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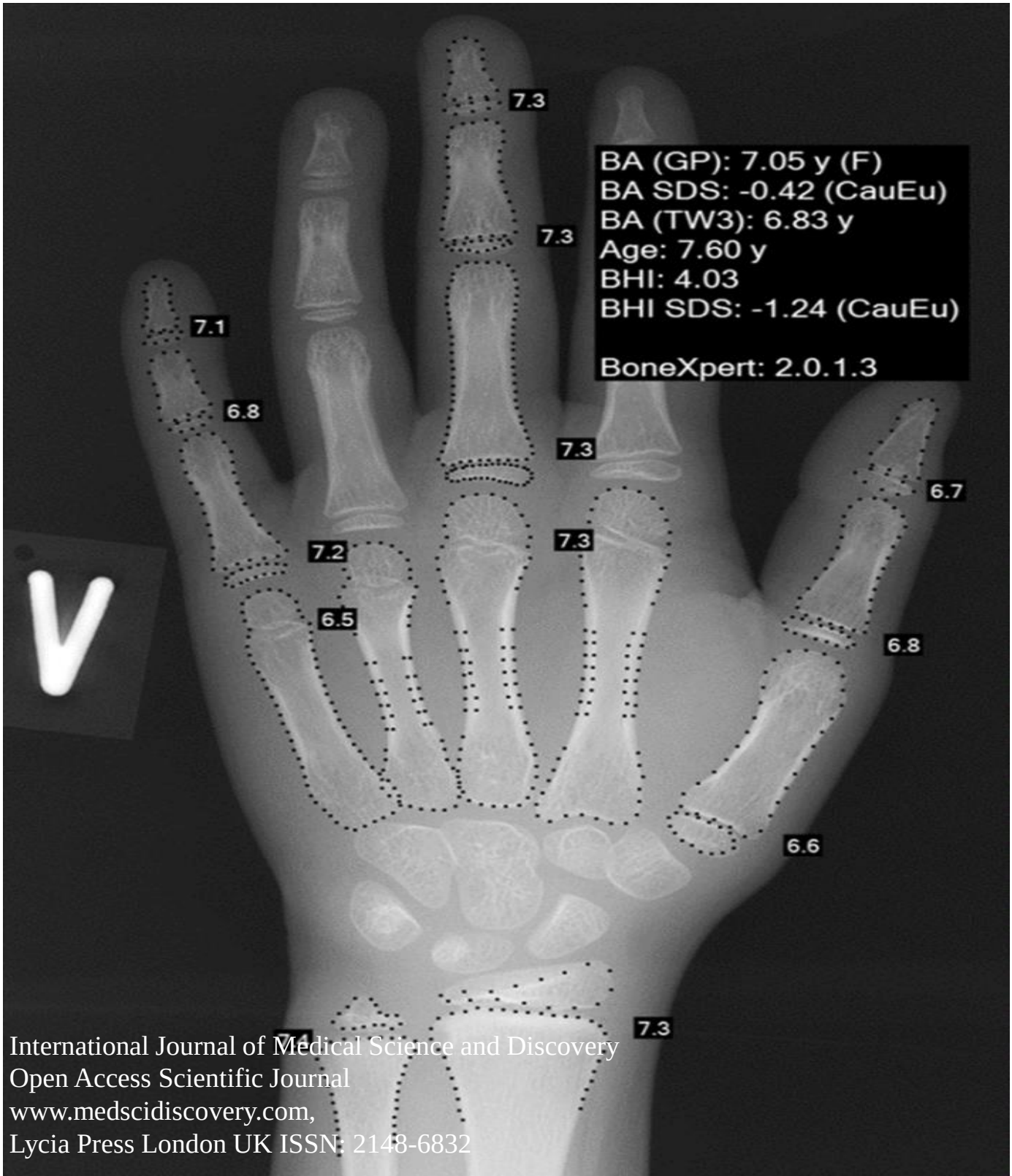

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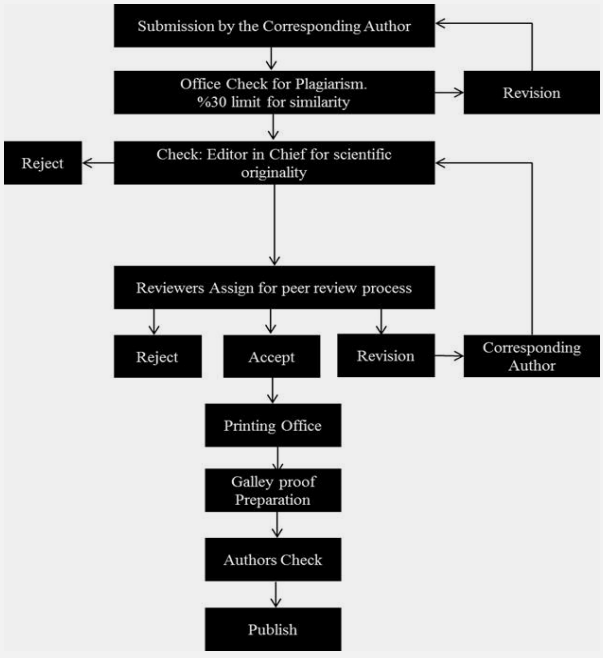

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Impact of iron status and inflammatory indices on atherosclerotic burden of patients with *Helicobacter Pylori* infection and coronary arterial disease

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ABSTRACT

Objective: Both inflammation and iron deficiency are suggested to be associated with coronary arterial diseases (CAD) and *H. Pylori* infection. The explanatory interaction depending on serum iron status and inflammatory biomarkers for the extent of atherosclerosis in H. Pylori infection is obscure. Therefore, we aimed to analyze the impact of iron Status and inflammatory indices on atherosclerotic burden of seropositive CAD patients with CagA (cytotoxin-associated gene A) strains of *H. Pylori*.

Materials and Methods: This was an observational study of patients' undergone elective and urgent coronary angiography due to CAD. Serologic *H. pylori* infection status and iron status was determined in all of 293 subjects. Further seropositive patients were divided into groups to evaluate the extent of coronary atherosclerosis according to Syntax scoring system. Propensity score matching and covariate- adjusted multivariate logistic regression were used to adjust for baseline differences between study groups.

Results: The odds ratio of positive serology for the presence of iron deficiency and acute coronary syndromes were 2.5 (95% CI (1.1-5.4); p = 0.02) and 3.0 (95% CI (1.3-7.0); p = 0.007) respectively. After controlling for diabetes mellitus, smoking, MPV, RDW and haemoglobin levels; Tsat ≤ 24.5 remained negatively associated with advanced atherosclerosis (OR:9.9, 95% CI (4.1-24.3); p < 0.0001). In our matched sample, multivariable linear regression analysis showed that association of syntax score with Tsat was independent of hs-CRP (p=0.001).

Conclusions: Irrespective of inflammatory status, transferrin saturation can be the decisive mirror indicator of advanced atherosclerosis in seropositive CAD patients with CagA strains of H. Pylori.

Keywords: H. Pylori, CagA, Coronary arterial disease, , Iron status, Inflammation

INTRODUCTION

Helicobacter pylori (H. Pylori) infection is one of the most common infectious diseases in the world, and it affects more than 50% of the world's population (1). *H.Pylori* infection is well known as responsible for an immune-inflammatory response, and CagA (cytotoxin-associated gene A) positive *H. pylori* strains are recognized as a marker of greater potential for inducing such a response (2-5). The occurrence and progression of atherogenesis is suggested to be linked to H. pylori infection through a low-grade, persistent inflammatory stimulation (6, 7). However, the relation between *H. Pylori* infection and stable or unstable forms of cardiac syndromes is controversial (8, 9). Recently, a meta-analysis showed a significant, positive association between CagA IgG seropositivity and the occurrence of acute coronary syndromes (ACS) (4). Also, prolonged exposure to CagA bearing strains of *H. Pylori* infection is suggested to be associated with H. Pylori related advanced disease manifestations such as more severe gastric injury, unexplained iron-deficiency anaemia, progression of gastric preneoplastic lesions and development of gastric adenocarcinoma (10, 11). In addition to the close link between *H. Pylori* infection and iron deficiency, it was defined that under conditions of iron depletion, *H. pylori* virulence had increased (10, 12, 13).

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Furthermore, low iron status was claimed as the eventual late sign of ischemic heart disease and Transferrin saturation (Tsat) was emphasized to be a good screening test for iron deficiency in patients with the coronary arterial disease (CAD) (**14, 15**). Lower iron levels may promote free-radical-induced lipid peroxidation that leads to vascular inflammation and atherosclerosis subsequently (16). In addition to circulating iron status biomarkers and high-sensitive C-reactive protein (hs-CRP); routinely reported, quickly obtained hematologic parameters are able to reflect systemic ongoing inflammation and may be helpful in cardiovascular risk stratification (**17**).

In addition to persistent inflammatory stimulation, the explanatory mechanisms for the progression of atherogenesis in *H. pylori* infection are uncertain. Therefore, the impact of simple hematologic and circulating iron status parameters on the extent of coronary atherosclerosis would be investigated in patients with positive CagA serology.

MATERIAL and METHODS

Study population

A total of 293 patients undergoing elective and urgent coronary angiography in our cardiology department with various manifestations of ischemic heart disease were included. In this observational study, all of the patients were suspected of having CAD due to clinical symptoms or the results of clinical tests including electrocardiography, blood test, treadmill exercise test and exercise 201-Tl myocardial scintigraphy. Subjects with angiographically normal coronary arteries were excluded. Patients with an identified risk for iron deficiency and a red cell disorder including heavy menstrual periods, known bleeding from a gastrointestinal site, known elevated levels of lead, thalassemia, sickle cell disease, sideroblastic anaemia, aplastic anaemia, vegan diet or a disorder associated with acute or chronic infection/inflammation and those taking iron supplements were excluded from the study. None of the subjects included in the study had clinical evidence of connective tissue disease, liver dysfunction, hypothyroidism, severe chronic heart failure (NYHA class III-IV), moderate or severe renal dysfunction (eGFR < 60 mL/min/1.73 m²) and malignant diseases. Additionally, patients with any surgery within the previous four weeks, prior upper gastrointestinal tract and coronary arterial bypass surgery, use of nonsteroid anti-inflammatory drugs, blood transfusion during the last three months and incomplete data were excluded. Also none of the 293 individuals recruited had a history of eradication therapy for *H. Pylori* infection or had received any antibiotic treatment during the study.

All subjects were screened with a questionnaire. Demographic data and risk factors for CAD were recorded in all participants. Participants were asked about medical history, including specific questions related to physician-diagnosed hypertension, diabetes, heart failure and hyperlipidemia. Furthermore, current medication, socio-demographic data, and lifestyle habits, including smoking, were recorded. Individuals whose income was lower than at least two times of the minimum wage in our country were defined as lower socioeconomic status. The education level was divided into <10 years and ≥10 years. Among the main cardiovascular risk factors, the presence of family history of CAD (in a first-degree relative <55 years of age), hypertension (systolic or diastolic blood pressure >140 and 90 mm Hg, respectively, or pharmacological therapy with antihypertensive drugs), diabetes mellitus (fasting glucose plasma concentrations >126 mg/dL or pharmacological therapy with antidiabetic drugs or insulin), hyperlipidemia (low-density lipoprotein (LDL) cholesterol levels ≥ 130 mg/dl or being treated with lipid-lowering medication) were considered definitions. Current smoking status was defined as at least 20 cigarettes per month for more than 6 months of usage. Exercise angina was determined by the presence of chest pain on walking that was relieved within 10 minutes after stopping or by ST-segment of ECG down-sloping in a standard 12-lead electrocardiogram during chest pain or by positive stress testing. The diagnosis of AMI was established by using American College of Cardiology/European Society of Cardiology criteria (18). Iron deficiency was defined as a serum iron < 40 µg/dL or Tsat < 20 % according to the definition previously used (**19**).

Participation was voluntary, and written-informed consent form was obtained from each subject. The study protocol was approved by the ethics committee of our Hospital. The inclusion period was six months.

Laboratory Methods

Indices of iron status and other laboratory measurements assessed in peripheral blood

All blood samples were drawn before the procedure after an overnight fasting under standardized conditions. Haematological parameters were measured using a Beckman Coulter LH780 Hematology Analyzer (Beckman Coulter, Inc).

Within 30 minutes, the remaining blood was centrifuged at 3000g for 10 minutes, immediately divided into aliquots, and frozen at -70°C until analysis. Serum samples were separated at the same time by centrifugation at room temperature for measurement of the following laboratory parameters: Iron, ferritin, transferrin saturation (Tsat) (the ratio of serum iron and total iron binding capacity) and hsCRP.

Iron status was assessed by serum iron (Beckman Coulter AU 5800 analyzer), ferritin (Beckman Coulter DXI 800 analyzer) and transferrin saturation (Beckman Coulter AU 5800 analyzer). HsCRP (Siemens BN-II kinetic nephelometry analyzer) was used as a marker of inflammation. Hs-CRP values were classified by American Heart Association (AHA) standards for risk for cardiovascular disease: Low (<1mg/L), Intermediate (1–3 mg/L), or High (>3mg/L) (**20**).

Specific *H pylori* anti-CagA IgG antibodies were measured by use of a commercial Enzyme-linked immune-sorbent assay (ELISA) (Radim Diagnostics, Rome, Italy) according to manufacturer's instructions. Titers were defined as positive or negative according to a cutoff value of 30 UR/mL. The sensitivity and specificity of the tests of Radim-TM was 88% and 93.8%, respectively (**21**). Patients were divided into 2 groups according to CagA IgG serostatus. All measurements were processed according to standard laboratory practice in a blinded fashion.

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Determination of Coronary Arterial Disease

Coronary angiography was performed by a femoral approach using the standard Judkins technique (Axiom Artis zee 2011; Siemens, Munich, Germany). Coronary arteries were demonstrated in the left and right oblique planes with cranial and caudal angulations. Left ventricular and aortic pressures were recorded. Coronary arteries were opacified with manual injections of Iohexol (Omnipaque, Nycomed Ireland, Cork, Ireland) at each position. Coronary artery disease has been defined as stenosis of at least one major epicardial coronary vessel at any degree. The evaluation of the degree of a stenosis relates to the percentage reduction in the diameter of the vessel. Abnormal angiograms were classified as one, two or three-vessel diseases according to the amount of stenosis higher than 50% in a major epicardial vessel. Furthermore, each angiographic lesion identified was scored according to the Syntax score (SXScore) system (**22, 23**). All lesions causing ≥50% stenosis in a coronary artery with a diameter ≥1.5 mm were included in SXScore calculation. For the analysis, the software on the Web site (http://www.syntaxscore.com) was used. The SXScore was evaluated separately by two interventional cardiologists blinded to the study protocol and patient characteristics. In the presence of a controversy between the two results, the opinion of a senior interventional cardiologist was applied, and a common consensus was obtained.

Further, in order to increase the statistical analytic reliability, patients with CAD and positive CagA IgG serology who had SXScore of 0 were excluded and remaining seropositive 183 patients were divided into two groups to evaluate the severity of CAD according to Syntax scoring system; mild for 0-22 scores and moderate-severe for > 22 scores.

Statistical Analysis

We used the Kolmogorov-Smirnov test to assess the normality of numeric variables and analyzed homogeneity of numeric variables using the Levene test. Continuous variables with a normal distribution were expressed as means with standard deviations. Continuous variables with a skewed distribution (Neutrophils, Lymphocytes, MPV, RDW, iron, ferritin, Tsat) were expressed as medians with lower and upper quartiles. The categorical variables were expressed as numbers with percentages.

The Student t test, the Mann–Whitney U test and the chi-square ($\chi 2$) tests (or Fisher's exact test if any expected cell count was <5) were used to compare baseline characteristics according to *H. Pylori* serology, atherosclerotic severity and CAD type. Comparisons of parameters among the hs-CRP risk groups were performed by the Kruskal-Wallis test due to the lack of parametric test assumptions. Bonferroni adjustment Mann-Whitney U test was used as a post hoc test for multiple comparisons between the groups.

Our study groups exhibited significant demographic and atherosclerotic risk factor differences (**Table 1 and 2**). To minimize the confounding effect of these factors and to obtain the best balance among groups, we performed a multivariate logistic regression model based on the significant variables (Propensity score-matched analysis) (**24**).

To evaluate the correlations of hematologic and iron status parameters with each other and between the SXScore, we used the Spearman's ρ correlation analysis. Furthermore, in order to estimate the ability of circulating blood markers to predict atherosclerosis severity, the receiver–operating characteristic (ROC) curve analysis was done to estimate area under curve (AUC). For the most accurate cut-off values of subsequent iron biomarkers, sensitivity (true positive/(true positive + false negative) and specificity (true negative/true negative + false positive) were calculated (and expressed in %).

Univariate logistic regression was used to investigate the relation between coronary disease severity and confounding parameters in our entire sample. After performing univariate analysis, significantly obtained variables (diabetes mellitus, smoking, Tsat ≤ 24%, MPV, RDW and haemoglobin levels) were used in multivariate logistic regression analysis.

To assess the effect of hs-CRP on the association between possible confounding laboratory parameters and the severity of coronary atherosclerosis, multivariable linear regression analysis was performed with and without including hs-CRP in the model in our matched sample.

Continuous variables with a skewed distribution were logarithmically transformed and results were expressed as odds ratio (OR) with 95% confidence intervals (CI). A P-value ≤0.05 was considered statistically significant. Statistical tests were two-sided. All analyses were performed with IBM SPSS 14 (SPSS Statistics version 14, IBM Corp.

RESULTS

Clinical variables, *H. pylori* infection, iron deficiency and coronary atherosclerotic disease.

The general features of patients according to specified groups for both entire and matched samples are summarized in **Tables 1, 2, 3** and **Supplementary Table 1**. Tests for CagA-positive strains of *H. pylori* infection were performed in all subjects with positive results in 81.2%. Prevalence of diabetes mellitus, family history of CAD and current smoking was higher in seropositive patients than the seronegative ones ($p < 0.05$).

Also SXScores, presence of acute coronary events and prevalence of iron deficiency were higher in seropositive patients ($p < 0.05$). The odds ratio of positive serology for the presence of iron deficiency and acute coronary syndromes were 2.5 (95% CI (1.1-5.4); p = 0.02) and 3.0 (95% CI (1.3-7.0); p = 0.007) respectively.

After balancing the groups for significant confounding CAD risk factors; higher lymphocyte counts, higher rates of iron deficiency and presence of acute coronary syndrome were observed in seropositive subjects compared to seronegatif ones ($p < 0.05$).

In the matched sample, while the difference of median SXScores was no longer significant between the groups; H. pylori infection was still increasing the likelihood of iron deficiency and acute coronary syndromes (OR:2.7, 95% CI (1.04-7.0); p = 0.03) and (OR:3.5, 95% CI (1.3-9.3); p = 0.02) respectively) (**Table-2**).

As we found significant associations between iron deficiency, CAD risk factors, acute coronary syndromes and CagA-positive strains of *H. pylori* infection, a second analysis was

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performed in seropositive CAD patients (n=183) according to the extent of coronary atherosclerosis. Overall, 75 (41%) subjects had more severe coronary atherosclerosis. Prevalence of diabetes mellitus and current smoking was higher in high SXScore group than the low SXScore group ($p < 0.05$). Also, levels of MCV, MPV, RDW, Hb, iron and Tsat differed significantly between the groups ($p < 0.05$) (**Supplementary Table 1**).

The ROC curve analysis showed that the Tsat at a cut point of 24.5 % had 81% sensitivity and 61% specificity to determine severe coronary atherosclerosis (AUC = 0.84, $p < 0.0001$). For iron, RDW and MPV; the values were 68.5 (AUC = 0.65, $p = 0.001$; 72% sensitivity, 64% specificity), 13.7 (AUC = 0.61, $p = 0.009$; 73% sensitivity, 47% specificity) and 8.5 (AUC = 0.60, $p = 0.02$; 77% sensitivity, 48% specificity) respectively. After balancing the groups for significant confounding CAD risk factors; levels of MPV, iron, Tsat and frequency of iron deficiency remained significantly different between the groups (**Table-3**).

CAD type

When the analyses was done according to CAD type, while none of the iron status or hematologic indices were different between the groups in our entire sample ($p > 0.05$); only median Tsat value for ACS patients was lower compared to stable CAD patients in our matched sample (n= 76)(21 (17-24) vs. 27 (18-29); $p = 0.04$). İron deficiency rates tended to be higher in patients with ACS according to stable CAD patients (85% vs.74%; $p = 0.09$). SXScore, anti-CagA IgG titer were higher and were independently associated with acute coronary events in entire and matched sample groups ($p < 0.05$) data not shown.

Hs-CRP

When the indices of haematology and iron status were analyzed according to classified hs-CRP levels; only Tsat values were significantly and gradually decreasing from low risk to high risk groups ($p<0.001$) (**Table-4**).

Medical treatments

Acetylsalicylic acid (ASA) treatment rate was higher in patients with iron deficiency compared to non-iron deficiency patients in both of our entire and matched samples (63% vs. 47% and 84% vs. 57%; $p < 0.05$ respectively). Positive CagA serology remained significant for the risk of iron deficiency after adjustment for ASA medication (OR:2.9, 95% CI (1.1-7.7); $p = 0.03$). Treatment rates with ACEI, beta-blocker, calcium channel blocker and statin were similar between the groups according to serostatus ($p > 0.05$). Also, medical treatment rates were identical in our patients according to the extent of coronary atherosclerosis for both entire and matched samples ($p > 0.05$; n=183 and n=76).

Correlations of variables with each other and the SXScore

Correlations of hematologic and iron status parameters with each other and between the SXScore for entire and matched group of patients (n= 293 and n=100) are presented in Supplementary Table 2. Tsat correlated negatively with SXScore ($r = - 0.22$, $p < 0.05$), number of diseased vessels ($r = - 0.26$, $p < 0.05$), white blood cell count ($r = - 0.45$, $p < 0.01$) and hs-CRP ($r = - 0.33$, $p < 0.01$) in both of our entire and matched samples. Hs-CRP levels correlated positively with SXScore ($r = 0.26$, $p < 0.05$) number of diseased vessels ($r = 0.66$, $p < 0.01$) and RDW ($r = 0.42$, $p < 0.01$) in both of our entire and matched samples. Also positive correlation between anti-CagA IgG titer and lymphocyte count was determined ($r = 0.55$, $p < 0.01$). Correlation between CagA titer and SXScore lost its significance after matching groups ($r = 0.22$, $p < 0.0001$ vs. $r = - 0.01$, $p > 0.05$).

Regression Analysis

The association of severe coronary atherosclerosis with confounding parameters was investigated by univariate and multivariate analyses. After controlling for diabetes mellitus, smoking, MPV, RDW and haemoglobin levels; Tsat ≤ 24.5 remained negatively associated with advanced atherosclerosis (OR:9.9, 95% CI (4.1-24.3); $p < 0.0001$). (**Table-5**). In our matched sample, multivariable linear regression analysis showed that association of SXScore with Tsat was independent of hs-CRP ($p=0.001$) (**Supplementary Table 3**).

Table 1. Demographic, clinical and laboratory characteristics of our study group according to CagA serostatus.

	Entire sample (n=293)			
	All n=293	CagA IgG seronegative n=55	CagA IgG seropositive n=238	*p*-value
Age , years	60±14	60±16	60±14	0.9
Female, n (%)	169 (58)	44 (88)	125 (51)	**<0.0001**
Family history of CAD, n (%)	174 (59)	22 (44)	152 (63)	**0.01**
Socioeconomic status, n (%)				
Low	112 (38)	16 (32)	96 (39)	0.32
Middle-High	181 (62)	34 (68)	147 (61)	
Education <10 years, n (%)	140 (48)	27 (54)	113 (46)	0.33
Diabetes, n (%)	129 (44)	10 (20)	119 (49)	**<0.0001**
Hypertension, n (%)	188 (64)	33 (66)	155 (64)	0.77
Dyslipidemia, n (%)	153 (52)	26 (52)	127 (52)	0.97
Smoking, n (%)	130 (44)	10 (18)	120 (50)	**<0.0001**
WBC (10^3 /μL)	9.4±3.1	10.2±3.8	9.3±2.9	0.07
Platelets (10^3 /μL)	228±61	211±56	231±62	**0.03**
Neutrophils (10^3 /μL)	6.3 (4.8-7.9)	7.4 (5.0-8.1)	5.6 (4.8-7.8)	**0.04**
Lymphocytes (10^3 /μL)	1.8 (1.3-2.4)	1.4 (1.2-1.8)	1.9 (1.4-2.7)	**<0.0001**
MCV, fL	89.6±5.9	88.7±6.5	89.8±5.6	0.21
MPV, fL	8.8 (7.8-9.4)	9.1 (8.2-9.3)	8.6 (7.7-9.5)	0.20
RDW, %	14.0 (13.4-15.1)	14.1 (13.2-15.1)	13.9 (13.4-15.0)	0.83
Creatinine, mg/dL	1.0±0.3	1.1±0.3	1.0±0.3	0.23

CAD, coronary arterial disease; **Cag A,** cytotoxin-associated gene product; **IgG,** immunoglobulin G; **MCV,** mean corpuscular volume; **MPV,** mean platelet volume; **RDW**, red cell distribution width; **WBC**, white blood cell.

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Table 2. Clinical, laboratory and angiographic characteristics of our study group according to CagA serostatus

	Entire sample (n=293)				*Matched Sample (n=100)*		
	All n=293	CagA IgG Seronegative n=55	CagA IgG seropositive n=238	*p*-value	CagA IgG seronegative n=50	CagA IgG Seropositive n=50	*p*-value
Hemoglobin, g/dL	13.9±1.7	14.1±1.6	13.8±1.8	0.29	14.1±1.6	14.3±1.5	0.63
Iron µg/dL	72 (46-91)	78 (68-118)	65 (45-91)	0.09	78 (68-87)	62 (66-94)	**0.01**
Ferritin, µg/L	100 (50-182)	183 (181-392)	98 (45-124)	**0.001**	183 (181-392)	120 (115-183)	0.10
Tsat, %, n (%)	22 (18-29)	21 (15-31)	22 (18-29)	0.52	21 (18-31)	20 (18-26)	0.12
Iron deficiency, n (%)	78 (27)	8 (15)	70 (29)	**0.02**	8 (16)	17 (34)	**0.03**
CAD type, n (%)							
Stable CAD	213 (73)	48 (87)	165 (69)	**0.02**	43 (86)	32 (64)	**0.01**
ACS	80 (27)	7 (13)	73 (31)		7 (14)	18 (36)	
Multivessel disease, n(%)	99 (34)	17 (31)	82 (34)	0.62	17 (34)	14 (28)	0.52
Syntax score	12 (0-23)	11 (0-15)	12 (4-24)	**0.001**	11 (0-17)	11 (0-12)	0.96
Syntax score, n (%)							
≤22	218 (74)	55 (100)	163 (68)	**<0.0001**	50 (100)	47 (94)	0.24
>22	75 (26)	0 (0)	75 (32)		0 (0)	3 (6)	
HsCRP, mg/L	5.9±4.0	6.7±4.2	5.8±4.0	0.13	6.9±4.1	6.4±3.5	0.55
Caga IgG titer UR/mL	115±91	8±7	137±85	**<0.0001**	8±7	124±76	**<0.0001**

ACS, acute coronary syndrome; **CAD,** coronary arterial disease; **Cag A,** cytotoxin-associated gene product; **HsCRP,** high sensitive C-reactive protein; **IgG,** immunoglobulin G; **Tsat,** transferrin saturation.

Table 3. Clinical, laboratory and angiographic characteristics of our study group according to severity and complexity of coronary atherosclerosis.

	Entire sample	*Matched Sample n=76*		*p*-value
	n=183	Syntax score ≤22 n=38	Syntax score >22 n=38	
WBC (10^3 /µL)	9.8±2.7	9.2±2.2	9.9±1.7	0.11
Platelets (10^3 /µL)	238±65	241±67	248±56	0.61
Neutrophils (10^3 /µL)	7.1 (5.1-8.2)	7.1 (4.4-8.1)	7.1 (5.5-9.0)	0.25
Lymphocytes (10^3 /µL)	1.9 (1.3-2.7)	2.1 (1.7-2.4)	1.9 (1.1-2.4)	0.58
MCV, fL	90.5±4.8	90.8±4.3	89.4±5.4	0.23
MPV, fL	8.6 (7.7- 9.4)	7.7 (7.3-9.1)	8.6 (8.2-9.7)	**0.02**
RDW, %	14.0 (13.4-15.0)	14.0 (13.5-15.0)	14.5 (14.0-16.0)	0.32
HsCRP, mg/L	6.2±4.0	6.0±4.6	7.3±3.5	0.17
Hemoglobin, g/dL	13.9±1.9	14.1±1.1	13.6±2.3	0.19
Iron µg/dL	70 (48-91)	87 (75-91)	61 (47-79)	**0.035**
Iron defficiency, n (%)	59 (32)	4 (11)	18 (47)	**<0.0001**
Ferritin µg/L	98 (50-124)	112 (40-122)	63 (59-172)	0.80
Tsat, %	22 (18-29)	28 (26-30)	18 (15-22)	**<0.0001**
Tsat ≤ 24.5, n (%)	84 (46)	7 (18)	29 (76)	**<0.0001**
CAD type, n (%)				
Stable CAD	110 (60)	26 (68)	27 (71)	0.80
ACS	73 (40)	12 (32)	11 (29)	
Multivessel disease, n (%)	82 (45)	11 (29)	31 (82)	**<0.0001**
Syntax score	16 (11-28)	12 (6-14)	35 (28-42)	**<0.0001**
LDL, mg/dL	112±33	125±27	116±36	0.21
Caga IgG titer UR/mL	143±81	116±66	117±80	0.94

ACS, acute coronary syndrome; **CAD,** coronary arterial disease; **Cag A,** cytotoxin-associated gene product; **HsCRP**, high sensitive C-reactive protein; **IgG,** immunoglobulin G; **LDL-C,** low-density lipoprotein cholesterol; **MCV,** mean corpuscular volume; **MPV,** mean platelet volume; **RDW,** red cell distribution width; **Tsat,** transferrin saturation; **WBC,** white blood cell.

Table 4. Comparison of some laboratory parameters by American Heart Association (AHA) risk for cardiovascular disease group.

Parameters	Low (hs-crp < 1 mg/L) n=10	Intermediate (hs-crp 1-3 mg/L) n=40	High (hs-crp > 3 mg/L) n=133	*p*-value#	*p*-value*
RDW, %	13.9 (13.4-13.9)	13.4 (12.8-15.0)	14.3 (13.6-15.1)	**0.005**	a
MPV, fL	8.0 (8.0-9.4)	8.6 (7.5-8.7)	8.9 (7.7-9.7)	**0.016**	c
Tsat, %	41 (0.05-0.46)	28 (0.06-0.58)	21 (0.07-1.00)	**<0.001**	a b c
Iron, µg/dL	142 (113-142)	80 (62-91)	65 (47-84)	**<0.001**	a c
Syntax score	5 (5-7)	17 (14-28)	19 (12-33)	**0.008**	a b

Hs-CRP, high sensitive C-reactive protein; **MPV,** mean platelet volume; **RDW,** red cell distribution width; **Tsat,** transferrin saturation.
AHA risk groups were compared by using Kruskal-Wallis test.
*Comparison of groups with each other by using Mann-Whitney test; Bonferroni-adjusted P value is 0.016.
a Difference between the low group and high group is statistically significant (p<0.016).
b Difference between the low group and intermediate group is statistically significant (p<0.016).
c Difference between the intermediate group and high group is statistically significant (p<0.016).

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DISCUSSION

This propensity score matched observational study has confirmed that CagA positive H. pylori infection prevalence is high and is associated with higher prevalence of iron deficiency, CAD risk factors and acute coronary events in CAD patients. The major and novel finding of this study is the independently association of Tsat with advanced coronary atherosclerosis in CagA positive *H. pylori* infection.

Nearly all of the samples collected from East Asian countries comprise CagA strains of *H. pylori*, contrary to western countries, in where it decreases to a half (25). In Turkey, a developing country, seroprevalence of *H. Pylori* IgG is between 41% to 83% (25). In a study regarding CAD patients from our country, seroprevalence of *H. Pylori* IgG was 80.2% (26). In another study from United States of America, 45% of CAD patients were seropositive for *H. Pylori* (27). However, the importance of anti-CagA seropositivity wasn't analyzed in these studies. Different prevalence rates of infected populations between developing and developed countries are explained partly by higher socioeconomic levels in developed countries (1, 25). In line with the literature, we found the prevalence of virulent *H. pylori* infection 81.2% in patients with CAD. The socioeconomic class was also recognized as a risk factor for atherosclerosis (1). Also, other factors predisposing to both of these diseases had been suggested to affect the association between CAD and *H. Pylori* infection (10, 28). Major factors responsible for the increasing rate of CAD occurrence like diabetes mellitus and smoking, were linked with higher prevalence of *H. Pylori* infection (28). This was supported by the findings in our study as male gender, family history of CAD, history of diabetes mellitus, and current smoking were the covariates associated with positive CagA IgG serology.

The relation between *H. Pylori* infection and stable or unstable forms of cardiac syndromes is controversial (2-5, 8, 9). Population-based cohort studies have not shown a significant association of *H. pylori* infection and CAD (3, 8). However, such a significant, positive association between CagA IgG seropositivity and the occurrence of ACS was concluded in a meta-analysis and a prospective, case-control study with a 12-year follow-up period (4, 9). Moreover, anti-CagA antibody titer was the only independent predictor of the extent of coronary atherosclerosis in a cross-sectional study with 60 patients by Niccoli et al. (5). Different from their research, that compared dissimilar groups for CAD risk factors; our study was carried out at a larger population by adjusting confounding factors. The predictive ability of anti-CagA antibody titer on the extent of coronary atherosclerosis lost its significance in our matched sample. In the literature, propensity score match analysis hasn't been used in this regard and discrepancy between the investigations may be attributed to cohort heterogeneity and differences in exposure time to the infection. Besides, most of these studies were based mainly on Enzyme-linked immune-sorbent assay (ELISA) that failed to confirm the existence of ongoing infection and the likelihood of different active *H. Pylori* infection rates might have affected the outcomes of studies. After ACS, ongoing infection by *H. Pylori* may be responsible for platelet aggregation and local inflammation within the vascular wall that diminish along with plaque stabilization during subsequent weeks (6, 7). The importance of acute infection had been claimed in unstable forms of cardiac syndromes by determining the significant association of seropositivity for *H. Pylori* with the risk of only short-term outcomes, but not with the risk of long-term outcomes (6, 7). Although the rate of acute infection was obscured due to the design of our study, the association of the presence of acute coronary syndromes with CagA titer independent from the SXScore strengthened this suggestion.

In addition to CAD; the prevalence of other *H. Pylori* related advanced disease manifestation rates such as more severe gastric injury, unexplained iron-deficiency anemia, progression of gastric pre-neoplastic lesions and development of gastric adenocarcinoma are suggested to be higher with CagA bearing strains (10, 11). Strain-specific virulence constituents may act in concert with environmental factors to influence pathogenic outcomes (11). Iron deficiency is also shown to be associated with an increased risk for neoplasms that arise within the gastrointestinal tract, including the stomach (12). *H. pylori* infection contributes to iron deficiency (12), and bacterial eradication results in reversal of this disorder (29). Virulent strains of *H. Pylori* translocate CagA into host cells by a bacterial type IV secretion system (T4SS) in order to affect and alter host cell morphology, signaling, and inflammatory responses. It was defined that under conditions of iron depletion *H. pylori* virulence had increased by enhancing assembly and function of the cag T4SS (13). *H. pylori* infection-related chronic mucosal inflammation, especially the presence of lymphoid follicles was shown to be reflected in the amount of higher peripheral blood lymphocytes and lower blood MPV levels (30). By determining 2.5 times higher iron deficiency risk and higher lymphocyte counts in our anti-CagA positive patients compared to seronegative ones, we emphasize the importance of interaction between inflammation, iron status and advanced disease manifestation.

As iron status, in conjunction with the presence of CagA positive *H. pylori* strains was considered as a risk factor for more advanced associated diseases; we investigated the association of iron deficiency with advanced coronary arterial diseases in terms of acute coronary events and advanced coronary atherosclerosis in seropositive patients. In the Hunt study, a large prospective-cohort study with a 11.4 year follow-up period, low iron status was demonstrated to be the eventual late sign of ischemic heart disease (14). In another study, the diagnostic accuracy of iron status parameters for determining iron deficiency in CAD patients was investigated. When iron stores in bone marrow aspirates were taken as the diagnostic gold standard test; it was observed that irrespective of concomitant anaemia many stable CAD patients had bone marrow iron deficiency. Tsat, as a more widely available screening tool, was emphasized to be a good alternative to serum soluble transferrin receptor for iron deficiency in patients with CAD (15). This evidence was confirmed by a systematic review and meta-analysis that examined the association between body iron status biomarkers and CAD by determining the only significant association between serum Tsat and CAD (16). Our results concerning the associations between advanced coronary arterial diseases and iron deficiency was in line with the current knowledge and also suggested the probable role of

CagA positive *H. Pylori* infection in the progression of both disorders.

Although DNA of *H. Pylori*, especially CagA positive, can be identified in atherosclerotic plaques of patients with severe CAD, low grade general inflammatory response is suggested to be the primary mechanisms linking *H. Pylori* infection to the development of atherosclerosis and ACS occurrence (**2, 6, 7**). Therefore, we evaluated the explanatory interactions depending on serum iron status and inflammatory biomarkers for the extent of atherosclerosis. Risk stratification of CAD by circulating blood biomarkers including direct or indirect inflammatory markers such as hs-CRP, Tsat, iron and more simple, inexpensive and widely available markers like RDW and MPV was asserted (**16, 17, 20**). However, the most critical limitation which could not be avoided in these studies was the inadequate adjustment of other cardiovascular risk factor effects. Although, in keeping with previous observations, we had found individual relationships between RDW, MPV, iron, Tsat and advanced coronary atherosclerosis; our study tried to solve most of this limitation. Therefore, these relationships were investigated after balancing the groups for significant confounding CAD risk factors. When hs-CRP was added in the model MPV lost its significance, while Tsat was still a significant variable for determining SXScore.

Strengths of our study include the large sample size relative to similar studies, fully matched compared samples for probable confounders and a detailed description of the quantitative coronary angiography method used. It is a limitation of our study that *H. pylori* diagnosis based on serology, which may reflect not only present but also recent or past *H. pylori* infection. So, the association of acute *H. Pylori* infection with the presence of acute coronary syndromes is uncertain. No history of eradication therapy for *H. Pylori* infection in none of the recruited 293 individuals points out this limitation at least partly. On the other hand, H. pylori status was determined among all cases with the same method reducing internal variability, and, although the test showed good correlation with previous *H. pylori* tests used in former studies, no specific local validation was performed. Our measurements of subsequent markers were based on a single determination, and the time-course relationship with vascular events cannot be extrapolated from the study. Observational studies are always open to residual confounding factors that cannot always be completely controlled. Here, we reported estimates of OR adjusted by the most widely recognized independent risk factors. In addition, although the study is not based on a random sample from the general population, in our country, CagA positive *H. Pylori* infection was also frequent in the general population as previously reported (**25**). When the higher rate of coexistent CAD risk factors with the infection is taken into account, our findings may have clinical importance for the general population.

CONCLUSIONS

In summary, these results may have clear implications for clinical practice in line with the previously reported studies (**10, 16, 26, 28**). The high seroprevalence of H. Pylori infection and the frequent coexistence of positive CagA IgG serology with covariates responsible for the occurrence of coronary atherosclerosis highlight the necessity of investigation for CAD in CagA positive *H. Pylori* infection. Besides, those more pathogenic strains can be responsible for higher rates of both ıron deficiency and more severe coronary disease. Also, we have shown that, Tsat might be the decisive mirror indicator of *H. Pylori* related advanced disease manifestations irrespective of inflammatory status. To better clarify that issue, larger prospective studies, including time-course relationship of Tsat with prognostic and progressive disease outcomes are needed.

Author Contributions: NOS: Data collection, Formal analysis, Methodology, Project administration, **BS**: Statistical Analyses, **BS:** Article writing and revisions

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Supplementary Table 1. Demographic, clinical, laboratory and angiographic characteristics of our entire study group according to severity and complexity of coronary atherosclerosis

	Entire sample n=183	Syntax score ≤22 n=108	Syntax score >22 n=75	*p*-value
Age, (years)	61±14	61±13	62±16	0.57
Female, n (%)	76 (42)	39 (36)	37 (49)	0.09
Family history of CAD, n (%)	123 (67)	70 (65)	53 (71)	0.41
Socioeconomic status, n (%)				
Low	69 (38)	46 (43)	23 (31)	0.10
Middle-High	114 (62)	62 (57)	52 (69)	
Education <10 years, n (%)	90 (49)	50 (46)	40 (53)	0.35
Diabetes, n (%)	103 (56)	54 (50)	49 (65)	**0.04**
Hypertension, n (%)	133 (73)	76 (70)	57 (76)	0.40
Dyslipidemia, n (%)	108 (59)	58 (54)	50 (67)	0.08
Smoking, n (%)	76 (42)	30 (28)	46 (61)	**<0.0001**
WBC (10^3 /µL)	9.8±2.7	9.9±3.2	9.6±1.9	0.26
Platelets (10^3 /µL)	238±65	239±66	236±63	0.76
Neutrophils (10^3 /µL)	7.1 (5.1-8.2)	7.1 (4.8-8.7)	6.8 (5.3-7.8)	0.80
Lymphocytes (10^3 /µL)	1.9 (1.3-2.7)	1.9 (1.2-2.9)	2.2 (1.7-2.7)	0.48
MCV, fL	90.5±4.8	91.6±4.3	88.9±5.1	**<0.0001**
MPV, fL	8.6 (7.7- 9.4)	8.6 (7.7-9.2)	8.7 (8.6-9.7)	**0.02**
RDW, %	14.0 (13.4-15.0)	13.9 (13.4-15.0)	14.3 (13.6-16.0)	**0.009**
HsCRP, mg/dL	6.2±4.0	5.8±4.3	6.7±3.6	0.24
Hemoglobin, g/dL	13.9±1.9	14.3±1.5	13.5±2.2	**0.011**
Iron µg/dL	70 (48-91)	84 (57-113)	61 (37-79)	**0.001**
Iron defficiency, n (%)	59 (32)	25 (23)	34 (45)	**0.002**
Ferritin µg/L	98 (50-124)	112 (40-124)	63 (55-100)	0.29
Tsat, %	22 (18-29)	28 (19-31)	20 (12-23)	**<0.0001**
Tsat ≤ 24.5, n (%)	84 (46)	34 (32)	50 (67)	**<0.0001**
Creatinine, mg/dL	1.1±0.3	1.1±0.3	1.0±0.2	0.25
LDL, mg/dL	112±33	111±29	112±37	0.83
Caga IgG titer UR/mL	143±81	144±77	142±88	0.90
CAD type, n (%)				
Stable CAD	110 (60)	61 (56)	49 (65)	0.23
ACS	73 (40)	47 (44)	26 (35)	
Multivessel disease, n (%)	82 (45)	31 (29)	51 (68)	**<0.0001**
Syntax score	16 (11-28)	12 (6-14)	33 (26-40)	**<0.0001**

ACS, acute coronary syndrome; **CAD,** coronary arterial disease; **Cag A,** cytotoxin-associated gene product; **HsCRP,** high sensitive C-reactive protein; **IgG,** immunoglobulin G; **LDL-C,** low-density lipoprotein cholesterol; **MCV**, mean corpuscular volume; **MPV,** mean platelet volume; **RDW,** red cell distribution width; **Tsat,** transferrin saturation; **WBC,** white blood cell.

Supplementary Table 2. Correlations of angiographic and laboratory parameters in our study group

characteristics	Correlation coefficient									
	Entire sample n=293					*Matched Sample n=100*				
	Hs-CRP	Lymphocytes	MPV	Iron	Tsat	Hs-CRP	Lymphocytes	MPV	Iron	Tsat
Age, (years)	**0.14***	**-0.38****	0.03	-0.09	-0.08	**0.31****	**-0.44****	0.32**	0.09	-0.22
WBC (10^3 /µL)	**0.38****	**0.37****	-0.09	**-0.16****	**-0.15***	**0.32****	**0.36****	-0.32**	**-0.32****	**-0.45****
Platelets (10^3 /µL)	-0.03	**0.17****	**-0.41****	-0.12*	-0.08	0.01	**0.32****	**-0.57****	-0.15	-0.36**
Neutrophils (10^3 /µL)	0.35**	-0.10	**-0.13***	-0.11	-0.10	0.17	-0.13	**-0.20***	-0.21*	-0.10
Lymphocytes (10^3 /µL)	0.02	-	**-0.16****	-0.07	0.04	0.10	-	**-0.33****	-0.08	-0.28*
MCV, fL	0.005	0.10	**-0.44****	-0.21**	0.16*	-0.18	0.41**	**-0.23***	0.19	0.02
MPV, fL	0.22**	**-0.16****	-	-0.05	-0.12	0.06	**-0.33****	-	0.01	-0.10
RDW, %	**0.32****	**-0.25****	**0.51****	-0.21**	-0.21**	**0.42****	**-0.46****	**0.41****	-0.18	-0.01
Iron µg/dL	-0.18**	-0.07	-0.05	-	**0.89****	-0.12	-0.08	0.02	-	**0.77****
Ferritin µg/L	0.20**	**-0.19****	-0.05	-0.04	0.11	0.21	**-0.27***	-0.21	0.34**	-0.28*
Tsat, %	**-0.23****	0.04	-0.12	**0.89****	-	**-0.33****	-0.28*	-0.09	**0.77****	-
Caga IgG titer UR/mL	0,07	**0.31****	**-0.17****	-0.22**	-0.1	-0.06	**0.55****	**-0.37****	-0.13	-0.06
Syntax score	**0.22****	-0.01	0.05	**-0.24****	**-0.28****	**0.26***	-0.13	-0.03	**-0.35****	**-0.22***
Diseased vessel number	**0.41****	0.05	0.07	-0.09	**-0.26****	**0.66****	-0.04	0.14	0.1	**-0.26***

Cag A, cytotoxin-associated gene product; **HsCRP,** high sensitive C-reactive protein; **IgG**, immunoglobulin G; **MCV**, mean corpuscular volume; **MPV,** mean platelet volume; **RDW,** red cell distribution width; **Tsat**, transferrin saturation; **WBC,** white blood cell.
Significance * p<0.05, ** p<0.01. Parameters significant in both samples are shown in bold.

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Supplementary Table 3. Univariable and multivariable linear regression analysis of Syntax score and potential confounding variables in our matched sample group.

Variables	Univariable linear regression analysis		Multivariable linear regression analysis		Standardized β	Collinearity statistics VIF
	β (95% CI)	*p*-value	β (95% CI)	*p*-value		
Analysis with hs-crp						
MPV	84 (24; 143)	**0.006**	53 (-7; 113)	0.08	0.21	1.18
RDW	93 (11; 177)	**0.03**	-13 (-96; 70)	0.75	- 0.04	1.32
hs-CRP	9 (2; 16)	**0.008**	4 (-3; 11)	0.24	0.15	1.29
LDL	-0.7 (0.2; 0.02)	0.14	-	-	-	-
Tsat	-38 (-55; -21)	**<0.0001**	-33 (-51; -14)	**0.001**	- 0.43	1.24
Iron	-4 (-18; 11)	0.63	-	-	-	-
Analysis without hs-crp						
MPV	-	-	64 (7; 121)	**0.03**	0.26	1,20
RDW	-	-	2 (-76; 81)	0.95	0.007	1.32
Tsat	-	-	-36 (-53; -19)	**<0.0001**	-0.46	1.10

β, Regression coefficient; **CI,** confidence interval; **hs-CRP,** high sensitive C-reactive protein; **HDL-C**, high-density lipoprotein cholesterol; **LDL-C**, low-density lipoprotein cholesterol; **LVEF**, left ventricular ejection fraction; **MPV**, mean platelet volume; **NLR**, neutrophil to lymphocyte ratio; **RDW**, red cell distribution width; **Tsat**, transferrin saturation; **VIF**, variance inflation factor.

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The relationship between working memory and expressed emotion in the related caregivers of psychotic patients

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ABSTRACT

Objective: To investigate the relationship between Expressed Emotion (EE) and working memory (WM) capacity in the caregivers of patients with psychosis, controlling for the potential confounds, namely, personality traits, subsyndromal psychotic symptoms, burden of care and the patient's illness severity.

Materials and Methods: The study covered 152 related caregivers of psychotic patients diagnosed with schizophrenia, schizoaffective disorder, or bipolar disorder with a psychotic component. The study continued with 120 participants who met the recruitment criteria. Patients were assessed with a Structured Clinical Interview for Axis I Disorders (SCID-I), the Brief Psychiatric Rating Scale (BPRS), and the Clinical Global Impression Scale (CGI). For related caregivers; SCID-I, Expressed Emotion Scale (EES), Temperament and Character Inventory (TCI); Magical Ideation Scale; Physical Anhedonia Scale; Social Anhedonia Scale; Zarit Caregiver Burden Scale (ZCBS), and Auditory Consonant Trigram Test (ACT) were used. A stepwise regression analysis was employed to analyze the relevant variables that had an independent impact on EES scores.

Results: There was a significant negative relationship between the ACT and EES scores ($r=-.25$, $p<0.01$). The ZCBS score (beta: 0.355, $p<0.01$), Harm Avoidance subscale of the TCI (beta: 0.231, $p<0.01$), and CGI overall improvement subscale (beta: 0.237, $p<0.01$) were independently associated with the EES score.

Conclusions: There have been few studies investigating the biological basis of this clinical characteristic. The present study found no significant relationship between WM and EE in terms of the effect of WM in the caregivers of patients with psychosis.

Keywords: Caregivers, Endophenotype, Expressed Emotion, Psychotic Disorders, Working Memory

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INTRODUCTION

The concurrent use of clinical and neurocognitive measurements is beneficial for identifying risk in individuals who are relatives of patients with psychotic disorders, and this method can improve the likelihood of a timely diagnosis (**1, 2**). The unaffected relatives of patients with schizophrenia or bipolar disorder exhibit cognitive deficits that are similar to those of the patients, which suggests that these deficits can be used as markers of a familial predisposition for psychotic disorders (**3, 4, 5**). Various types of cognitive dysfunction in patients with schizophrenia and bipolar disorder have been evaluated as candidates for the endophenotypes of psychotic disorders (**2**). Attention, verbal memory, and working memory (WM) emerged as crucial factors that meet the criteria for a potential endophenotype, and WM, a primary neurocognitive function that stores limited information for later use in more complex cognitive tasks, appears to be the most critical component (**2, 6, 7**).

Communication, interpersonal relationships, social interaction, problem-solving, and behavioral preferences are information-processing operations that require intact WM (**8, 9**). These functions are the products of the cognitive and emotional processing of social stimuli that precede the choice of a corresponding response (**8, 9**).

The high levels of expressed emotion (EE) exhibited by the relatives of psychotic patients in clinical settings and during laboratory investigations have typically garnered a great deal of attention. This is likely because impairments in the emotions, thought processes, and behaviors of patients diagnosed with a psychotic disorder affect the family as a whole (**10**). On the other hand, tuning of the EE is a complex cognitive process determined by the caregiver individual's cognitive capacity alongside their experience with the patient. As basic cognitive abilities are among the endophenotypes of the disorder, the average caregiving family member with no history of psychosis could also have subtle cognitive deficits that impact the interactions with the patient. To our knowledge, the potential association between the levels of EE and cognitive abilities of the caregiver individuals has not been addressed in a controlled study before.

MATERIAL and METHODS

The present study included psychotic patients and their relatives who act as primary caregivers. All subjects were recruited from among patients who presented to the Schizophrenia and Other Psychotic Disorders Unite of Psychiatry outpatient Clinic in Ankara Oncology Training and Research Hospital and were diagnosed with schizophrenia, schizoaffective disorder, or bipolar disorder with manic–depressive episodes with a psychotic component and whose symptoms had subsided for at least three months. The patients and relatives who agreed to participate in this study were given information about the research, and all provided informed consent. The Ethics Committee of the Ankara Oncology Training and Research Hospital approved (Document No: 8329, Date: 13/06/2012) all aspects of this study. In total, 152 related caregivers of psychotic patients presented to the outpatient unit between June and December 2012 and who met the inclusion criteria were enrolled in this study.

The inclusion criteria for patients diagnosed with a psychiatric disorder were as follows: a diagnosis of schizophrenia, schizoaffective disorder, or bipolar disorder with manic–depressive episodes with a psychotic component (all diagnoses were based on the Structured Clinical Interview for Axis I Disorders [SCID-I] of the Diagnostic and Statistical Manual of Mental Disorders-IV[DSM-IV]); disease symptoms that had subsided for at least three months as the Clinical Global Impression (CGI) scale scores of 1, 2, or 3 were included; not hospitalized at the time of the study (if discharged, the patient must have been monitored regularly for at least one month by the treating physician); aged 18–65 years; not diagnosed with a psychotic disorder based on substance abuse or general medical status; and agreement that their relative may participate in the study. The inclusion criteria for the related caregivers of the patients were as follows: the patient had been diagnosed with schizophrenia, schizoaffective disorder, or bipolar disorder with manic–depressive episodes with a psychotic component; the relative providing primary care for the patient (together at least 35 hours per week); between 18 and 65 years of age; not diagnosed with dementia; agreement to participate; ability to read and write; and not diagnosed with schizophrenia, schizoaffective disorder, a psychotic disorder based on substance abuse or general medical status, or schizophreniform disorder.

For this study, the patients and their related caregivers were invited for a single interview in which the sociodemographic data of the patients and their relatives and the clinical history of the patients were recorded. The pharmacological treatments used by the patients did not interfere with the study objectives. Of the 152 patients included in the present study, 14 decided to resign, and 18 left some of the questions blank or completed the form without a complete understanding of the content (e.g., answering all questions with "yes" or "no"); thus, 32 patients were excluded from the study sample. Of the remaining 120 patients, 85 were diagnosed with schizophrenia or schizoaffective disorder, and 35 were diagnosed with bipolar disorder with psychosis. The sociodemographic and clinical data of the patients and related caregivers who refused to take part in the study or did not complete the forms properly were comparable to those of the patients and related caregivers who participated. As part of the study, the related caregivers were given 1) a sociodemographic data form and the following measures: 2) SCID-I; 3) Expressed Emotion Scale (EES); 4) Temperament and Character Inventory (TCI); 5) Magical Ideation Scale (MIS); 6) Physical Anhedonia Scale (PAS); 7) Social Anhedonia Scale (SAS); 8) Auditory Consonant Trigram Test (ACT); and 9) Zarit Caregiver Burden Scale (ZCBS). The patients were given 1) a sociodemographic data form and the following measures: 2) the Brief Psychiatric Rating Scale (BPRS) and 3) the CGI scale (assessed over the previous three months).

Scales and Measures

Expressed Emotion Scale (EES):

The EES is a 41-item scale (11); includes items regarding how the relatives of patients perceive the patient and themselves. Of the 41 items, 29 identify critical/hostile (CH) behavior, and 12 indicate over-involvement/protective/defensive (OIPD) attitudes. All questions are answered as "True" or "False" and are rated from 0 to 1, with higher scores indicating higher levels of EE. Some items are reverse scored so that the answer "False" is given one point (items 3, 8, 14, 28, 36, 38, 39, 40, and 41). Thus, the total score is between 0 and 41, with CH scores ranging from 0 to 29 and OIPD scores from 0 to 12. Examples of the items measuring CH behavior include "I don't believe he/she is sick" and "His/her presence makes me mad"; examples of the items evaluating OIPD attitudes include "I overindulge him/her" and "I am concerned that he/she will suffer from even minor things." No cut-off point for the scale has been established. The strength of the scale lies in the unique social and cultural characteristics considered when developing the items (**11, 12**).

Temperament and Character Inventory (TCI): The present study employed the 240-item version of the TCI based on the seven-factor personality model of Cloninger (**13, 14**).

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Physical Anhedonia Scale (PAS), Social Anhedonia Scale (SAS), and Magical Ideation Scale (MIS): PAS (15), the SAS (15, 16), and the MIS (17) assesses individual dimensions of schizotypy and evaluates an individual's risk of psychosis.

Auditory Consonant Trigram Test (ACT): The ACT measures short-term memory, split attention, and information-processing capacity in adults (18, 19) as well as verbal processing memory.

Zarit Caregiver Burden Scale (ZCBS): The ZCBS is used to measure the stress experienced by the caregivers of patients (20).

Statistical analysis

For all descriptive statistics, the mean ± standard deviation (SD) was used for variables with a normal distribution, and the median (min-max) was used for variables with a non-normal distribution. For nominal variables, the n and the percentages are presented. Significant differences between mean group values were assessed using Student's t-test, and significant differences between median group values were analyzed using the Mann–Whitney U-test. Pearson's Correlation test was used to evaluate the relationship between two continuous variables if the distribution was normal, and Spearman's Correlation test if it was non-normal. A stepwise regression analysis was employed to analyze the relevant variables that had an independent impact on EES scores. Statistical significance for all tests was set at $p < 0.05$.

RESULTS

Of the 120 patients included in the present study, 59 (49.2%) were female, and 61 (50.8%) were male; their mean age was 30.00±10.32 years, and mean time since diagnosis was 6.00±6.98 years. Of the patients, 95 (79.2%) were single, 17 (14.2%) were married, and 8 (6.7%) were divorced or widowed. There were no significant differences in the sociodemographic and clinical characteristics between patients with schizophrenia/ schizoaffective disorder and patients with bipolar disorder.

Of the relatives providing primary care included in the present study, 77 (64.2%) were female, and 43 (35.8%) were male; their mean age was 50 ± 11.141 years.

Their relationships to the patients were as follows: 52 (43.3%) were the patient's mother; 27 (22.5%) were the patient's father; 34 (28.3%) were a sibling of the patient, and seven (5.8%) were a child of the patient. There were no significant differences in the sociodemographic and clinical characteristics between the relatives providing primary care for patients with schizophrenia/schizoaffective disorder and those providing care for patients with bipolar disorder (**Table 1**).

When the mean scores of all scales were analyzed for caregivers, related caregivers of the patients with schizophrenia/schizoaffective disorder exhibited higher EES scores compared with the related caregivers of patients with bipolar disorder (p=0.02). However, the groups did not show any other significant differences except on ZCBS sub-factor 4 (Economic Burden) (p=0.01) and on the Harm Avoidance-1 subscale (p=0.00), the Harm Avoidance total score (p=0.01), the Self-Directedness-2 subscale (p=0.03), and the Self-Directedness total score (p=0.04) on the TCI.

Comparisons of the EES scores and sociodemographic characteristics of the related caregivers and the relationships among these factors: For relatives providing care to patients with bipolar disorder, the time from the emergence of symptoms to the presentation of the disorder (r = 0.56, p=0.00), the previous history of psychiatric disorders (p=0.03), and the marital status (p=0.01) of the patient were significantly related to the EES score. The age of the patient (r=0.29, p=0.00) and regularity of medication (p=0.00) in patients with schizophrenia/schizoaffective disorder were significantly related to the EES score of their related caregivers.

Analyses of the correlations of the EES scores with the age of the patient, time from the emergence of symptoms to the presentation of the disorder, and scores on the BPRS, CGI, ACT, MIS, SAS, PAS, ZCBS, and TCI are provided (**Tables 2**). There was a significant negative relationship between the ACT and EES scores for the entire group (r=-.25, p=0.00). When siblings were removed from the sample to obtain a group with greater genetic similarity and the analysis was repeated using only parent and child caregivers (n=86), the relationship between the ACT and EES scores was no longer statistically significant (r=-0.89, p=0.414). Similarly, there was no longer a significant relationship between the ACT and EES scores for the genetically first-degree relative caregivers from the schizophrenia/schizoaffective disorder group (r=-0.360, p=0.091) and the bipolar disorder group (r=0.022, p=0.0864).

To determine which of the factors that were found to be related to EE independently predicted EES score, the EES values for all caregivers were used as dependent variables in a stepwise regression analysis. The ZCBS score (beta: 0.355, p<0.01), Harm Avoidance subscale of the TCI (beta:0. 231, p<0.01), and CGI overall improvement subscale (beta: 0.237, p<0.01) were independently associated with the EES score.

Figure1. The relationship between ACT scores and EES scores. R2 Linear= 0.06

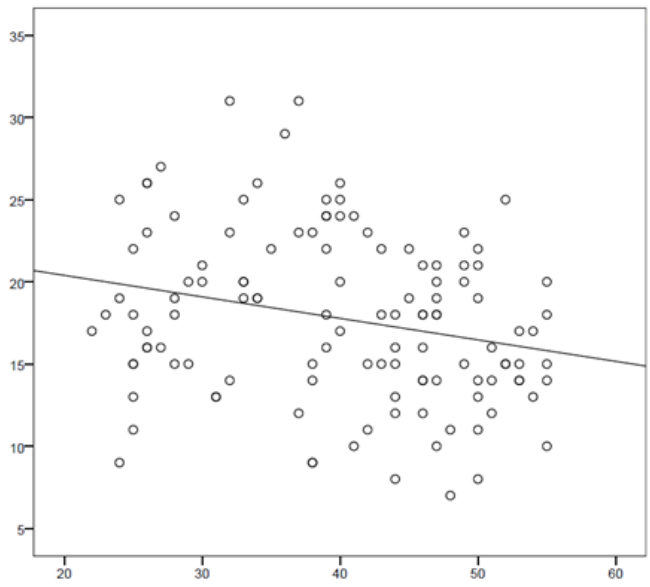

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Table 1. Sociodemographic and clinical characteristics of relatives providing primary care for patients with schizophrenia/schizoaffective disorder or patients with bipolar disorder.

	Overall group (*n*=120)	Bipolar disorder (*n*=35)	Schizophrenia/Schizoaffective üdisorder (*n*=85)	*P*-values
	mean ± SD (min–max) *n* (%)			
Age	50.00 ± 11.14 (18–68)	48.00 ± 11.43 (19–59)	50.00 ± 10.86 (18–68)	0.097
Sex[§] (Male/Female)	43/77 (35.8/64.2%)	11/24 (31.4/68.6%)	32/53 (37.6/6.4%)	0.518
Type of relationship[§]				
Mother	52 (43.3%)	12 (34.3%)	40 (47.1%)	0.534
Father	27 (22.5%)	8 (22.9%)	19 (22.4%)	
Sibling	34 (28.3%)	12 (34.3%)	22 (25.9%)	
Child	7 (5.8%)	3 (8.6%)	4 (4.7%)	
Education[§]				
Primary/secondary school	60 (50.0%)	13 (37.1%)	47 (5.3%)	0.145
High school	39 (32.5%)	13 (37.1%)	26 (30.6%)	
University and higher	21 (17.5%)	9 (25.7%)	12 (14.1%)	
Marital status[§]				
Single	28 (23.3%)	12 (34.3%)	16 (23.3%)	0.104
Married	82 (68.3%)	22 (62.9%)	60 (70.6%)	
Divorced/widowed	10 (8.3%)	1 (2.9%)	9 (10.6%)	
Occupation[§]				
Student	6 (5.0%)	3 (8.6%)	3 (3.5%)	0.064
Civil servant/worker	40 (33.3%)	14 (40.0%)	26 (30.6%)	
Housewife/unemployed	49 (40.8%)	8 (22.9%)	41 (48.2%)	
Retired	25 (20.8%)	10 (28.6%)	15 (17.6%)	
Currently employed[§]				
Employed	37 (30.8%)	13 (37.1%)	24 (28.2%)	0.337
Unemployed	83 (69.2%)	22 (62.9%)	61 (71.8%)	
Average income[§]				
≤minimum wage	34 (28.3%)	9 (25.7%)	25 (29.4%)	0.683
>minimum wage	86 (71.7%)	26 (74.3%)	60 (70.6%)	
Time from patient's symptoms to presentation (yr)[ε]	4.00 ± 13.77 (0.1–60.0)	3.00 ± 12.87 (0.1–36.0)	6.00 ± 14.19 (0.1–60.0)	0.656
Place lived[§]				
Metropolis/City	108 (90.0%)	30 (85.7%)	78 (91.8%)	0.329
Village/Town/District	12 (10.0%)	5 (14.3%)	7 (8.2%)	
Time spent with patient/week (hours)	168.00 ± 44.94 (40–168)	168.00 ± 46.33 (50–168)	168.00 ± 44.24 (40–168)	0.146
Past psychiatric disorder[§]				
Yes	31 (25.8%)	5 (14.3%)	26 (30.6%)	0.064
No	89 (74.2%)	30 (85.7%)	59 (69.4%)	

[§]Chi-square analysis; [ε]Mann–Whitney *U*-Test

Table 2. Analysis of the correlations between EES scores and BPRS, CGI, ACT, MIS, SAS, PAS, ZCBS, and TCI scores.

	ACT	EES
MIS	*r* = -.23.; *p* = 0.00	*r* = .12; *p* = 0.18
SAS	*r* = -.34; *p* = 0.00	*r* = .21; *p* = 0.01
PAS	*r* = -.19; *p* = 0.03	*r* = .11; *p* = 0.22
ZCBS-F1	*r* = -.10; *p* = 0.25	*r* = .49; *p* = 0.00
ZCBS-F2	*r* = -.05; *p* = 0.56	*r* = .44; *p* = 0.00
ZCBS-F3	*r* = -.16; *p* = 0.07	*r* = .43; *p* = 0.00
ZCBS-F4	*r* = -.22; *p* = 0.01	*r* = .49; *p* = 0.00
ZCBS-F5	*r* = -.09; *p* = 0.32	*r* = .43; *p* = 0.00
ZCBSTOTAL	*r* = -.12; *p* = 0.19	*r* = .53; *p* = 0.00
TCI-NSTOTAL	*r* = .01; *p* = 0.86	*r* = -.09; *p* = 0.29
TCI-HATOTAL	*r* = -.31; *p* = 0.00	*r* = .34; *p* = 0.00
TCI-RDTOTAL	*r* = .00; *p* = 0.94	*r* = -.22; *p* = 0.01
TCI-SDTOTAL	*r* = .23; *p* = 0.00	*r* = -.22; *p* = 0.01
TCI-COTOTAL	*r* = .20; *p* = 0.02	*r* = -.08; *p* = 0.34
TCI-STTOTAL	*r* = -.17; *p* = 0.055	*r* = .04; *p* = 0.61
TCI-PS	*r* = .10; *p* = 0.24	*r* = .13; *p* = 0.15
BPRS	*r* = -.02; *p* = 0.78	*r* = .24; *p* = 0.00

ACT, Auditory Consonant Trigram Test; **EES,** Expressed Emotions Scale; **BPRS,** Brief Psychiatric Rating Scale; **MIS,** Magical Ideation Scale; **PAS,** Physical Anhedonia Scale; **SAS,** Social Anhedonia Scale; **TCI,** Temperament and Character Inventory (**NS**, Novelty seeking; **HA**, Harm Avoidance; **RD**, Reward dependence; **SD**, Self-Directedness; **CO**, Cooperativeness; **ST**, Self-transcendence; **PS**, Persistence); **ZCBS**, Zarit Caregiver Burden Scale (**Factor 1**: Mental strains and impaired private life, **Factor 2**: Nervousness and restrictedness, **Factor 3**: Impaired social relationships, **Factor 4**: Financial burden, **Factor 5**: Dependency).

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DISCUSSION

Relatives providing primary care to patients with schizophrenia, schizoaffective disorder, or psychosis bipolar disorder who have been in remission for at least three months were included in this study. The primary goal of this study was to investigate the relationship between EE and WM in related caregivers that is independent of the care burden, personality traits, and subsyndromal psychotic symptoms of the related caregivers and the psychotic symptoms of the patients. The present findings revealed a negative correlation between scores on the ACT score, which assesses WM, and scores on the EES, which measures EE. This correlation disappeared when parent and child caregivers (groups with higher genetic similarity) were only analyzed.

Working memory can be considered as the basis of all cognitive functioning because it plays an active role in interpersonal communication, relationships, socializing, problem-solving skills, and the choice of behaviors, and it is required for the cognitive and emotional processing of social stimuli (**21**).

Therefore, it can be expected that impaired WM in a caregiver may interfere with that individual's communication and/or relationship with the patient. Impairments in WM are commonly observed among schizophrenic patients and their first-degree relatives and are thought of as a core deficit that contributes to other manifestations of the disorder (**22, 23, 24**). Additionally, WM deficits have been described as a key endophenotype in patients with schizophrenia (**25, 26, 27**). Schizophrenic patients from families with high EE levels perform better on cognitive function tests than patients from families with low EE levels (**28, 29, 30**).

To date, no studies have investigated the relationship between cognitive functioning and EE in caregivers. The present study identified a significant negative correlation between the ACT score, which assesses WM, and the EES score, which evaluates EE. However, of the variables that were correlated with EES, only the ZCBS total score, the Harm Avoidance subscale of the TCI, and the overall improvement subscale on the CGI were independently related with EES scores.

The present findings also revealed a positive correlation between EE and caregiving burden, which supports the findings of several studies that observed a high caregiving burden in the high-EE relatives of schizophrenic patients (**31, 32, 33**). Levels of EE and caregiving burden may or may not be causally related; our findings or those from previous studies do not allow us to conclude the direction of potential causality. In the present study, an overall improvement over the course of the disease was positively correlated with the EE of caregivers independent of other factors.

The Harm Avoidance subscale of the TCI correlated with the EES score in the present study has been proposed as an endophenotype candidate because the relatives of patients with schizophrenia and bipolar disorder exhibit high subscale scores. Considering that temperament is at least partially inherited (**34, 35**), the existence of a relationship between the harm-avoidance component of temperament and EE supports the hypothesis that EE possesses a neurobiological origin. On the other hand, the relationship between EE and WM, another endophenotype candidate, was not significant.

Additional studies using cognitive functioning tests, an additional endophenotype candidate, will contribute to the further characterization of these relationships.

There are several limitations to the present study. The fact that a majority of the related caregivers included in this study were mothers may have influenced the results. Furthermore, the number of relatives caring for patients with bipolar disorder was lower than the number of relatives caring for those with schizophrenia/ schizoaffective disorder. Moreover, the patients with bipolar disorder who were included in this study were required to have a history of psychosis, which precludes the extrapolation of these findings to all types of bipolar disorder. Studies using larger populations of patients with bipolar disorder are needed. Another limitation of the present study was that the included patients with schizophrenia had relatively favorable CGI scores compared with the general schizophrenia population because the former consisted of patients who had been treated on an outpatient basis and who had received continuous psychosocial support in a unit specializing in psychosis. The large number of scales and the number of questions in the scales was another limitation because the reliability of the responses may have been compromised considering the significant time necessary to complete them. However, this was considered, and the ACT, which measures WM, was the first scale administered due to its primary importance in this study.

One of the strengths of the present study is that no previous studies have investigated the relationship between EE and WM in related caregivers of patients with psychotic disorders. However, some studies have evaluated temperament, personality traits, schizotypy, care burden associated with these traits, cognitive functioning, and EE in patients with psychotic disorders.

CONCLUSIONS

Expressed emotion is a clinical characteristic that clinicians have monitored for a significant period and has been shown to negatively affect the course of a disorder. However, there have been few studies investigating the biological foundations of this clinical characteristic. The present study assumed EE to be associated with the mental processes that support WM. Working memory is involved in the basics of cognitive functioning and is thought to be one of the strongest candidates for an endophenotype of psychotic disorders because it is a fundamental cognitive ability in the basic components of the thought process. The present study found no significant relationship between WM and EE in terms of the effect of WM on the course of a disorder. However, the present findings indicate that EE in the related caregivers of psychotic patients is associated with disease severity, caregiver burden, and caregiver temperament rather than the patient's cognitive capacity.

Author Contributions: PE, EŞC, SUK, AHS, ECA: Data collection, Formal analysis, Methodology, Project administration, Statistical Analyses, **PE:** Article writing and revisions

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Artificial Intelligence in the endocrine clinic: Automated bone age analysis in children from UAE

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ABSTRACT

Objective: Artificial intelligence (AI) is playing an increasing role in patient assessment. AI bone age analysis is such a tool, but its value in Arabic children presenting to an endocrine clinic has not been explored. We compared results from an experienced pediatric radiologist and the AI bone age system, BoneXpert (BX), (Visiana, Denmark) to assess its utility in a cohort of children presenting to the Al Jalila Children's Specialty Hospital endocrine service.

Materials and Methods: We conducted a retrospective chart review of 47 children with growth disorders, initially assessed by a single experienced radiologist and subsequently by BX, to confirm the usefulness of the BX system in our population. The results of the analyses were analysed using a Bland-Altman plot constructed to compare differences between the radiologist's interpretation and BX across the available range of bone age.

Results: Forty-four of the patient x-ray images were analysed by BX. Three X-ray images were rejected by BX due to post-processing artifacts, which prevented computer interpretation. For the remaining 44 X-rays, there was a close correlation between radiologist and BX results ($r=0.93$; $p<0.00001$). Two radiographs were identified with a large discrepancy in the reported bone ages. Blinded, independent re-evaluation of the radiographs showed the original manually interpreted bone age to have been erroneous, with the BX results corresponding closely to the amended bone age. A small positive bias was noted in bone age (+0.39 years) in the BX analyses, relative to manual interpretation.

Conclusions: AI bone age analysis was of high utility in Arabic children from UAE presenting to an endocrine clinic, with results highly comparable to an experienced radiologist. In the two cases where a large discrepancy was found, independent re-evaluation showed AI analysis was correct.

Keywords: Bone Age, Greulich & Pyle, Growth, Puberty

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INTRODUCTION

Assessment of bone age is a critical tool in the investigation of disorders affecting growth and puberty. (**1, 2, 3**) A child growing as expected for age will have a bone age close to his chronological age. However, a child with pathological short stature or delayed puberty is likely to have significant bone age delay. In contrast, a child who has precocious puberty will have a relatively advanced bone age. (**4**) Accuracy in the determination of bone age is crucial to correct diagnosis and may be used to determine the height prognosis, which is a major concern to parents and children. (**5**)

Additionally, serial bone age assessment is invaluable in disease monitoring, as in congenital adrenal hyperplasia, where excessive steroid replacement retards growth and delays bone age, whereas the inadequate replacement is reflected in the advanced bone age and compromised final height. (**6**) Similarly, the effect of treatments such as gonadotropin releasing hormone agonists (used for central precocious puberty) or growth hormone can be monitored through serial measurements of bone age. (**4**)

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In order for bone age measurement to have maximum utility, accuracy and precision of bone age assessment is critical. This is particularly true for serial measurements. There are two widely used approaches to determine bone age from a hand and wrist radiograph – the Greulich & Pyle method (G&P) – in which, most commonly, bone age is determined by comparing a hand-wrist radiograph of a child with the age-matched standard radiographs shown in the Greulich & Pyle atlas. (7) This method is straightforward and quick and hence widely used, but when bone age is assessed in this way, it is typically somewhat imprecise (e.g. 'the bone age is between 7 and 7.5 years'). The Tanner Whitehouse method, now in its third iteration (TW3) depends on assessing and scoring the skeletal maturity of each individual bone of the hand, (8) and hence is time-consuming and laborious compared with the G&P method and while more precise, it has less utility for determining height prognosis, which is a major objective of bone age assessment. (9) The G&P bone age method was originally compiled from bone age assessments of largely Caucasian children from Ohio, USA, of good socioeconomic status, but subsequently has been validated in many populations worldwide, although important ethnic differences do exist.(10)

Use of Artificial Intelligence (AI) to assess bone age has been attempted for over 30 years. The first step in the use of AI was the HANDX system developed in 1989. (11) HANDX was a semi-automated system able to detect skeletal growth abnormalities in children. The PROI system (1991) (12) and Computer-based Skeletal Aging Scoring (CASAS) system (1994) (13) allowed assessment of bone age in a highly reproducible and accurate way. However, although of increased accuracy, compared with manual assessment of bone age, CASAS was more labor-intensive than manual reading, and hence not viable for clinical practice. Improvements in computational power and AI analysis techniques finally led to the realization of viable commercial solutions, such as BoneXpert™ (BX) (Visiana, Copenhagen, Denmark) in 2008. (14) BX is an AI system that calculates bone age by analyzing the shape and density of 21 bones (ulna, radius, metacarpals and phalanges). The borders of the bones are detected using a machine-learning algorithm which has learned to locate landmarks on the bones, and the normal anatomy of each bone. The information is used to generate a G&P and TW3 bone age, and more recently, BX version 3.0 introduced in September 2019 also reports carpal bone age. BX is now widely used in Europe and has additionally been validated in multiple ethnic populations, worldwide. (15) This system uses AI assessment of bone age to derive G&P and TW bone ages, with a high degree of precision. It has been progressively refined since its introduction and has attained a level of precision and accuracy superior to conventional radiographical interpretation. Indeed, the current software (version 3.03) is equivalent to the combined assessment of five expert radiologists. (16)

An additional utility of the BX system is that it includes a measure of bone density – Bone Health Index – which has been found to correspond very closely to measurements by dual energy X-ray absorptiometry (DXA). (17, 18)

The use of BX to determine bone age also yields actionable results immediately, allowing real-time decision-making on the basis of the result, rather than awaiting a manual report, and aids the radiologist, the clinician and the patient. Having introduced BX, we conducted a retrospective validation of the previously obtained bone age results.

MATERIAL and METHODS

We examined bone age x-rays from 47 children with disorders of growth and puberty attending endocrine clinics between August 2017 until December 2018, who required bone age assessment. The children were aged between 3.75 and 14.95 years, (27 males) assessed by a single experienced radiologist (EA) before the introduction of BX, using the G&P method. We compared the results to those obtained with BoneXpert (BX), (version 2.0.1.3, Visiana, Denmark), in December 2018. Hand radiographs for bone age were identified retrospectively from the Picture Archiving and Communication System (PACS) in standard digital imaging (DICOM) format. **Figure 1** shows an example of an AI generated BX report.

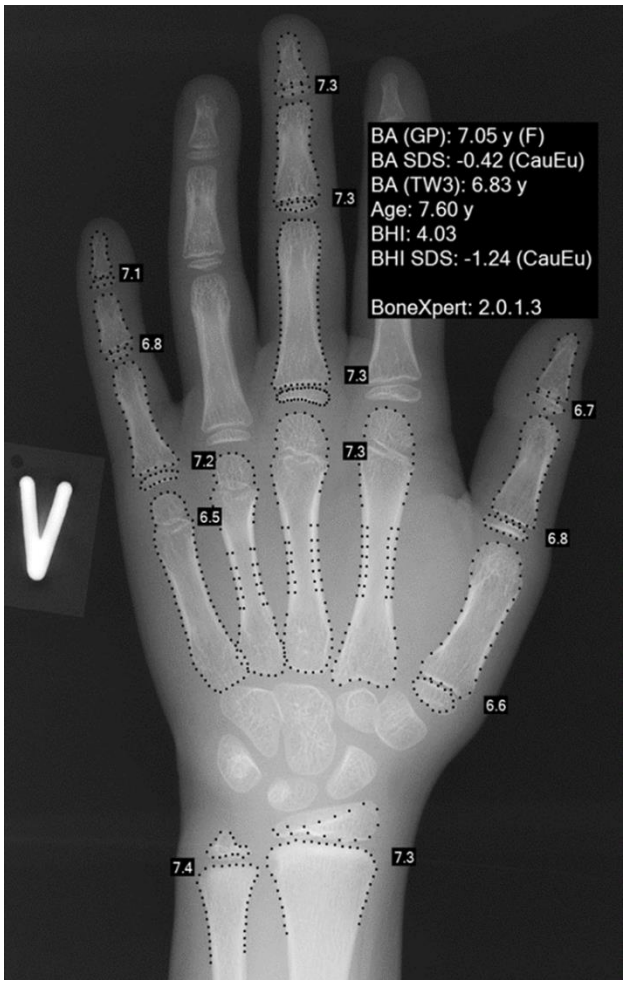

Figure 1. An example BX report. The annotated image is the result of the analysis, containing the following: **BA (GP):** Greulich-Pyle bone age (gender, M or F), **BA SDS:** Bone Age Standard deviation score of GP bone age, **BA (TW3):** Tanner-Whitehouse bone age; Chronological age, **BHI:** Bone health index – a measure of bone density, **BHI SDS:** Standard deviation score of BHI

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Statistical analysis was performed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA). The bone ages obtained by the two methods were compared and the correlation coefficient and statistical significance were determined. A Bland-Altman plot was constructed to determine analogy between the manual bone age and BX results and to identify any systematic bias.

The analysis was a retrospective review of routine radiographic data obtained as part of normal clinical practice and as such, ethical approval was deemed unnecessary.

RESULTS

Of the 47 X-rays analysed, three could not be evaluated by BX due to image processing artifacts. Image processing ('edge enhancement') is commonly employed to improve the clarity of radiographs for manual interpretation, but the resultant digital "noise" may render the X-ray unsuitable for AI analysis. (**14**)

Of the 44 analyses where comparison was possible, there was a high correlation between the two bone age measures ($r= 0.93$, $p<0.00001$). There were 2 outliers, which differed by more than 3 years. The outliers were independently reviewed by co-author, APD, who was blinded to the original bone age assessments. He reported bone ages that corresponded closely to the bone age determined by BX. The range excluding these two outliers was -1.22 years to +2.30 years, which were within the expected range of bone age, as shown by the Bland-Altman plot (**Figure 2**).

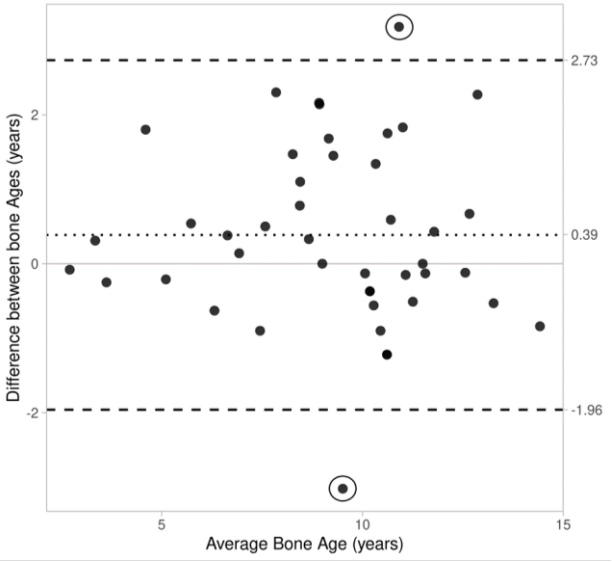

Figure 2. Bland-Altman plot demonstrating difference between bone age measurements plotted against the average bone age. The dashed lines indicate the Limits of Agreement between which 95% of measurements are expected to lie. Two outliers (highlighted with circles) are clearly evident – one above, and one below the upper and lower limits of agreement, respectively. There is a small positive bias of 0.39 years indicating that, on average, the bone age determined by BX is 0.39 years greater than manual reading

This clearly demonstrated the two outlying values, (circled) but the remaining observations were in close agreement, all lying within the limits of agreement, albeit that there was a systematic positive bias of +0.39 years, indicating that BX systematically scored bone ages 0.39 years higher than manual reading. The data spread and bias were similar when the results were analysed by gender (data not shown).

DISCUSSION

In our retrospective analysis of bone ages of children attending an endocrine clinic, we found a close correlation between an experienced radiologist's interpretations and AI Bone age analysis. Two bone age results differed markedly from those determined by BX and following independent review of the images, blinded to the original bone age estimation, the original bone age was amended and was in agreement with that given by BX. With the exception of these two outliers, there was close agreement between the bone ages, indicating that the discrepancy arose from erroneous manual reading of bone age and not from software error. This suggests that for practical purposes, AI bone age analysis using BX is a suitable tool for the evaluation of bone age in Arabic children attending an endocrine clinic in UAE.

To a certain extent, bone age is an artificial construct. The assessment of bone age using a largely Caucasian reference population from the 1940s takes no account of secular changes in bone age over time, (**19**) nor of important ethnic differences. (**10**) The mean age of puberty is falling worldwide, in both boys and girls and this is reflected by a relative advance in bone age compared with chronological age. (**19**) Nonetheless, BX has been validated in multiple ethnic populations and despite such systematic differences, has nevertheless been found suitable for clinical use. (15, 20, 21, 22)

The finding of a small systematic bias in bone age assessment between the software and an experienced radiologist (+0.39 years) could simply represent the skills of the assessor, secular trend in bone age or a true ethnic difference in the application of the G&P bone age to an Arabic population. Ethnic variation in bone age using the G&P method is well-recognised (10) and this seems the most likely explanation.

Recently, the validity of the G&P and TW3 methods of bone age measurement was assessed in a large, predominantly Arabic Saudi population of children attending an emergency Dept. for reasons unrelated to growth or puberty. (23) The authors found that there were small systematic differences in bone age estimation using either TW3 or G&P. They manually reviewed hand and wrist radiographs from 420 children and compared the bone age with the patient's chronological age. They concluded that G&P bone age consistently underestimated true chronological age in girls but overestimated it in boys. Analysis using BX was possible in only 210 children (50%) of the cohort and they found the same pattern, with a mean difference of -2 months in girls and +2.5 months in boys. H owever, we would observe that although the bone age significantly differed from the chronological age in statistical terms, in clinical practice this difference is inconsequential.

Moreover, the endocrinologist is not seeking to estimate chronological age, but to identify discrepancies between bone age and chronological age, which may highlight the presence of an endocrine disorder.

Potentially, a significant limitation of our study was the relatively small number of radiographs available for analysis, which was inevitable in a newly established children's hospital – the endocrine service was established only in August 2017. However, the correlation between assessment by an experienced radiologist and BX was highly statistically significant ($p<0.00001$) and corroborated by the Bland-Altman analysis (**Figure 2**).

Clearly, children attending an endocrine clinic with concerns regarding growth and puberty are not representative of all children in our population. However, our primary purpose in conducting the study was to assure ourselves that AI bone age analysis produced comparable results to manual reading in a cohort of Arabic children undergoing bone age analysis for the evaluation of endocrine disorders, and in this respect it excelled. Our data gave us confidence that BX is indeed a suitable tool in the assessment of bone age in our local population. Since its introduction, we have come to rely on BX and at the time of writing (July 2021) we have analysed a further 931 x-rays with BX. The added efficiency of the AI system which provides results in moments, means we can make immediate decisions based on the bone age. This convenience and speed aids clinical management, to the benefit of the patient clinician and radiologist alike. Moreover, serial bone age assessments may be performed to monitor progress, with the added confidence of a precise bone age measurement. This is in contrast to relying on often conflicting measurements of different human observers, based on subjective impressions from a bone age atlas. While experts may achieve intra-observer error as low as 0.25 years (**24**), Bull et al. found that in clinical practice, intra-observer error averaged 0.82 years for G&P bone age. (**25**) In comparison, even the first iteration of BX achieved a precision of 0.17 years, and this has been progressively improved with subsequent versions. Indeed, with the latest version (3.0.3), BX is equivalent to the combined assessment of 5 expert raters. (**16**) Thus, for routine clinical use, bone age assessment by BX is superior to manual bone age interpretation. (**26**) The additional benefit of an estimate of bone density (Bone Health Index) provides a valuable extra dimension to the assessment. We are now at a point where AI bone age analysis is so clearly superior to manual reading that it should become the tool of choice for bone age analysis. (**27**) For the present, BX cannot replace radiological review of bone age X-rays as it is not capable of identifying morphological abnormalities such as rickets or features of skeletal dysplasia, but it has replaced the onerous and burdensome task of manual bone age evaluation.

CONCLUSIONS

AI bone age assessment gives a speedy, accurate and precise result and does away with subjective visual interpretation and obviates the issue of inter- and intra-observer variability, thereby reducing the reporting burden on radiologists and facilitating patient care, through enabling a "one stop" visit. Our experience shows that AI bone age analysis is of high utility in evaluating bone age in Arabic children from UAE presenting to our endocrine service, with results highly comparable to those obtained by an experienced consultant pediatric radiologist.

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Can tissue nitric oxide synthesis 2 (iNOS) levels play a role in the pathophysiology of reflux esophagitis?

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ABSTRACT

Objective: Nitric oxide (NO) is a strong dilatator, playing an important role in inflammatory events. Its production is regulated by NO synthase 2 (NOS2/iNOS). Our aim was to compare iNOS in esophageal tissues of patients with erosive or non-erosive reflux esophagitis to that of normal cases.

Materials and Methods: The study was conducted in 2019–2020 on patients undergoing upper gastrointestinal (UGI) endoscopy. Study included 30 patients who had no reflux symptoms and were not diagnosed with reflux esophagitis in the UGI endoscopy (control), 22 who had pronounced reflux symptoms but could not be diagnosed with reflux esophagitis in the endoscopy (non-erosive reflux), and 51 who had reflux esophagitis in the endoscopy (erosive reflux esophagitis). Using the enzyme-linked immunosorbent assay, tissue iNOS levels were assessed on samples from the lower end of the esophagus.

Results: Average iNOS level was 5.02±1.51 picogram/milliliter (pg/mL) in the normal group and 5.04±1.68 pg/mL in all reflux esophagitis cases. iNOS levels were higher in non-erosive reflux and lower in erosive reflux than in controls. In erosive reflux A, B, and C, iNOS levels were 5.03±1.64, 5.10±2.23, and 4.06±0.02 pg/mL, respectively. The level in erosive reflux C is considerably lower than in the normal group. However, none of the differences between the groups was significant.

Conclusions: NO synthase was higher in patients with non-erosive reflux esophagitis and considerably lower in those with erosive reflux C, compared to the normal cases. Although not significant, the differences suggest that NO and iNOS levels may be important in reflux physiopathology.

Keywords: Nitric oxide synthase, Nitric oxide, Gastroesophageal reflux

INTRODUCTION

Among the most common disorders in the gastrointestinal tract is gastroesophageal reflux disease (GERD) (1, 2). Research has revealed that about 20% of adults in American culture have reflux symptoms, such as burning in the chest and acid regurgitation, at least once every week (3, 4). Gastroesophageal reflux disease occurs if the content in the stomach gets back to the esophagus, causing the person to experience a number of disturbing symptoms and/or health issues (5). Reflux esophagitis, in other words, is a reflux condition that develops in cases where the damage done by acid, pepsin, and bile cannot be mitigated by the mechanisms of the mucosal defense system. The mucosa may be usual or mildly erythematic in non-erosive reflux disease (NERD). There is evident mucosal damage in the case of erosive esophagitis. The damage is characterized with redness, friability, superficial linear ulcers, and exudation (6). Reflux esophagitis is, in other words, the consequence of an inflammatory process in the mucosa of the esophagus.

Smooth muscles and vascular structures may be affected by the strong dilator characteristic of nitric oxide (NO). While its mechanism is not yet clearly known, it may have a role to play in the development of reflux esophagitis. Moreover, NO is a soluble endogenous gas. It has a variety of biological functions, including signaling. It functions as an effector molecule or metabolic regulator. Immune myeloid cells like macrophages

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stimulate the supply of cytokines and NO in response to inflammation, causing signals that are important for the eradication of pathogens (7).Nitric oxide synthases (NOSs) are the isoform family responsible for NO synthesis. Inducible NOS (iNOS) expression takes place in inflammatory conditions, and under such conditions, NO is generated in high amounts. INOS is viewed to be a harmful enzyme in pathological conditions and is believed to be one of the major parameters affecting the development of cardiovascular system diseases like atherosclerosis (8). Nitric oxide generated by the inducible isoform of nitric oxide synthase (iNOS) has very complicated role. The induction of iNOS expression and, therefore, NO creation has been identified to have desirable affects that destroy viruses, parasites, microbes, and tumors and that are immunomodulatory. But iNOS leads to harmful consequences and seems to play a role in the pathophysiology of different human diseases if induced in the wrong spot and at the wrong time (9). The role of NO and iNOS in the inflammatory process of esophageal mucosa in GERD is not a subject that has been sufficiently investigated. In our study, we explored whether iNOS production in patients with reflux esophagitis differs from that in normal people due to the dilatator effect of NO and its responsibility in inflammatory processes. Thus and so, we assessed the role of iNOS levels of esophageal tissues in the pathophysiology of reflux esophagitis and attempted to identify its potential to become a parameter for diagnosis if it had a role to play.

MATERIAL and METHODS

The study was carried out between 2019 and 2020 on 103 patients who presented to our clinic and underwent upper gastrointestinal (UGI) endoscopy for various of reasons. Prior to the study, approval was obtained from the ethics committee of our institution. Ethical approval for this study (Ethical Committee decision No;198) was provided by the Ethical Committee of İstanbul Medipol University Hospitals, on 22 March 2019. The study was designed and carried out as a prospective study. Patients were included in the study voluntarily, and each patient was required to sign an informed consent form. The study included 30 patients who had no reflux symptoms and were not diagnosed with reflux esophagitis in the UGI endoscopy as the control group, 22 who had pronounced reflux symptoms but could not be diagnosed with reflux esophagitis in the endoscopy as the non-erosive reflux group, and 51 who had considerable reflux esophagitis in the endoscopy as the erosive reflux esophagitis group.

The Los Angeles (LA) classification was used to screen the patients endoscopically and stage reflux esophagitis. Patients in the non-erosive reflux group were included in the study after 24-hour pH-metry confirmed the diagnosis of reflux. Samples of tissue were taken from all patients in the case and control groups using biopsy forceps from the lower end of the esophagus about 5 cm above the Z-line. The samples were put in a tube without subjecting to any solution or processing and kept at –80 degrees until the study was completed. At the final phase of the study, iNOS levels of the tissue samples were studied using commercial kits produced by Cloud-Clone Corp. and the enzyme-linked immunosorbent assay (ELISA) method.

Statistical Analysis

IBM SPSS 22 statistics program was used for statistical analysis to assess the findings obtained in the study. Descriptive statistical methods (means, standard deviations, medians, frequencies, ratios, and minimum and maximum values) were used when assessing the data of the study. Whether the quantitative data were normally distributed was tested using Kolmogorov-Smirnov test, Shapiro-Wilk test and graph analyses. The student t test was used for pairwise comparisons of quantitative data with normal distribution, and the Mann Whitney U test was used for pairwise comparisons of data not normally distributed. One-Way ANOVA test was used in comparisons of three or more groups with normal distribution, and Bonferroni test was used in their pairwise comparisons. Kruskal Wallis test was used in comparisons of three or more groups that were not normally distributed, and Bonferroni-Dunn test was used in their pairwise comparisons. Pearson's Chi-Square test was used to compare qualitative data. Significance was assessed at least at the $p < .05$ level.

RESULTS

This study was conducted on a total of 103 cases, 37.9% (n = 39) female and 62.1% (n = 64) male. The ages of the cases ranged from 19 to 61 years, and the mean age was 37.50 ± 10.72 years. **Table 1** illustrates the percentage-wise distribution of cases by group.

The demographic data of the control group and the reflux esophagitis group by gender and age are shown in **Table 2.**

With regard to age, there was no statistically significant difference between the normal cases in the control group and the cases in all groups of reflux esophagitis ($p > .05$). No statistically significant age difference was found between the patients in the normal group and the non-erosive and erosive reflux groups either ($p > .05$). A statistically significant difference was found between the gender distributions of the normal cases and the patients with reflux esophagitis ($p = .003$; $p < .01$). Regardless of gender, the rate of male reflux esophagitis patients is higher among randomly recruited cases.

The average iNOS level of esophageal tissues in all cases is 5.04 ± 1.62 picogram/milliliter (pg/mL). The average was 5.02 ± 1.51 pg/mL in the normal group, while it was 5.04 ± 1.68 pg/mL in the group with all reflux esophagitis cases. There was no statistically significant difference between tissue iNOS measurements of normal and all reflux esophageal group patients ($p > .05$).

A separate evaluation of the normal, non-erosive reflux and erosive reflux groups revealed that the average iNOS level was 5.02 ± 1.51 pg/mL in the normal group, 5.39 ± 1.62 pg/mL in the non-erosive reflux group, which was high, and 4.89 ± 1.70 pg/mL in the erosive reflux group, which was low. However, the differences between the tissue iNOS levels of these cases are not statistically significant ($p > .05$).

Table 3 shows the presentation of data from sub-types of reflux esophagitis and control group cases.

When the cases in the groups were examined according to the type of reflux, the iNOS level was 5.02 ± 1.51pg/mL in the

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normal group and 5.39 ± 1.62 pg/mL in the non-erosive group, the latter of which was considerably higher. Next, the cases in the erosive reflux group were evaluated among themselves. While the iNOS level was 5.02 ± 1.51 pg/mL in the normal group, it was 5.03 ± 1.64 pg/mL in erosive reflux A, and 5.10 ± 2.23 pg/mL in erosive reflux B, and 4.06 ± 0.02 pg/mL in erosive reflux at C. The iNOS level was considerably lower in erosive reflux C. However, the differences between the iNOS measures of these different groups are not statistically significant either ($p > .05$). **Figure 1** shows the graphical representation of the iNOS levels in tissues of normal cases and cases in non-erosive reflux and erosive reflux subgroups.

At the beginning of the study, it was planned that patients with LA Stage D and those with Barret esophagitis who had complications would also be included in the erosive reflux esophagitis group in this study. However, no patients of this group could be included in the study because they did not present to our center during the period the study was conducted.

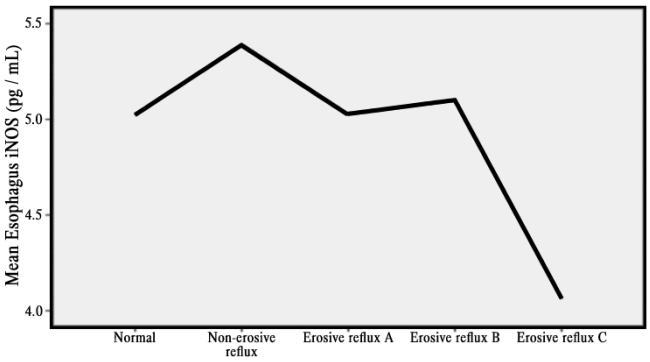

Figure 1. INOS levels in tissues of normal, non-erosive reflux and erosive reflux group cases

Table 1. Case distribution by group

Groups	Total (n = 103) n	%
Control Group	30	29.1
Non-Erosive Reflux Group	22	21.4
Erosive Reflux LA Stage A	27	26.2
Erosive Reflux LA Stage B	15	14.6
Erosive Reflux LA Stage C	9	8.7

Table 2. Demographic data of cases in the control and reflux esophagitis groups

		Reflux esophagitis (n = 73)	
	Normal (n = 30)	Non-Erosive Reflux (n = 22)	Erosive Reflux (n = 51)
Age (Years)			
Min–Max (Median)	19–51 (33.00)	19–56 (36.50)	24–61 (38.00)
Mean ± SD	33.00 ± 10.51	37.32 ± 9.88	39.15 ± 10.63
Gender, *n (%)*			
Female	18 (60.00)	12 (54.50)	9 (17.60)
Male	12 (40.00)	10 (45.50)	42 (82.40)

Table 3. Analysis of data according to reflux esophagitis sub-groups

		Reflux esophagitis (n = 73)				
	Normal (n = 30)	Non-Erosive Reflux (n = 22)	Erosive Reflux A (n = 27)	Erosive Reflux B (n = 15)	Erosive Reflux C (n = 9)	*p*
Age (Years)						
Min–Max (Median)	19–51 (33)	19–56 (36.5)	26–61 (37)	24–55 (33)	30–52 (50.5)	[a]***0.214***
Mean ± SD	33.00 ± 10.51	37.32 ± 9.88	38.33 ± 10.58	37.31 ± 10.99	44.88 ± 9.46	
Gender, *n (%)*						
Female	18 (60.0)	12 (54.5)	4 (14.8)	2 (13.3)	3 (33.3)	[b]***0.001****
Male	12 (40.0)	10 (45.5)	23 (85.2)	13 (86.7)	6 (66.7)	
Esophageal iNOS (pg/mL)						
Min–Max (Median)	3–7.9 (4.9)	2.7–10 (5.3)	3.1–9.2 (4.5)	1.8–9.1 (5.9)	4.1–4.1 (4.1)	[a]***0.197***
Mean ± SD	5.02 ± 1.51	5.39 ± 1.62	5.03 ± 1.64	5.10 ± 2.23	4.06 ± 0.02	

*[a]Kruskal Wallis Test; bPearson Chi-square Test *p < .01*

DISCUSSION

Nitric oxide is an important mediator of processes that are physiological and pathological. NO has direct and indirect effects. Direct effects occur between NO and its specific biological molecules, while indirect effects are mediated by the reactive types of nitrogen oxide (RNOS) consisting of NO reactions with oxygen or superoxide (**10**). NO is synthesized from L-arginine in the living organism. It is a soluble enzyme, and its production is catalyzed by iNOS and active in its dimeric form. It is an important biological mediator. However, it is cytotoxic if produced too much. Cytokines stimulating the immune system and bacterial pathogens activate iNOS and generate high amounts of NO by activating inducible nuclear factors (**11**). In macrophages, NO is generated by iNOS as an outcome of stimulation by microbes and cytokines. NO is required for the protection of the host and immune regulation against pathogens (**12**). INOS can be most easily detected in monocytes or macrophages of people suffering from inflammation or infections. Continuous NO generation provides macrophages with activity that inhibits cell growth or is toxic to living cells, against viruses, bacteria, fungi, protozoans, parasitic worms, and tumor cells. The high-output NO pathway is likely to have evolved to defend the host from being infected; nevertheless, it grants iNOS the dilemma of both protective and destructive immune response through its ability to suppress lymphocyte multiplication and to harm other normal cells of the host (**13**). For this reason, the production of NO through iNOS stimulation appears to lead to different and contradictory consequences for different organ and tissue systems. In Barret esophagus and esophageal adenocarcinoma, which develops on the basis of gastroesophageal reflux, inflammatory disorders are thought to have the potential cause carcinogenesis through activation of genes that are prosurvival, including cyclooxygenase-2 (COX-2) and iNOS. Yet, Heather et al. determined no significant relationship between iNOS polymorphism and Barret esophagus or reflux esophagitis (**14**). NO, generated by iNOS, has had a role to play not only in the induction of DNA damage but also in abnormal signaling of cells in a variety of previous tissue and cell studies. Inflammatory mediators like NO may have a major role in the development of esophageal cancer because esophageal adenocarcinoma is caused by gastroesophageal reflux disease in the context of Barrett's esophagus. McAdam et al. have confirmed in their study that iNOS protein levels are increased in esophageal adenocarcinoma emerges on the background of reflux esophagitis and therefore in the development of neoplasms in the esophagus (**15**). NO level in the intestinal tract increases during inflammation, possibly contributing to intestinal injuries. It is unclear, however, how the expression of two forms of messenger ribonucleic acid (mRNA) — iNOS and endothelial NO synthase mRNA — in the esophageal mucosa adds to the damage to the mucosa that reflux esophagitis causes. Inamori et al. has found that iNOS mRNA expression in the mucosa of the esophagus is intensified as a function of the gravity of esophagitis and argued that the buildup of NO induction by iNOS has something to do with reflux esophagitis exacerbation (**16**).

In our study, the patients with non-erosive reflux esophagitis were found to have higher levels of iNOS in the esophageal tissue than those in the control group but lower in comparison to those with erosive reflux C. In addition, these iNOS values did not differ statistically significantly. However, if the cases in the erosive reflux D and Barret esophagus groups could be included in the study, the results could have changed, which is a limitation of our study. Moreover, there are no specifically defined thresholds for iNOS and NO amounts in normal esophageal tissues. In our study, the iNOS levels in esophageal tissues were about 5 pg/mL, which is probably within sensitive limits. If we take the average level of 5.39 pg/mL observed in patients with non-erosive reflux in our study to be elevated, the potential high NO levels due to high iNOS levels could cause patients to have more reflux symptoms due to the dilator effect of NO on the smooth esophageal muscles. In patients with erosive reflux C, however, the iNOS levels were found to be 4.06 pg/mL, which was lower than normal. Minimal increase in expression of NOS may increase extensive production of NO. So, minimal changes in NOS may effect different metabolic processes (**17**). These results suggest the necessity of further research into the role of lower esophageal sphincter relaxation, which may occur via the NO pathway in the pathophysiology of reflux esophagitis as well as other mechanisms.

CONCLUSIONS

Low iNOS and NO levels may be impairing the blood supply to the microvascular bed in the mucosa of esophagus in these patients, contributing even more to the gastric acid-induced damage to the esophagus. Moreover, a low microvascular supply of blood to esophageal tissues may be leading to a decrease in their acid clearance. Comprehensive studies involving a more detailed and broader group of cases are needed to prove all these theories and to reveal the role of iNOS in reflux esophageal pathophysiology.

Human Rights Statement and Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. Informed consent or substitute for it was obtained from all patients for being included in the study.

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Effectiveness and safety of orally administered silymarin (milk thistle) for Pegylated Interferon unresponsive chronic Delta Hepatitis patients

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ABSTRACT

Objective: Silymarin is a natural extract from milk thistle (Silybum marianum), a natural herb that contains flavonoids. Silymarin also has anti-inflammatory properties and lipid peroxidation effects on human hepatocytes. It has also been used for the treatment of acute alpha-amanitin poisoning and chronic hepatitis C infection. Chronic Hepatitis D virus (HDV) infection is a severe health problem leading to fibrosis and hepatocellular carcinoma. Patients with chronic HDV infection can be treated with Peg-IFN with lower treatment success. Most patients with chronic HDV are unable or unwilling to use interferon (IFN)-based treatment due to liver cirrhosis. Our objective was to establish the long-term clinical outcomes with silymarin for interferon-experienced chronic HDV patients.

Materials and Methods: We studied ten patients from one centre with interferon who experienced chronic HDV, of which 8 had cirrhosis, and 2 had chronic hepatitis who received HDV treatment with silymarin 600 mg/day after a median period of 12 months. Information collected included demographic, clinical, virologic, and outcomes data. MELD and Child-Pugh (CP) scores were also obtained. Friedman test was used to evaluate the laboratory parameters during the study period.

Results: 10 chronic HDV patients (median age 54 yrs, six female, all of them previous null responders to Peg-IFN with mildly decompensated cirrhosis [CP 7 (range 6-11), MELD 11 (range 6-20] were followed for 12 months from the start of silymarin 600 mg/day. There was no decompensation of both MELD and CP scores among patients at the end of therapy. In addition, no patients stopped silymarin treatment early due to side effects. At the end of treatment, there was no significant change in prothrombin time (p= 0.949), AST (p=0.662) and AFP (p=0.983) levels and platelets counts (p=0.988) compared to the pre-treatment period (all p>0.005). Finally, HDV-RNA suppression was seen in all patients at the end of treatment (p=0.009).

Conclusions: In the light of the presented data, silymarin seems to be effective in treating chronic HDV infection. Further research is needed for validation. The study is ongoing with a collection of data on sustained viral response.

Keywords: Silymarin, Interferon therapy, Delta hepatitis

INTRODUCTION

Silymarin, an extract from the seed of the milk thistle plant (Silybum marianum [S. marianum]), is widely known for its hepatoprotective functions, mainly due to its anti-oxidative, anti-inflammatory, and immunomodulatory effects (1). The primary bioactive components of the extract consist of several flavonolignans (silybin, silychristin, silydianin, is silybin, and dehydrosilybin), and a few flavonoids, mainly taxifolin (2). The mixture of silybin A and silybin B (1:1) is also known as silibinin (C25H22O10, PubChem CID: 31553; **Figure 1**), which makes up the major active ingredient (roughly 50%) of silymarin (2,3).

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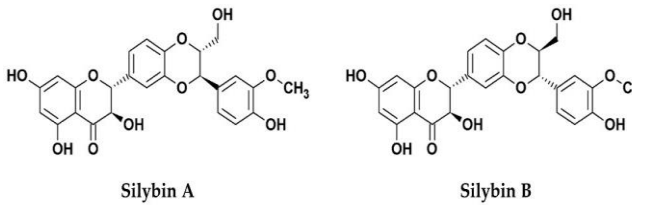

Figure 1. Chemical structures of silibinin, the 1:1 mixture of silybin A and silybin B.

Silymarin also has anti-inflammatory properties and lipid peroxidation effects on human hepatocytes. It has also been used for the treatment of acute alpha-amanitin poisoning and chronic hepatitis C infection.

Chronic Hepatitis D virus (HDV) infection is a serious health problem leading to fibrosis and hepatocellular carcinoma. Hepatitis D virus is an RNA virus that needs hepatitis B surface antigen for infection. That's why it doesn't cause infection on its own. He needs the hepatitis B virus for his infection. The main sensitive patients are chronic hepatitis B patients who become superinfected with this virus (4). Spreads parenterally like HBV in HDV (5).

HDV infection has been shown to worsen the natural course of underlying HBV infection. Hepatitis D is considered the most severe form of viral hepatitis in humans, accelerates progression to cirrhosis, and leads to earlier deterioration in liver function compared to the infection HBV alone. Approximately 5% of hepatitis B patients are infected with the hepatitis D virus (6).

Patients with chronic HDV infection can be treated with Peg-IFN therapy with lower treatment success. The majority of patients with chronic HDV are unable or unwilling to use Peg-IFN -based treatment due to liver cirrhosis. Recent studies have also documented the antiviral activities of silymarin and its derivatives against several viruses, including the flaviviruses (hepatitis C virus and dengue virus), togaviruses (Chikungunya virus and Mayaro virus), influenza virus, human immunodeficiency virus, and hepatitis B virus (7).

Silymarin acts as a free radical cleanser and modulates enzymes associated with the development of cellular damage, fibrosis, and cirrhosis.

These hepatoprotective effects have been observed in clinical trials in patients with alcoholic or non-alcoholic fatty liver disease, including patients with cirrhosis (8). At the same time, the intravenous form of Silybinin Amanita phalloides is used in fungal poisoning. Today, there is no direct effective antiviral agent for the hepatitis D virus. Interferon treatments currently used are the only treatment option for hepatitis D. However, both the serious side effects of interferons and the fact that their effectiveness around 30%, directs the researchers to new treatment modalities (9).

Although there are studies that report the positive effects of silymarin in patients with acute hepatitis, liver fat, and metabolic syndrome, there are no studies on the use of treatment in patients with unanswered hepatitis D. Our objective was to establish the long-term clinical outcomes with silymarin for interferon-experienced chronic HDV patients.

MATERIAL and METHODS

We studied ten patients from one center who experienced chronic HDV, of which 8 had cirrhosis, and 2 had chronic hepatitis who received HDV treatment with silymarin 600 mg/day after a median period of 12 months. Information collected included demographic, clinical, virologic, and outcomes data. MELD and Child-Pugh scores were also obtained. Friedman test was used to evaluate the laboratory parameters during the study period.

RESULTS

Ten chronic HDV patients (median age 54 yrs, six female, all of them previous null responders to Peg-IFN with mildly decompensated cirrhosis [Child Pugh 7 (range 6-11), MELD 11 (range 6-20) were followed for 12 months from the start of silymarin 600 mg/day. There was no decompensation of both MELD and Child-Pugh scores among patients at the end of therapy. In addition, no patients stopped silymarin treatment early due to side effects. At the end of treatment, there was no significant change in prothrombin time (p= 0.949), AST (p=0.662), and AFP (p=0.983) levels and platelets counts (p=0.988) compared to the pre-treatment period (all p>0.005). Finally, HDV-RNA suppression was seen in all patients at the end of treatment (p=0.009).

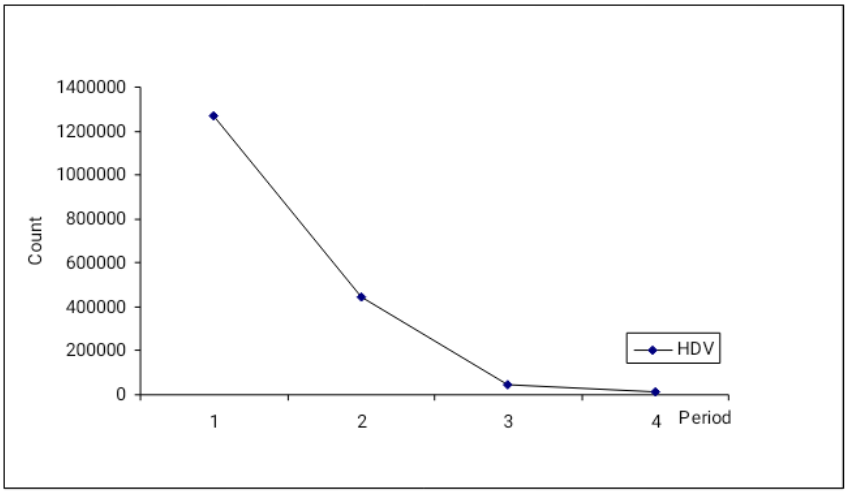

Figure 2. HDV RNA levels During 12 months of Silibinin IV Monotherapy and Subsequent Follow-up (HDV RNA Levels Measured by Abbott Real Time HDV Assay)

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Table 1. Baseline characteristics of patients

	Time periods (months)	Median	Mean	St. Dev.	Mak.	Min.	p
HDV-RNA	0	567536	1269254,40	1487630,76	58586	4446111	0,009
	4	334147	443303,50	449171,35	0	1366552	
	8	23170	48821,56	62699,59	0	172000	
	12	12990	15071,43	15403,27	0	41000	
AST	0	64,50	71,50	41,68	39	177	0,662
	4	62,50	66,70	30,76	21	132	
	8	88,00	83,22	34,73	39	149	
	12	89,00	84,86	35,86	40	138	
PLT	0	113,50	129,40	82,92	17	262	0,988
	4	107,00	130,00	79,16	19	262	
	8	90,00	119,67	79,08	19	254	
	12	92,00	120,57	84,61	19	241	
Prothrombin time	0	14,00	15,40	2,84	12	20	0,949
	4	15,00	15,80	3,46	13	23	
	8	15,00	16,11	3,22	13	22	
	12	14,00	16,29	4,03	13	22	
AFP	0	6,50	24,90	53,34	3	176	0,983
	4	5,50	25,30	62,87	2	204	
	8	6,00	34,33	83,60	2	257	
	12	7,00	34,29	73,17	2	200	

DISCUSSION

Hepatitis D virus is a defective virus that alone does not cause infection without hepatitis B virus. In patients affected by this virus, which is infected with hepatitis B virus, both the progression to cirrhosis is accelerated, and the risk of hepatocellular cancer increases.

In Western societies, it often leads to infection with hepatitis B virus in IV drug addicts and often individuals infected with Hepatitis C and HIV virus. While Delta hepatitis in the west has decreased to the degree that it almost forgets itself, it has become a major problem again in recent years with the increase in the flow of migrants to the west from the geographies where it is endemic. In this new wave, the disease is both more severe and faster to spread than before due to the fact that the patients are young populations compared to their predecessors (**4**).

There has been a search for new treatments due to the fact that there is no treatment option outside of interferon yet, and interferon is both limited in effectiveness and due to its frequent side effects. Silymarin preparation is an over-the-counter preparation that is recommended as a support treatment for various diseases. It is used in many liver and pancreatic disorders.

In a study conducted by Ferenci and his colleagues, they suggested that intravenously administered silymarin caused a decrease in viral load in hepatitis C patients who did not respond to Peg-IFN (**10**).

However, since no significant clinical effects were detected in many subsequent studies, intravenous silymarin therapy is not included in clinical practice in these patients. In a 2016 study on mice, Ni X. and his colleagues showed that silymarin causes intrahepatic lipid accumulation and positive effects on LDL, HDL, and Triglyceride lipid profiles. For this reason, silymarin is one of the promising treatments for non-alcoholic liver fat (**11**).

Song and his colleagues found that in alcoholic liver disease, silymarin had a positive effect on oxidative stress parameters and limited ALT growth (**12**). A 2017 report stated that silymarin in meta-analysis led to a minimal decrease in AST and ALT levels, but this decrease was not clinically significant (**13**).

In our study, AST decrease was observed but not statistically significant. What is interesting about our research is that it significantly reduces the level of HDV RNA. This result is promising for future treatment plans. These findings provide compelling evidence to explore the use of silymarin and derivatives in combination with existing antivirals as a potential treatment strategy, particularly for the treatment of chronic viral hepatitis.

Author Contributions: MA, EE, ETT, YD, SO, HG and ACD Data collection, Formal analysis, Methodology, Project administration, Statistical Analyses, **MA:** Article writing and revisions

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CONCLUSION

In the light of the presented data, silymarin seems to be effective in the treatment of chronic HDV infection. Further research is needed for validation. The study is ongoing with a collection of data on sustained viral response. Further research to improve the bioavailability, delivery, as well as elucidating the main mechanism of antiviral activity of silymarin and derivatives, could help to boost our understanding of these drugs and accelerate their development as hepatoprotective, antiviral agents.

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Determination of risk factors using Nonlinear Principal Component Analysis in patients with breast tumour

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ABSTRACT

Objective: Breast cancer, which is the most common among women in the world and constitutes approximately 30% of all cancers, takes places near the top among the diseases that threaten women's health. The purpose of this study is to determine the risk factors in patients with breast tumours using nonlinear principal component analysis.

Materials and Methods: During the application process, a data set of 569 (357 benign, 212 malign) patients with breast tumours was used. To find independent features, the data set was reduced to two dimensions via nonlinear principal component analysis. The results were evaluated by comparing the success of the method with the ROC curve.

Results: The cut-off values for the radius, perimeter, area, smoothness and texture of the tumour were 14.19, 656.10, 0.09, 2.87 and 0.11, respectively. The sensitivity of the current values according to the results of ROC analysis was determined as 84% for radius, 80% for perimeter, 86% for the area and 94% for texture. It is seen that the method has an overall success of over 80% in detecting malignant tumours.

Conclusion: It is hoped that this method, which is used to reveal risk factors and identify distinctive features in breast tumours, will reduce medical costs and provide a second opinion to physicians. In terms of decision making, it is predicted that the method can recognize malignant tumours and reduce the need for unnecessary biopsy for benign tumours.

Keywords: Breast Tumours, Dimensional reduction, Nonlinear principal omponent analysis, Optimal Scalling

INTRODUCTION

Breast cancer occurs when cells in the breast divide and grow without reasonable control (1). Breast cancer mostly begins with the malfunction of the milk-producing ducts (invasive ductal carcinoma), and cancer cells can spread to lymph nodes and even to other parts of the body such as the lungs (2). Breast cancer, which ranks first among diseases that threaten women's health and constitutes approximately 23% of all cancers, is the most common type of cancer in women in the world (3, 4). The annual incidence of breast cancer is approximately 1.7 million cases in the world, with ~ 231,840 cases in the US and ~ 100,000 cases in Europe. Considering the risk of breast cancer, which has a substantial morbidity and mortality rate, especially in terms of women's health/life, and the early stage, effective treatment and good prognosis, the importance of implementing early diagnosis studies becomes clear (5, 6).

Nonlinear principal component analysis (NLPCA) is a descriptive dimension reduction method that provides numerical and visual results for data sets containing continuous, categorical or discrete variables with a linear or nonlinear relationship between them (7).

The aim of this study is to determine risk factors for patients with breast tumours using nonlinear principal component analysis.

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MATERIAL and METHODS

In the study, from the free-access data site as application material (*http://mlr.cs.umass.edu/ml/machine-learningdatabase*. Access date: 04.05.2020) 11 variables data of the patient with breast tumour of 569 (357 benign, 212 malign) provided were used and the variables and their features are given in following.

The variables and their properties are as follow.

Radius: The radius of an individual nucleus is measured by averaging the length of the radial line segments defined by the centroid of the snake and the individual snake points.

Perimeter: The total distance between the snake points constitutes the nuclear perimeter.

Area: Nuclear area is measured simply by counting the number of pixels on the interior of the snake and adding one-half of the pixels in the perimeter.

Compactness: Perimeter and area are combined to give a measure of the compactness of the cell nuclei using the formula perimeter2/area.

Smoothness: The smoothness of a nuclear contour is quantified by measuring the difference between the length of a radial line and the mean length of the lines surrounding it.

Concavity: Chords are drawn between non-adjacent snake points, and how far the true boundary of the core extends within each chord is measured.

Concave Points: This feature is similar to Concavity but measures only the number, rather than the magnitude, of contour concavities.

Symmetry: The difference in length between lines perpendicular to the main axis is measured in both directions to the cell border.

Fractal Dimension: The perimeter of the nucleus is measured using increasingly larger 'rulers'. As the ruler size increases, with decreasing the precision of the measurement, the observed perimeter decreases. Plotting these to values on a log scale and measuring the downward slope gives (the negative of) an approximation to the fractal dimension.

Texture: The texture of the cell nucleus is measured by finding the variance of the gray scale intensities in the component pixels (**8**).

Methods

Breast tumour, which is common in many parts of the world, occurs in breast cells. Data of 569 patients with breast tumours, 212 malignant and 357 benign, were used for the study. This data was taken from the study conducted by Street et al. (**8**). The data set reached on 04.05.2020 was evaluated via NLPCA.

Nonlinear principal component analysis: The nonlinear principal component analysis aims to find x object scores and yj mean values in various ways under some limitations. Thus, the following function is minimized.

$$\sigma\,(X;Y) = n_w^{-1} \sum_j c^{-1}\ tr\left(\left(X - G_j Y_j\right)' M_j\, W\left(X - G_j Y_j\right)\right)$$

$$j = 1, \dots, m \quad (1)$$

Variance explanation rates for each dimension for multiple nominal variables;

$$VAF1_s = n_w^{-1} \sum_{j \epsilon J} v_j tr(Y_{js}'\, D_j\, Y_{js})$$

$$s = 1, \dots, p \quad (2)$$

For multiple non-nominal variables, it is calculated as follow;

$$VAF2_s = \sum_{j \notin J} v_j a_{js}^2$$

$$s = 1, \dots, p \quad (3)$$

Eigenvalues for each dimension are calculated by the following formula;

$$\sqrt{\lambda_s} = VAF1_s + VAF2_s$$

$$s = 1, \dots, p \quad (4)$$

And λ_s is the diagonal element of Λ. Total explained variance for multiple nominal and non-multiple nominal variables over the means of dimensions is calculated by the equation below;

$$tr\left(\sqrt{\Lambda}\right) = p^{-1} \sum_s VAF1_s + \sum_s VAF2_s$$

$$s = 1, \dots, p \quad (5)$$

This equation is known as total eigenvalues. Vector coordinates for NLPCA are calculated by the following equation;

$$VAF_{js} = v_j a_{js}^2 \quad s = 1, \dots, p \quad \text{ve}$$

$$\text{j} \notin \text{J} \quad (6)$$

If the analysis is made for non-multiple variables, there are no missing observations or if it is determined passively, the correlation matrix is $q_j = G_j y_j$R and $R = n_w^{-1}\, Q'WQ$. The first p eigenvalue of R is equal to $\sqrt{\Lambda}$. If there are multiple nominal variables in the analysis, the p correlation matrices are calculated by equation 7;

$$R_s = n_w^{-1}\, Q_s' \text{W} Q_s$$

$$s = 1, \dots, p \quad (7)$$

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It is calculated by equation 7. In Equation 7, q_{js} is calculated as $G_j y_j$ for multiple non-nominal variables and as

$$\frac{G_j Y_{js}}{\sqrt{Y_{js}' D_j Y_{js}}}$$

for multiple nominal variables (**9**).

The 1st eigenvalue of the R_s matrix is generally higher and is equal to $\sqrt{\lambda_s}$. Lower values of $\sqrt{\Lambda}$ usually belong to the 2nd and later eigenvalues of Rs. In calculating eigenvalues; if variable j is the complementary variable for the singular value decomposition of the R matrix, first the first column from the R matrix and j-th the row is removed, then R_{ij} is multiplied by $\sqrt{v_i v_j}$ (**10**).

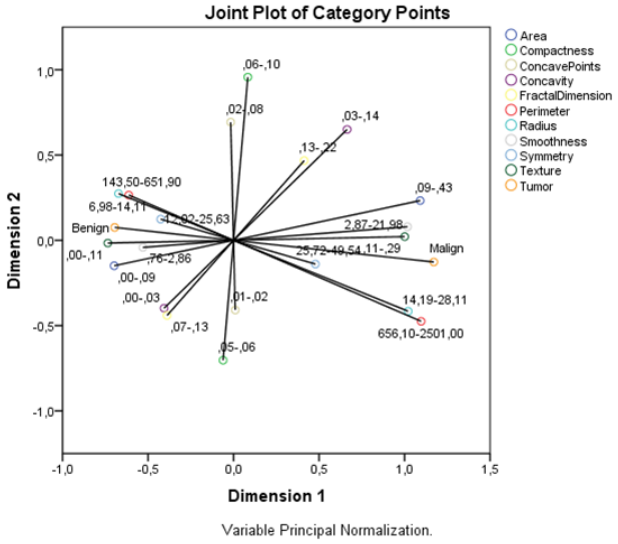
Receiver Operating Characteristics Curve Analysis: One of the common methods used to distinguish patients and healthy individuals by finding a cut-off point to determine the performance of continuous variables as a diagnostic test is the ROC (Receiver Operating Characteristics) curve. The ROC curve is the curve obtained by taking the measured values of the continuous variable (respectively) as the cut-off point, plotting the Sensitivity values on the Y-axis, and 1-Specificity values on the X-axis. The total area remaining under the curve is "1". If the area under the curve is 0.50 then the feature has no discriminating power, "1" indicates that it is 100%. The ROC curve summarizes the accuracy of the test with a single numerical value.

RESULTS

As a result of the applied principal components analysis, the results of the first two main components are given in **Table 1**. As seen in **Table 1**; 44.095% of the total variance was explained with the first main component, 15.686% with the second main component. Thus, eleven original variables have been reduced to two (basic) components that explain 59,781% of the total variance. When the principal component loads that express the correlations between original variables and principal components are examined; a high correlation between radius, perimeter, area, smoothness, texture, and tumour variables and the first major component; A moderate correlation was found with concave, symmetry, and fractal dimension. The contribution of compactness and concave point variables to the first fundamental component is almost negligible. Compactness is the variable that contributes the most to the second principal component. When the correlations between the variables analyzed with NLPCA are examined, there is a high correlation between the tumour and the variables of radius, perimeter, area, smoothness and texture; There is a moderate correlation between the tumour and the variables of concave, symmetry, and fractal size. No relationship was found between tumour and the variables of compactness and concave points. Parallel to the increase in the contribution of the categories to the dimensions and the increasing power of separation, the coefficient values of the dimensions also increase. In other words, moving away from the origin of the values of any category in the dimensions indicates that the effect of the said category in determining the size is higher. Accordingly, the "malign" category of the tumour variable with a value of 1.171 in the first dimension, the "0.06-0.10" category of the compactness variable with a value of 0.957 in the second dimension received the highest positive value, while in the first dimension with -0.735 the texture variable "0.00-0.11" category, “0.05-0.06” category of compactness variable has the highest negative value with -0.703 value.

In Figure 1, it is seen that the "malignant category" of tumour variable is highly associated with the "14.19 - 28.11" category of radius variable, "656.10-2501.00" category of perimeter variable, "0.09-0.43" category of area variable, "2.87-21.98" category of smoothness variable, and "0.11-0.29” category of texture variable, and it is moderately positively associated with the “0.03-0.14” category of concavity variable, “25.72-49.54” category the symmetry variable, “0.13-0.22” category of fractal dimension variable. Similarly, it was determined that the benign category of the tumour variable and the “6,98-14,11” category of the Radius variable, the “143.50-651.90” category of the perimeter variable, the “0.00-0.09” category of the area variable, the “0.76-2.86” category of the smoothness variable, and the “0.00-0.11” category of the texture variable were positively correlated.

Figure 1. Joint Plot of Category Points



It can be said that the categories that are close to the origin have low effects and they have no relation with other categories. In this context, it was observed that the effect of the concave points variable is very low and not related to other variables (**Figure 1**).

According to the ROC analysis results in the study; the area under the curve was found as 0.938 ± 0.010 for radius, perimeter and area, 0.876 ± 0.015 for smoothness, and 0.967 ± 0.007 for texture. Cut-off values for radius, perimeter, area, smoothness and texture are respectively seen as; 14.1950 (Sensitivity 84.4%, Specificity 87.1%), 656.25 (Sensitivity 80.7%, Specificity 91%), 0.0905 (Sensitivity 86.3%, Specificity 89.4%), 2.8780 (Sensitivity 71.2%, Specificity 88.5%), 0.1102 (Sensitivity 94.3%, Specificity 85.2%) (Table 2).

Table 1. Analysis results for the first two principal components

	Total (Vector Coordinates) Dimension		
	1	2	Total
Tumour	0,814	0,010	0,824
Radius	0,687	0,114	0,801
Perimeter	0,672	0,126	0,798
Area	0,762	0,035	0,797
Compactness	0,005	0,673	0,678
Smoothness	0,539	0,003	0,542
Concavity	0,270	0,259	0,529
ConcavePoints	0,000	0,283	0,283
Symmetry	0,204	0,017	0,221
FractalDimension	0,161	0,205	0,366
Texture	0,736	0,000	0,736
Active Total	4,850	1,725	6,576
% of Variance	44,095	15,686	59,781

Table 2. ROC analysis summary

	Group	Cut-Off Value	Area Under The Curve	St. Error	Sensitivity	Specificity	*P*
Radius	Malign-Benign	14.1950	0.938	0,010	0.844	0.871	0,001
Perimeter	Malign-Benign	656.25	0.938	0,010	0.807	0.910	0,001
Area	Malign-Benign	0.0905	0.938	0,010	0.863	0.894	0,001
Smoothness	Malign-Benign	2.8780	0.876	0.015	0.712	0.885	0,001
Texture	Malign-Benign	0.1102	0.967	0.007	0.943	0.852	0,001

DISCUSSION

Breast cancer is a very common cancer; It has the second-highest incidence rate worldwide among all types of cancer and is ranked as the fifth leading cause of cancer-related death (**11**). Various risk factors have been identified for breast cancer. Sun et al. listed these risk factors as an agent, family history, reproductive factors, estrogen, and lifestyle, respectively. In their studies where they emphasized the importance of preventing breast cancer, they stated that current prevention methods such as screening, chemoprevention and biological prevention are more accurate and more effective than previous methods (**12**). Stating that the risk of breast cancer is lower in breastfeeding women than other women, Turkoz et al. pointed out that obesity and overweight may also be considered as risk factors at later ages (**13**). Early diagnosis is the cornerstone of preventing mortality in breast cancer (**12**). Therefore, the importance of imaging for the detection and diagnosis of breast cancer cannot be denied. The chance of treating cancer depends primarily on early diagnosis, and treatment choice depends on the level of malignancy. For this reason, it is very important to detect cancer, to separate cancerous from benign and healthy ones, and determine the level of malignancy. The geometric organization of cells in tissue can affect proliferation, propagation, branching, stem cell properties and cancer cell survival and invasion (**14**). Traditionally, pathologists use histopathological images of biopsy samples taken from patients, examine them under a microscope, and make decisions based on personal experience. However, these decisions are subjective and often lead to variability (**15**).

Grove et al. in their study, in which they stated that tumour shape and intratumoral density variation reflect tumour biology and may affect patient survival, they show that quantitative imaging biomarkers can be used as an additional diagnostic tool in the treatment of lung adenocarcinomas (**16**). In a study, it was stated that the variation in the size and shape of the tumour can be used as an indicator of the presence of cancer (**17**). Using the Bayesian network inference approach, Hussain et al. found significant associations between morphological features extracted from prostate cancer images (**18**).

Computer-aided diagnostic systems based on tissue and morphological analysis have proven to be extremely sensitive in evaluating breast tumours. Zhou et al. were able to distinguish benign breast tumours with high accuracy and short training time in their study (**19**).

In a standard principal component analysis, the aim is to find fewer new variables consisting of combinations of these variables that can explain the total variance of the original variables as much as possible (**20**). The size reduction feature of principal components analysis has been used in this article. The risk factors affecting the malignancy of the tumour were determined by reducing the data set into two dimensions. Accordingly, the radius, perimeter, area, softness and tissue of the tumour were found to be significantly effective on malignancy. In many studies, it has been determined that the radius, perimeter and area of the tumour are effective. However, in this study, it has been shown that the above-mentioned characteristics with high effects on the malignancy of the tumour increase the malignancy after which values.

It can be said that the tumour can now be a malignant tumour when the radius of the tumour is 14.19, the perimeter is 656.10, the area is 0.09, the smoothness is 2.87 and the texture is above 0.11. The sensitivity of the current values according to the results of ROC analysis was determined as 84% for radius, 80% for perimeter, 86% for the area and 94% for texture. It is seen that the method has an overall success of over 80% in detecting malignant tumours.

In particular, radius, perimeter, area and texture variables can be shown as important risk factors, but it can be said that the variables of compactness, concavity, concave points, symmetry and fractal dimension have a low effect on malignancy signs.

CONCLUSIONS

The results show that the use of morphological features is effective and safe. Determining the morphological features and risk factors in breast tumours can be seen as an advantage. In terms of decision making, it is predicted that the method can recognize malignant tumours and reduce the need for unnecessary biopsy for benign tumours. It has been suggested that breast tumours start early in life and are shaped by the number of cells at risk, the integrity of these cells and the environment they are exposed to (21). Therefore, it is hoped that this method, which is used to reveal risk factors and distinguish features in breast tumours, will reduce medical costs and provide a second opinion to physicians.

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Cognitive rehabilitation effectiveness for severe to moderate traumatic brain injury: Case Series

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ABSTRACT

Objective: This case series study aimed to investigate the effectiveness of a holistic approach of a computer-assisted and traditional neuropsychological rehabilitation program in improving some cognitive functions in patients who sustained a traumatic brain injury (TBI).

Methods: The case series study followed a single-case design, with an A-B-A-B design and was conducted in the rehabilitation center at King Fahad Medical City-Saudi Arabia between Aug 2015 and March 2016. Participants comprised 5 males with moderate-to-severe TBI and persistent cognitive impairments. The computerized model included known software programs for cognitive rehabilitation to improve this rehabilitation process. The program period was six weeks for each case, all focusing on executive functions, memory, and attention.

Results: three out of the five cases improved remarkably in their attentional, executive, and related memory functions; with one showing moderate improvement and the five-case showing little improvement.

Conclusion: The holistic approach of the neuropsychological rehabilitation program is effective for some TBI cases in improving their cognitive and psychosocial functioning, alongside vocational outcomes, as reported in the follow-up interviews of the patients and their families. More research is required to contribute to the current literature and for the study's findings to be further analyzed for these interventions.

Keywords: Cognitive Rehabilitation, Traumatic Brain Injury, Case Series, neuropsychological.

INTRODUCTION

For many, traumatic brain injury (TBI) exceeds other diseases as a major cause of death and disability, with an estimated 10 million individuals affected annually by it. Saudi Arabia is no exception, especially with the Kingdom's rapid urbanization and huge development in construction, transportation, communication, and changing lifestyles (1). This has led to different types of health problems and huge losses, among which TBI is eminent on the list; yet, it remains largely unmeasured due to being considered the main cause of death and disability in Saudi Arabia. One of the several treatment modalities used to improve cognitive functions in patients with TBI is neuropsychological or cognitive rehabilitation. Therefore, it would be extremely valuable and helpful to examine this aspect in Saudi Arabia. The relationship between clinical severity measures and various types of outcome measures, such as neuropsychological, functional disability, and levels of handicap, have been well established (1, 2).

Researchers have analyzed cognitive rehabilitation to enhance the recovery of brain-injury survivors. They developed a range of therapies for patients with non-traumatic brain injuries, such as a stroke, that causes language (aphasia) or visuospatial skill impairments. Cognitive remediation therapy (CRT) is explained based on the preferred result of the treatment, such as improved memory or attention tasks or by the method or provider giving the treatment. CRT is like occupational therapy, speech-language pathology, and physical treatment. All these treatments are used to reduce or compensate for an underlying cognitive disorder (3).

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TBI severity is generally graded from mild to moderate or severe. There are multiple ways to classify severity, and each measure has a different predictive utility, including determining injury, death, or long-term functional outcomes. The degree of severity is often based on the acute effects of the injury, such as an individual's stimulation level or duration of amnesia (**3-6**).

The current study aimed to evaluate the effectiveness of a mixed holistic neuropsychological program which was computer-assisted and combined with the traditional cognitive training program to (i) improve attentional function in cases of TBI and (ii) examine whether this improvement, if any, would be generalized to related executive functions, as measured by neuropsychological tests.

MATERIAL and METHODS

Research Design

The present study is case series study performance at rehabilitation center at King Fahad Medical City-Saudi Arabia, between Aug 2015 and March 2016, that used single-case design (SCD).

Sample Population

Five participants were selected from the rehabilitation center in KFMC in Riyadh, Saudi Arabia, with severe to moderate TBIs, and cognitive disabilities for 3-12 months post-injury.

Brief Background on the 5 Cases

Case (1): SA is a 16-year-old male who showed paroxysmal asthma until 4 months prior to admission due to a road traffic accident (RTA) in June 2015. The accident caused severe TBI, and the initial Glasgow Coma Scale score (GCS) admission was August 15.

Case (2): AA is an 18-year-old male student, he was involved in an RTA, causing a fractured skull, intracranial hemorrhage, and clavicle fracture, where he was admitted on December 10, 2015.

Case (3): MH is a 22-year-old male, he experienced an RTA in October 2015, which resulted in a TBI with subarachnoid hemorrhage, left clavicular fracture, and a left acetabular fracture. MH was admitted to the rehabilitation hospital on March 6, 2016.

Case (4): BA is a 24-year-old male patient who experienced an RTA on November 23, 2015. With the GCS score of 15/15, the patient was eventually intubated in the ICU.

Case (5): AS a 22-years-old male patient, who have fractured skull, intracranial hemorrhage, and clavicle fracture due to a road traffic accident (RTA) in Aug. 2015, he stayed in the ICU for 10 weeks, then he transferred to KFMC rehabilitation hospital, where he was admitted on Nov. 2015.

Research Procedures

The consecutive duration of treatment was 6-weeks. Subjects were evaluated thrice (during the course of treatment) i.e. prior to the start (baseline), after 3-weeks (midway), and until the end of the 6th week.

Materials and Instruments

Cognitive Training Tools: The program was tailored to each participant's cognitive status while maintaining a systematic approach to attention and executive functions by following the treatment manual. Cognitive training was conducted through 3 therapeutic sessions per week at the rehabilitation center. Each session lasted approximately 120 minutes.

The concentration Attention and Mental Speed Rehabilitation Task (CAMSART) program combines structured tasks with performance measurement. (**7, 8**).

RESULTS

Case Study (1): SA. The Therapeutic Procedure and Results

SA showed significant impairment in selective and sustained attention, immediate and delayed verbal memory, and executive functions during week 1 of the baseline assessment (**Table 1**). In the same week, we started the Cognitive Rehabilitation Program as a multicomponent cognitive training program, targeted largely at attention-related executive functions training including worksheet exercises and CAMSART.

Case Study (2): AA. The Therapeutic Procedure and Results

Compared to the baseline, AA did not achieve any notable improvement after week 3 and week 6 (**Table 2**), in any of the computerized training tasks. He demonstrated little improvement in his cognitive flexibility, attention control, and orientation to place. It was obvious that he had difficulties with attention, concentration, poor motivation, and delaying the acquisition.

Case Study (3): MH. The Therapeutic Results and Discussion

MH received intensive therapy by the rehabilitation team, including physical, occupational, speech, and psychological therapies. When cognitive rehabilitation started, MH was still suffering from attention and concentration difficulties, alongside forgetfulness. He forgets the date and time and suffers from low confidence (**Table 3**). During week 1 of CRT, his concentration was raised to the level of awareness, and his weak points now included poor attention and his ability to perform the required tasks and remember instructions.

Case Study (4): BA. The Therapeutic Procedure and Discussion

In week 1, BA was not able to remember the time, date, or days due to the difficulty in maintaining recording words for more than a minute. We started training with a computer and paper-based cognitive rehabilitation program to improve his attention, concentration, and orientation to time, date, and days (**Table 4**). However, he did not show a sufficient degree of cooperation for his cognitive functions to improve due to quick dispersion, monotony, and much laughter. There was minor improvement in attention, concentration, and orientation to time and memory.

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Case Study (5): A.S the Therapeutic Procedure and Discussion

The results of pre- and post-treatment scores on computerized training tasks for case 5 are shown in **Table 5** as raw scores. Compared to the baseline, As did not achieve any notable improvement after week 3 and week 6, in any of the computerized training tasks.

Although we showed a little improvement in his cognitive flexibility and attention control. In addition to, he required more time to achieve daily tasks. It was obvious that he had difficulties with attention, concentration, and delaying the acquisition. The later assessment showed clear impairment in his executive functioning, which also might have affected his cognitive flexibility and level of motivation.

Table 1: Changes in Performance during the Training Program for Case 1

Trained TBI Patients							
CAMSART Training	**Days/ Sessions**	**Weeks**					
		1	**2**	**3**	**4**	**5**	**6**
Task (1): A symbols Response	**Day (1)**	25	+28	+35	+39	+40	+41
	Day (2)	-24	+28	+38	+39	+40	+41
	Day (3)	+26	+30	+38	+41	-40	+43
Task (2): A word Response	**Day (1)**	25	-15	+30	+30	+33	+38
	Day (2)	+26	-22	+32	+32	+35	+38
	Day (3)	+29	-26	+35	+38	-37	+39
Task (3): Color and word combination	**Day (1)**	23	+35	+36	+36	+39	+41
	Day (2)	+24	+38	-33	+35	+39	+40
	Day (3)	+27	+29	+38	+39	+40	+40
Task (4): Same word ignoring words' color	**Day (1)**	23	+25	+30	+30	+34	+40
	Day (2)	+25	+28	+29	+29	+35	+39
	Day (3)	+29	-26	+33	+33	+37	+41
Task (5): Color description	**Day (1)**	7	+17	+29	+30	+36	+36
	Day (2)	+17	+20	+28	+29	+36	+38
	Day (3)	+18	+22	+29	+33	+34	+39
Task (6): The color in the word	**Day (1)**	27	+32	+33	+35	+38	+38
	Day (2)	+32	+33	+35	-33	+34	+40
	Day (3)	+35	+36	-35	+35	+35	+39

Table 2: Changes in Performance during the Training Program for Case 2

Trained TBI Patients							
CAMSART Training	**Days/ Sessions**	**Weeks**					
		1	2	3	4	5	6
Task 1: A symbols Response	**Day (1)**	22	+23	+24	-23	+28	+29
	Day (2)	+23	+25	+25	+26	+29	+29
	Day (3)	+25	-24	+26	+28	+28	-27
Task 2: A word Response	**Day (1)**	15	+23	+23	+24	+29	+30
	Day (2)	+21	+24	+25	+26	+27	+33
	Day (3)	+23	+26	+28	+29	+31	-30
Task 3: Color and word combination	**Day (1)**	23	+30	+31	-30	+33	+34
	Day (2)	+25	+28	+32	-30	+30	+31
	Day (3)	+28	+30	+30	-29	+32	+35
Task 4: Same word; ignoring words' color	**Day (1)**	12	+22	+30	+32	+33	+39
	Day (2)	+17	+20	+28	+30	-29	+35
	Day (3)	+22	+23	+27	+30	+31	+38

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Table 3: Changes in Performance during the Training Program for Case 3

Trained TBI Patients							
CAMSART Training	**Days/ Sessions**	**Weeks**					
		1	**2**	**3**	**4**	**5**	**6**
Task 1: A symbols Response	**Day (1)**	28	+ 36	-35	+36	+39	+42
	Day (2)	+29	+30	+30	+32	+36	+38
	Day (3)	+33	+32	+36	-32	+38	+40
Task 2: A word Response	**Day (1)**	37	+41	+39	+39	+39	+40
	Day (2)	+38	+41	-39	+40	+41	+41
	Day (3)	+39	+40	+40	-38	+39	+40
Task 3: Color and word combination	**Day (1)**	34	+40	+40	-38	+40	+40
	Day (2)	+35	+45	+42	+40	+40	+42
	Day (3)	+37	+47	+45	+42	-39	+43
Task 4: Same word; ignoring words' color	**Day (1)**	30	+34	+40	-38	+39	+40
	Day (2)	30	+35	-39	-30	-38	+41
	Day (3)	+33	+33	+39	+41	-40	+44
Task 5: Color description	**Day (1)**	26	+25	+30	+33	+37	+39
	Day (2)	26	+28	+30	+31	+32	+35
	Day (3)	+29	+33	+33	-30	+33	+40
Task 6: The Color in the word	**Day (1)**	18	+25	+33	+36	+36	+39
	Day (2)	+25	+30	+35	+35	+37	+40
	Day (3)	+24	+32	+37	+37	+37	+38

Table 4: Changes in Performance during the Training Program for Case 4

Trained TBI Patients							
CAMSART Training	**Days/ Sessions**	**Weeks**					
		1	**2**	**3**	**4**	**5**	**6**
Task 1: A symbols Response	**Day (1)**	8	+9	+12	+18	+21	+23
	Day (2)	-6	-8	+10	+11	+20	+21
	Day (3)	-5	8	+10	+12	+22	+24
Task 2: A word Response	**Day (1)**	8	-7	+9	+10	+18	+20
	Day (2)	+9	9	+9	+10	+15	+22
	Day (3)	-7	-5	+7	+9	+13	+19
Task 3: Color and word combination	**Day (1)**	9	+10	+12	+14	+20	+23
	Day (2)	9	9	+10	+10	+14	+18
	Day (3)	-8	+11	-9	+13	+17	+20
Task 4: Same word; ignoring words' color	**Day (1)**	4	4	+7	+7	+11	+15
	Day (2)	+6	6	+6	+9	+13	+18
	Day (3)	-5	-4	+7	+8	+12	+19

Table 5: Changes in Performance during the Training Program for Case 5

Trained TBI Patients							
CAMSART Training	**Days/ Sessions**	**Weeks**					
		1	**2**	**3**	**4**	**5**	**6**
Task 1: A symbols Response	**Day (1)**	21	+22	+24	-25	+28	+27
	Day (2)	+21	+23	+25	+28	+28	+28
	Day (3)	+24	-23	+25	+29	+28	-25
Task 2: A word Response	**Day (1)**	15	+22	+22	+23	+29	+29
	Day (2)	+20	+24	+25	+25	+26	+32
	Day (3)	+22	+26	+27	+29	+31	-29
Task 3: Color and word combination	**Day (1)**	24	+30	+30	-31	+32	+34
	Day (2)	+26	+29	+31	-31	+30	+30
	Day (3)	+27	+30	+31	-28	+31	+34
Task 4: Same word; ignoring words' color	**Day (1)**	15	+20	+30	+31	+32	+37
	Day (2)	+19	+22	+29	+32	-29	+36
	Day (3)	+23	+24	+29	+31	+32	+39

DISCUSSION

The main objective of this study was to explore the effectiveness of a combined CACR and paper and pencil cognitive rehabilitation program, in improving the impairment of cognitive functions following TBI. The results of this study partially support the objective that individuals with TBI may improve functional and impairment level measures following direct attention training. Specifically, the findings demonstrated that the two cases with moderate TBI, (Cases 1 and 3) showed remarkable improvement in their attentional, executive, and verbal memory functions, which is supported by other studies (**9, 10**). This is shown in both the scored computerized training tasks and the objective neuropsychological measures of these functions. The holistic cognitive rehabilitation program, which implements the combined approach of attention exercises, executive functions, and memory training strategy, can be effective in remediating attention deficits and executive dysfunctions following TBI. For both SA and MH cases, areas of weakness were determined at the baseline through formal testing and their report of deficits. Thus, the SAC, the SDMT, and the CTT part (A) identified attention as the main problems of these two cases. In addition, the CTT, the VD and DF, and verbal memory indicated that these two patients were functioning at a deficient level at the first baseline assessment. For the other three cases (cases 2, 4 and 5), the recorded improvement in both the computerized training tasks and the neuropsychological measure was not as expected. Case 2 and 5 is described as moderate and limited only to some tests, while case 4 is described as slow and small. Two main reasons could explain this result. The first was related to the duration of stay in the ICU following a TBI for each patient. Compared to Case 1 and 3, who showed better improvements, Cases 2, 4 and 5 had a relatively longer stay in the ICU.

Thus, Case 2 and 5 spent nearly 3 months, Case 4 stayed for more than 6 weeks, while the other two cases needed less than 3 weeks in the ICU. This interpretation is consistent with many other studies, which reported that patients with severe TBI and a mass lesion on admission in their head CT, were found to have a prolonged ICU stay independent of the indicators of injury severity and intracranial pressure course. These studies also reported that the length of stay is a predictor of severity and diffuseness of the injury alongside being a strong predictor of the outcome (**11, 12**). Thus, the length of stay could be considered as an indicator of the diffuseness of the injury, which has consequently caused the slow progress of Cases 2, 4 and 5 in this study.

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