important to an athlete's sporting successas regular exercise and adequate nutrition. The aim of this study was to investigate the relation between oral health and sporting performance.
Methods: This prospective study was carried out with male athletes. Athletes' demographic characteristics, dental care habits, number of decayed, missing or filled teeth, and sporting performance data were recorded. The Decayed Missing Filled Teeth (DMFT) index, Significant Caries index (SCI) and Plaque index (PI) were used to assess oral health. The T-Drill, Zig-Zag, Lateral Change of Direction (LCD) and 505 tests to assess agility, and 10-, 20- and 30-m short sprint tests were used to assess speed. The results were analyzed on SPSS software, and p values < 0.05 were regarded as significant.
Results: Ninety-six athletes were included in the study. Active caries was determined in 70.8%. The mean DMFT index value was 3.9 ± 3.7, mean SCI 10.2, and mean PI 0.9 ± 0.4. DMFT was ≥ 4 in 45.8% of athletes and < 4 in 57.2%. No difference was determined between subjects with DMFT < 4 and DMFT ≥ 4 in terms of age, height, body weight, or years engaged in sports. Agility and speed tests results were superior in subjects with DMFT<4 than in those with DMFT ≥ 4, and times to completion of performance tests were shorter ($p < 0.05$). Pearson correlation analysis revealed that DMFT was positively correlated with agility tests [T-Drill test ($r = 0.428$), Zig-Zag test ($r = 0.428$), LCD test ($r = 0.286$) and 505 test ($r = 0.529$)], and speed tests [short sprint, 10-m ($r = 0.309$), 20-m ($r = 0.336$), 30-m ($r = 0.449$)] ($p < 0.05$).
Conclusion: Impairment of oral health has an adverse effect on sporting performance, and this can lead to poorer results in performance tests such as agility and speed.

Keywords: Athletes, athletic performance, oral health, tooth

Received: June 8, 2018; Accepted: October 13, 2018; Published Online: October 15, 2018

An athlete's success is closely associated with physical and mental health [1]. Oral and dental health is one of the principal indicators of quality of life and general physical health, not only in athletes but in the general population [2]. In addition to gingival bleeding, dental caries, and tooth loss, poor oral health can also lead to several systemic diseases [3, 4]. Studies have shown that poor oral health is associated with weight loss, growth delay, coronary artery disease, constipation, and immune system weakness

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e-ISSN: 2149-3189

[5-8]. Oral health evaluation is based on various parameters, including numbers of decayed, missing or filled teeth, dental erosion, plaque levels, and gingival bleeding. Frequently employed tools include Decayed Missed Filled Teeth (DMFT) index, the Significant Caries index (SCI) and Green and Vermillion's Plaque index (PI) [9-12].

Studies have reported poor oral and dental health in both professional and amateur athletes [13-15]. While this is linked to dental decay occurring in association with frequent consumption of carbohydrates, a good energy source, or energy drinks containing high acid and carbohydrate levels, particularly during exercise, it is also related to individual factors such as nutrition or tooth brushing habits [16, 17]. Compromise of oral health in athletes can affect performance in terms of speed, strength, power, flexibility, and agility, and these changes in performance can be shown by means of tests, both in the field and in the laboratory [18-20].

The aim of this study was to investigate the relation between oral health and athletic performance.

METHODS

Study Design

Approval for the study was granted by the local ethics committee (No. 2012/12-02). This prospective study of male athletes was performed by the Kırıkkale University Department of Physical Education and Sports, Department of Oral Health and Dentistry, and Department of Emergency Medicine in November 2013. Information about the study aim was given to all athletes participating, and all provided informed consent. The study was performed in accordance with the Declaration of Helsinki and the Good Clinical Practice Directive.

Demographic data (age, height, body weight, sports age, heart rate, and fat measurements), oral health, numbers of decayed, missed and filled teeth, teeth brushing behaviors, and speed and agility test results were recorded for all subjects.

Assessment of Oral Health and Dental Care

Oral health and dental care examinations were conducted separately for all subjects. All oral and dental health examinations were carried out at the Kırıkkale University Faculty of Dentistry and by the same experienced dentist. The Green and Vermillion PI was used to evaluate oral hygiene, while assessment of decayed, missed or filled teeth was based on the DMFT index. The SCI indexwas used to evaluate the prevalence of caries in groups of individuals. Care was taken during oral health examinations to adhere to World Health Organization oral health and dental care rules[10, 11, 21-23].

Assessment of Speed and Agility

Athletes' performance tests were conducted at the Kırıkkale University Faculty of Physical Education and Sports gymnasium. Calibration and linearity settings of the instruments used in the measurements were completed before the performance tests. Height, weight and body composition measurements were performed using a Tanita body composition analyzer (Tanita Body Composition Analyzer BC 418 professional model, USA). All athletes were familiarized with the performance test procedures through practice in the gymnasium before the final tests. In order to prevent fatigue, athletes were instructed to avoid intense exercise for the 24-h period before the testing session. We used 10-, 20-, and 30-m short sprint tests to assess speed, and the T-Drill test, Zig-Zag test, 505 test, and Lateral Change of Direction (LCD) test to evaluate agility [24]. All tests were performed three times, and the best results were recorded.

Statistical Analysis

All data were analyzed on SPSS 18.0 (Statistical Package for the Social Sciences Inc., Chicago, IL, USA) software. Data obtained from the performance tests exhibited normal distribution and are expressed as mean ± standard deviation (SD). The independent samples t-test was used to compare the test results between the DMTF < 4 and DMTF ≥ 4 groups. We also determined relations between DMTF and different performance tests using Pearson correlations (r) and determination (r^2) coefficients. The p values < 0.05 were regarded as statistically significant.

RESULTS

Ninety-six male athletes were included in the

Table 1. Relations between athletes' demographic data and the DMFT index

	DMFT	n	mean ± SD	*p value*
Age (years)	≥ 4	41	20.29 ± 1.15	0.308
	< 4	55	20.58 ± 1.51	
Height (cm)	≥ 4	41	1.77 ± 0.76	0.698
	< 4	55	1.78 ± 0.61	
Weight (kg)	≥ 4	41	72.48 ± 14.04	0.344
	< 4	55	70.30 ± 8.29	
Years in sport	≥ 4	41	7.1 ± 2.5	0.76
	< 4	55	6.2 ± 2.6	

DMFT = Decayed missing filled teeth, SD = standard deviation

study. The mean age of the athletes was 20.4 ± 1.3 years, mean body mass index (BMI) was 22.01 ± 2.3 kg/m^2, mean body weight was 71.2 ± 11.0 kg, mean height was 178.4 ± 0.7 cm, mean body fat was 12.6 ± 2.5%, and mean heart rate was 68.2 ± 5.2 beats/min (resting state). The participants had been actively engaged in sports for a mean 6.6 ± 2.6 years and trained 3-4 times a week (for a total of 6-8 hours a week). Twenty-five (26.1%) attended regular dental check-ups. Twenty-eight (29.2%) brushed their teeth twice or more a day. However, only thirty-eight (39.6%) used dental floss or mouth rinses. The most frequently reported symptom was gingival bleeding (66.8 %).

The mean DMFT index value was 3.9 ± 3.7 based on the following component measures: mean number of teeth with active caries 2.7 ± 2.9, missing teeth 0.6 ± 1.1, and filled teeth 0.5 ± 1.4. Forty-one athletes (42.7%) had DMFT ≥ 4 and 55 (57.3%) had DMFT < 4. The mean SCI value was 10.2, and the prevalence of caries was 66%. The mean PI score was 0.9 ± 0.4. Twenty-eight (%29.2) athletes had non-carious teeth and 70.8% carious teeth.

There was no significant difference between the DMFT ≥ 4 and DMFT < 4 groups in terms of age, height, weight or years engaged in sport ($p > 0.05$) (Table 1).

A difference was determined between the DMFT ≥ 4 and DMFT < 4 groups in terms of decayed, missing, and filled teeth ($p < 0.01$, $p < 0.01$, and $p < 0.05$, respectively). The most important factor affecting DMFT values was the number of decayed teeth (Table 2).

The DMFT < 4 group was more successful than the DMFT ≥ 4 group in the agility tests (T-drill, Zig-Zag, LCD, and 505) and speed tests (10-, 20-, and 30-m short sprint) ($p < 0.05$). Success rates decreased as DMFT values increased. Increased DMFT values were associated with prolonged times to completion of agility and speed tests. Analysis of t values in the agility tests revealed that the most significant difference was in the Zig-Zag tests, while the greatest difference in the speed test was observed in the 20-m sprint test (Table 3).

Table 2. Comparison of athletes with DMFT < 4 and with DMFT ≥ 4 in terms of numbers of decayed, missing and filled teeth

n = 96	DMFT	min-max	mean ± SD	*p value*
Decayed	≥ 4	0-11	4.97 ± 3.12	**< 0.01**
	< 4	0-3	1.04 ± 1.02	
Filled	≥ 4	0-7	1.24 ± 1.93	**< 0.01**
	< 4	0-1	0.09 ± 0.29	
Missing	≥ 4	0-4	1.20 ± 1.29	**< 0.05**
	< 4	0-3	0.18 ± 0.51	
Total	≥ 4	4-15	7.39 ± 3.00	**< 0.01**
	< 4	0-3	1.31 ± 1.10	

DMFT = Decayed missing filled teeth, SD = standard deviation, min = minimum, max = maximum

Table 3. Analysis of performance test results by DMFT index scores

n = 96		DMFT	min - max	mean ± SD	*p* value*
Agility	**T-Drill test** (sec)	≥ 4	9.14 - 13.78	11.33 ± 1.06	**< 0.05**
		< 4	9.06 - 13.50	10.64 ± 0.91	
	Zig-Zag test (sec)	≥ 4	5.64 - 7.34	6.47 ± 0.40	**< 0.05**
		< 4	5.08 - 6.86	6.03 ± 0.39	
	505 test (sec)	≥ 4	2.27 - 4.44	3.02 ± 0.47	**< 0.05**
		< 4	2.31 - 3.53	2.77 ± 0.36	
	LCD test(sec)	≥ 4	4.70 - 7.44	6.12 ± 0.61	**< 0.05**
		< 4	4.22 - 7.12	5.64 ± 0.73	
Speed	**10 m sprint best** (sec)	≥ 4	1.59 - 2.75	1.99 ± 0.29	**< 0.05**
		< 4	1.56 - 2.38	1.85 ± 0.18	
	20 m sprint best (sec)	≥ 4	2.34 - 3.10	2.63 ± 0.21	**< 0.05**
		< 4	1.75 - 2.81	2.47 ± 0.21	
	30 m sprint best (sec)	≥ 4	3.24 - 5.56	4.25 ± 0.60	**< 0.05**
		< 4	3.16 - 4.78	3.89 0.50	

DMFT = Decayed missing filled teeth, SD = standard deviation, min = minimum, max = maximum, *The independent samples t-test

The results showed that the correlation coefficient values of the agility and speed tests were all significant ($p < 0.01$). Positive correlations were observed between the DMFT index and performance tests. This correlation was especially significant in the Zig-Zag test (among the agility tests) and in the 30-m sprint test (among the speed tests) (Table 4).

DISCUSSION

A successful athlete must always take regular exercise andreceive balanced nutrition, and must always keep his body healthy and prepared. As also reported in previous studies, considering the link between oral and dental health, general health, and adequate nutrition, these play a significant role in athletic performance, and impairment of oral health has a negative effect on sporting performance [2, 3, 25, 26]. The findings from our study show that an increasing number of decayed, missing and filled teeth has an adverse impact on performance, and prolongs the completion of both agility and speed tests.

Studies of professional and non-professional athletes in various different spheres have evaluated athletes in terms of oral health, nutritional habits, and saliva profiles [13-15, 27, 28]. Ashley *et al*. [25] reported poor oral health in professional athletes, with a dental caries rate of 15-75%, and a dental erosion rate of 36-85%, while Gay-Escoda *et al*. [14] reported a gingival bleeding rate of 60%. Using a similar method to that in our study, Forrest [29] evaluated numbers of decayed, missing, and filled teeth in Olympic and non-Olympic athletes and reported DMFT indices of 2.8 to 16.8. In two different studies of footballers, de Sant'Anna *et al*. [15] reported

Table 4. Correlation between performance tests and the DMFT index

Relation to DMFT		r	r^2	*p* value*
Agility	T- Drill test	0.428	0.18	**< 0.01**
	Zig-Zag test	0.529	0.28	**< 0.01**
	505 test	0.286	0.08	**< 0.01**
	LCD test	0.398	0.15	**< 0.01**
Speed	10-m sprint best	0.281	0.08	**< 0.01**
	20-m sprint best	0.309	0.09	**< 0.01**
	30-m sprint best	0.336	0.11	**< 0.01**

DMFT = Decayed missing filled teeth, Pearson correlations (r) and determination (r^2) coefficients. *The independent samples t-test

DMFT = 8, and SCI = 12, while another study reported findings of DMFT = 5.7 ± 4.1, and SCI = 10 in the Barcelona soccer team [14]. In our study, dental decay was determined in 70.8% of athletes and gingival bleeding in 66.8%, whilethe mean DMFT score was 3.9 ± 3.7, mean SCI = 10.2, and mean PI = 0.9 ± 0.4. Our results are in agreement with those of previous studies, and suggest that Turkish athletes, like those in other parts of the world, do not attach sufficient importance to oral and dental health.

Several studies have investigated the effect of oral health on physical performance. However, these studies have reported general conclusions that poor oral health has an adverse impact on physical performance. Needleman *et al*. [30] reported that approximately half of footballers in the United Kingdom were troubled by poor oral health, and that some experienced decreased quality of life and physical performance due to poor oral health. In studies of Olympic athletes, most subjects have reported decreased performance due to poor oral health, while some have even reported being prevented from training due to problems deriving from oral and dental health impairment [2, 29, 31, 32]. This led the International Olympic Committee to take action, and obliged it to issue calls to all relevant countries concerning oral health protection [33]. One more specific investigation of oral health and performance examined the link between oral health and grip strength and showed that poor oral health had an adverse effect on grip strength [34]. Iwasaki *et al*. [35] considered the subject from a different perspective and showed that poor oral health also caused compromise of cognitive functions. Our study investigated the relation between male athletes' oral health and sporting performances using previously developed performance tests [24]. In one previous study, Reilly *et al*. reported a mean 30-m short sprint time of 4.46 ± 0.21 sec, while in our study the relevant timings were 4.25 ± 0.60 sec in subjects with DMFT ≥ 4 and 3.89 ± 0.50 sec in those with DMFT < 4 [36]. In their study of 106 professional athletes, Little *et al*. reported a 10-m short sprint time of 1.83 sec, a 20-m sprint time of 2.40 sec and a mean Zig-Zag agility test time of 5.34 sec, while in our study these values were similar to those of professional athletes only in the subjects with DMFT < 4, but were longer in those with DMFT ≥ 4 [37]. Similarly,in their study of agility in footballers, Hoffman *et al* reported a mean T-Drill test time of 9.36 ± 0.44 sec, while in our study this time was only approached by athletes withDMFT < 4 (T-drill agility test results; DMFT < 4 = 10.64 ± 0.91 sec; DMFT ≥ 4 = 11.33 ± 1.06 sec) [38]. It would appear that an increased number of decayed, missing or filled teeth has an adverse impact on physical performance. This thesis is supported by all agility and speed test results being better in athletes with better oral health, with DMFT < 4, than in those with DMFT ≥ 4.

Limitations

There are a number of limitations to our study. First, although athletes were asked to rest in the period before the tests were to be performed, some may not have complied. This may have affected the results obtained in the performance tests. Second, since only male athletes were included in the study, the results obtained cannot be generalized to all athletes. Wide-ranging performance studies also including female athletes are therefore needed.

CONCLUSION

In conclusion, the data we obtained show that impairment of oral health in male athletes, in other words an increasing number of decayed, missing or filled teeth or gingival bleeding, has an adverse effect on physical performance and that this results in slower times in tests of physical performance, such as speed and agility, and to poorer outcomes.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2019;5(6):1014-1019 ORIGINAL ARTICLE

Effect of cardiac rehabilitation on neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in ST elevation myocardial infarction patients

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DOI: 10.18621/eurj.447027

ABSTRACT

Objectives: Cardiac rehabilitation is known to have positive effects on the inflammatory processes. The neutrophil-to-lymphocyte ratio (NLR) and the platelet-to-lymphocyte ratio (PLR) were found to be indicative of inflammation. The purpose of this study is to determine the effects of cardiac rehabilitation on the NLR and PLR ratios of ST elevation myocardial infarction (STEMI) patients.

Methods: The study includes 101 STEMI patients that underwent primary percutaneous coronary intervention (PCI). The patients were randomized into two groups: the cardiac rehabilitation group (CR group, n = 68), and the control group (n = 33). One month after primary PCI, cardiac rehabilitation was applied to CR group with cycle ergometer for 8 weeks (30 sessions). The NLR and PLR parameters were calculated from the complete blood count results from before and after the cardiac rehabilitation application for both groups.

Results: When the baseline values of the two groups were evaluated, the hemoglobin value of the control group (13.10 ± 1.52 g/dL vs. 13.79 ± 1.26 g/dL; $p = 0.03$) and the PLR value of the CR group (122.50 ± 43.89 vs. 92.41 ± 23.70; $p = 0.001$) were significantly higher. The post-cardiac rehabilitation complete blood count parameters, and the NLR and PLR values were similar in both groups. The NLR (3.11 ± 1.95 vs 2.39 ± 1.03; $p = 0.003$) and PLR (122.50 ± 43.89 vs. 108.68 ± 41.83; $p = 0.025$) parameters significantly decreased after the cardiac rehabilitation application in the CR group, whereas there wasn't a change in the control group.

Conclusion: It was found that cardiac rehabilitation applied in STEMI patients caused a significant decrease in NLR and PLR parameters, which are indicators of inflammation.

Keywords: cardiac rehabilitation, myocardial infarction, inflammation, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio

Received: July 23, 2018; Accepted: July 25, 2018; Published Online: July 27, 2018

Acute myocardial infarction (MI) is a serious disease that develops due to sudden decrease or discontinuation of coronary blood flow due to various causes and results in different degrees of ischemic necrosis of the myocardial tissue associated with the artery. Restoration of coronary blood flow is of great importance in the early stage of the disease, within 12 hours of the onset of symptoms and in patients with persistent ST-segment elevation or possible new left bundle branch block (LBBB). For this purpose, primary percutaneous coronary intervention (PCI) is applied.

Chronic inflammation has an important role in atherosclerosis, coronary artery disease, and STEMI.

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e-ISSN: 2149-3189

This inflammation is related to the onset and progression of the disease, and plaque rupture and thrombus formation in the AMI process. Leukocytes play an important role in this inflammatory process [1]. The neutrophil-to-lymphocyte ratio (NLR) is one of the important parameters that can be used to determine the severity of this inflammatory process. High NLR levels are one of the most important in-hospital and long-term mortality indicators in acute coronary syndrome (ACS). Platelet-to-lymphocyte ratio (PLR) is also a parameter showing chronic inflammation.

Cardiac rehabilitation (CR) can be defined as all of the activities necessary to ensure that cardiovascular patients are able to regain their physical, mental and social status as early as possible [2]. Numerous studies have documented the efficacy of exercise-based CR after extensive MI. Exercise training in CR after acute coronary syndromes has multifaceted effects. Exercise training appears to be associated with endothelial function, inflammation, cardiovascular autonomic function regulation and risk factor control. Exercise also has benefits related to antithrombotic and ischemic preconditioning.

We have recently published a study about the effects of CR on cardiac autonomic functions; we re-analyzed the study data and evaluate NLR, and PLR. Using these data, we examined the effect of CR on the NLR, PLR and on the inflammatory process in STEMI patients.

METHODS

Patient Selection and the Procedure

The study includes 101 STEMI patients that admitted to a public University hospital in Turkey in 2014 with STEMI diagnosis, who underwent primary PCI (aged 35-85). CR was applied to 68 of these patients at least 1 month after primary PCI, and 33 were randomised in the control group. The exclusion criteria were as follows: patients with severe valve disease, who were over 85 years of age, respiratory dysfunction, decompensated heart failure, uncontrolled hypertension (HT) and diabetes mellitus (DM), patients with a cerebrovascular disease with orthopedic restriction, and who did not accept the CR program for various reasons. Patients who did not have a sinus rhythm, or were on a pacemaker were also excluded from the study. Patients' anamnesis and physical examination findings, atherosclerosis risk factors, demographic data, height, weight, body mass indexes, biochemical parameters, lipid parameters were recorded. NLR and PLR values before rehabilitation were calculated from the complete blood counts taken at least 1 month after STEMI in patients (CR and control groups). Patients in the CR group were taken to the 8-week CR program at least 1 month after STEMI. Eight weeks of cycling ergometry was performed for an average of 4 sessions per week for 30 minutes (5 minutes warming, 20 minutes aerobic exercise to reach 70-85% of heart rate, 5 minutes cooling) in the direction of heart rate and the watt level. At the end of the rehabilitation, the complete blood count measurement was repeated and the NLR and PLR values were recalculated. Again blood samples were taken from the patients in the control group 1 month after the first blood samples and the NLR and PLR values were calculated. All patients were echocardiographically evaluated using standard techniques using the GE Vivid 7 pro (General Electric Company, Connecticut, USA) instrument with a 3.5 MHz phase-adjusted transducer and in accordance with the recommendations of the American Echocardiography Society guideline.

Statistical Analysis

The data were analyzed using the SPSS (IBM SPSS Statistics Version 22) program. Continuous variables were expressed as mean ± standard deviation, and categorical variables as percent. The normal distribution of continuous variables was assessed by the Shapiro-Wilk normality test. Independent sample T-test or Mann-Whitney U test was used to compare parametric data, and Chi-square or Fisher's Exact test was used to compare categorical variables. Repeated measurements (pre- and post-rehabilitation) were performed with the Wilcoxon signed-rank test. $p < 0.05$ was accepted as significant in all statistical measures.

RESULTS

When the demographic and laboratory findings of the patients were examined, there were no significant differences in age, gender, DM, HT, hyperlipidemia.

Table 1. Baseline characteristics of the study patients

	CR Group (n = 68)	Control Group (n = 33)	*p* value
Age (years)	58.23 ± 9.69	55.48 ± 9.99	0.21
Gender			
Male, n (%)	54 (79.4)	27 (81.8)	0.77
Female, n (%)	14 (20.6)	6 (18.2)	
Diabetes mellitus, n (%)	13 (19.4)	7 (21.9)	0.77
Hypertension, n (%)	24 (35.8)	6 (18.8)	0.08
Smoking, n (%)	31 (46.3)	21 (70)	0.03
Previous PCI, n (%)	7 (10.4)	4 (12.1)	0.80
LVEF (%)	42.13 ± 11.07	42.12 ± 9.13	0.90
Systolic BP (mmHg)	115.80 ± 9.79	111.77 ± 13.81	0.10
Diastolic BP (mmHg)	71.02 ± 7.94	69.03 ± 8.4	0.28
Average HR (beats per minute)	66.23 ± 8.04	69.69 ± 10.31	0.069
WBC count ($\times 10^3$)	9.61 ± 2.80	9.5 ± 2.34	0.97
Neutrophil count ($\times 10^3$)	6.26 ± 2.39	5.84 ± 2.07	0.40
Lymphocyte count ($\times 10^3$)	2.29 ± 0.87	2.62 ± 0.72	0.07
Eosinophil count ($\times 10^2$)	2.63 ± 2.21	2.06 ± 1.63	0.20
Hemoglobin (g/dL)	13.10 ± 1.52	13.79 ± 1.26	**0.03**
Thrombocyte count ($\times 10^3$)	256.70 ± 83.85	230.41 ± 42.69	0.27
NLR	3.11 ± 1.95	2.41 ± 1.15	0.065
PLR	122.50 ± 43.89	92.41 ± 23.70	**0.001**
Urea (mg/dL)	15.77 ± 4.51	15.51 ± 5.43	0.49
Creatinine (mg/dL)	0.8 ± 0.17	0.79 ± 0.18	0.55
Total colesterol (mg/dL)	179.96 ± 47.41	182.48 ± 41.33	0.51
LDL-C (mg/dL)	120.36 ± 44.61	117.84 ± 35.26	0.70
Sodium (mEq/L)	140.07 ± 2.57	140.64 ± 2.25	0.19
Potassium (mEq/L)	4.43 ± 0.39	4.27 ± 0.45	0.10
Rehabilitation start (days)	62.21 ± 29.33	-	
Drugs			
ASA, n (%)	68 (100)	33 (100)	1.0
Clopidogrel, n (%)	42 (61.8)	17 (51.5)	0.32
Ticagrelor, n (%)	26 (38.2)	16 (48.5)	0.32
Beta blocker, n (%)	66 (98.5)	33 (100)	0.99
ACEI, n (%)	60 (89.6)	30 (90.9)	0.83
ARB, n (%)	6 (9.1)	2 (6.1)	0.60
Statin n (%)	68 (100)	32 (97)	0.99
Spironolactone, n (%)	27 (40.9)	9 (27.3)	0.18
Myocardial infarct area			
Anterior, n (%)	34 (50)	15 (45.5)	0.66
Inferior, n (%)	34 (50)	18 (54.5)	

Data are shown as mean±standard deviation or number (%). ACEI = angiotensin converting enzyme inhibitor, ARB = angiotensin receptor blocker, ASA = acetilsalysilic acid, BP =blood pressure, CR = cardiac rehabilitation, HR =heart rate, LDL-C= low density lipoprotein cholesterol, LVEF = left ventricular ejection fraction, NLR = neutrophil-to-lymphocyte ratio, PCI = percutaneous coronary intervention, PLR = platelet-to-lymphocyte ratio, WBC = white blood cell

Table 2. Comparison of the hemogram parameters after rehabilitation between groups

	CR Group (n = 68)	Control Group (n = 33)	*p* value
WBC count (×10^3)	8.75 ± 2.36	8.85 ± 1.32	0.81
Neutrophil count (×10^3)	5.40 ± 1.49	5.23 ± 1.63	0.61
Lymphocyte count (×10^3)	2.47 ± 0.83	2.49 ± 0.68	0.91
Eosinophil count (×10^2)	2.30 ± 1.49	1.93 ± 1.12	0.22
Hemoglobin (g/dL)	13.54 ± 1.26	13.95 ± 1.23	0.13
Thrombocyte count (×10^3)	245.74 ± 57.29	230.74 ± 39.09	0.19
NLR	2.39 ± 1.03	2.23 ± 0.96	0.48
PLR	108.68 ± 41.83	99.65 ± 32.88	0.29

Data are shown as mean±standard deviation. CR = cardiac rehabilitation, NLR = neutrophil-to-lymphocyte ratio, PLR = platelet-to-lymphocyte ratio, WBC = white blood cell

There was no significant difference in echocardiographic findings. Among the complete blood count parameters, the hemoglobin value was significantly higher in the control group (13.10 ± 1.52 g/dL vs 13.79 ± 1.26 g/dL; $p = 0.03$), and the PLR was significantly higher in the CR group (122.50 ± 43.89 vs 92.41 ± 23.70; $p = 0.001$) (Table 1). There was no difference between the groups in terms of MI region and applied treatments (Table 1). NLR and PLR values of the whole blood count parameters after the rehabilitation period were similar in both groups (Table 2).

Complete blood count parameters, NLR and PLR values of the CR group and the control group were analyzed before and after rehabilitation. Leukocyte and neutrophil counts in the CR group were significantly higher than those before CR, while hemoglobin values were significantly lower (Table 3). NLR (3.11 ± 1.95 vs 2.39 ± 1.03; $p = 0.003$) and PLR (122.50 ± 43.89 vs. 108.68 ± 41.83; $p = 0.025$) ratios of the CR group were significantly decreased with CR (Table 3). There was no significant difference in NLR and PLR values of the control group before and after CR period (Table 3).

Table 3. Rehabilitation group and control group; comparison of hemogram parameters before and after rehabilitation

	CR Group (n = 68)			Control Group (n = 33)		
	Before rehabilitation	After rehabilitation	*p* value	Initial	Control	*p* value
WBC count (×10^3)	9.61 ± 2.80	8.75 ± 2.36	**0.016**	9.5 ± 2.34	8.85 ± 1.32	**0.017**
Neutrophil count (×10^3)	6.26 ± 2.39	5.40 ± 1.49	**0.007**	5.84 ± 2.07	5.23 ± 1.63	0.180
Lymphocyte count (×10^3)	2.29 ± 0.87	2.47 ± 0.83	0.067	2.62 ± 0.72	2.49 ± 0.68	0.068
Eosinophil count (×10^2)	2.63 ± 2.21	2.30 ± 1.49	0.219	2.06 ± 1.63	1.93 ± 1.12	0.395
Hemoglobin (g/dL)	13.10 ± 1.52	13.54 ± 1.26	**< 0.001**	13.79 ± 1.26	13.95 ± 1.23	0.059
Platelet count (×10^3)	256.70 ± 83.85	245.74 ± 57.29	0.38	230.41 ± 42.69	230.74 ± 39.09	0.739
NLR	3.11 ± 1.95	2.39 ± 1.03	**0.003**	2.41 ± 1.15	2.23 ± 0.96	0.686
PLR	122.50 ± 43.89	108.68 ± 41.83	**0.025**	92.41 ± 23.70	99.65 ± 32.88	0.161

Data are shown as mean±standard deviation. CR = cardiac rehabilitation, NLR = neutrophil-to-lymphocyte ratio, PLR = platelet-to-lymphocyte ratio, WBC = white blood cell

DISCUSSION

In our study, the NLR and PLR values in patients who had STEMI had significantly decreased after a CR program that was applied one month after the primary CPI. When this value is evaluated as an inflammation parameter, it can be said that CR has a positive effect on the cardiac inflammatory process.
Chronic inflammation plays an important role in the pathogenesis of atherosclerosis. As a result, a large number of intracoronary atherosclerotic plaques form. Plaques include a fibrous capsule, a nucleus composed of necrotic tissue and cholesterol, smooth muscle cells, calcification, and cells involved in inflammation. Mild or moderate stenotic plaques were detected in 3/4 of the patients with myocardial infarction in the responsible lesion area, and the concept of vulnerable plaques become prominent as this condition does not develop only in areas with severe stenosis. Vulnerable plates are probably already in an inflammatory process that has already begun. This process facilitates the plaque rupture [3]. It is known that calcification is not a feature of vulnerable plaques. In a study published in 2014, it was found that NLR value correlated with the presence of noncalcified plaques in the patient [4].

In a meta-analysis published in 2018; patients with higher NLR values had more frequent in-hospital angina, advanced cardiac insufficiency, arrhythmia, major cardiovascular events, death due to cardiac causes, all-cause mortality, stent thrombosis, non-fatal myocardial infarction, and no-reflow phenomena. In addition, long-term mortality, cardiac mortality and major cardiovascular events were significantly more frequent; but there was no difference in non-fatal myocardial infarctions [5]. Thus, cardiovascular outcomes correlate with inflammation level. The ability of the NLR level to predict this may suggest that this parameter may be useful in terms of clinical follow-up.

The NLR used in various chronic diseases is an important parameter showing the chronic inflammation used in patients with coronary artery disease and ACS. Different cut-off levels were determined for this value in different studies. In general, a high value has been shown to be associated with poor cardiovascular outcome. In a study published in 2014 that was conducted with 250 patients, it was determined that an NLR value above 7.4 is an independent indicator of short- (< 30 days) and long-term (< 2 years) deaths [6]. In our study, the NLR value was initially 3.11, and 2.39 afterward. These findings were statistically significant. Cardiac rehabilitation is known to decrease proinflammatory cytokine levels and consequently has an inflammation-reducing effect by reducing oxidative stress. When evaluated in this respect, this value can not be considered as high risk in the beginning or after rehabilitation. However, it might have a positive effect on chronic inflammation and its cardiovascular effects. This decline in value supports this notion. It can be said that; the higher the NLR value, the greater the efficacy of cardiac rehabilitation. Additional research is needed to confirm this.

PLR value is also a parameter showing chronic inflammation like NLR. Azab *et al*. [7] conducted a study in 619 patients with non-STEMI. It has been shown that high PLR value increases mortality; and that among patients with PLR > 176, double-antiplatelet therapy reduced mortality compared to single-antiplatelet treatment. In our study, PLR value decreased from 122 to 108 after rehabilitation, and this result is statistically significant. From this point of view, it can be predicted that chronic inflammation can be regressed with cardiac rehabilitation. Further studies with larger samples are required to support these findings.

Limitations

Among the limitations of our study were as follows: the relatively low number of patients, not examining other inflammatory parameters (hs-CRP, etc.), evaluating only STEMI patients.

CONCLUSION

In our study, the NLR and PLR values in patients who had STEMI had significantly decreased after a CR program that was applied one month after the primary PCI. This suggests that CR has a positive effect on inflammation. Our study is the first in the literature to assess the effect of CR on the cardiac inflammatory process as a guideline for studies to be performed in larger patient populations.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2019;5(6):1020-1023 CASE REPORT

Unusual uterine metastasis of plasmablastic lenfoma: a case report

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DOI: 10.18621/eurj.455365

ABSTRACT

Plasmablastic lenfoma (PBL) is a diffuse large B-cell lymphoma (DLBCL)'s variant form that is especially reported in HIV-infected patients. PBL is a rare entity seen most commonly in the setting of immunocompromise. However, this disease may present in extraoral sites in which the genital tract appears to be the less common. Here we present a patient with uterine and fallopian mass diagnosed PBL. A 51-year-old multipara (G6P4C2) woman with a chief complaint of irregular vaginal bleeding was presented to our outpatient clinic. She had a prior history of breast cancer treated by right mastectomy and adjuvant chemotherapy.Transvaginal ultrasonography and magnetic resonance imaging both showed an uterine mass thought to be leiomyoma, which 4×3 cm in size on the anterior wall of uterin corpus. The pathology result of the mass was found to be plasmablastic lymphoma. Genital involvement also appears to be last but possible site option to find PBL focus in immunocompetent patients.

Keywords: Plasmablastic lymphoma, uterine mass, surgery

Received: August 27, 2018; Accepted: June 1, 2019; Published Online: July 29, 2019

Plasmoblastic lenfoma (PBL) is a diffuse large B-cell lymphoma(DLBCL)'s variant form that is especially reported in HIV-infected patients. After time this illness is also reported in immunocompetent patients [1-3]. Despites PBL most frequently presents in oral cavity, in the literature it was reported that many other parts of the body are affected by this illness [3]. On rare occasions, female genital tract organs can be the initial site of lymphoproliferative malignancies [4, 5].

It generally presents earlier in life in HIV-infected patients compared with immunocompetent patients (median age, 38 years vs. 58 years, respectively). PBL has a clear male predominance, and the incidence rises in HIV-infected patients with decreased CD4 count [1]. We present an interesting case of plasmablastic lymphoma, which is a rare type of non-Hodgkin lymphoma (NHL) that is typically diagnosed in HIV-positive patients and has an immunophenotype that overlaps with multiple myeloma [6]. NHL and it is estimated incidence of PBL accounts for approximately 5% of all HIV-positive NHL cases. Incidence of HIV-negative PBL is still unclear [7, 8]. With regard to the management of PBL, the common treatments are chemotherapy, radiotherapy with or without surgical excision, or the combination of chemotherapy and radiotherapy. PBL has a poor prognosis and in the first year illness relapses at the rate of approximately 60% [8, 9].

Here we report a rare case of patient with plas-

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e-ISSN: 2149-3189

moblastic lymphoma of uterus and fallopian tubes.

CASE PRESENTATION

A 51 year-old multipara (G6P4C2) woman with a chief complaint of irregular vaginal bleeding was presented to our outpatient clinic. She had a prior history of breast cancer treated by right mastectomy and adjuvant chemotherapy. Chronic renal failure treated first by peritoneal dialysis and eventually renal transplant. Physical examination did not show any abdominal or cervical mass. Transvaginal ultrasonography and magnetic resonance imaging (MRI) (Figure 1) both showed an uterine mass thought to be leiomyoma, which 4×3 cm in size on the anterior wall of uterin corpus and tubular-cyctic mass sized approximately 5 cm on right ovary. A complete blood count revealed the following: hemoglobin, 10.8 g/L; white blood cell count, 12.15×103/uL with a differential count of 75.9% segmented neutrophils, 20.3% lymphocytes, 2.9% monocytes, 0.4% basophils, and 0.5% eosinophils. There was no abnormality in Pap-smear results. Total abdominal hysterectomy and bilateral salphingo-oophorectomy was performed and the specimen was sent to the Department of Pathology for pathological-anatomical analysis.

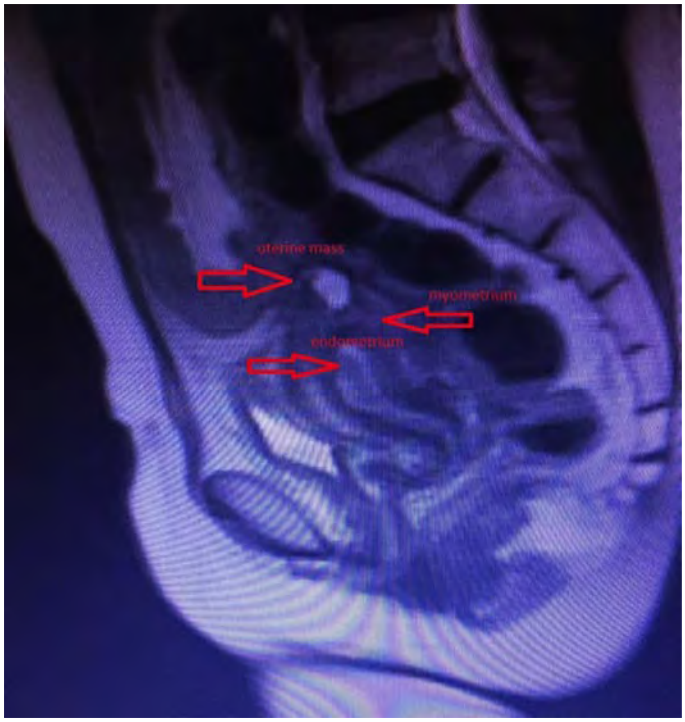

Figure 1. Magnetic resonance image of Uterine mass. Arrows shows uterine mass; endometrium and myometrium.

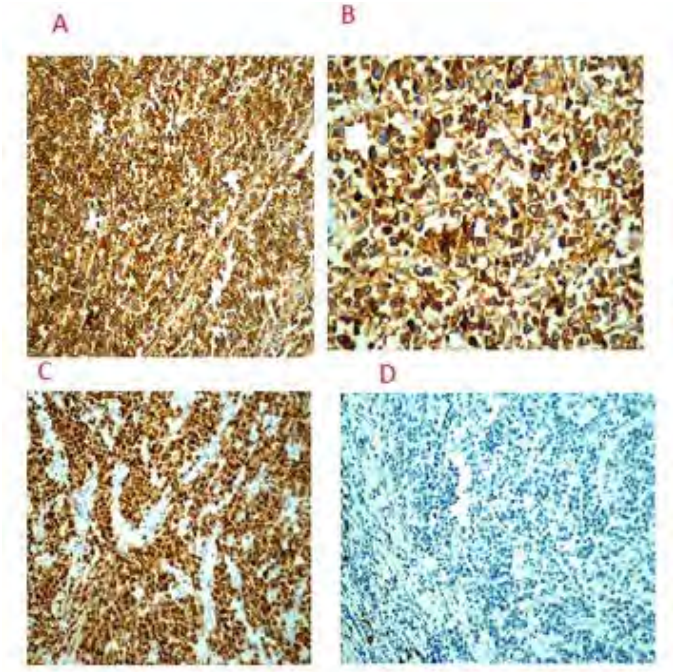

Figure 2. Immunohistochemical markers. (A) CD 38 with membranous positive (CD38×200), (B) CD 138 with membranous positive (CD138×200), (C) MUM-1 with nuclear positive (MUM-1×200), and (D) CD 20 with negative (CD20×200).

On postoperative period, hemoglobin and hematocrit levels began to drop, in spite of blood transfusion. On the first day, hemoglobin was 8.9 g/L, transfusion with 2 units of erithrocyte suspension was made. However, there were no increase on levels, on the contrary, until pos-operative day 18, hemoglobin levels continued to drop with the last value being 7.6 g/L. Transvaginal ultrasonography and computed tomography (CT) scan were performed. They showed a loculated, hyperdense lesion on operation site, sized 7.5×5 cm thought to be an abscess or hematoma. CT scan also showed a hyperreactive lymph node sized 2.7×1.5 cm on right inguinal region. Ongoing ampiric antibiotherapy was changed to piperacillin-tazobactam 4.5 g per three times a day. In the meantime, pathology report of operation specimen was completed and result came as malignant. A plasmablastic neoplasm showing lambda light chain monotype was localized diffusely on serosal surfaces of the uterus, paracervical and parametrial tissues, external surface and muscular layer of both fallopian tubes, omentum was detected. On microscopic examination, morphologically plasmablastic, multinuclear with large, pleomorphic

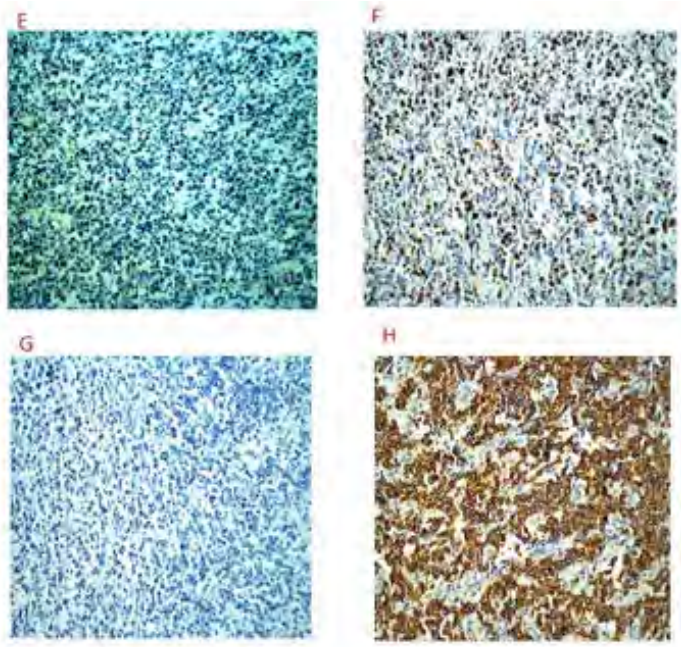

Figure 3. Immunohistochemical markers of speciment. (E) CD 79alfa with negative (CD 79alfa), (F) EBER with positive (EBER CISH×200), (G) Kappa with negative (Kappa CISH×200), and (H) Lambda positive (Lambda CISH×200).

nuclei and eccentric nuclei localization cells were seen. In immunohistochemical studies, neoplastic cells were CD38(+), CD138(+), MUM-1(+), CD20(-) (Figure 2), CD79a(-), EBER(+), KAPPA(-), LAMBDA(+) (Figure 3), CD56(+), CD117(+), CD3(-), CD15(-), LCA(+), bcl-1(-), bcl-2(-),ALK(-), pancytokeratin(-), cytokeratin 7(-), cytokeratin 20(-). Ki67 proliferation index was 80-90%. Bone marrow aspiration was performed, however, the result was normocellular marrow. A lymph node biopsy was planned, tru-cut biopsy of the right inguinal lymph nodes was resulted in confirmation of the previous report, neoplastic lymphoid infiltration matching with lymphoplasmacytic lymphoma. PET CT scan showed increased FDG uptake on little curvature of the stomach and various peritoneal serosal surfaces. Antibiotic therapy ended and the patient was transferred to hematology clinic on postoperative 32nd day.

DISCUSSION

Plasmablastic lymphoma (PBL) is a very rare and an aggressive type of lymphoma. It displays some features of multiple myeloma (MM), and some features of diffuse large B-cell lymphoma (DLBCL). This tumor was described in the oral cavity of HIV-positive patients firstly in 1997. Subsequently, the tumor has been described in regions other than the oral cavity, particularly in HIV negative patients [3]. Differences between HIV-positive and HIV-negative cases of PBL are significant. Clinically, HIV-positive patients have a stronger male predominance, early lifetime onset, and classically manifest with EBV positive oral cavity lesions. Additionally, HIV-positive patients with PBL respond to antiretroviral medications and have improved overall survival [6].

In the literature, it was mentioned that a weak male predominance (male/female ratio of 1.8) was noticed upon the PBL cases. HIV-associated PBL cases male/female ratio was reported as a 4:1. All the Chinese PBL cases included in this study were HIV-negative, suggesting HIV might be more easily affected in male patients with PBL. Actually, most PBL cases were HIV-positive. As a result of the study, the advanced clinical stage was associated with the poor survival and identified as a prognostic factor for HIV-negative PBL patients in Chinese [7].

PBL has been less frequently described in immunocompetent patients and in extraoral locations like our case. The GI tract appears to be the most common site of involvement (20%) in HIV-negative PBL. Soft tissue (17%), bone marrow (15%), and skin (12%) are the other sites that could be seen. Uterus and the fallopian tubes are the rare localizations. In chemotherapy protocols mostly used CHOP or CHOP-like regimen in PBL. Hyper-CVAD, dose-adjusted EPOCH and CODOX-M/IVAC regimen is also used in PBL. Unfortunately aggressive regimens have not shown a statistically significant improvement in outcome and survival [2, 3, 8].

HIV-negative patients was associated with worse outcomes. Despite this opinion, comparative studies have shown that HIV status does not appear to affect the outcomes in PBL. In the literature, HIV-positive patients with PBL that added antiretroviral therapy seemed to do better to chemotherapy [6, 7].

PBL has a characteristic immunophenotype, where in they are positive for the plasma cell markers such as CD38, CD138, and MUM1; and negative for the typical B-cell antigens such as CD20 and CD79

[6, 9]. As the pathological examination of our case showed that CD38, CD138 and MUM1 markers were positive and CD20 and CD79 were negative. There is usually a high level of proliferation as detected by high Ki-67 levels in PBL.6We also determined in our case that Ki67 proliferation index was 80-90%. In two studies they suggested that Ki-67 values more than 80% have been seen to be an independent poor prognostic factor [6, 10].

In HIV-negative patients, the median overall survival was 19 months compared with 15 months in HIV-infected patients. There is no clear role for transplant in these patients. This case illustrates an interesting presentation of PBL and the challenges faced in treating these patients, because of the paucity of reported cases and the absence of a standard therapy. Exploring new therapeutic pathways by targeting activated pathologic pathways might be in the interest of these patients [1].

CONCLUSION

In conclusion, PBL is a rare entity seen most commonly in the setting of immunocompromise. The most common site involved in these patients is the oral cavity. However, this disease may present in extraoral sites in which the genital tract appears to be the less common. Genital involvement also appears to be last but possible site option to find PBL focus in immunocompetent patients. The prognosis is uniformly poor even with optimal management with cytotoxic agents.

Informed consent

Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

Conflict of interest

The authors declared that there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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The European Research Journal 2019;5(6):1024-1027 CASE REPORT

A new etiology for variant of Guillain-Barré syndrome: bariatric surgery

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DOI: 10.18621/eurj.461760

ABSTRACT

Bariatric surgery is an effective treatment for obesity. However, the number of acute or chronic neurological complications after bariatric surgery, including Guillain-Barré syndrome, is increasingly reported. We present here two cases which developed acute motor sensory polyneuropathy a couple months after bariatric surgery which rapidly progressed over the following month. Both patients used received parenteral vitamin B complex replacement after surgery. The first case responded well to intravenous immunoglobulin (IVIg) treatment. However, the second case required plasmapheresis and physical rehabilitation for recovery after IVIg treatment. It is thought that minerals, vitamins, and trace element deficiencies can develop after bariatric surgery. These deficiencies may trigger inflammatory and autoimmune mechanisms and cause acute polyneuropathies. In such cases, it should be kept in mind that immune therapies may be beneficial, as well as vitamins.

Keywords: Bariatric, complication, neuropathy, malnutrition, Guillain-Barré syndrome

Received: September 20, 2018; Accepted: December 11, 2018; Published Online: August 2, 2019

Obesity is a growing problem and the necessity for bariatric intervention is apparent worldwide [1-3]. Neurological complications are increasingly recognized by the number of bariatric surgeries performed. These complications such as encephalopathy, optic neuropathy, myelopathy, radiculoplexopathy, and neuropathy can involve any level of the central and peripheral nervous system. Neuropathies after bariatric surgery have been reported in 5-16% of various studies [4-6]. Neurological complications may result from immune and inflammatory mechanisms, and some authors claim that the real underlying pathology originates from micro-nutritional deficiencies, such as a lack of vitamins B and E and trace elements [5, 6]. Changes in incretins secreted from intestinal endocrine cells after gastrectomy are also blamed on a pathological mechanism [7, 8]. In addition, there are very few studies reporting that polyneuropathy develops as a result of an autoimmune reaction after bariatric surgery [9, 10]. Although the underlying mechanisms are still debated, there is, as yet, no general consensus for treatment [10-13]. We discuss the clinical findings and treatment response of two cases developing acute axonal sensory motor neuropathy (AMSAN) which as a variant of Guillain-Barré syndrome (GBS) related bariatric surgery.

CASE PRESENTATION

Case 1

A 27-year-old female with 59.7 kg/m2 body mass index (BMI) (height: 152 cm, weight: 138 kg), underwent a sleeve gastrectomy and was discharged

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e-ISSN: 2149-3189

on a special diet including oral multivitamins and mineral and protein supplements. She had also been using intramuscular 1000 mcg hydroxocobalamin, 100 mg thiamine and 100 mg pyridoxine per week. Three months after surgery, she developed a burning sensation in her feet, and lower back pain. After this, weakness in both lower limbs increased progressively. Weight loss was almost 20 kg over this duration. Her examination showed normal mental function, intact cranial nerves, dystal muscle weakness (a grade of muscle strength of 4/5) with areflexia in lower extremities. Complete blood counts, vitamin B12, folate levels, and metabolic panels, electro-neuromyography (ENMG) and magnetic resonanace imaging (MRI) of brain, cervical, thoracic and lumbar regions were found to be normal. Routine cerebrospinal fluid (CSF) analysis including protein, glucose, sodium and potassium levels were normal, in addition no inflammatory cell reaction was found. Her complaints got progressively worse. Over the following month she was unable to get up and walk without full physical assistance. Laboratory studies were repeated. Vasculitis panel, protein electrophoresis, and tumour markers were found negative. The new ENMG showed a severe, diffuse reduction in the amplitudes of the motor and sensory action potentials, particularly in the lower extremities. No conduction block or increase in distal latencies were noted. Needle examination showed fibrillation potentials and positive sharp waves in distal limb muscles. It was thought to be a case of AMSAN. Intravenous immunoglobulin (IVIg) treatment was started 0.4 g/kg/day for 5 consecutive days and 2 g/kg/month for 6 consecutive months. Also, treatment with intramuscular B-complex vitamins were continued. She began walking short distances after six months. Weight loss was 45 kg over the 6 month period.

Case 2

A 19-year-old male with 50.5 kg/m2 BMI (height: 1.78 m, weight: 160 kg) was submitted to a sleeve gastrectomy four months previous. A liquid high protein/low carbohydrate diet was instituted and intramuscular vitamine B complex per week was administered after surgery. He had numbness and pain in his legs and hands for 2 weeks. Muscle weakness in the lower extremities started in the feet and gradually ascended over the following days. A cranial nerve examination was normal. The predominant distal symmetric paresis was observed in the arm and leg muscles (grade of muscle strength of 3/5). Deep tendon reflexes were absent in the lower extremities. Routine blood chemistry analyses, including vitamin B12, folate levels, and vasculitis panel, were normal. An MRI of the brain, cervical and lumbar spine was normal. Routine CSF findings were also normal. ENMG showed a severe, diffuse reduction in the amplitudes of the motor and sensory action potentials, prolongation in distal latencies, and slowing of conduction velocities - particularly in the lower extremities. Needle examination showed acute denervation potentials in the muscles of the lower extremities. AMSAN has been established as a diagnosis. IVIg treatment was started at a dose of 0.4 g/kg/day for 5 consecutive days. However, his muscle strength reduced to a grade of 1/5 in the distal part of the extremities. Plasmapheresis was started 5 times daily on alternate days. In addition, treatment with intramuscular B-complex vitamins was maintained. Six weeks of physiotherapy was admistered after plasmapheresis. At his last examination his neurological condition was nearly normal. He had lost 90 kg over the 6 months.

DISCUSSION

Several bariatric procedures have been applied in the treatment of obesity, such as Roux-en-Y gastric bypass, adjustable gastric banding, and sleeve gastrectomy. Selection criteria for surgery includes BMI $\geq$ 40 kg/m^2 without co-morbidities. If patients have diabetes mellitus, or obstructive sleep apnea, the BMI = 35-40 kg/m^2 can be accepted for surgery [1-3]. However, the incidence of neurological complications is increasing with the widespread use of bariatric surgery, especially in cases of acute axonal polyneuropathies [6].

AMSAN, a variant of GBS, is characterized by acute onset of distal weakness, loss of deep tendon reflexes, sensory symptoms, and is confirmed by ENMG [12-15].

Altered diet, reduced absorption, dysmotility, loss of gastric acid and intrinsic factors are important reasons in developing complications. Among them,

B12 deficiency is the most common. A low B12 level has been reported at a ratio of 70% in these patients [4, 5]. B12 levels were normal in our patients. In addition, deficiencies in B-group vitamins (niacin, pyridoxine and especially thiamine), vitamin E, copper, zinc, selenium, and folic acid are responsible for other nutritional factors in neurologic complications [6]. A deficiency in our evaluation was the lack of analysis of other B group vitamins and trace elements as this is not a part of our work routine. It has been reported that obese patients have pre-existing micronutrient deficiencies and these patients' conditions deteriorate after surgery [6, 7]. Also, a history of repeated attacks of vomiting have been reported as a risk factor for complications in bariatric cases [4]. Our patients had already taken vitamin B supplements and had no history of repeated attacks of vomiting after surgery.

Rapid and excessive weight loss may cause malnutrition related polyneuropathy [12-15]. In the first case, BMI decreased from 59.7 kg/m^2 to 40.3 kg/m^2 in six months. In the second case, it decreased from 50.5 kg/m^2 to 22.1 kg/ m^2 in six months.

It is reported that neurological complications generally developed 3-20 months after bariatric surgery. This interval may be related to immunological disturbance caused by deficits of gastric incretins, and microelements deficiencies [11-14]. In our patients, AMSAN developed 3-4 months after surgery.

Diaz *et al*. [10] showed inflammatory changes in nerve biopsy in two cases with acute polyneuropathy after bariatric surgery. They also reported good response to IVIg in these cases. Also, Chang *et al*. [11] reported one patient who after being treated with plasmapheresis no response to IVIg followed. IVIg is a gold standard treatment of autoimmune neuropathies including GBS, chronic inflammatory demyelinating neuropathy and multifocal motor neuropathy. In addition, plasmapheresis may require a second line therapy in cases which are not responding to IVIg [9]. In light of these data, we preferred IVIg as the first line treatment. However, in the second case, plasmapheresis was needed because of no response to IVIg.

Currently, there is confusion in the naming of cases with post-bariatric polyneuropathies, using terms such as nutritional polyneuropathy, acute/subacute axonal polyneuropathy, and variants /mimics of GBS [12-14]. Using the term 'GBS variants related to malnutrition' could be more suitable in order to have a generally accepted term for these cases. Nutritional support before and after bariatric surgery, and routine biochemical evaluation at frequent intervals are very important to prevent these complications [7-10].

CONCLUSION

Our cases showed that IVIg should be kept in mind as a reliable treatment option for GBS variants caused by bariatric surgery.

Informed consent

Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

Conflict of interest

The authors declared that there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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The European Research Journal 2019;5(6):1028-1030 CASE REPORT

Toxic effects of herbal medicines: *Teucrium polium* and acute kidney injury

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DOI: 10.18621/eurj.461829

ABSTRACT

Herbal medicines are believed to be effective in the treatment of many diseases. The genius *Teucrium* is a member of *Lamiaceae* family, which contains more than 300 species. Antimutagenic, anticancer, hypoglycemic, antibacterial, anti-inflammatory, antipyretic, antiulcer, hypolipidemic and antidiarrheal effects of *Teucrium polium* have been reported in the studies. In this article, we describe an acute kidney injury (AKI), which is thought to have developed as a result of *T. polium* use for the treatment of diabetes mellitus. It should not be forgotten that herbal medicine is an important reason for AKI. In addition, community should be made aware of the danger of herbal medicines which especially have potential renal and hepatic side effects.

Keywords: acute kidney injury, *Teucrium polium*, diabetes mellitus, bariatric surgery

Received: September 20, 2018; Accepted: March 22, 2019; Published Online: August 2, 2019

Diabetes mellitus (DM) is a chronic disease that occurs due to impairment of insulin secretion and decrease in insulin function; it leads to increase in blood glucose. Thus, it causes retinopathy, nephropathy, neuropathy and cardiovascular complications. Since DM taking place among oxidative stress related diseases; various antioxidants, herbal medicines thought to have antioxidant effect are believed to be effective in the treatment of the disease and its complications. Therefore, uncontrolled use of herbal medicines is increasing all over the world. The most important plants believed to be effective in DM treatment are different species of *Lamiaceae, Asteraceae* and *Apiaceae* families [1]. Various toxic effects, especially renal and hepatic damages, can occur with the increase in the use of these plants that we do not yet fully understand the mechanisms of action and side effects. Acute kidney injury (AKI) can be defined as a decline in the glomerular filtration rate that results rapid renal function impairment.

AKI cases due to exposure to nephrotoxins such as contrast agent and herbal medicine are considerably higher. Exposure to contrast agent and herbal medicine can be the cause of nephrotoxicity and AKI.

In this article, a diabetic patient refused oral antidiabetic treatment and required hemodialysis because of AKI after using T*eucrium polium*, has been described.

CASE PRESANTATION

A 46-year-old female was admitted to the emergency service with fatigue continuing about 10 days. The patient has had metrorrhagia for the last 2 years but has not been examined for this reason before.

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e-ISSN: 2149-3189

Hemoglobin was found 6.1 gr/dl on hemogram evaluation and she was admitted to gynecology clinic. Endometrial thickening detected in ultrasonographic evaluation. Endometrial curettage was performed to stop vaginal bleeding and to take a diagnostic sample. Then, norethisterone 15 mg/day treatment started. Blood urea nitrogen (BUN): 112 mg/dl, creatinine: 15.9 mg/dl, potassium: 6.4 meq/L were detected in the biochemical tests, performed approximately 10 hours after hospitalization. The patient was urgently consulted with nephrology clinic. It was learned from the patient history that she was diagnosed with DM 3 months ago. She used oral antidiabetic therapy for only one mont hand did not use the last two months. She boiled a pinch of meryem hort plant with water and drank iteveryday. Apart from that, there was no chemical, herbal medicine and contrast agent exposure. Creatinine level was 0.8 mg/dl in the endocrinology outpatient clinic 2 months ago.

On the physical examination of the patient consciousness, co-operation and orientation were normal. Arterial blood pressure was160/90 mmHg, body temperature was 36.7 °C, pulse rate was 80/min. Crepitant crackles were heard in the lower zones of lung in the respiratory system oscultation. There was no additional pathology other than vaginal bleeding, suggesting metrorrhagia for 2 years. Femoral hemodialysis catheter was inserted urgently, and the patient was hemodialized without heparin for 2 hours. In other biochemical assays, leukocyte: 12100/mm^3, platelet: 131000/mm^3, sedimentation: 88 mm/h, C-reactive protein: 17.2 mg/l were detected and there were 37 leukocytes, 16 erythrocytes in urine analysis. 2 grams of proteinuria was seen in the 24-hour urine analysis, but his result was not reliable due to urinary system infection. Thyroid function tests, hepatitis markers, lipid profile were normal. No pathological findings were found in renal doppler ultrasonography. The long axis of the right kidney was 13 cm and the left kidney was 11 cm.

The patient was alerted not to use meryem hort plant again. Treatment with norethisterone 15 mg/day for vaginal bleeding continued. There was no evidence of malignancy in endometrial curettage pathology and vaginal bleeding did not continue. Hemodialysis was performed 6 times in the first 8 days after admission. Due to the detection of Streptococcus agalactia in urine culture, linezolid therapy was initiated with the recommendation of the infectious diseases clinic and renal biopsy could not performed. Because of high sedimentation, anemia and rapid progressive acute renal injury the patient was evaluated in terms of multiple myeloma and vasculitis (protein electrophoresis, serum and urine immunization electrophoresis; p-ANCA, c-ANCA, anti-glomerular basement membrane antibody). The results were normal. She was followed only with oral and intravenous hydration from the 8th day of admission and creatinine value was 0.8 mg on the 21st day. AKI was thought to be caused by teucrium polium. Kidney function tests returned to normal after 21 days. The patient is still in routine control at our nephrology clinic.

DISCUSSION

The genus *Teucrium* is a member of *Lamiaceae* family, which contains more than 300 species [2]. In Turkey, it is known as meryem hort plant. It grows up to 1000 meters above sea level and mostly in the arid rocky areas of South West Asia, North Africa, Mediterranean and Europe. This plant was used for thousands of years, considering that it is effective in the treatment of cough and asthma. Numerous effects of *T. polium* such as antimutagenic, anticancer, hypoglycemic, antibacterial, anti-inflammatory, antipyretic, antiulcer, hypolipidemic and antidiarrheal effects have been reported in many studies [3]. For this reason, up to now, it has been used in the treatment of many diseases such as pain, DM.

However, for all known positive effects, the potential organ side effects, liver and kidney effects must be known for a 'safe use' of herbal or chemical drugs. Otherwise, herbal medicines, which are recommended for therapeutic purposes, may cause many unwanted side effects. Essential oils constitute 0.45% of *T. polium*. It is also thought that the sephenolic compounds in these essential oils have antidiabetic effects [4].

In a study investigated whether *T. polium* hydroalcoholic extract caused renal toxicity, 100 male Wistar rats were examined and the rats were divided into two groups [5]. The first group was given intraperitoneal *T. polium* for 28 days to examine possible renal damage. The second group was given

intraperitoneal *T. polium* for 28 days and after 28 days without medication, renal examination was performed in the second group. Renal damage was not seen in the first group but in the second group, renal damage findings such as vacuolization, destruction and degeneration were detected. According to our investigations, there is no study except only one study [5] in the literature which examines the relationship between *T. polium* and AKI. In our case, AKI occurred at approximately 2 months after the use of *T. polium*. However, pathologic findings of renal injury could not be detected because of the development of urinary tract infections in our patient and the inability to perform renal biopsy.

CONCLUSION

In this article, we describe an AKI due to unconsciously used *T. polium* to control blood glucose. It should not be forgotten that the use of herbal medicines is an important reason for AKI and it should be asked every patient admitted due to AKI. Inaddition, community should be warned not to use herbal medicines whose especially renal and hepatic side effects are not known.

Informed consent

Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

Conflict of interest

The authors declared that there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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The European Research Journal 2019;5(6):1031-1035 CASE REPORT

Rectus femoris tendinopathy: a case report

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DOI: 10.18621/eurj.461932

ABSTRACT

Tendinopathy may not be noticed in the differential diagnosis due to the complaint of pain spreading to the leg in the presence of nerve radiculopathy in the lumbar discopathy which is seen more frequently in the clinic shows similarity to the leg pain of musculus rectus femoris tendinopathy which is rarely seen. This situation leads to time, labor force and economic loss for both the patient and the health professionals. The case referred to the hospital with severe lower extremity pain and the complaint of incapability to walk. Despite the absence of findings in the imaging reports supporting a discopathy; conventional physiotherapy, intramuscular injection, and nerve blockage treatments were administered for the discopathy due to the clinical presentation. However, the complaints of the patient did not recover. The patient who had pain with a maneuver during exercise training was evaluated regarding tendinopathy and m.rectus femoris tendinitis was diagnosed with ultrasonography. The pain, quality of life and lower extremity functions of the patient were evaluated before and after treatment. The isolated deep transverse friction massage was applied on the tendon for the treatment. A positive change in pain, quality of life and lower extremity function scores was obtained after the five sessions of treatment.

Keywords: tendinopathy, rectus femoris, friction massage

Received: September 20, 2018; Accepted: March 20, 2019; Published Online: March 21, 2019

Tendinopathy is a general term and is named differently according to the histological changes that occur in the tendon structure. Tendonitis, which is examined in the tendinopathy class, is a condition characterized by the inflammatory reaction in the tendon structure. Micro-tears occurred as a result of overpressing on the musculotendinous junctionusually cause to this process. The tendon is sensitive and painful to the stretching, pressing on and palpation [1]. Lower extremity pain and complaints of incapability to walk might be seen. Another cause of the lower extremity pain is lumbar discopathy (LD) [2]. Particularly in LD with radiculopathy, there is a complaint of pain spreading to the lower extremity [2]. Lower extremity tendinopathies are conditions that can go unnoticed because of the pain that spreads to the leg, which can also be seen due to radiculopathy in LD; therefore may lead to time, labor force and financial loss for both the patient and the healthcare economics. Achieving success in treatment is possible with the correct treatment approach to the correct diagnosis. For this reason, it should be emphasized that the pain complaints related to LD and tendinopathy may be similar in patients with lower extremity pain and that this condition should be taken into consideration in the differential diagnoses. Treatment in tendinopathies consists of conservative phytotherapy agents, corticosteroidor non-steroid injections, and surgical treatment

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options [1]. Deep transverse friction massage (DTFM) is a form of conservative treatment and should be considered as a cheap treatment technique that allows rapid and practical reduction of complaints that reduce the quality of life, such as pain and functional limitation [1]. In this case report, it was aimed to draw the attention to the fact that the patient who was followed by Physical Medicine and Rehabilitation (PMR), algology, neurosurgery and emergency outpatient clinics due to the severe pain in the lumbar and lower extremity areas was evaluated as LD because of the pain in the lumbar region and spreading to the leg, the treatment was planned for this diagnosis as the tendinopathy was ignored, and isolated DTFM without the combination of other physiotherapy agents in the treatment of tendinopathies was effective.

CASE PRESENTATION

A 42-year-old female patient referred to the PMR out patient clinic with severe left lower extremity pain and the complaint of incapability to walk which started acutely after heavy lifting at home. The patient had no chronic comorbidities. The patient was a housewife with sedentary lifestyle.

On the first examination of the patient regarding her complaint, there was the pain in the forward flexion of the back, and the straight leg lift test was positive. There was severe pain in the entire leg and around the left knee joint. The patient could not walk without any help. According to the Visual Analogue Scale (VAS), the lower extremity pain score was nine at rest. Table 1 summarizes the time line of the case,

Table 1. Summary of the patient's history and follow up with interventions given

Date	Patient history and applications
02th April 2018 - 11th May 2018	A 42-year-old female patient was admitted to the PMR out patient clinic with acute onset of severe left lower extremity pain and difficulty walking, subsequent to heavy lifting at home.
	The patient had no accompanying chronic illness. Lumbosacral and knee MRI was requested. 30 sessions of conventional physiotherapy and medical treatment were applied.
14 th May 2018 – 17 th May 2018	The patient was admitted to the adult emergency, brain and neurosurgical outpatient clinic with complaints of lumbar and lower extremity pain. Intramuscular (IM) analgesic injection was made in the adult emergency department and the patient was discharged with the recommendations. Neurosurgery department suggested surgical treatment for discopathies due to clinical complaints.
18 th May 2018	In the algology service, medial branch blockage was applied to the patient's L4-5 and L5-S1 intervertebral spaces. The patient was called for control 1 week later
25 th May 2018	On the day of the control the patient's pain was continuing. Exercise training provided.
	The patient was counseled because of the pain while training the exercise by the therapist.The PMR physician performed ultrasonography (USG) of the patient's musculoskeletal system and was diagnosed with Rectus Femoris Tendonitis.
	The treatment of he patient was planned and informed consent form was signed.
	VAS, SF-36 and Lower Extremity Function Scales were applied before the treatment.
25 th May 2018 – 01 th June 2018	DTFM treatment was applied for 5 sessions every other day.
01th July 2018	VAS, SF-36 and Lower Extremity Function Scale were applied at 1 month after treatment

interventions given, and the progress of procedure conducted on the patient.

Magnetic resonance imaging (MRI) of the lumbosacral and knee regions was taken for the diagnostic evaluation of the patient. In the lumbosacral MRI, the vertebral corpus, intervertebral discs, and neural foramen were regular. As in the intervertebral discs, there were no abnormal findings besides the minimal hydration loss at L4-5 and L5-S1 levels. In the knee MRI report, there was a mild fluid increase around the knee joint, a minimal degeneration in the posterior horn of the medial meniscus, while the knee joint ligaments and lateral meniscus were normal. Thirty sessions of conventional physiotherapy including transcutaneous electrical nerve stimulation (TENS), ultrasound and superficial heating agents for the lumbar region, and TENS and cold agents for the knee region. The patient whose treatment was arranged with analgesic and anti-inflammatory medication at the end of the therapy was discharged. Within one week following the physiotherapy treatment, the patient was found out to refer to the outpatient clinics of the emergency department, neurosurgery, and algology on different days with the complaint of the lumbar and lower extremity pain. While the patient was discharged with the suggestions after intra muscular analgesic injection in the adult emergency department, the neurosurgery clinic offered surgical treatment for discopathy due to the patient's clinical complaints. The patient who did not accept the surgical treatment option, underwent medial branch blockage to the intervertebral spaces of L4-5 and L5-S1 in the algology clinic. She was reported to have decreased back pain by 50% after the blockage treatment and was discharged with a control planned after one week. Due to the continuing lower extremity pain in the control examination, the patient was directed to the PMR outpatient clinic. Here the MRI of the patient's sacroiliac region was requested. The MRI of the patient which was evaluated according to The Assessment of Spondyloarthritis Society (ASAS) criteria [3] had no pathology. The patient was directed to a physiotherapist for exercise advice. Due to the occurrence of severe pain in the complete passive flexion of the patient's hip during the exercise training, the physiotherapist consulted with a physician. There was tenderness in the examinationby palpation on the tendon which was approximately 8 cm distal to the spina iliaca anterior superior (SIAS). The pain associated with the compression of the tender part in the complete passive flexion of the hips was indicative of the presence of tendinopathy [1]. The diagnosis was confirmed by USG of the musculoskeletal system made by a specialist physician. In the USG examination; there was hypoecotic edema in the synovial sheath and around the rectus femoris tendon; the tendon was thickened and there was mai adjacent to the tendon. These findings obtained by USG indicated tendinopathy. In the evaluation of soft tissues such as tendons USG is accepted as a reliable, non-invasive and economical method [4].

Before starting the study, patient was informed about the treatments and evaluations to be made by the physician and physiotherapist, and a written consent form was signed. Afterward, the treatment of the patient was planned and applied. Manual therapy techniques have an essential role in the treatment of tendinopathies [1]. Transverse friction application was made in the treatment; while the patient was in the semi-supine position, the physiotherapist located the tendon approximately 8 cm below the SIAS and placed the distal phalanges of the second and third fingers on the tendon. The first finger was placed on the trochanter major of the femur to applycounter pressure. DTMF was applied with the rhythmic flexion-extension movements of the wrist. DTFM was continued to be applied for 10 minutes after the sensation of numbness in the region was obtained. Figure 1 presents the technic of DTFM application. This application was repeated as five sessions as on

Figure 1. Application of DTFM on musculus rectus femoris tendon.

every other day [1]. During this period, no additional medication was given to the patient.

The efficacy of the applied treatment was assessed regarding pain, quality of life, and lower extremity functions before the treatment (BT) and after the treatment (AT) at the first month. The pain was assessed with VAS, the quality of life with Short form-36 (SF-36) [5], lower extremity functions with Lower Extremity Function Scale [6] According to VAS, the pain of the patient was 9 at rest BT, immediately AT 4, and 0 after one month AT. The lower extremity function scale score was 10 BT and 71 at first month AT. According to the SF-36, physical functioning score of the patient was 0 BT and 90 at first month AT, physical role functioning score was 0 BT and 100 at first month AT, emotional role functioning score was 0 BT and 100 at first month AT, energy-fatigue-vitality score was 5 BT and 75 at first month AT, emotional well-being score was 16 BT and 80 at first month AT, social functioning score was 12,5 BT and 87,5at first month AT, pain score was 0 BT and 90 at first month AT, and general health perception score was 5 BT and 70 at first month AT. Considering the scores obtained, it is seen that there is a significant improvement in the patient's pain, quality of life and lower extremity function after the treatment.

DISCUSSION

Rectus femoris tendinopathy is an uncommon condition even for the athletes. It is even rarer in the sedentary individuals [7]. Symptoms start as a slow and progressive pain and discomfort on the anterior aspect of the hip and are typically exacerbated by exercise, especially during jumping and running [7]. Patient history and clinical evaluation involving direct palpation of the tendon and resistant muscle activation are necessary to make the appropriate diagnosis [7]. USG and MRI techniques provide useful and valuable diagnostic information for planning the therapeutic strategies. Conservative treatment includes oral and topical anti-inflammatory drugs and physical therapies with a wide range of modalities [7]. Due to the similar complaints, it is necessary to distinguish tendinopathy from the lumbar and neck problems. Pain caused by the problems in the lumbar or neck region mayspread to the extremities and may be confused with other symptoms such as the motor or sensory disorders [2]. Our case study aimed to draw attention to the possibility of tendinopathy diagnosis in patients with lower extremity pain due to our patient was evaluated as LD, had a treatment in this direction and eventually was diagnosed as tendinopathy. There are limited studies on the differential diagnosis of tendinopathy. Literature report that, rectus femoris tendinopathy was more frequently seen as calcific tendinitis [8]. In our case, acute onset tendinopathy was present. The literature also reports treatment options for rectus femoris tendinopathy as options for nonsteroidal anti-inflammatory drugs, extracorporeal shock wave therapy (ESWT), local TENS and other electrotherapeutic modalities [8]. A review study indicated that steroid-non steroid injections frequently used in the treatment of sport injuries and tendinopathies but long-term use of these drugs should be limited by the reason for preparing new injuries [9]. DTFM application with no known reported side effects, was used as a treatment option in our case and the pain, quality of life and lower extremity function scores of the patient were significantly improved at the end of 5 sessions. DTFM holds a significant place in the conservative treatment of tendinopathies [1]. In a systematic review study of DTFM in the treatment of tendinopathies, it was reported that there was insufficient evidence for DTFM which was used alone without combining with other conventional treatment agents [10]. The researchers preferred to apply the DTFM combined with other modalities more frequently in the treatment of tendinopathy [11, 12]. In fact, DTFM alone may be an excellent alternative treatment as a non-invasive method which is cheap and give results in a short-term.

CONCLUSION

It can be seen from the results that the evaluation of the patients with lower extremity pain in terms of tendinopathy should not be overlooked in the differential diagnosis and DTFM can be used as a treatment option alone in the treatment of tendinopathy. Yet it is clear that there is a need for more controlled studies involving more samples and isolated applications to demonstrate the efficacy of DTFM in tendinopathies.

Informed consent

Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

Conflict of interest

The authors declared that there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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