Volume 3 Issue 2, April 2021
ISSN: 2687-4245

Turkish Journal of Internal Medicine

Turkish Journal of Internal Medicine

http://www.tjim.org

e-ISSN: 2687-4245

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Turkish journal of Internal Medicine

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Turkish Journal of Internal Medicine

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ISSN:2687-4245
Review

COVID-19 and Its Implications for Thrombosis

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Turk J Int Med 2021;3(2):37-39
DOI: 10.46310/tjim.851367

Keywords: *SARS-CoV-2; COVID-19; thrombosis*

COVID-19 is a systemic infection with a significant impact on the hematopoietic system and hemostasis. Reported findings indicate that immunosuppression, endothelial activation, and direct viral-mediated tissue damage rather than hyperinflammation-related injury mediates COVID-19 induced organ dysfunction. If direct infection drives injury, the vascular tissue is expected to be quite susceptible as it highly expresses angiotensin converting enzyme-2 (ACE-2), which is essential for coronavirus uptake. Viral injury, disordered cytokine release, and damage-associated molecular patterns (DAMPs) induce localized microvascular inflammation, which triggers endothelial activation, leading to vasodilation and pro-thrombotic conditions.[1-3] It has been shown that lymphocytes express the ACE-2 receptor on their surfaces thus, SARS-CoV-2 may directly infect those cells and ultimately lead to their lysis. Furthermore, the cytokine storm is characterized by markedly increased levels of interleukins and TNF –alpha, which may promote lymphocyte apoptosis.[2] Apoptosis mediates lymphocyte depletion and inhibitory effects of lactic acid on lymphocyte proliferation.[3]

Coagulation disorders are relatively frequently encountered among COVID-19 patients, especially among those with severe disease. The venous thromboembolism (VTE) risk in hospitalized COVID-19 patients is an emerging issue. The rate of symptomatic VTE in acutely ill hospitalized medical patients gets as high as 10%.[4] Thrombotic complications were first reported from intensive care units (ICU) in China and the Netherlands in up to 30% of patients. There is also emerging evidence of thrombosis in intravenous catheters and extracorporeal circuits, and arterial vascular occlusive events, including acute myocardial infarction, acute limb ischemia, and stroke, in severely affected people in studies from the USA, Italy, and France.[3]

COVID-19 associated coagulopathy is marked by elevated D-dimer and fibrinogen levels, with minor abnormalities in prothrombin time, activated partial thromboplastin time, and platelet counts in the initial stage of infection.[3,5] In a multicenter retrospective study during the

Received: December 31, 2020; Accepted: January 15, 2021; Published Online: April 29, 2021

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first two months of the epidemic in China, 260 of 560 patients (46.4%) with laboratory-confirmed COVID-19 infection had elevated D-dimer (<0.5 mg/L), whereas, the elevation was more pronounced among severe cases (59.6% vs. 43.2% for mild ones).[4] In COVID-19, the typical findings include high fibrinogen and high Factor VIII activity, suggesting that significant consumption of coagulation factors is not occurring. In contrast, acute decompensated disseminated intravascular coagulation is associated with low fibrinogen due to consumption of clotting factors.[6]

Although older age and comorbidity such as cardiovascular disease confer a higher risk for severe disease, young and otherwise healthy patients are also at risk for complications.[7] Prolonged immobilization during illness, dehydration, acute inflammatory state, presence of other cardiovascular risk factors, previous history of VTE, and classical hereditary thrombophilia, such as heterozygous Factor V Leiden mutation are common comorbidities in hospitalized COVID-19 patients, which potentially increase VTE risk.[4]

Tang et al.[7] assessed 183 patients with COVID-19, 21 (11.5%) of whom died. Among the notable differences between patients who died and those who survived were increased levels of D-dimer and fibrin degradation products (~3.5 and ~1.9 fold, respectively) and prothrombin time prolongation (by 14%). A recent study from China reported that 40% of hospitalized patients with COVID-19 were at high risk of VTE.[7]

In sepsis, thrombocytopenia is usually more profound, and D-dimer concentrations do not reach the high values seen in patients with COVID-19.[6] In critically ill patients, the incidence of thromboembolic complications in patients with COVID-19 is 35-45%.[8-13]

An autopsy study revealed deep venous thrombosis in 7 of 12 patients (58%) in whom VTE was not suspected before death; pulmonary embolism was the direct cause of death in 4 patients.[14] Autopsy studies of patients who died due to COVID-19 have shown high rates of microvascular and macrovascular thromboses, especially in the pulmonary circulation. A post-mortem series of seven patients from Germany showed that alveolar-capillary microthrombi were nine-fold common in people who died of COVID-19 than in those who died of influenza.[3]

There are variations in prophylaxis regimens, and these variations thromboprophylaxis regimens and screening schedules may help explain this variation in event rates across published studies. When we look at the studies regarding the dose and duration of heparin administration, we see the following: COVID-19 infected patients, whether hospitalized or ambulators, are at high risk for VTE an early and prolonged pharmacological thromboprophylaxis with low molecular weight heparin ((LMWH) is highly recommended. Although no data specific to COVID-19 exist, it is reasonable to employ individualized risk stratification for thrombotic and hemorrhagic complications, followed by consideration of extended prophylaxis (for up to 45 days) for patients with an elevated risk of VTE. Recently published interim consensus-based guidelines for the prevention and management of thrombotic disease in patients with COVID-19 recommended routine risk assessment for VTE for all hospitalized patients with COVID-19. Standard dose pharmacological prophylaxis should be considered in the absence of absolute contraindications in such patients. Empiric use of higher than routine prophylactic dose or therapeutic dose anticoagulation in patients admitted to the ICU in the absence of proven thromboses has also been implemented in some institutions. This is an area of ongoing intense discussions among experts, particularly for those patients who exhibit marked COVID-19 associated coagulopathy.[3,11,15-17] There is currently not sufficient evidence to recommended such a strategy.

The World Health Organization interim guidance statement recommends prophylactic daily LMWHs or twice-daily subcutaneous unfractionated heparin (UFH).[7] Parenteral anticoagulants (such as LMWH or UFH) are preferred to oral anticoagulants in the inpatient setting, given their short half-life and the presence of ready availability of reversal agents, due to the possibility of drug-drug interactions when they are taken with antiviral treatments (such as ritonavir) and antibiotics (such as azithromycin).[3] However, the existing evidence, including studies on thrombotic complications, is very limited and derived primarily from small and retrospective analysis.[18,19] The pathogenesis of hypercoagulability in COVID-19 is incomplete.

We believe that more and more quality data are needed to learn the relationship between COVID-19 and thrombosis.

Conflict of Interest

All authors declare that they have no confict of interest.

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TURKISH JOURNAL OF INTERNAL MEDICINE
ISSN:2687-4245
Original Article

Investigation of the Relationship Between Altruism Levels of Terminal Patients' Relatives and their Ego Status Based on Transactional Analysis

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Abstract

Background This study was conducted to examine the relationship between altruism levels of terminal patients' relatives and their ego states based on transactional analysis.

Material and Methods This research, which is planned as descriptive-correlational type, was carried out between March and May 2020 in a hospital located east of Turkey. The target population of the study consisted of individuals with terminal stage patients in a hospital located east of Turkey. The sample of the study consisted of individuals who met the research criteria and agreed to participate in the study.

Results According to the findings obtained from the study, the Critical Parent (CP) score mean of the individuals was 0.17±0.49, the Nurturing Parent (NP) score mean was 0.22±0.03, the Adult (A) ego score mean was 0.21±0.19, the Adapted Child (AC) score mean was 0.19±0.02, Natural Child (NC) score mean was found to be 0.19±0.03. Altruism Scale Total score mean was found to be 67.53±9.06, the Family Sub-Dimension was 17.42±2.49, the Social Sub-Dimension was 14.94±4.56, the Helpful Sub-Dimension was 17.50±3.10, and the Responsibility Sub-dimension was 17.65±2.75.

Conclusions It has been determined that individuals have a high level of altruism and get the highest ego score from the Nurturing Parent ego state. The lowest mean ego score was found to be Critical Parent Ego Condition (CP). It is recommended to carry out studies to reduce the critical ego state and to conduct the study in larger groups.

*Turk J Int Med 2021;3(2):*40-48
DOI: 10.46310/tjim.800460

Keywords: *altruism, ego states, terminal period*

Received: September 26, 2020; Accepted: November 25, 2020; Published Online: April 29, 2021

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Introduction

Although the importance of the family as social support has been proven, families that care for patients also need support and empowerment. The long duration of this disease affecting the family, life-threatening, loss of the usual daily life order, being unable to return to work and social life can lead to self-sadness, mourning of their own losses, feelings of anxiety helplessness hopelessness and depressive for the patient may cause depressive affect in the relatives of the patient, and grief reactions may be observed in family members due to these losses. Being in a caregiver position places roles and responsibilities on the patient's relatives other than they are used to, and increases emotional burden.[1,2] The nurses explained that family relatives want to control everything because of their distrust of the healthcare worker, do not want to leave the patient at all, the patient relatives do not believe in the treatment, reflect their anger on the healthcare worker, do not pay attention to the general care of the patient, and the relatives of the patient are afraid of being alone with the patient.[2]

Since altruism serves personal well-being, it is possible to say that altruism constitutes its own reward. Even if it has rewards such as feeling good for altruistic people, the main thing in altruism should be the well-being of the other person.[3] It can be said that a kind of gift is offered to other individuals with altruistic behavior. While other individuals experience positive emotions with this gift, this situation becomes a reason for happiness for the individual who acts altruistically.[4] Even if it seems to be done for other people, behaviors involving assistance are essentially shown with the intention of strengthening the ego. The ego feeds these helping behaviors and helping behaviors feed the ego.[5] Transactional Analysis is defined as an approach that tries to explain the changing relationship and communication between individuals, the individual's difference from another individual and how he behaves when communicating with other individuals.[6,7]

Basic elements of Transactional Analysis approach; Ego States, Transaction, Contact Messages, Psychological Games and Life Positions.[6,7] Berne defines the ego state, which is one of the elements that make up the Transactional Analysis approach, as a consistent pattern of emotions and thoughts associated with a behavioral model.[7] Berne described each of the subjective experiences as an ego state, beginning with birth and prenatal, and stated that they all constitute personality.[6] Ego states are examined in two different models, structural and functional. According to the structural analysis, there are three ego states in the personality of each individual, namely Parent, Child and Adult. Structural analysis is concerned with what is inside each of the Parent, Adult, and Child ego states.[6,8]

The relationship between altruism and the ego states based on transactional analysis of individuals caring for terminal stage patients' relatives has not been studied before, and this study was conducted to determine the level of ego state and altruism in terminal stage patient relatives and the relationship between them based on transactional analysis.

Material and Methods

Study Design

This research, which is planned as descriptive-correlational type, was carried out between March and May 2020 in a hospital located east of Turkey.

The target population of the study consisted of individuals with terminal stage patients in a hospital located east of Turkey. The sample of the study consisted of individuals who met the research criteria and agreed to participate in the study.

Collection of Data

An Introductory Information Form, Altruism Scale and Ego States Scale (ESS) were used to collect research data. Volunteers among the patients' relatives of terminal-stage patients and those who could use a telephone/computer were included in the study. After explaining the purpose of the study, verbal consent was obtained from those who voluntarily accepted to participate in the study, and the data were collected online using the Google form prepared by the researchers.

Data Collection Tools

Introductory Information Form: It consists of questions created by the researchers and containing the introductory characteristics of the individuals.

Altruism Scale: The altruism scale is a scale developed by London and Bower (1968) to

measure altruistic behavior. Its adaptation and standardization to Turkish was made by Akbaba et al. (1991) to be used in Akbaba's study "The effect of group counseling on altruism, which is a social psychological concept".[9] The scale consists of four sub-dimensions. There are five items for each sub-dimension. 1th, 2nd, 3rd, 4th and 5th items are on the family dimension, Items 6th, 7th, 8th, 9th and 10th are on social dimension, 11th, 12th, 13th, 14th and 15th items are on benevolence dimension, 16th, 17th, 18th, 19th and 20th items belong to the dimension of responsibility. Each item has 5 answer options. The altruism score of the individual is determined by gathering the marked options. In the adaptation studies of Akbaba (1991), the Cronbach Alpha internal consistency coefficient was found to be .85. In our study, the Cronbach Alpha internal consistency coefficient was found to be .75.

Ego States Scale (ESS)

It was developed by Williams in 1978.[10] The adaptation to Turkish was made by Ari in 1989.[11] ESS is a list of 95 adjectives that qualify human. The test subject is asked to mark the adjectives that "define himself and see as a feature of himself" with free selection technique. There is no restriction on the number of adjectives to mark. In the scale; Each adjective and ego state is measured with five different standard values (Critical Parent (CP), Nurturing Parent (NP), Adult (A), Adapted Child (AC), Natural Child (NC)) ranging from 0-4. The scores obtained from the referees for the five ego states of each marked adjective are added to reach five separate total scores. These scores are then divided by the highest score (coefficient) that can be obtained from the scale for each ego state. Five ego state scores from this process are added and by dividing each division result by this general sum, ego state scores showing the proportions of each ego state in a whole are obtained.

Analysis of Data

The analysis of the data was done on the computer using the Statistical Package for the Social Sciences (SPSS-22) statistical software. Frequency, descriptives, percentage, mean, standard deviation, explore and normality plots with tests were used as descriptive statistical methods. Kolmogorov–Smirnov test was used to test normality distribution with analytical tests. Mann-Whitney U test was used for binary groups. Kruskal-Wallis test was used for groups more than two. Spearman correlation test was used to determine whether there is a linear relationship between the two numerical measurements, the direction and severity of this relationship, if any. In our study ($p<0.05$), it was accepted as statistically significant difference.

Ethical Principles

Consent from the Scientific Research Ethics Committee (Date: 27.02.2020 and number: 14) and written permission from the institutions where the study will be conducted was obtained. The necessary explanations were made to the individuals included in the study and verbal permission was obtained from those who wanted to participate in the study.

Table 1. Descriptive Characteristics of Individuals (N=134)

Variables		n	%
Gender	Female	39	29.1
	Male	95	70.9
Marital status	Single	80	59.7
	Married	54	40.3
Education Level	Illiterate	17	12.7
	Primary education	30	22.4
	High school education	47	35.1
	High education	40	29.9
Income rate	Less than income	94	70.1
	Income equal to expense	15	11.2
	More than income	25	18.7
		$\bar{X}$ **±SD**	
Age		35.14±16.02 (min.19,max 68)	

(Frequency, descriptives, mean, standard deviation)

Results

It was determined that 70.9% of the individuals participating in the study were male, 59.7% were single, 35.1% were graduates of high school education, 70.1% of their income is less than their expenses and the average age of the group was 35.14±16.02 *(Table 1)*.

According to the findings obtained from the study, it was determined that the CP total score mean of the individuals was 0.17±0.49, the total score mean of NP was 0.22±0.03, the A total score mean was 0.21±0.19, the AC total score mean was 0.19±0.02, and the NC total score mean was 0.19±0.03. Altruism Scale Total score mean was 67.53±9.06 and individuals were found to have a high level of altruism. Among the Sub-Dimensions of the Altruism Scale, it was determined that the Family Sub-Dimension was 17.42±2.49, the Social Sub-dimension was 14.94±4.56, the Helpful Sub-Dimension was 17.50±3.10, and the Responsibility Sub-dimension was 17.65±2.75 *(Table 2)*.

The total score mean of CP was found to be statistically significantly higher in single, higher education graduates, and those with higher income than expenses ($p<0.05$).

In the advanced analysis (Games Howell) conducted to determine which group originated the difference between the groups in terms of education level, the total score mean of CP was found that the score mean of those who graduated from high school education and higher education was higher than those who were illiterate and graduated from primary education.

In the advanced analysis (Games Howell) conducted to determine which group originated the difference between the groups in terms of the income level, the CP total score mean was found that the score mean of those whose income is more than their expenses are higher than both groups.

The total mean score of NP was found to be statistically significantly higher in married, illiterate, and low-income group ($p<0.05$).

In the advanced analysis (Games Howell) conducted to determine which group originated the difference between the groups in terms of education level, the total score mean of NP was found that the score mean of the illiterate and primary school graduates was higher than the groups with high school education and higher education.

In the advanced analysis (Games Howell) conducted to determine which group originated the difference between the groups in terms of the income level , the total score mean of NP was determined that the score mean of those whose income was higher than the expense of both groups.

The total score mean of A was found to be statistically significantly higher in males, married, illiterate, and those whose income was less than their expenses ($p<0.05$).

In the advanced analysis (Games Howell) conducted to determine which group originated

Table 2. Individuals' Altruism Scale Total and Sub-Dimension Mean Scores and their Ego Status Mean Scores

	Mean ± SS	Min-Max
Critical Parent Ego Condition (CP)	0.17±0.49	0.11-0.31
Nurturing Parent Ego State (NP)	0.22±0.03	0.13-0.26
Adult Ego State (A)	0.21±0.19	0.18-0.25
Adapted Child Ego State (AC)	0.19±0.02	0.13-0.23
Natural Child Ego State (NC)	0.19±0.03	0.11-0.24
Altruism Total Score	67.53±9.06	45-84
Family Sub-dimension	17.42±2.49	12-22
Social Sub-Dimension	14.94±4.56	5-25
Benevolent Sub-Dimension	17.50±3.10	12-23
Responsibility Sub-Dimension	17.65±2.75	14-23

(Mean, standard deviation)

the difference between the groups in terms of education level, A total score mean was found that the score mean of those who graduated from high school education was lower than that of the illiterate group.

In the advanced analysis (Games Howell) conducted to determine which group originated the difference between the groups in terms of the income level, the A total score mean was determined that the score mean of those whose income is more than their expenditure is lower than both groups.

The total score mean of AC was found to be statistically significantly higher in those who graduated from higher education and those whose income was less than their expenses ($p<0.05$).

In the advanced analysis (Games Howell) conducted to determine which group originated the difference between the groups in terms of educational level, the AC total score mean was determined that the score mean of the higher education graduates is lower than the illiterate and primary school graduates.

In the advanced analysis (Games Howell) conducted to determine which group originated the difference between the groups in terms of income level , the AC total score mean was found that the score mean of those whose income was less than their expenses were higher than both groups.

It was found that the total score mean of NC was statistically significantly higher in those who graduated from higher education and those whose income was equivalent to their expenses ($p<0.05$).

In the advanced analysis (Games Howell) conducted to determine which group originated the difference between the groups in terms of educational level, the total score mean of NC was found that the score mean of the higher education graduates was higher than the illiterate group.

In the advanced analysis (Games Howell) conducted to determine which group originated the difference between the groups in terms of income level, the total score mean of NC was found that the score mean of those whose income was less than their expenses were lower than both groups *(Table 3)*.

The total score mean of the altruism scale was found to be statistically significantly higher in singles, those who graduated from higher education, and those whose income was higher than their expenses ($p<0.05$).

In the advanced analysis (Games Howell) conducted to determine which group originated the difference between the groups in terms of educational level ,the altruism scale total score mean was found that the score mean of the graduates of higher education is higher than all groups.

In the advanced analysis (Games Howell) conducted to determine which group originated the difference between the groups in terms of income level, the altruism scale total score mean was found that the score mean of those whose

Table 3. Comparison of Individuals' CP, NP, A, AC, NC Mean Scores According to Socio-Demographic Features (N=134)

Variables			CP		NP		A		AC		NC	
		N	$\overline{X}$ ±SD	Test and Significance	$\overline{X}$ ±SD	Test and Significance	$\overline{X}$ ±SD	Test and Significance	$\overline{X}$ ±SD	Test and Significance	$\overline{X}$ ±SD	Test and Significance
Gender	Female	39	0.18±0.04	U=1534.0 p=0.118	0.21±0.03	U=17044.0 p=0.466	0.21±0.01	U=1141.0 **p=0.001**	0.19±0.02	U=1531.0 p=0.115	0.19±0.04	U=1807.0 p=0.823
	Male	95	0.17±0.05		0.22±0.03		0.22±0.01		0.18±0.02		0.18±0.03	
Marital status	Single	80	0.19±0.05	U=1171.0 **p=0.000**	0.21±0.03	U=1387.0 **p=0.000**	0.21±0.02	U=1503.0 **p=0.003**	0.18±0.02	U=1914.0 p=0.264	0.19±0.04	U=2026.0 p=0.543
	Married	54	0.15±0.01		0.23±0.01		0.22±0.01		0.19±0.01		0.19±0.02	
Education Status	Illiterate	17	0.13±0.01	KW=69.360 **p=0.000**	0.25±0.01	KW=56.437 **p=0.000**	0.23±0.01	KW=27.950 **p=0.000**	0.20±0.01	KW=27.136 **p=0.000**	0.17±0.03	KW=8.657 **p=0.034**
	Primary education	30	0.14±0.02		0.23±0.01		0.22±0.01		0.20±0.01		0.18±0.02	
	High School Education	47	0.20±0.03		0.21±0.03		0.20±0.01		0.18±0.02		0.19±0.02	
	High education	40	0.21±0.02		0.20±0.02		0.22±0.02		0.17±0.02		0.20±0.04	
Income status	My income is less than my expenses	94	0.16±0.03	KW=49.340 **p=0.000**	0.23±0.02	KW=57.694 **p=0.000**	0.22±0.01	KW=56.667 **p=0.000**	0.19±0.02	KW=20.745 **p=0.000**	0.17±0.03	KW=29.545 **p=0.000**
	My income is equal to my expenses	15	0.15±0.01		0.21±0.01		0.21±0.01		0.17±0.01		0.22±0.01	
	My income is more than my expenses	25	0.24±0.05		0.17±0.02		0.19±0.01		0.16±0.02		0.21±0.04	

(Mann-Whitney U, Kruskal-Wallis) Critical Parent Ego Condition (CP), Nurturing Parent Ego State (NP), Adult Ego State (A), Adapted Child Ego State (AC), Natural Child Ego State (NC)

income is equivalent to their expenditure was lower than both groups *(Table 4)*.

A statistically significant negative correlation was found between the total score of CP and the the Responsibility sub-dimension of Altruism Scale, , NP, A, AC, and age (p<0.05). A positive statistically significant relationship was found between the total score of CP and the family and social sub-dimensions of the Altruism scale (p<0.05).

A statistically significant negative correlation was found between the total NP score and the Social sub-dimension of the Altruism Scale, CP and NC (p<0.05). A positive statistically significant correlation was found between total NP score and A, AC, responsibility sub-dimension of Altruism scale and age (p<0.05).

A statistically significant negative correlation was found between the A total score and the Social and benevolent sub-dimensions of Altruism Scale, CP, NC (p<0.05). A positive statistically significant relationship was found between A total score and the responsibility sub-dimension of the Altruism Scale, NP, AC, and age (p<0.05).

A statistically significant negative correlation was found between the total score of AC and the Social sub-dimension of Altruism scale, CP, NC (p<0.05). It was found that there was a statistically significant positive correlation between AC total score and the responsibility sub-dimension of Altruism Scale, NP, A, and age (p<0.05).

It was found that there was a statistically significant negative correlation between NC total score and the responsibility sub-dimension of the Altruism Scale, NP, A, and AC (p<0.05). A positive statistically significant relationship was found between the total score of NC and the social and benevolent sub-dimensions of the Altruism scale, NP, A (p<0.05).

A positive statistically significant relationship was found between the total score of the Altruism Scale and the Altruism Scale sub-dimensions (p<0.05). A statistically significant negative correlation was found between altruism scale total score and age (p<0.05).

A statistically significant negative correlation was found between age and the total score of Altruism and CP (p<0.05). A statistically significant positive correlation was found between age and benevolence sub-dimensions of Altruism, NP, A, AC (p<0.05) *(Table 5)*.

Discussion

For centuries, scientists have attempted to define the natural and dynamic tension of the relationship between supporting one's own wants and needs (self-interest) and meeting other people's wants and needs (altruism). People who focus on meeting their own needs are generally defined by negative concepts (eg, egocentric, hedonistic, selfish), while those who focus on the needs of others are defined by positive concepts (eg, generous, altruistic).[12] Regarding the balance between self-interest and

Table 4. Comparison of Altruism Scale Mean Scores According to Socio-Demographic Features (N=134)

Variables		Altruism Scale Mean Scores		
		N	$\bar{X}$ ±SD	Test and Significance
Gender	Female	39	68.48±5.53	U=1689.0 p=0.420
	Male	95	67.14±10.16	
Marital status	Single	80	72.22±6.77	U=519.50 **p=0.000**
	Married	54	60.59±7.44	
Education Status	Illiterate	17	53.47±8.23	KW=42.785 **p=0.000**
	Primary education	30	67.73±6.06	
	High School Education	47	67.82±6.68	
	High education	40	73.02±7.42	
Income status	My income is less than my expenses	94	68.36±9.63	KW=25.497 **p=0.000**
	My income is equal to my expenses	15	59.40±1.54	
	My income is more than my expenses	25	69.32±6.82	

(Mann-Whitney U, Kruskal-Wallis) Critical Parent Ego Condition (CP), Nurturing Parent Ego State (NP), Adult Ego State (A), Adapted Child Ego State (AC), Natural Child Ego State (NC)

social commitment, Freud (1960) suggested that simple self-interest (id) must come to terms with the expectations of society (ego). On the other hand, Maslow (1950) found that self-actualized individuals are both altruistic and self-interested, based on the assumption that self-actualized individuals openly enjoy their altruistic behaviors and that these behaviors also serve them.[13] Studies on prosocial behavior reveal that egoistic processes play an important role in helping other people.[12] The emotional states of individuals who care for terminal stage patients' relatives may affect the care they provide. In this study, the level of altruism and ego states of individuals is discussed in the light of the literature.

In our study, it was found that there was a statistically significant negative correlation between the total score of CP and the Responsibility sub-dimensions of the Altruism scale ($p<0.05$). CP is the prejudiced thoughts, feelings, and beliefs of the personality learned from parents or parent figures. The Critical Parent is to protect social rules/values and to criticize those who do not obey them.[14] Our study suggests that the reason for such a result is that critical caregiving individuals adopt the stubborn, strong, principled, punitive and task-bearing position as a principle[15], and they approach negatively to the responsibilities of individuals other than their own.

A positive statistically significant relationship was found between the total score of CP and the family and social sub-dimensions of the Altruism scale ($p<0.05$). Using the critical parental aspect, a person aims to keep the social rules that he learned from those who convey the culture of the society to him and gradually adopt them correctly and to transfer them to future generations.[14] Because of these features, it is thought that critical caregivers approach familial and social altruism more positively.

A statistically significant negative correlation was found between the total NP score and the Social sub-dimension of the Altruism Scale ($p<0.05$). A Nurturing Parent is protective and guardian. He speaks affectionately when he thinks harm will come. He is based on social values and thinks that if social values go beyond, they will suffer. The Nurturing Parent is curious, caring, forgiving, supportive, permissive, compassionate, protective and anxious.[15] A high score in the social dimension indicates that the person participates in social activities and undertakes duties and responsibilities in these activities, while low scores indicate that the social side of individuals is weak. Because of these features, it makes us think that caregivers with higher social aspect have less

Table 5. The Relationship Between Individuals' CP, NP, A, AC, NC and Altruism Scale Total Score Means and Scale Sub-Dimensions and Age

		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
(1) CP	r p	- -										
(2) NP	r p	-.902** **.000**										
(3) A	r p	-.528** **.000**	.548** **.000**									
(4) AC	r p	-.412** **.000**	.482** **.000**	.194* **.025**								
(5) NC	r p	.112 .196	-.288** **.001**	-.401** **.000**	-.805** **.000**							
(6) Altruism	r p	.093 .285	-.069 .431	-.032 .712	.077 .375	.009 .919						
(7) Family Sub-dimension	r p	.230** **.007**	.178 .373	.064 .460	.048 .579	-.165 .057	.616** **.000**					
(8) Social Sub-Dimension	r p	.350** **.000**	-.324** **.000**	-.205* **.018**	-.190* **.028**	.174* **.045**	.765** **.000**	.263** **.002**				
(9) Benevolent Sub-Dimension	r p	.042 .629	-.107 .220	-.220* **.011**	.010 .908	.189* **.029**	.890** **.000**	.477** **.000**	.660** **.000**			
(10) Responsibility Sub-Dimension	r p	-.421** **.000**	.354** **.000**	.411** **.000**	.528** **.000**	-.383** **.000**	.320** **.000**	.288* **.001**	-.192* **.026**	.124 .153		
(11) Age	r p	-.813** **.000**	.795** **.000**	.519** **.000**	.315** **.000**	-.146 .093	-.242** **.005**	-.097 .267	.598** **.000**	-.148 .089	.306** **.000**	

(Spearman Correlation Test) (* $p<0.05$, ** $p<0.01$) Critical Parent Ego Condition (CP), Nurturing Parent Ego State (NP), Adult Ego State (A), Adapted Child Ego State (AC), Natural Child Ego State (NC)

protective thoughts.

A positive statistically significant correlation was found between the total NP score and the responsibility sub-dimension of the Altruism scale ($p<0.05$). The Nurturing Parent is curious, caring, forgiving, supportive, permissive, compassionate, protective and anxious.[15] It is thought that protective individuals have a positive attitude towards taking the responsibility of the individual they care for because of their supportive and compassionate attitude. Similar results have been found in the literature.[16-18]

A statistically significant negative correlation was found between the total score of A and the social and benevolent sub-dimensions of the Altruism scale ($p<0.05$). The adult parent is defined as the current set of realistic and autonomous emotion, thought and behavior patterns.[15] It is thought that they display a negative attitude towards the social and benevolent aspects of the other person due to having autonomous feelings and thoughts.

A positive statistically significant relationship was found between the total score of A and the responsibility sub-dimension of the Altruism scale ($p<0.05$). Adult ego state can also be viewed as a data processing center. This part of the personality correctly processes the data heard, seen and thought, proposes solutions to problems, and evaluates existing data without relying on biased thoughts or emotions.[19] This suggests that individuals due to this situation evaluate the events rationally and look positively to take responsibility. Similar results have been found in the literature.[16-18]

It was found that there was a statistically significant negative correlation between AC total score and Social sub-dimension of Altruism scale ($p<0.05$). Adapted Child is part of our personality, which consists of parent messages.[16] The adapted child ego state manifests itself with the behaviors of submission or rebellion. Unlike the natural child, Adapted Child reacts as if his parents are watching him.[8] Because of these features, it is thought that among the caregivers who have a high social aspect, they use the less adapted child side. Similar results were found in Akar's study.[16]

A positive statistically significant relationship was found between AC total score and responsibility sub-dimension of Altruism scale ($p<0.05$). The Adapted Child reacts as if his parents are listening or watching him. He is hardworking, well-behaved, rebellious, or uses any of his parental figures as a reference.[6] Due to these characteristics, it is thought that caregivers have the feeling that they should take responsibility.

It was found that there was a statistically significant negative correlation between NC total score and responsibility sub-dimension of the Altruism scale ($p<0.05$). The natural child is spontaneous, behaves as he/she intends to, is active, creative, is the untrained side of personality.[14,6,8] Due to these characteristics, it is thought that caregivers do not want to take responsibility.

A positive statistically significant correlation was found between the total score of NC and the social and benevolent sub-dimensions of the Altruism scale ($p<0.05$). The natural child takes care of the physical needs of the person and is creative.[14,6,8] It is thought that individuals due to these characteristics have the feeling that they should help each other's needs.

Conclusions

It has been determined that individuals have a high level of altruism and get the highest ego score from the Nurturing Parent ego state. The lowest mean ego score was found to be Critical Parent Ego Condition (CP). It is recommended to carry out studies to reduce the critical ego state and to conduct the study in larger groups.

Conflict of interest

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

Limitations

The fact that the study was conducted in only one city is a limitation of the study.

Authors' Contribution

Study Conception: MY, GO; Study Design: YS; Supervision: MSY, GO; Funding: MY; Materials: MSY, GO; Data Collection and/or Processing: YS; Statistical Analysis and/or Data Interpretation: MY, GO; Literature Review: MSY; Manuscript Preparation: YS, GO; and Critical Review: MY.

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TURKISH JOURNAL OF INTERNAL MEDICINE
ISSN:2687-4245
Original Article

The Evaluation of The Relationship Between Antibody Response and COVID-19 Disease Severity

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Abstract

Background World Health Organization (WHO) reported COVID-19 as a pandemic, on March 11th, 2020. The quick and accurate diagnosis is crucial to provide the appropriate treatment and isolation process. Immunity against COVID-19 is essential for disease control. There is scant information about antibody response and disease severity. In this study, we aimed to investigate the relationship between clinical severity and COVID antibody response.

Material and Methods Hospitalized PCR (n=10) and/or radiologically (n=31) proven 35 COVID-19 patients were included in the study. The blood samples were collected at least eight days after the onset of symptoms and studied by using the COVID-19 IgG/IgM Rapid Test. Patients were divided as mild (n=14), severe (n=12) and critical (n=9) according to COVID-19 disease severity. The results were compared among the groups.

Results A total of 35 COVID-19 patients' (mean age: 54.65±16.51 years, Male/Female: 23/12) rapid test results were compared according to clinical severity. A significant correlation was observed between disease severity and IgG results in both PCR positive (p=0.007) and whole patients (p=0.026). The positive IgG ratio was significantly low in the mild patient group while it was higher in severe and critical patients.

Conclusions Our study reveals that the greater antibody response occurs with the more serious COVID-19 disease. The application of the rapid test, in addition to PCR, may be used as a clue to foresee the clinical progression. These tests not only have an important role in diagnosis with PCR tests but also are associated with disease severity.

Turk J Int Med 2021;3(2):49-55
DOI: 10.46310/tjim.839313

Keywords: *antibody; SARS-CoV-2; COVID-19; disease severity; immune response*

Received: December 14, 2020; Accepted: January 16, 2021; Published Online: April 29, 2021

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Introduction

The outbreak of the Coronavirus disease 2019 (COVID-19) in Wuhan spread rapidly all over the world. World Health Organization (WHO) reported COVID-19 as a pandemic, on March 11th, 2020.[1] The virus was first observed in China, but European and American content countries are the most affected. From the first case, which was reported on March 11th, 2020, to July 8th, 2020, the total number of reported cases in Turkey was 207.897, with 5.260 deaths.[2]

Immunity against COVID-19 is crucial for disease control. There is scant information about antibody response and disease severity. COVID-19 can be diagnosed by using clinical, radiological, and laboratory tests. The most frequently encountered symptoms are fever, cough and fatigue. Progression to acute respiratory distress syndrome (ARDS), septic shock, bleeding, coagulation dysfunction might be observed in severe cases.[3] These symptoms are not specific features of COVID-19 since they are similar to that of other viral diseases or pneumonia with other respiratory tract pathogens. The chest computed tomography (CT) of COVID-19 patients revealed the ground-glass opacities and bilateral patchy shadowing predominantly located peripherally.[4] Currently, the only way for diagnosis of COVID-19 is polymerase chain reaction (PCR) based viral RNA detection from the nasopharyngeal and oropharyngeal swabs. These real-time Reverse Transcription Polymerase Chain Reaction (rRT-PCR) test requires certified laboratories, expensive equipments, trained technicians, and it has false-negative results for rRT-PCR of COVID-19.[5] False-negative results may be due to the inadequate education of health professionals for sample collection, different stages of infection (in some radiologically typical patients for COVID-19, the RNA remains negative), quality of the test.[6] Due to these disadvantages, radiologic and laboratory tests were also employed for COVID-19 diagnosis.

Publications on severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS) revealed that specific antibodies were found in 80-100% of patients two weeks after the onset of symptoms.[7,8] Antibody responses to SARS-CoV-2 are not fully understood, and the clinical benefits of serological tests are still uncertain.[9,10] Serological tests have more advantages over PCR; such as easy access to the specimen, faster test results, minimal need for equipment, specialized laboratories and workload. However, we still have many obscures about the disease; the immune response plays a crucial role in the course of the disease. As we all know, immunoglobulin M (IgM) is the first antibody to be produced in response to infections before the generation of humoral IgG responses that are crucial for long term immunity.[11] In this study, we aimed to investigate the relationship between clinical severity and COVID antibody response.

Material and Methods

This study was conducted on 35 hospitalized COVID-19 patients [PCR (n=10) and/or radiologically (n=31) proven] in the pandemic clinic of Ondokuz Mayıs University, Faculty of Medicine. After the local ethics committee approval (approval date: April 10, 2020; approval number:2020/149) consent given patients were recruited to the study. Combined oropharyngeal and nasopharyngeal swab samples were taken from all participants upon admission, 48th hours, and 96th hours of hospitalization. SARS-CoV-2 (2019-nCoV) qPCR Detection Kit" (Bioeksen Bio-Speedy R&D Co, Ltd, Turkey) was used to demonstrate the presence of SARS-CoV-2.

The blood samples that collected at least eight days after the onset of COVID symptoms were studied with COVID-19 IgG/IgM Rapid Test Cassette (Citus Diagnostic Inc. Ovios). The COVID-19 IgG/IgM Rapid Test Cassette was compared with confirmed clinical diagnosis. The study included 446 specimens for IgG and 456 specimens for IgM *(Table 1 and Table 2)*. Clinical information including travel and exposure history, starting of clinical symptoms and radiological and laboratory findings (Lactate dehydrogenase, D-Dimer, Ferritin, Creatine phosphokinase, C-reactive protein, Troponin-I, Procalcitonin, and Lactate) were collected. Patients were divided as mild (non-pneumonia and mild symptoms, n=14), severe (dyspnea, respiratory rate≥30/min, blood oxygen saturation≤93%, the partial pressure of arterial oxygen to fraction of inspired oxygen ratio 50% within 24 to 48 hours, n=12) and critical (respiratory failure, septic shock, or multiorgan dysfunction, n=9) according to COVID-19 disease severity.[12,13] The results were compared among these groups.

Table 1. Validity of the COVID-19 IgG/IgM Rapid Test for IgG results

Method		Confirmed Clinical Diagnosis		**Total Results**
		Positive	Negative	
COVID-19 IgG/IgM Rapid Test Cassette for IgG	Positive	75	2	77
	Negative	0	369	369
Total Results		75	371	446

Table 2. Validity of the COVID-19 IgG/IgM Rapid Test for IgM results

Method		Confirmed Clinical Diagnosis		**Total Results**
		Positive	Negative	
COVID-19 IgG/IgM Rapid Test Cassette for IgM	Positive	78	3	81
	Negative	7	368	375
Total Results		85	371	456

Statistical Analysis

The research data were evaluated by using "SPSS (Statistical Package for Social Sciences) for Windows 21.0 (SPSS Inc, Chicago, IL)" licensed to Ondokuz Mayıs University. Definitive statistics were given as average±standard deviation, median (minimum-maximum), frequency distribution, and percentage. Chi-square, Fisher's precision test was used for qualitative evaluations.

Results

The demographics and results of 35 patients (age=54.65±16.51, Male/Female=23/12) were collected. Most of these patients had co-morbid diseases including cardiovascular disease (n=9), cancer (n=6), hypertension (n=3), chronic renal failure (n=2), diabetes mellitus (n=1), chronic obstructive pulmonary disease (n=1), and asthma (n=1) while 12 patients had no comorbidity *(Table 3)*. No significant difference was observed between having comorbidity and disease severity (p=0.236).

The most frequently encountered complaints during admission were fever (65.7%), cough (57.1%) and shortness of breath (SOB,57.1%). Except SOB no significant difference was observed between the admission complaints and disease severity. SOB was observed 3 of 14 mild patients while it was observed 9 of 12 severe patients and 8 of 9 critical patients (p=0.002).

Radiological evaluation reveals bilateral ground glass appearance in 11 patients (31.4%) at Chest X-ray and 19 patients (54.3%) in Chest CT. Other chest CT findings including messy infiltration (n=4), pleural effusion (n=3), consolidation (n=3) and unilateral ground glass appearance (n=2) were also observed.

Ten patients have positive PCR test results on admission, while the remaining 25 patients had three consecutive negative PCR test results. Five of the triple-negative PCR patients' COVID-19 IgG/IgM rapid test was resulted as positive for IgG. The remaining 20 patients with negative antibody test results were considered as a probable case since they had typical clinical symptoms and radiological findings. The evaluation of the COVID-19 IgG/IgM rapid test results revealed that eight patients (22.9%) were positive for IgM, and ten patients were positive for IgG (28.5%).

In the whole patient group and particularly PCR positive group, the IgM and IgG positivity rate were significantly higher in severe and critical patients compared to the mild group *(Table 4)*.

Table 3. Socio-demographic data of patients

Study Parameter	Value
Age	54.65±16.51
Gender, n (%)	
Male	23 (65.7)
Female	12 (34.3)
Health personnel, n (%)	9 (25.7)
Suspected contact history, n (%)	9 (25.7)
Co-morbid diseases, n (%)	
None	12 (34.2)
Cardiovascular disease	9 (25.7)
Cancer	6 (17.14)
Hypertension	3 (8.5)
Chronic renal failure	2 (5.7)
Diabetes mellitus	1 (2.8)
Chronic obstructive pulmonary disease	1 (2.8)
Asthma	1 (2.8)
Smoking History, n (%)	
Never smoked	20 (57.1)
Ex-smoker	12 (34.3)
Active smoker	2 (5.7)
Passive smoker	1 (2.9)

Table 4. Comparison of groups in terms of IgG and IgM results in all patients and PCR positive patients

	Disease Severity			
	Mild (n=14)	**Severe (n=12)**	**Critical (n=9)**	**p**
Immunoglobulin G (Positive/Negative)	0/14	5/7	5/4	0.007
Immunoglobulin M (Positive/Negative)	0/14	4/8	4/5	0.026
PCR positive patients, (n=10)				
	Mild (n=5)	**Severe (n=2)**	**Critical (n=3)**	**p**
Immunoglobulin G (Positive/Negative)	0/5	0/2	0/3	0.007
Immunoglobulin M (Positive/Negative)	0/5	1/1	3/0	0.019

The evaluation of laboratory results reveals that SpO2, LDH, Ferritin, and CPK measurement on admission were significantly different among the groups *(Table 5)*.

Discussion

Our study reveals that COVID IgM/IgG Rapidtest results were significantly correlated with disease severity. In our study we have mild, severe and critical disease group but we have no moderate disease group. Since patients with pneumonia had respiratory distress at the time of admission, we put them in severe disease group. Laboratory results, including acute phase reactants, were significantly higher in critical patients, while oxygen saturation was significantly higher in mild patients.

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) can initially be detected with PCR test 1 to 2 days prior to the onset of symptoms in the upper respiratory samples and can persist for 7 to 12 days in moderate cases and up to 2 weeks in severe cases. Molecular tests that detect viral RNA may have false-negative results due to sample types, sampling time, accurate sampling technique, sample quality, transport, and storage conditions. In a study of 205 patients with COVID-19, bronchoalveolar lavage fluid specimens demonstrated the highest positive PCR rates (93%), followed by sputum (72%), nasal swabs (63%), bronchoscopy brush biopsy (46%) and pharyngeal swabs (32%) (14). Although PCR is the gold standard for confirming the infection, rapid antibody tests can be used, especially in severe and critical cases, as a diagnostic tool. Our results show that 28.5% of our COVID patients have a positive

Table 5. The laboratory results of patients on admission

	Mild (n=14)	Severe (n=12)	Critical (n=9)	p
SpO_2 (%)	96.79±1.76[a,b]	92.25±4.39[a]	93.78±3.15[b]	**0.004**
White Blood Cells	7.51±2.76	8.37±5.44	8.22±5.72	0.879
Neutrophils	5.04±2.46	6.56±5.07	5.73±5.34	0.670
Lymphocyte	1.73±0.83	1.13±0.97	1.67±0.85	0.205
Lymphocyte (%)	28.4±18.21	23.48±21.38	23.46±12.97	0.739
Hemoglobin (mg/dL)	13.55±2.39	12.45±2.25	12.38±3.8	0.503
Platelet	224.5±88.23	172.92±64.45	253.67±159.57	0.210
C-reactive Protein	35.91±59.21	92.16±129.67	97.55±78.94	0.208
Lactate	1.36±0.81	42.03±139.81	1.7±1.49	0.392
Procalcitonin	0.22±0.43	2.41±7.05	0.14±0.11	0.336
Lactate Dehydrogenase	272.5±145.54[c]	321.25±83.96	802.89±817.01[c]	**0.014**
Ferritin	387.35±497.22	452.4±546.24	2493.29±4039.56	**0.047**
Creatine phosphokinase	132.5±179.77[d]	93.67±88.06	496.78±603.08[d]	**0.017**
Troponin I	0.1±0	0.17±0.24	2781.29±8332.02	0.241
D-dimer	348.24±295.95[e]	1910.17±3244.74	3129.67±3979.47[e]	0.070

SpO2: Oxygen saturation, a: mild vs. severe, p=0.002, b: mild vs. critical, p=0.008, c: mild vs. critical, p=0.026, d: mild vs. critical, p=0.044, e: mild vs. critical, p=0.015

PCR test on a nasopharyngeal swab.

Detection of antibodies has been shown to improve the diagnosis of positive cases and also helpful for PCR negative patients' diagnosis. Guo et al.[15] reported a significantly increased diagnosis rate with PCR and IgM ELISA assay combination (98.6%) compared to PCR alone (51.9%). The IgM detection rate was reported to be higher at least five days after the onset of the symptoms. We studied the Elisa test at least eight days after the onset of the symptoms. In accordance with the literature, the COVID-19 diagnosis rate was increased (14.2% (n=5) more patients) with the addition of the Elisa test.

A Chinese study about antibody response demonstrated a significant relationship between antibody titer and clinical severity 2-week after the onset of symptoms.[15] Another study from China shows significantly lower virus-specific IgG levels in the asymptomatic group compared to the symptomatic group in the acute phase.[17] Similar to the literature, our results show a significant relationship with Elisa test positivity and disease severity. All PCR positive patients were not developed positive IgM (n=6) and IgG (n=5) response. Since it cannot reach the statistical significance, our results show that radiologically proven, PCR and Elisa negative patients mostly have mild symptoms (p=0.170). In light of the literature, we can assume that patients with mild symptoms develop lower antibody response to SARS-CoV-2. The individual time variability to develop an antibody response to the disease may have a role in a lower rate of antibody response.

An ongoing study by Sweden Public Health Agency revealed that a total of 7.3% of the blood samples collected from people in Stockholm were positive in the antibody study, which can be compared with a total of 4.2% in Skåne and 3.7% in Västra Götaland.[18] Innate immunity plays an essential role in SARS-CoV-2 clearance.[19] Moreover, innate immunity alone might be enough to clear the virus. In the current study, PCR positive five mild patients were discharged without any complaints with negative IgM and IgG results 15 days after the onset of the symptoms. Furthermore, we found that positive IgG rates were significantly lower in mild patients, whereas in critical cases, it was found to be significantly high.

We found a significant relationship between D-dimer, ferritin, and LDH levels and disease severity. Zhang et al.[20] reported that elevated D-dimer on admission correlates with hospital mortality in patients with Covid-19, which indicates D-dimer as an early and helpful prognostic marker to improve management of Covid-19 patients. In our study, we found that d-dimer levels were significantly lower in mild patients compared to critical patients (p=0.015,*Table 5*). Sun et al.[21] also reported that the inflammatory markers such as ferritin, CRP level, and erythrocyte sedimentation rate were elevated in severe or critically ill groups, and D-dimer was an independent predictor of disease severity. In our study, we observe that CPK and LDH levels were significantly higher in critical patients' groups in addition to ferritin and d-dimer *(Table 5)*.

Although we have presented some interesting results, our study has some limitations. First, we performed a single rapid antibody test for each patient varying 8 to 20 days after the onset of the symptoms. We did not perform a second control test that could be positive 30 days after onset of symptoms. And also, our study sample size group is small and further studies with more patients should be conducted.

Conclusions

The results of current study reveals the importance of antibody tests in severe or critical COVID-19 patients. These tests not only have an essential role in diagnosis with PCR tests but are also associated with disease severity. Severe and critical COVID-19 patients had more positive IgM and IgG antibody compared to mild patients. However, further studies should be conducted to clarify the potential association between disease severity and antibody response with more patients.

Conflict of Interest

All authors declare that they have no confict of interest.

Authors' Contribution

Study Conception: YTG, NTT, MNG; Study Design: YTG, NTT, TGK; Supervision: YTG, NTT, TGK; Funding: YTG, NTT; Materials: YTG, NTT; Data Collection and/or Processing: YTG, NTT, TGK; Statistical Analysis and/or Data Interpretation: YTG, NTT, TGK; Literature Review: YTG, NTT, TGK; Manuscript Preparation: YTG, NTT, TGK; and Critical Review: YTG, TGK.

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TURKISH JOURNAL OF INTERNAL MEDICINE

ISSN:2687-4245

Original Article

Self-Reported Olfactory Function According to The Severity of COVID-19

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Abstract

Background Establishing a relationship between COVID-19 severity and olfactory dysfunction may be beneficial in-patient follow-up. Thus, in this study, we aimed to evaluate the association between self-reported olfactory dysfunction and the clinical stages of COVID-19.

Material and Methods The patients included in this study were divided into three groups according to the severity of the novel coronavirus disease as mild, severe, and critical (life-threatening) patients. Patients were then contacted by phone and asked questions with the help of structured documentation form that evaluated their general status, sense of smell, taste and compared the data within the three groups.

Results Among the 126 subjects evaluated in the present study (mild, n=51; severe, n=53, critical, n=22), 61 of the participants were males, and 65 were females. The findings showed that olfactory loss was the most prominent feature of the COVID-19's mild clinical course and the majority of the patients with loss of smell were female and young patients.

Conclusions The findings obtained from clinically mild cases suggest that more olfactory dysfunction, indicating that the effects of viral load alone, is not decisive for olfactory dysfunction.

Turk J Int Med 2021;3(2):56-61
DOI: 10.46310/tjim.817623

Keywords: *COVID-19; olfactory dysfunction; clinical severity; gustatory dysfunction*

Received: October 31, 2020; Accepted: January 16, 2021; Published Online: April 29, 2021

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Introduction

The clinic of COVID-19 has a wide spectrum, ranging from an asymptomatic form to acute respiratory distress syndrome and multiorgan failure. The clinical stages of the disease are categorized as mild, severe, and life-threatening (critical).[1] In 80% of the patients, while the disease has a mild symptom and uncomplicated course, approximately 14% of the patients experience respiratory distress requiring oxygen treatment need to be to hospitalized, and an estimated 5% of patients need treatment in intensive care unit.[2]

Olfactory loss is prominent in patients with COVID-19 and varies in its general clinical appearance. In addition to the sudden and isolated form of olfactory loss, there are forms that occur together with typical signs of disease, such as fever or cough, or it occurs immediately after these findings.[3,4] The frequency of occurrence of olfactory dysfunction in COVID-19 positive patients varies between studies but reaches 80%.[4] In previous studies, it was discussed that olfactory dysfunction might also have predictive value for clinical outcomes of COVID-19 based on observations that olfactory dysfunction was more prevalent in individuals with a milder clinical course.[5-10]

COVID-19 is associated with significant morbidity and mortality, and many prognostic factors, such as older age, presence of comorbidity, history of smoking and significantly elevated C-reactive protein, have been identified.[11] In addition to these parameters, the olfactory loss is investigated concerning its prognostic value. It is a specific finding that will likely provide an opportunity for patients to start treatment early or to isolate these patients.[6] However, establishing relationships in COVID-19 that may be related to olfactory loss and disease severity can be beneficial in-patient follow-up, as well as providing a parameter to help healthcare providers answer their concerns while answering questions about patients' conditions. Thus, we aimed to evaluate the association between self-reported olfactory dysfunction and the severity of COVID-19. Thus, unlike previous studies, our study included patients who were classified as mild, severe and critical stages of COVID-19. We have taken the studies conducted in outpatient and hospitalized patient groups one step further. To our knowledge, for the first time, we included patients with critical stage disease in the literature.

Material and Methods

Study Group and Clinical Evaluation

This cross-sectional study consisted of confirmed COVID-19 (+) patients who underwent treatment between March 2020 and April 2020. We retrospectively reviewed electronic medical records of the patients, and the patients were classified as mild, severe, and critical according to the clinical severity of the COVID -19.

The patients were then contacted by phone and asked questions using a structured documentation form. This form was used to assess the general and otolaryngology symptoms of the patients. In addition, data such as the presence of olfactory loss in patients, onset time and duration were also recorded on this form. In order to evaluate their changes in taste, the patients were asked if they experienced any disturbances in sweet, salty, sour and bitter flavors. The sample form was given as a supplementary tool.

The definition of mild level referred to outpatients, who showed general symptoms, such as fever, cough, fatigue, with don't have dyspnea or abnormal chest radiography. Severe level was related to hospitalized patients with widespread findings of pneumonia in chest radiography or computed tomography, SpO^2 <94% on room air at sea level, a respiratory rate of >30 breaths/min, PaO2/FiO2 <300 mmHg. At the critical level, patients were in severe respiratory distress and mechanical ventilation support required or shock, multiple organ dysfunction syndromes developed, and patients treated by in an intensive-care unit.[1]

Inclusion and Exclusion Criteria

The diagnosis of COVID-19 was made by the confirmed polymerase chain reaction (PCR) positive test for SARS-CoV-2 viral nucleic acid from nasopharyngeal swabs. The inclusion criteria for the study group were as follows: 18 years or older and the presence of olfactory loss due to the acquisition of COVID -19.

The exclusion criteria for the study group were pregnancy, malignant tumors and/or a history of oncology treatment, history of nasal or paranasal surgery, history of olfactory loss with another reason, such as head trauma, sinonasal disease, postinfectious anosmia or neurodegenerative diseases.

Ethical Concerns

The study protocol was approved by the local medical research ethics committee (No: 2020/14, Date: 19.08.2020). This study complied with the Declaration of Helsinki. Verbal informed consent was obtained from all the participants in this study.

Statistical Analysis

Statistical analyses were carried out using SPSS for Windows, version 21 (SPSS, Chicago, IL, USA). Shapiro-Wilk test was used to check the normality of the variables. Continuous variables were presented as mean±standard deviation (mean±SD) and categorical variables as frequency (n) and percentage (%). The Kruskal–Wallis test and Mann–Whitney U test were used to compare the continuous variables among groups. Two-sided P-value ≤0.05 was interpreted as statistically significant.

Results

In this study, 163 cases were evaluated. However, eleven patients were excluded from this study because they could not be reached by phone, five patients reported having an olfactory problem before, eighteen patients stated to have chronic sinonasal or allergic rhinitis disease and the information provided by the three patients was contradictory, so they were not included in this study. Among the 126 subjects evaluated in the present study (mild, n=51; severe, n=53, critical, n=22), 61 of the participants were males, and 65 were females. The mean age of the female subjects was 40.2±14.61 years, and the mean age of the male subjects was 45.32±13.11 years. The descriptive statistics of the study groups are shown in Table 1.

Also, the frequency of smell and taste dysfunction and other symptoms according to the groups are shown in Table 2.

Among patients with olfactory dysfunction in all groups, 70.4% (n=38) were female and 29.6%

Table 1. Demographic and clinical characteristics

	Mild Group n=60	p^1 value	Severe Group n=61	p^2 value	Critical Group n=23	p^3 value
Age (mean±SD, years)	33.9±8.9	<0.001	44.9±13.9	0.053	52.7±14.4	p<0.001
Sex n (%)		<0.001		0.03		p<0.001
Male	26 (44.3%)		28 (45.9%)		16 (69.6%)	
Female	34 (56.7%)		33 (54.1%)		7 (30.4%)	
Education(years)	7.9±4.4	0.058	6.4±5.1	0.45	6.8±2.8	0.168
Smoking n (%)		0.019		0.013		p<0.001
No	44 (73.3%)		50 (81.9%)		13 (56.5%)	
Former	0		4 (6.5%)		7 (30.4%)	
Current	16 (26.7%)		7 (11.5%)		3 (13%)	
Comorbidities n (%)		0.001		0.662		0.048
None	54 (90%)		36 (59%)		16 (63.6%)	
Diabetes mellitus (DM)	1 (1.7%)		5 (8.2%)		1 (4.3%)	
Hypertension (HT)	0		7 (11.5%)		3 (13%)	
Asthma	2 (3.3%)		1 (1.6%)		1 (4.3%)	
DM and HT	3 (5%)		12 (19.7%)		2 (8.7%)	
Chronic or allergic rhinosinusitis	9 (15%)	0.765	8 (13.1%)	0.247	1 (4.3%)	0.182
Length of hospital stay (mean±SD, days)			6.4±3.2	0.001	11.6±4.9	

p^1 Group Mild vs. Group Severe; p^2 Group Severe vs. Group Critical; p^3 Group Mild vs. Group Critical

(n=16) were male (p<0.001). Of the 54 patients with olfactory dysfunction, 53 patients also had gustatory dysfunction at the same time (98.2%).

Olfactory Dysfunction Features

Mean duration of olfactory dysfunction was as follows: In the mild group, it was 10.2±4.9 days; in the severe group, it was 9.2±2.2 days (p=0.623). Olfactory dysfunction started as a first symptom; in the mild group, it was 57.1% (n=18); in the severe group, it was 57.1% (n=12).

The other symptoms started 3.8±0.8 days later in the mild group; 4.7±1.7 days later in the severe group (p=0.17).

Olfactory dysfunction started with other COVID -19 symptoms: in mild group, it was 16.1% (n=5); in severe group, it was 19% (n=4). Olfactory dysfunction started after the other COVID -19 symptoms: in mild group, 25.8% (n=8); in severe group, it was 23.8% (n=5).

5.8% (n=3) patients in the mild group had olfactory dysfunction without other symptoms.

In the accompanying neurological symptom evaluation, 19.8% of the patients had a headache, 19.8% tinnitus, 15.9% dizziness, 11.1% ocular discomfort and 2.4% hearing loss. Although no statistically significant difference was detected, hearing loss, tinnitus, and dizziness were more in the severe and critical group. In all groups, olfactory loss with patients and related general and neurological symptoms are shown in Figures 1 and 2.

Discussion

This study had two major findings: Olfactory loss is the most prominent feature of COVID-19's mild clinical course and the majority of patients with loss

Table 2. Symptoms in the groups

	Mild Group n=60	p^1 value	Severe Group n=61	p^2 value	Critical Group n=23	p^3 value
Olfactory dysfunction	39 (65%)	0.008	25 (41%)	0.005	2 (8.7%)	<0.001
Gustatory dysfunction	40 (66.7%)	0.013	27 (44.3%)	0.023	4 (17.4%)	<0.001
Fever	21 (35%)	0.298	27 (44,3%)	0.517	12 (52.2%)	0.152
Dyspnea	16 (26.7%)	0.882	17 (27.9%)	0.323	4 (17.4%)	0.377
Cough	12 (20%)	0.004	27 (44.3%)	0.058	5 (21.7%)	0.861
Fatigue	18 (30%)	0.274	13 (21.3%)	0.390	3 (13%)	0.112
Myalgia	23 (38.3%)	0.909	24 (39.3%)	0.000	0	0.000
Appetite loss	6 (10%)	0.004	19 (31.1%)	0.034	2 (8.7%)	0.857
Nausea	10 (16.7%)	0.094	18 (29.5%)	0.476	5 (21.7%)	0.591
Diarrhea	11 (18.3%)	0.021	3 (4.9%)	0.913	4 (4.3%)	0.105
Throat pain	1 (1.7%)	0.054	6 (9.8%)	0.119	0	0.533
Nasal congestion	3 (11.7%)	0.523	5 (8.2%)	0.157	0	0.087
Headache	18 (30%)	0.024	8 (13.1%)	0.068	0	0.003
Ocular discomfort	1 (1.7%)	0.003	11 (18%)	0.945	4 (17.4%)	0.007
Tinnitus	9 (15%)	0.497	12 (19.7%)	0.293	7 (30.4%)	0.111
Hearing loss	0	0.157	2 (3.3%)	0.814	1 (4.3%)	0.104
Dizziness	7 (11.7%)	0.454	10 (16.4%)	0.568	5 (21.7%)	0.243

p^1 Group Mild vs. Group Severe; p^2 Group Severe vs. Group Critical; p^3 Group Mild vs. Group Critical

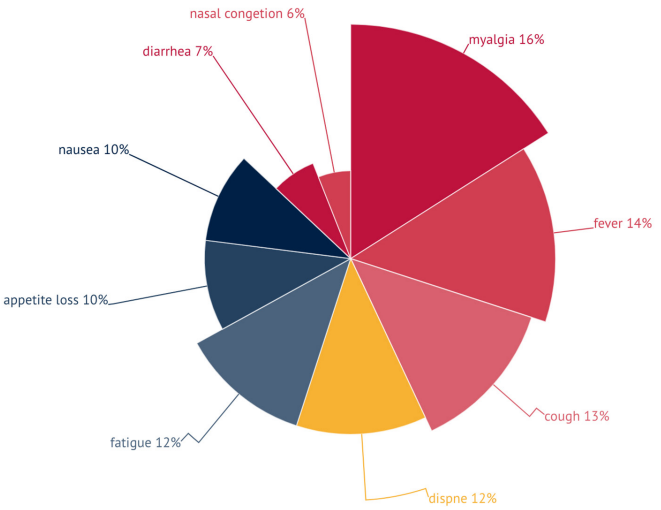

Figure 1. Olfactory dysfunction with symptoms

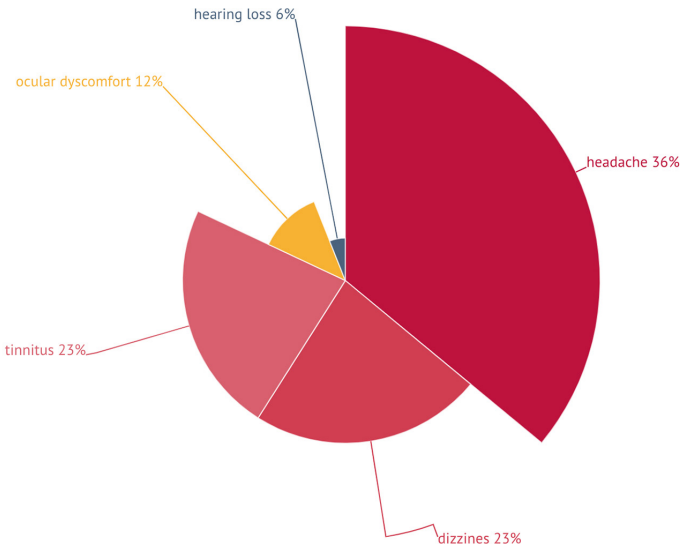

Figure 2. Olfactory dysfunction with neurological symptoms

of smell are female and young patients.

According to the current literature, in the presence of olfactory symptoms, patients recover from mild symptoms.[9,10] Studies report that 59 to 86% of COVID-19 positive patients receiving outpatient treatment have an olfactory loss, whereas, in inpatients, this rate is (5-35%).[4,5,12] Clues are beginning to accumulate that anosmia is not only a clinical finding in COVID-19 disease, but is also directly related to the clinical process of the disease.[6-8]

In the previous studies conducted in China, it has been reported that the frequency of COVID-19 infection varies depending on gender.[13,14] COVID-19 is more common in men and has a more severe clinical condition, whereas COVID-19-related olfactory loss is more common in women, and the disease has a milder course in these patients.[4,15,16]

The factors that investigate the severity of the clinical course in COVID-19 seem to be a viral load in addition to personal factors, such as age and comorbidity.[17] In a study where the viral load was measured immediately after the symptoms started, the viral load was reported at a higher rate in the nose than in the throat.[18] In addition, the viral load of severe cases was, on average, 60 times higher than mild cases, suggesting that higher viral loads may be associated with severe clinical outcomes.[17] More severe cases are often older and have risk factors, such as comorbidity. In addition, clinically mild cases show more olfactory dysfunction, and patients with severe clinics have less olfactory dysfunction, indicating that the effects of viral load alone are not decisive for olfactory dysfunction. Of course, it is possible that patients with a more severe clinical course do not notice changes in their sense of smell and taste. However, interestingly, in some patients, changes related to smell and taste may emerge before other signs of the disease. Then, they are more likely to notice these changes. While a viral load of SARS-CoV-2 might be a useful marker for assessing disease severity and prognosis, we do not yet know the meaning of this for the olfactory function.[17] However, the nasal respiratory epithelium has a higher expression of CoV-2 entry genes than the respiratory epithelium of the trachea or lungs.[19] Although it seems that the decrease in the expression of viral receptors due to changes in the nasal and olfactory epithelium with age, the cell entry and replication of the virus is disadvantageous, local immune responses decreasing with age may be related to less damage in the olfactory area.[20,21] This may be the inability to limit the virus with the disruption of the first-line antiviral immune response, leading to viremia. Moreover, as the age progresses, the "immunosenescence", including decreased immune responses, plays a role in the innate and acquired immune system, possibly causing increased infections and more severe consequences of infections.[22,23]

Conclusions

In the present study, the olfactory loss is less reported in patients with severe and critical diseases. Olfactory dysfunction and clinical status seem to be the projection of local and systemic immune responses.

Conflict of interest

All authors declare that they have no confict of interest.

Authors' Contribution

Study Conception: OS, AA, DETS, ANS, MNG; Study Design: OS, AA, DETS, ANS, MNG; Supervision: AA, NY, BY; Funding: OS, EA, AI, OAD; Materials: OS, EA, AI, OAD; Data Collection and/or Processing: OS, EA, AI, OAD; Statistical Analysis and/or Data Interpretation: OS, DETS, ANS; Literature Review: OS, DETS, ANS, NY, BY; Manuscript Preparation: OS, DETS; and Critical Review: OS, DETS, NY, BY.

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TURKISH JOURNAL OF INTERNAL MEDICINE
ISSN:2687-4245
Original Article

The Relationship Between Thyroid Ultrasonography and Cytopathology

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Abstract

Background Thyroid fine needle aspiration biopsy (FNAB) is a method performed under ultrasound guidance for diagnosis. The nodule is described according to EU-TIRADS (European Thyroid Imaging and Reporting Data System). FNAB results are classified according to Bethesda system. The aim of this single center retrospective study was to investigate which EU-TIRADS groups had no malignancy based on FNAB results.

Material and Methods Ultrasonography and pathology reports of the patients whom FNAB was performed at the State Hospital between January 2016 and December 2018 were reviewed. 251 patients (201 female, 50 male) who were over 18 years of age (mean age 52.62±12.29) were included. Distribution of EU-TIRADS categories by Bethesda Classification was shown. Numbers and percentages, means, and standard deviation, minimum and maximum for variables were used for descriptive statistics. The level of significance was set at $p<0.05$.

Results Of the 7 cases in Bethesda group V, which were 'suspicious for papillary carcinoma', 42.9% were in EU-TIRADS-5 and 57.1% were in EU-TIRADS-4. None of the EU-TIRADS-2 were in the Bethesda IV, V and VI groups. EU-TIRADS category 4 and 5 ($p=0.003$) and Bethesda category V ($p=0.008$) were significantly higher in the papillary carcinoma diagnosed group as a result of thyroid surgery.

Conclusions With larger number of cases, it can be investigated whether it will be considered safe to follow-up the cases in EU-TIRADS-2 group without applying FNAB.

Turk J Int Med 2021;3(2):62-72
DOI: 10.46310/tjim.822949

Keywords: *thyroid, ultrasonography, biopsy, needle, pathology*

Received: December 1, 2020; Accepted: January 17, 2021; Published Online: April 29, 2021

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Introduction

The European Thyroid Association describes thyroid nodules as space-occupying lesions in the thyroid gland which can be distinguished sonographically from environmental parenchyma.[1]As a result of the widespread use of ultrasonography, the incidence of nodules and malignancies in the thyroid has increased. Thyroid nodules can be detected in 19-68% of randomly selected individuals and are more common in women and the elderly.[2,3] Thyroid fine needle aspiration biopsy (FNAB) results are classified according to the Bethesda system.[4] The prevalence of thyroid nodules in healthy adults is 20–76%.[5] In ultrasonography, the characteristics of the suspicious nodule are determined by the European Thyroid Imaging and Reporting Data System (EU-TIRADS) category developed by the European Thyroid Association.[1,6] The ultrasonography report should include the size of the nodule, its location, structure, echogenicity, calcification, margin and shape, halo presence, colloid content and vascularity, and if any, lymph nodes should also be interpreted. EU-TIRADS categories are: No thyroid nodule is found in EU-TIRADS 1 (Normal); there are pure/ anechoic cysts or entirely spongiform nodules in EU-TIRADS 2 (benign); oval shape, smooth margins, isoechoic or hyperechoic, without any feature of high risk are seen in EU-TIRADS 3 (Low-Risk); oval shape, smooth margins, mildly hypoechoic, without any feature of high risk in EU-TIRADS 4 (Intermediate-Risk); there are nodules with at least 1 of the following high-risk features: non-oval shape, irregular margins, microcalcifications, and marked hypoechogenicity in EU-TIRADS 5 (High-Risk).

The Bethesda System for Reporting Thyroid Cytopathology was first published in 2009 to provide standardization in thyroid cytopathology results and was renewed in 2017.[4] It is recommended to follow-up the patients who had benign cytology at FNAB. Malignancy rate was found to be 54.2% in cases of Bethesda III/IV class who subsequently underwent surgical intervention.[7]

The aim of this study was to investigate which EU-TIRADS groups had no malignancy as a result of FNAB

Material and Methods

In this retrospective study, the hospital records between January 2016 and December 2018 were examined. A total of 251 patients (201 women, 50 men) who had a thyroid FNAB older than 18 years of age (range:18-85 years) were included in the study. Before the biopsy procedure, the patients were questioned for contraindications (anticoagulant use or anxiety not to allow FNA). Informed consent of patients were obtained. FNAB was performed under ultrasound guidance with a 22 Gauge spinal needle using a 20 cc injector. The radiologist sit in front of the screen of the ultrasound equipment, on the right side of patient. The patient was placed supine with the neck hyperextended during the procedure. High-resolution 7.5-14 MHz linear-array transducer was used. The needle tip was placed within the target nodule, 4-5 passes with a negative suction was applied. After FNAB, hemostasis was achieved, after a while bleeding control was done with control USG. The patients were observed for a while and discharged. 1 drop of aspirated material was forced onto several glass slides and smears are prepared by using a second glass slide. The slides were fixed immediately in 95% alcohol. The cytological specimens were stained with the dyes of PAP and Hematoxylin and eosin stain (HE). The FNAC samples were double-read by two experienced pathologists.

Thyroid ultrasonography findings and pathology reports were examined. In the pathology report, the size of the nodule which FNAB obtained was mentioned. Those who were previously diagnosed with thyroid malignancy were excluded.

Nodules were categorised according to EU-TIRADS. Gender ratio and average age for each group were found. Ultrasonography and pathology results were compared according to gender.

The Bethesda System for Reporting Thyroid Cytopathology was used to categorise thyroid fine-needle aspiration (FNA) specimens: (I) nondiagnostic or unsatisfactory; (II) benign; (III) atypia of undetermined significance (AUS) or follicular lesion of undetermined significance; (IV) follicular neoplasm or suspicious for a follicular neoplasm; (V) suspicious for malignancy; and

(VI) malignant.[4]

Regional Ethics Committee's Approval and informed consent of the patients were obtained (23.07.2019, 1362).

Statistical Analysis

Statistical analysis was performed using SPSS 15.0 software (SPSS Inc, Chicago, IL, USA).

Percentage, frequency, distribution were used to determine the distribution of patients according to

Table 1. Age and gender distribution of patients by EU-TIRADS categories, Bethesda Classification, and having a thyroid surgery condition

			Gender n (%)		Age		
A: Age							
Mean.±SD (min-max)		52.6±12.3 (18-85)					
B: Gender		n (%)					
Male		50 (19.9%)					
Female		201 (80.1%)					
C: EU-TIRADS Categories		n (%)	F	M	min	max	mean±SD
	2	4 (1.6)	1 (25.0)	3 (75.0)	49	73	59.3±10.0
	3	204 (81.3)	164 (80.4)	40 (19.6)	18	84	52.4±12.2
	4	37 (14.7)	30 (81.1)	7 (18.9)	35	85	54.6±12.0
	5	6 (2.4)	6 (100.0)	0 (0.0)	29	62	43.2±14.5
D: Bethesda Classification							
	I	32 (12.7)	26 (81.3)	6 (18.8)	30	85	53.7±12.0
	II	180 (71.7)	141 (78.3)	39 (21.7)	18	84	52.8±12.5
	III	31 (12.4)	26 (83.9)	5 (16.1)	27	72	51.9±11.0
	IV	1 (0.4)	1 (100.0)	0 (0.0)	39	39	39.0
	V	7 (2.8)	7 (100.0)	0 (0.0)	29	63	47.4±13.4
	VI	0 (0)	0 (0)	0 (0)	-	-	-
E: Whether had a thyroid surgery							
	A	179 (91.3)	144 (80.4)	35 (19.6)	26	85	53.8±12.4
	B	8 (4.1)	7 (87.5)	1 (12.5)	29	63	45.9±11.6
	C	8 (4.1)	6 (75.0)	2 (25.0)	32	65	48.3±9.5
	D	1 (0.5)	1 (100)	0 (0)	39	39	39.0±0
F: The group with no information about whether they have thyroid surgery		55 (21.9)	43 (78.2)	12 (21.8)	*18*	*85*	52.6±12.3

TIRADS -4: Intermediate-Risk, EU-TIRADS-5: High-Risk; Bethesda I:Nondiagnostic or unsatisfactory, II:Benign, III: Atypia of Undetermined Significance or Follicular Lesion of indetermined Significance, IV: Follicular Neoplasm or Suspicious for a Follicular Neoplasm, V: Suspicious for papillary carcinoma, VI: Malignant; Whether had a thyroid surgery A: Has not been operated, B:Operated and diagnosed with papillary carcinoma, C:Operated and diagnosed with adenomatous hyperplasia, D: Operated and diagnosed with follicular adenoma

gender, Bethesda class and EU-TIRADS category. Descriptive statistics were used to examine the age of the patients for gender, Bethesda class and EU-TIRADS category. Kruskal-Wallis H test was used to compare the largest nodule size according to the Bethesda class. Cross-tables were prepared to determine the Bethesda classes of the patients according to the EU-TIRADS category, nodule's size, shape, echogenicity, margins, colloid content, vascularity, and thyroid section where the nodule was located. Descriptive statistics, numbers and percentages for each value of a variable, means, standard deviation, minimum and maximum for all variables. p values of <0.05 were considered statistically significant.

Results

The mean age was 52.62±12.29. Age and gender distribution of patients by EU-TIRADS categories, Bethesda classification,and having a thyroid surgery condition are shown in Table 1, and correlation of EU-TIRADS categories with Bethesda classification is shown in Table 2. The patients were divided in groups according to whether they had thyroid surgery or not and diagnosis after tyroid surgery, according to EU-TIRADS category and Bethesda classification *(Table 3 and 4, respectively)*. The distribution of those patients who had thyroid surgery and not according to EU-TIRADS categories and Bethesda Classification are shown in Table 5 and 6 respectively. The gender distribution and correlation of those who underwent thyroid surgery according to EU-TIRADS categories and Bethesda classification are shown at Table 7. Correlation between the findings on EU-TIRADS categories with Bethesda classification is shown at Table 8.

There were no results in the Bethesda class VI. 42.9% of the 7 cases in the Bethesda class V (Suspicious for papillary carcinoma) were in the EU-TIRADS-5 and 57.1% were in the EU-TIRADS-4 categories. The only case in the Bethesda IV class was in the EU-TIRADS-3 category. None of those in EU-TIRADS-2 category were in Bethesda classes V and VI *(Table 2)*.

No information could be obtained in 55 of 251 patients whether they had thyroid surgery or not. Of the 196 patients whose information

Table 2. Correlation of EU-TIRADS categories with Bethesda Classification

		EU-TIRADS Categories				
n (%)		**2**	**3**	**4**	**5**	**Total**
Bethesda Classification	**I**	2 (50.0%)	21 (10.3%)	8 (21.6%)	1 (16.7%)	32 (12.7%)
	II	1 (25.0%)	154 (75.5%)	23 (62.2%)	2 (33.3%)	180 (71.7%)
	III	1 (25.0%)	28 (13.7%)	2 (5.4%)	0 (0.0%)	31 (12.4%)
	IV	0 (0.0%)	1 (0.5%)	0 (0.0%)	0 (0.0%)	1 (0.4%)
	V	0 (0.0%)	0 (0.0%)	4 (10.8%)	3 (50.0%)	7 (2.8%)
	VI	-	-	-	-	-
	Total	4 (1.6%)	201 (80.1%)	37 (14.7%)	6 (2.4%)	251

EU-TIRADS: Thyroid Imaging Reporting and Data System, EU-TIRADS-2: benign, EU-TIRADS-3: Low-Risk, EU-TIRADS-4: Intermediate-Risk, EU-TIRADS-5: High-Risk; Bethesda Classification I: Nondiagnostic or unsatisfactory, II: Benign, III: Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance, IV: Follicular Neoplasm or Suspicious for a Follicular Neoplasm, V: Suspicious for papillary carcinoma VI: Malignant.

Table 3. The distribution of the groups formed according to whether the patients had thyroid surgery and the diagnosis of those who underwent thyroid surgery, according to EU-TIRADS category

		EU-TIRADS Categories			
n (%)		**2**	**3**	**4**	**5**
Whether had a thyroid surgery	Has not been operated	2 (100%)	154 (94.5%)	21 (80.8%)	2 (40.0%)
	Papillary carcinoma	0 (0.0%)	1 (0.6%)	4 (15.4%)	3 (60.0%)
	Adenomatous hyperplasia	0 (0.0%)	7 (4.3%)	1 (3.8%)	0 (0.0%)
	Follicular adenoma	0 (0.0%)	1 (0.6%)	0 (0.0%)	0 (0.0%)
	No information	2 (50.0%)	41 (20.1%)	11 (29.7%)	1 (16.7%)

EU-TIRADS-2: Benign, EU-TIRADS-3: Low-Risk, EU-TIRADS-4: Intermediate-Risk, EU-TIRADS-5: High-Risk

Table 4. The distribution of the groups formed according to whether the patients had thyroid surgery and the diagnosis of those who underwent thyroid surgery, according to Bethesda Classification

		Bethesda Classification					
n (%)		**I**	**II**	**III**	**IV**	**V**	**VI**
Whether had a thyroid surgery	Has not been operated	25 (100%)	135 (95.1%)	19 (86.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Papillary carcinoma	0 (0.0%)	1 (0.7%)	1 (4.5%)	0 (0.0%)	6 (100%)	0 (0.0%)
	Adenomatous hyperplasia	0 (0.0%)	6 (4.2%)	2 (9.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Follicular adenoma	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100%)	0 (0.0%)	0 (0.0%)
	No information	7 (21.9%)	38 (21.1%)	9 (29.0%)	0 (0.0%)	1 (14.3%)	0 (0.0%)

Bethesda Classification I: Nondiagnostic or unsatisfactory, II: Benign, III: Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance, IV: Follicular Neoplasm or Suspicious for a Follicular Neoplasm, V: Suspicious for papillary carcinoma, VI: Malignant

Table 5. The distribution of those who had not thyroid surgery by EU-TIRADS Categories and Bethesda Classification

		n (%)
Gender	M	35 (19.6)
	F	144 (80.4)
EU-TIRADS Categories	2	2 (1.1)
	3	154 (86.0)
	4	21 (11.7)
	5	2 (1.1)
Bethesda Classification	I	25 (14.0)
	II	135 (75.4)
	III	19 (10.6)

F: Female, M: Male; EU-TIRADS-2: Benign, EU-TIRADS-3: Low-Risk, EU-TIRADS-4: Intermediate-Risk, EU-TIRADS-5: High-Risk; Bethesda I: Nondiagnostic or unsatisfactory, II: Benign, III: Atypia of Undetermined Significance or Follicular Lesion of indetermined Significance

Table 6. The distribution of those who had thyroid surgery by EU-TIRADS Categories and Bethesda Classification

		n (%)
Gender	M	3 (17.6)
	F	14 (82.4)
EU-TIRADS Categories	3	9 (52.9)
	4	5 (29.4)
	5	3 (17.6)
Bethesda Classification	II	7 (41.2)
	III	3 (17.6)
	IV	1 (5.9)
	V	6 (35.3)

F: Female, M: Male; EU-TIRADS-2: Benign, EU-TIRADS-3: Low-Risk, EU-TIRADS-4: Intermediate-Risk, EU-TIRADS-5: High-Risk; Bethesda I: Nondiagnostic or unsatisfactory, II: Benign, III: Atypia of Undetermined Significance or Follicular Lesion of indetermined Significance

Table 7. The distribution of those who underwent thyroid surgery according to EU-TIRADS Categories, Bethesda Classification and gender

		Diagnosis of Malignancy in Pathological Examination After Surgery				
		Yes		No		
		n	%	n	%	p
Gender	M	1	12.5	2	22.2	1.000
	F	7	87.5	7	77.8	
EU-TIRADS Categories	3	1	12.5	8	88.9	0.003
	4	4	50.0	1	11.1	
	5	3	37.5	0	0.0	
Bethesda Classification	II	1	12.5	6	66.7	0.008
	III	1	12.5	2	22.2	
	IV	0	0.0	1	11.1	
	V	6	75.0	0	0.0	

was available, only 17 of them had undergone thyroid surgery. Of these patients, 8 had papillary carcinoma, 1 had follicular adenoma, 8 had adenomatous hyperplasia.

Of the 8 patients diagnosed with papillary carcinoma, 6 of them had a suspicion of papillary carcinoma in FNAB, one patient was a 55-year-old female patient with a diagnosis of atypia of indeterminate significance in FNAB, classified in Bethesda III and EU-TIRADS 3 categories, with a nodule of 17×10 mm in the left lobe inferior. The other patient who was diagnosed with papillary carcinoma was a 42-year-old male patient who was in the Bethesda II and EU-TIRADS 4 category and had an isoechoic nodule with coarse calcifications in the left lobe, without atypical cells in FNAB. No information could be obtained whether 1 out of 7 patients with suspected papillary carcinoma in FNAB was operated.

One patient with follicular neoplasia in FNAB was diagnosed histopathologically as follicular adenoma.

In 8 patients who were histopathologically diagnosed as adenomatous hyperplasia after surgery, 6 had no atypical cells in FNAB, 1 was compatible with follicular lesion, 1 had atypia of indeterminate significance.

Among the patients who underwent thyroid surgery, there was a significant difference between benign and malignant cases, both EU-TIRADS categories (p=0.003) and Bethesda classifications (p=0.008). EU-TIRADS categories 4 and 5 and Bethesda category V were significantly higher in papillary carcinoma group than the others.

Discussion

Thyroid nodules are more common in women,[2] 80.1% of our cases were women.

Statistically significant malignant features include microcalcification, irregular or amorphous morphology, long but not wide shape, irregular margins, vascularity and presence of a pathological-looking lymph node.[8,9] In a retrospective cohort study, 495 nodules with a non-diagnostic result were followed-up for 2.7 years and thyroid cancer was found in 3%. The presence of nodular calcifications was the strongest predictor of thyroid malignancy. Initial nodule size was inversely associated with malignancy. Nodules

Table 8. Correlation of the findings on EU-TIRADS with Bethesda Classification

Nodule's			Bethesda					
			I	II	III	IV	V	Total
Size	0.5-0.9 cm	n	3	10	3	0	0	16
		%	(18.8%)	(62.5%)	(18.8%)	(0.0%)	(0.0%)	(100.0%)
	1.0-2.0 cm	n	20	97	16	1	6	140
		%	(14.3%)	(69.3%)	(11.4%)	(0.7%)	(4.3%)	(100.0%)
	≥ 2.1 cm	n	7	73	12	0	1	93
		%	(7.5%)	(78.5%)	(12.9%)	(0.0%)	(1.1%)	(100.0%)
	Total	n	30	180	31	1	7	249
		%	(12.0%)	(72.3%)	(12.4%)	(0.4%)	(2.8%)	(100.0%)
Composition	Solid	n	0	8	0	0	0	8
		%	(0.0%)	(100.0%)	(0.0%)	(0.0%)	(0.0%)	(100.0%)
	Predominant solid	n	1	2	0	0	0	3
		%	(33.3%)	(66.7%)	(0.0%)	(0.0%)	(0.0%)	(100.0%)
	Predominant cystic	n	13	129	22	0	1	165
		%	(7.9%)	(78.2%)	(13.3%)	(0.0%)	(0.6%)	(100.0%)
	Cystic	n	2	4	1	0	0	7
		%	(28.6%)	(57.1%)	(14.3%)	(0.0%)	(0.0%)	(100.0%)
	Total	n	16	143	23	0	1	183
		%	(8.7%)	(78.1%)	(12.6%)	(0.0%)	(0.5%)	(100.0%)
Echogenicity	Markedly hypoechoic	n	1	10	3	0	3	17
		%	(5.9%)	(58.8%)	(17.6%)	(0.0%)	(17.6%)	(100.0%)
	Mildly hypoechoic	n	1	5	3	0	0	9
		%	(11.1%)	(55.6%)	(33.3%)	(0.0%)	(0.0%)	(100.0%)
	Isoechoic	n	25	153	24	0	4	206
		%	(12.1%)	(74.3%)	(11.7%)	(0.0%)	(1.9%)	(100.0%)
	Hyperechoic	n	0	7	0	0	0	7
		%	(0.0%)	(100.0%)	(0.0%)	(0.0%)	(0.0%)	(100.0%)
	Total	n	27	175	30	0	7	239
		%	(11.3%)	(73.2%)	(12.6%)	(0.0%)	(2.9%)	(100.0%)
Margins	Irregular	n	1	2	2	0	3	8
		%	(12.5%)	(25.0%)	(25.0%)	(0.0%)	(37.5%)	(100.0%)
	Smooth	n	2	11	2	0	1	16
		%	(12.5%)	(68.8%)	(12.5%)	(0.0%)	(6.3%)	(100.0%)
	Ill-defined	n	1	1	0	0	0	2
		%	(50.0%)	(50.0%)	(0.0%)	(0.0%)	(0.0%)	(100.0%)
	Total	n	4	14	4	0	4	26
		%	(15.4%)	(53.8%)	(15.4%)	(0.0%)	(15.4%)	(100.0%)
Calcifications	Microcalcifications	n	1	5	0	0	1	7
		%	(14.3%)	(71.4%)	(0.0%)	(0.0%)	(14.3%)	(100.0%)
	Macrocalcifications	n	7	29	2	0	2	40
		%	(17.5%)	(72.5%)	(5.0%)	(0.0%)	(5.0%)	(100.0%)
	Rim calcifications	n	1	3	0	0	0	4
		%	(25.0%)	(75.0%)	(0.0%)	(0.0%)	(0.0%)	(100.0%)
	Total	n	9	37	2	0	3	51
		%	(17.6%)	(72.5%)	(3.9%)	(0.0%)	(5.9%)	(100.0%)
Colloid inclusions	Yes	n	1	14	2	0	1	18
		%	(5.6%)	(77.8%)	(11.1%)	(0.0%)	(5.6%)	(100.0%)
	No	n	0	0	29	0	6	35
		%	(0.0%)	(0.0%)	(82.9%)	(0.0%)	(17.1%)	(100.0%)
	Total	n	1	14	31	0	7	53
		%	(1.9%)	(26.4%)	(58.5%)	(0.0%)	(13.2%)	(100.0%)
Vascularity	No	n	1	3	n	0	1	5
		%	(20.0%)	(60.0%)	%	(0.0%)	(20.0%)	(100.0%)
	Peripheral	n	1	1	n	0	0	2
		%	(50.0%)	(50.0%)	%	(0.0%)	(0.0%)	(100.0%)
	Mildly intranodular	n	0	4	n	0	0	4
		%	(0.0%)	(100.0%)	%	(0.0%)	(0.0%)	(100.0%)
	Marked intranodular	n	6	15	n	0	3	24
		%	(25.0%)	(62.5%)	%	(0.0%)	(12.5%)	(100.0%)
	Total	n	8	23	n	0	4	35
		%	(22.9%)	(65.7%)	%	(0.0%)	(11.4%)	(100.0%)
Location	Right lobe	n	22	86	9	0	6	123
		%	(17.9%)	(69.9%)	(7.3%)	(0.0%)	(4.9%)	(100.0%)
	Left lobe	n	9	87	17	0	1	114
		%	(7.9%)	(76.3%)	(14.9%)	(0.0%)	(0.9%)	(100.0%)
	Isthmus	n	1	7	5	0	0	13
		%	(7.7%)	(53.8%)	(38.5%)	(0.0%)	(0.0%)	(100.0%)
	Total	n	32	180	31	0	7	250
		%	(12.8%)	(72.0%)	(12.4%)	(0.0%)	(2.8%)	(100.0%)

Bethesda I- Nondiagnostic or unsatisfactory II- Benign III- Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance IV- Follicular Neoplasm or Suspicious for a Follicular Neoplasm V- Suspicious for papillary carcinoma VI- Malignant

containing calcifications should be followed-up.10

Calcification status of the nodule was mentioned in 20.3% of our cases. 25% of patients with rim calcification were in the nondiagnostic or unsatisfactory and 75% in the 'Benign' group. Calcification was observed in 28.1% of our nondiagnostic patients and 11.1% of them were rim calcification. Malignancy was detected in 27% of those with peripheral calcification in the literature.[11]

The largest nodule size measurements of our cases did not differ significantly between Bethesda classes (p>0.05). In a retrospective cohort analysis at Boston, of those 1.0 to 1.9 cm in diameter, 10.5% were cancerous, of those >2.0 cm, 15% were cancerous, no graded increase in risk beyond the 2-cm threshold. When malignant, the proportion of papillary carcinoma decreased (nodules 1.0-1.9 cm, 92% of cases; >4 cm, 74% (p<.01).[12] In 85.7% of our 'suspicious for papillary carcinoma' cases, the nodule size was between 1.0-2.0 cm. 1,104 patients who underwent thyroid FNAB and subsequent thyroidectomy retrospectively reviewed, it was found that nodule size alone was not predictive of malignancy in patients except for Hürthle cell neoplasms.[13] As the nodule is low/intermediate/high-risk, the probability of malignancy may increase as the size of the nodule increases.[14,15]

In this study, 49.2% of the nodules were located in the right lobe, 45.6% in the left lobe and 5.2% in isthmus. Six of the 7 nodules in the group 'suspicious for papillary carcinoma'were in the right lobe and 1 in the left lobe. In the literature, it has been reported that the prevalence of malignancy in isthmus, right or left lobe was not significantly different.[16] According to the location of the nodules, the incidence of malignancy was higher in those located in the upper pole,[17] however, in our cases, no information was found about the pole where the nodule was located.

There are studies linking echogenicity and vascularity of the nodule with malignancy.[18,19,20] The majority of malignant thyroid tumors are 62.5-87.2% hypoechoic and hypoechoic nodules have a higher risk of malignancy (%20.6-70.4).[3] In our cases, 57.1% isoechoic and 42.9% markedly hypoechoic nodules were detected in the 'suspicious for papillary carcinoma' group.

Vascularity status was noted in 13.9% of our cases, 11.4% of all cases whose vascularization status was stated were 'suspicious for papillary carcinoma', and 75% of them showed significant intranodular vascularization, while 25% did not show vascularization. To draw conclusions from vascularity is not feasible as only 35 cases have been studied here. There are publications showing that there is a relationship between intranodular vascularization and malignancy.[21,22]

Irregular shapes and margins differ significantly between groups in a study compared with Bethesda II to III-IV.[23] In our study, nodule margins were determined in 10.3% of cases, 25% of patients with irregular nodule margins were 'AUSor follicular lesion of undetermined significance', and 37.5% of them were 'suspicious for papillary carcinoma'. Margins were noted in 57.1% of cases with 'suspicious for papillary carcinoma', of which 75% were irregular margins.

The presence of colloid-filled cyst on ultrasonography showed 100% benignity in a prospective study conducted among American elderly veterans.[8] In 21.1% of our cases, there was information about the colloid content of the nodule. 5.6% of patients with nodule colloid were nondiagnostic or unsatisfactory, 77.8% of patients with nodule colloid were benign, 11.1% were 'AUS or follicular lesion of undetermined significance, and 5.6% were 'suspicious for papillary carcinoma'.

In 27.1% of our cases, solid or cystic composition of the nodule was not specified in the ultrasonography report. Only 1 of the 7 cases with 'suspicious for papillary carcinoma' has been identified, and it is predominant cystic. Of the nodules whose composition was mentioned, 4.4% were solid and all of them were Bethesda II. When we searched the literature, 81.6-93% of malignant thyroid tumors were solid.[3]

In the axial plan, the definition of nodules as height>width was not made in any of our cases.

According to the Bethesda classification, 12.7% of patients were nondiagnostic or unsatisfactory. In the literature, this rate is up to 20%.24 71.7% of them were 'benign', 12.4% were of 'AUS or 'follicular lesion of undetermined significance' and were compatible with the literature.[25] Papillary thyroid carcinoma is the most common thyroid malignancy in the literature,[3] it can be found at a rate of 9.2 -13% after FNAB,26 2.8% of our cases were in the group 'suspicious for papillary carcinoma'. Other malignancies were not found.

In an article published in 2018, 184 patients were prospectively included, and malignancy risk

in EU-TIRADS was 0, 2.2, 38.5 and 77.8% in benign, low risk, intermediate risk, and high risk groups, respectively.[27]

In our study, none of the cases in the benign group in EU-TIRADS was found to be malignant as a result of FNAB (0%). In EU-TIRADS, 0.49% of those in the low risk group, 10.8% of the intermediate risk, and 50% of the high risk were found at Bethesda IV and V. The malignancy rate of the intermediate risk group is 22 times higher than that of the low risk group, and the malignancy rate of those in the high risk group is 102 times higher than the low risk group.

Conclusions

None of the cases in the 'Benign group' in EU-TIRADS were found to be malignant as a result of FNAB. By carrying out studies with larger number of cases, it can be investigated whether it will be considered safe to follow-up the cases in 'Benign' EU-TIRADS group without applying FNAB.

Conflict of Interest

All authors declare that they have no confict of interest.

Limitations

According to EU-TIRADS, it was seen that detailed information was not included in every ultrasonography report in our cases. This may be due to the excessive workload of radiologists. While we were making the statistical evaluation, we took into account what was stated in the report. Other limitations are that it is a retrospective study and the number of cases is low. There are many studies on this subject, but this findings can be considered as local data.Better results can be obtained in a prospective study with higher number of cases.

Authors' Contribution

Study Conception: AA, RK, SK, MNG; Study Design: AA, RK, SK, MNG; Supervision: AA, RK, SK, MNG; Funding: AA, RK; Materials: AA, RK, SK, MNG; Data Collection and/or Processing: AA, RK; Statistical Analysis and/or Data Interpretation: AA, RK; Literature Review: AA, RK; Manuscript Preparation: AA, RK; and Critical Review: AA, RK.

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TURKISH JOURNAL OF INTERNAL MEDICINE

ISSN:2687-4245

Original Article

Results and Adverse Effect Evaluations in Localized Prostate Cancer Patients Undergoing Intensity Modulated Radiotherapy with Tomotherapy

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Abstract

Background: The aim of this study is assess the dosimetric results and early and late adverse effects of radiotherapy with tomotherapy in localized prostate cancer patients.

Material and Methods: Treatment results and early and late adverse effects in 60 patients who had undergone curative radiotherapy due to prostate cancer and who had been followed up for at least 6 months in the post-treatment process were assessed retrospectively. 28 of the patients were in the low-intermediate risk group, whereas 32 were in the high-risk group. 74 Gy radiotherapy was delivered to the prostate with simultaneous integrated boost strategy, 60 Gy to seminal vesicles, and 52 Gy to pelvic lymph nodes of the cases. Patients with at least 6 months of post-treatment follow-up were assessed in terms of early and late adverse effects.

Results: Twenty patients had grade 1, and two patients had grade 2 acute genitourinary toxicity, whereas 15 patients had grade 1, and 4 patients had grade 2 acute gastrointestinal toxicity. Twelve patients had grade 1, and 3 patients had grade 2 late genitourinary toxicity, 6 patients had grade 1, and two other patients had grade 2, and grade 3 late gastrointestinal toxicity. Biochemical recurrence developed in four patients. One of the patients with recurrence died in the 14th month of recurrence due to organ metastasis.

Conclusions: Image-guided dose-escalated radiotherapy with IMRT technique is a reliable method in prostate cancer treatment. Increased toxicity was not observed in the cases with lymph node irradiation despite the increased radiotherapy field.

Turk J Int Med 2021;3(2):73-79
DOI: 10.46310/tjim.871471

Keywords: *Prostate cancer, Intensity modulated radiotherapy, Acute-late toxicity.*

Received: February 01, 2021; Accepted: February 28, 2021; Published Online: April 29, 2021

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Introduction

Prostate cancer is the second most frequent malignancy in males following lung cancer.[1] Radiotherapy (RT) is one of the main treatment methods of localized prostate cancer and developments in RT techniques in recent years have enabled safe application of higher doses of RT.[2] RT can be used alone for early-stage prostate cancer, whereas it is used with hormonotherapy with locally advanced tumors.[3,4]

The main purpose in RT is to reduce the radiation dose to normal tissues while increasing the dose to tumor tissue.[5] Dose escalation in prostate RT can lead to interruption of RT by increasing normal tissue toxicity and especially causing rectal and urinary adverse effects.[6] Long lasting studies on localized prostate cancer have demonstrated that there is an increasing dose-response relationship in RT.[7,8] In many studies it has been shown that better biochemical control is achieved with doses between 74-80 Gy compared to conventional doses under 70 Gy.[9-11] Toxicity, which is the most important restraint of high dose RT delivery, is controlled with developments in RT techniques. It has become possible to significantly reduce the irradiated normal tissue volume with engagement of intensity-modulated radiation therapy (IMRT) following three-dimensional conformal radiotherapy (3D-CRT).[12-14] In some studies comparing 3D-CRT and IMRT it has been observed that acute gastrointestinal (GI) and genitourinary (GU) toxicity are significantly decreased with the IMRT technique with reduction of high dose receiving volumes of bladder and rectum.[15-17]

Some recent studies have also shown that dose fractionation schemes used in RT significantly affect prostate specific antigen (PSA) control.[8,18] There are randomized trials showing that radiation therapy with increased fraction (fx) dose and reduced fx quantity (>75 Gy biologically equivalent dose) improves prostate cancer control and it has become the standard treatment for prostate cancer.[19-21] Therefore, currently high dose IMRT is the recommended standard treatment in early and locally advanced prostate cancer. It is of great importance to predict organ movements in the treatment field and to pay attention to bladder and bowel filling rates and regulation of eating habits during RT in order to increase IMRT success and reduce adverse effects.

In this study we evaluated the efficacy and early and late toxicity outcomes of localized prostate cancer patients who had undergone IMRT with a tomotherapy device using image-guided simultaneous integrated boost.

Material and Methods

60 patients who had undergone curative RT due to T1-3N0M0 stage prostate cancer diagnosis between the years 2012-2019 and who had been followed-up for at least 6 months in the post-treatment period were included in this study. All of the patients were histologically diagnosed with prostate cancer. Patients were classified in three risk groups before treatment according to D'Amico risk classification by assessing PSA value, Gleason score and T stage; low risk (T1-T2a, Gleason ≤6, PSA ≤10 ng/mL), intermediate risk (patients who are not in low or high-risk groups) and high risk (≥T2c or Gleason >7 or PSA >20 ng/mL) groups. Patients in intermediate and high-risk groups were assigned androgen deprivation therapy (ADT) comprised of LHRH and anti-androgen, 2-3 months prior to RT. Anti-androgen therapy was interrupted at the end of RT. ADT was applied for 6 months in the intermediate risk group and for 2-3 years in the high-risk group. ADT was not delivered to patients in the low-risk group.

All cases were recommended a diet therapy to avoid flatulent foods and prevent constipation and increase water consumption and physical activity. Patients' planning tomographies were scanned prior to the RT in a computed tomography (CT) simulator in supine position by fixing the patient with knee and feet supports, with a full bladder and empty rectum, covering the whole pelvis with 3 mm intervals. CT data were transferred to the treatment planning system and then clinical target volume (CTV), planned target volume (PTV) and adjacent organs at risk were identified. Three separate target volumes were created. CTV consisted of prostate and proximal seminal vesicles in the low-risk group, prostate and all seminal vesicles in the intermediate risk group and prostate and all seminal vesicles and lymph nodes in the high-risk group. Bladder, rectum and femur heads were determined as organs at risk.

Small bowel was added to organs at risk in the high-risk group to be irradiated with pelvic lymph nodes. During PTV establishment, 7 mm margins were given to CTV for each direction, whereas a 5 mm margin was given posterior. During pelvic lymph node PTV establishment, 5 mm margins were given to each direction to external, internal iliac and obturator lymph nodes referring to the iliac vessels. It was assured that the patients underwent the treatment with the same bladder and rectum volume every day.

All patients underwent IMRT with a Tomotherapy Hi art device with simultaneous integrated boost technique. Three separate target volumes were established in total. The prostate was irradiated with 74 Gy (2.24 Gy/fx), prostate + seminal vesicles were irradiated with 60 Gy (1.81 Gy/fx) and lymph nodes were irradiated with 52 Gy (1.57 Gy/fx) doses. In the planning, it was provided that 95% of PTV delivered 100% of the target dose. The whole treatment was completed at 33 fx.

Patients were called for weekly outpatient clinic control during the treatment. In the post-treatment period, the patients were evaluated with complete blood count, total PSA, biochemistry and yearly pelvic tomography in the 1st month, every 3 months within the following first 2 years, and then every 6 months. GI and GU adverse effects were graded according to Radiation Therapy Oncology Group (RTOG) toxicity scoring.

Statistical Analysis

Statistical analysis was performed with SPSS 20.0 (SPSS Inc. Chicago, IL, USA) software. Descriptive statistics of all variables in the study were calculated. Normality of the data distribution was evaluated with Shapiro-wilk test, and its homogeneity was assessed with Levene's test. Continuous variables are expressed as mean±standard deviation. For inter-group comparisons of numerical data, Kruskal-Wallis test was used for non-parametric data and student-t test was used for parametric data. Paired comparisons in case of significancy were done with Mann-Whitney U test. Categorical variables were compared with chi-square test, Pearson chi-square and Fisher's exact chi-square test. Survival analysis was performed with Kaplan-Meier survival analysis. $P<0.05$ was considered significant.

Results

Mean age of all patients was calculated as 69.19 (49-80) years. Pre-treatments mean PSA level was 31.19 (4.2-201) ng/mL and Gleason score were 7 (4-10). Clinical features of the cases by risk groups were shown in Table 1.

Mean follow-up time of the cases was 36.36 (6-96) months. It had been observed that in the dose volume histogram, at least 95% of the PTV volume of all cases received 100% of the target dose, whereas in the adjacent organ doses, an excess of less than 15% was detected in 3 patients

Table 1. Patient characteristics by risk groups

	Low-Intermediate risk	High risk
Age (mean-years)	68.07 (52-80)	70.28 (49-79)
Clinical T1-T2b (n)	24	4
T2c-T3	0	32
Mean Gleason score	5.89 (4-7)	7.66 (5-10)
Mean PSA (ng/mL)	11	48.86
Androgen suppression treatment (n)	23 (6 months)	32 (2-3 years)
Disease free survival (months)	35.66	37.48
Overall survival (months)	39.64	43.55

in the low-intermediate risk group and in 7 patients in the high-risk group in V40 criteria for the bladder. Mean V40 value for the bladder was 41.3 (17-57) and mean V65 value was 13.6 (3-21). V40 value for the rectum exceeded less than 20% in 4 patients in the low-intermediate risk group and 7 patients in the high-risk group. Mean V40 and V65 values for the rectum were respectively 44.6 (33-59.6) and 12.4 (4-27). Femur head mean doses were 23.08 Gy for the right femur head and 23.24 Gy for the left femur head.

In general, the treatment was well tolerated. As acute adverse effects, 5 patients in the low-intermediate risk group (17.8%) experienced grade 1 and 1 patient (3.5%) grade 2 GI toxicity; 10 patients in the high-risk group (31.2%) experienced grade 1 and 3 patients (9.3%) grade 2 GI toxicity. The most frequent GI toxicity was proctitis. An increase in preexisting hemorrhoidal complaints was observed in 2 patients. As acute GU adverse effect, grade 1 toxicity was observed in 8 patients in the low-intermediate risk group (28.5%), grade 1 toxicity in 12 patients in the high-risk group (37.5%) and grade 2 toxicity in 2 patients (6.2%) in the same group. The most frequent GU toxicities were pollakiuria, nocturia and dysuria. Symptoms regressed by using alpha blockers, anti-inflammatory medications and spasmolytic agents in the treatment. Grade 3 and higher acute toxicity were not detected. Statistically no significant difference was detected in terms of toxicity in any of the groups. Hematologic toxicity was not observed in any patient. Acute adverse effect rates by risk groups are shown in Table 2.

In late adverse effect evaluation, grade 1 GI toxicity was observed in 2 patients (7.1%) and grade 3 GI toxicity was observed in 1 patient (3.5%) in the low-intermediate risk group, whereas in the high-risk group, grade 1 GI toxicity developed in 4 patients (12.5%) and grade 2 GI toxicity was seen in 1 patient (3.1%). Grade 3 toxicity was not observed. As GU adverse effects, grade 1 toxicity developed in 5 patients (17.8%) and grade 2 toxicity was observed in 3 patients (10.7%) in the low-intermediate risk group, whereas in the high-risk group 7 patients (21.8%) experienced grade 1 toxicity, however grade 2 and greater toxicity was not observed in this group. In the whole group, grade 1 toxicity was observed in 12 patients (20%) and grade 2 in 3 patients (5%). Late adverse effects by risk groups are shown in Table 3.

During the follow-up period of the patients, PSA recurrence developed in a total of 4 patients by the 31st month on average. All patients with recurrence were in the high-risk group. Follow-up of one patient with recurrence continued at an external center. This patient was declared excitus in the post-recurrence 14th month due to organ

Table 2. Acute Adverse Effect Rates by Risk Groups

	Acute Gastrointestinal			Acute Genitourinary		
Grade	1	2	3	1	2	3
Low-Intermediate risk	5 (17.8%)	1 (3.5%)	0	8 (28.5%)	0	0
High risk	10 (31.2%)	3 (9.3%)	0	12 (37.5%)	2 (6.2%)	0
Total	15 (25%)	4 (6.6%)	0	20 (33.3%)	2 (3.3%)	0

Table 3. Late Adverse Effect Rates by Risk Groups

	Late Gastrointestinal			Late Genitourinary		
Grade	1	2	3	1	2	3
Low-Intermediate risk	2 (7.1%)	0	1 (3.5%)	5 (17.8%)	3 (10.7%)	0
High risk	4 (12.5%)	1 (3.1%)	0	7 (21.8%)	0	0
Total	6 (10%)	1 (1.6%)	1 (1.6%)	12 (20%)	3 (5%)	0

metastasis. There was no organ metastasis in the other 3 patients during recurrence and androgen deprivation treatment was started. One of these patients received chemotherapy for hormone refractory prostate cancer during the follow-ups. Follow-up of the other two patients still continues with ADT.

Discussion

Several studies have demonstrated that treatment applied in prostate cancer RT with IMRT is superior to 3-dimensional conformal RT in terms of local, biochemical control and adverse effect aspects, and high dose IMRT application in prostate cancer RT has become a standard treatment method.[22-24] Therefore, accurate evaluation of treatment-related toxicities is essential for clinicians.[25]

Zelefsky et al.[26] have reported acute grade 2 and grade 3 GU toxicity rates of 28% and 0.1%, and acute grade 2 GI toxicity rate of 4.5% in 772 prostate cancer cases who received high doses with IMRT (81 Gy-86.4 Gy). Grade 2 and greater rectal toxicity have not been reported in this study. In the randomized trial performed by Pollack et al.[27] comparing IMRT applying 76 Gy and hypo-fractionated 70.2 Gy, in multivariate analysis the combined rectal DVH parameter of V65 Gy/V50 Gy for GI toxicity and bladder volume for GU toxicity was significant.

In the study of Ozdemir et al.[28] consisting of 101 patients in which they delivered a median of 76 Gy with IMRT/VMAT, grade 1 GU adverse effects developed in fifty-seven (56.4%) patients and grade 2 GU adverse effects developed in three (3%) patients. In this study, grade 1 GI adverse effects were observed in 15 (15%) patients. Grade 2 and greater GI early adverse effects were not reported in any of the cases.[28]

In this study, we evaluated the results of localized prostate cancer patients who underwent image guided IMRT with tomotherapy. Our fraction dose was higher than conventional fractionation (2.24 Gy/fx) and our total length of treatment period was 6.5 weeks. Acute grade 1 and grade 2 GU toxicity was 33.3% and 3.3%, respectively. Grade 3 and greater acute toxicity were not detected.

According to the randomized dose escalation trial (68 Gy-78 Gy) of Heemsbergen et al.[29] conducted in the Netherlands, 28% late rectal toxicity was reported. Massive rectal bleeding occurred in 6% of these patients. In the study of Al-Mamgani[30], grade 2 and greater early GI toxicity after 78 Gy in prostate cancer cases was reported as 20%. In this study, conformal RT and IMRT therapies have been compared and bladder dose reduction has been provided by IMRT. However, similar adverse effect rates have been reported in both groups. This situation was associated with similar urethra doses with both techniques.[30]

In some studies, it has been demonstrated that bladder, rectum and small bowel doses can be significantly reduced with IMRT in cases with pelvic lymph node irradiation.[31,32] In a study evaluating 230 high risk prostate cancer patients, significantly less grade 2 GI and grade 3 GU adverse effects were observed in the group treated with IMRT with respect to four-field delivery. In multivariate analysis, bladder fullness was found as the dominant factor determining acute GI adverse effect.[34]

In another randomized trial, hypo-fractionated dose-escalated intensity-modulated radiation therapy (HIMRT) and conventional fractionated intensity-modulated radiotherapy (CIMRT) were compared. Patients were randomly assigned to 75.6 Gy with 1.8 Gy/fx delivered over 8.4 weeks (CIMRT) or 72 Gy with 2.4 Gy/fx fractions delivered over 6 weeks (HIMRT). In this trial, 10.7% recurrence was observed in HIMRT and 15.4% recurrence was observed in CIMRT. In terms of toxicity, GU toxicity rates were similar with both techniques, whereas there was a non-significant increase in late grade 2 and 3 GI toxicity with HIMRT. A lower rectal toxicity was reported when the rectal volume receiving 65 Gy of HIMRT was ≤15.[34]

In our study, statistically no significant difference was detected in terms of adverse effects between the high risk group irradiated in pelvic lymph nodes and the low-intermediate risk group, which was not irradiated ($p>0.05$). This situation is related to the possibility to reduce normal tissue doses provided by IMRT despite larger field of irradiation in patients assigned to pelvic nodal irradiation. This advantageous

situation in IMRT planning requires a sensitive accuracy of daily fractions. Otherwise, planned target volume doses may decrease or adjacent organ doses may increase.

Conclusion

We observed that image-guided dose-escalated IMRT with tomotherapy is well tolerated in prostate cancer treatment. In terms of early and late adverse effects, our results are within acceptable limits compatible with the literature. Moreover, an increase in adverse effects has not been observed in pelvic lymph node irradiation patients despite enlargement of the RT field. Therefore, dose-escalated RT can be safely applied in localized prostate cancer treatment. Long-term studies are needed in terms of late adverse effects.

Conflict of Interest

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

Authors' Contribution

Study Conception: GAO, MO; Study Design: GAO, MO; Supervision: GAO, MO; Funding: GAO; Materials: GAO; Data Collection and/or Processing: GAO, MO; Statistical Analysis and/or Data Interpretation: GAO, MO; Literature Review: GAO, MO; Manuscript Preparation: GAO, MO; and Critical Review: GAO, MO.

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TURKISH JOURNAL OF INTERNAL MEDICINE
ISSN:2687-4245
Case Report

Markedly Elevated Lipase as The First Manifestation of Celiac Disease: A Case Report

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Abstract

Lipase is a hydrolytic enzyme and commonly used for the diagnosis of pancreatitis with amylase. Except for pancreatitis, lipase is elevated in many clinical conditions such as hepatobiliary disorders, bowel diseases, malignancies, renal impairment. Celiac disease (CD) should be considered as one of the causes. In patients with CD, the frequency of pancreatic hyperenzymemia and possible pathophysiological mechanisms are not well studied. To date, several mechanisms explaining pancreatic hyperenzymemia in CD are reported. Malnutrition, disease bowel induced pancreatic dysfunction, autoimmune pancreatic inflammation, and macroenzymemia are the main ones. Herein, we report a patient with newly diagnosed CD, representing markedly elevated serum lipase level with normal amylase.

Turk J Int Med 2021;3(2):80-84
DOI: 10.46310/tjim.814732

Keywords: *celiac disease, hyperlipasemia, macrolipasemia, pancreatic hyperenzymemia*

Introduction

Lipase is an enzyme that catalyzes the breakdown of triglycerides into glycerol and free fatty acids. It is produced by various cells in many organs such as the pancreas, liver, bowel, tongue, and stomach. Lipase is mostly found in the pancreas and crucial test for the diagnosis of pancreatitis. Except pancreatitis, serum lipase levels may also increase in a wide range of conditions, including renal impairment, hepatobiliary disorders, gastroduodenal perforations and ulcers, bowel necrosis and obstruction, certain neoplasms, critical illness, and other diseases such as diabetic ketoacidosis and celiac disease (CD).[1]

Celiac disease is a small bowel disorder characterized by mucosal inflammation, villus atrophy, and crypt hyperplasia dependent on gluten ingestion. The mainstay of the treatment is a gluten-free diet, which concerns most of the

Received: October 28, 2020; Accepted: January 14, 2021; Published Online: April 29, 2021

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patients' social life. Therefore, diet adjustment is commonly problematic—gluten tolerability changes from person to person.[2] Although the time to the beginning of patients' treatment response is different, clinical improvement is observed in two weeks for 70%.[3] Trace elements, vitamin levels, and serological markers are used for patient follow-up.[4] The level of anti-tissue transglutaminase IgA antibody reaches optimum for 75% of patients having a gluten-free diet in almost one year.[5] The other indicator for an appropriate gluten-free diet is intestinal fatty acid-binding peptide, which was of working response to treatment quicker than any serological marker.[6]

Although the digestive tract is the main target organ, CD may present with extraintestinal manifestations and atypical laboratory findings such as pancreatic hyperenzymemia.[7,8] The frequency of pancreatic hyperenzymemia (PH) in CD is not well-known, but CD is suggested to be on the checklist of differential diagnosis in case of unexplained hyperenzymemia.[8,9] Isolated elevation of lipase is a rare manifestation in celiac disease.[8] Herein, we report a CD representing markedly elevated serum lipase levels.

Case Report

A 45-year-old female patient was admitted to the state hospital with abdominal pain. Laboratory evaluation revealed hyperlipasemia, but serum amylase level was within the normal range. In computed tomography (CT) imaging of the pancreas, enlargement of the pancreas was observed, and there were no inflammatory changes, peripancreatic fluid collections, or necrosis. She was diagnosed with acute pancreatitis and treated for 20 days. Notwithstanding treatment, the level of serum lipase remained high. Therefore, he was referred to the gastroenterology department of Bursa Uludag University.

The patient complained of fatigue and slight abdominal pain persisting for six months while taking her detailed history. She did not smoke, get any medications, or use alcohol. Likewise, she did not report nausea, vomiting, or diarrhea. Physical examination showed neither rebound nor tenderness. Laboratory examination revealed markedly elevated serum lipase, normal amylase (upper limit of normal range), minimal elevation of liver enzymes, and slight anemia consistent with iron deficiency *(Table 1)*. The levels of serum glucose, creatinine, triglyceride, calcium, total bilirubin, and C-reactive protein were all in normal range (92 mg/dL, 0.79 mg/dL, 148 mg/dL, 9.4 mg/dL, and 0.33 mg/dL, respectively). The amylasetocreatinine clearance ratio was 1.5.

Further laboratory studies revealed normal serum levels of tumor markers (CEA, AFP, CA125, CA15-3, CA19-9) and immunoglobulins (IgA, IgG, IgM, IgG4). Hepatitis B, C, and HIV were negative. Magnetic resonance cholangiopancreatography and CT imaging

Table 1. Laboratory findings at diagnosis and after one year of the gluten-free diet

	At the time of diagnosis	After one-year gluten-free diet	Normal range
AST	33	18	11-25 IU/L
ALT	32	15	7-28 IU/L
Amylase	124	85	25-125 IU/L
Lipase	**943**	39	8-78 IU/L
Hemoglobin	**11.9**	13.3	12.20-18.0 g/dL
ESR	**57**	40	0-32 mm/h
Ferritin	**14.5**	44	4.63-204 ng/mL
IgA-anti-tG	**266**	8	<20 EU/mL

ESR: erythrocyte sedimentation rate, **IgA-anti-tG**: anti-tissue transglutaminase A

of the abdomen were performed to exclude pancreatic disease, hepatobiliary disorders, and malignancy. Imaging studies revealed no pathological findings. Endoscopic examination revealed that duodenal mucosa appeared atrophic with loss of fold and had a nodular appearance. Then, anti-tissue transglutaminase A and G were studied and found positive at high titer *(Table 1)*. Human leukocyte antigen (HLA) typing was positive for HLA-DQ2. On histopathological examination of duodenal biopsy, increased intraepithelial lymphocytes, mucosal atrophy was observed, immunohistochemical C3 and C8 staining revealed intraepithelial lymphocytosis *(Figure 1)*.

The patient was diagnosed with CD and started on a gluten-free diet (GFD). After a strict one-year GFD, serum lipase level decreased into the normal range *(Table 1)*.

Discussion

Increased serum lipase level is commonly an expression of pancreatic disease. Nevertheless, a wide range of clinical conditions, including CD was reported in previous publications.[1,9] To our knowledge, we present the first case that had more than ten-fold isolated lipase elevation as the first manifestation of CD.

To date, there are a few conflicting reports on the frequency of PH in CD. Carrocio et al.[8] reported that 40 of 202 newly diagnosed CD patients had elevated pancreatic enzymes, and in 14 of them (6.9%), the isolated elevation of lipase

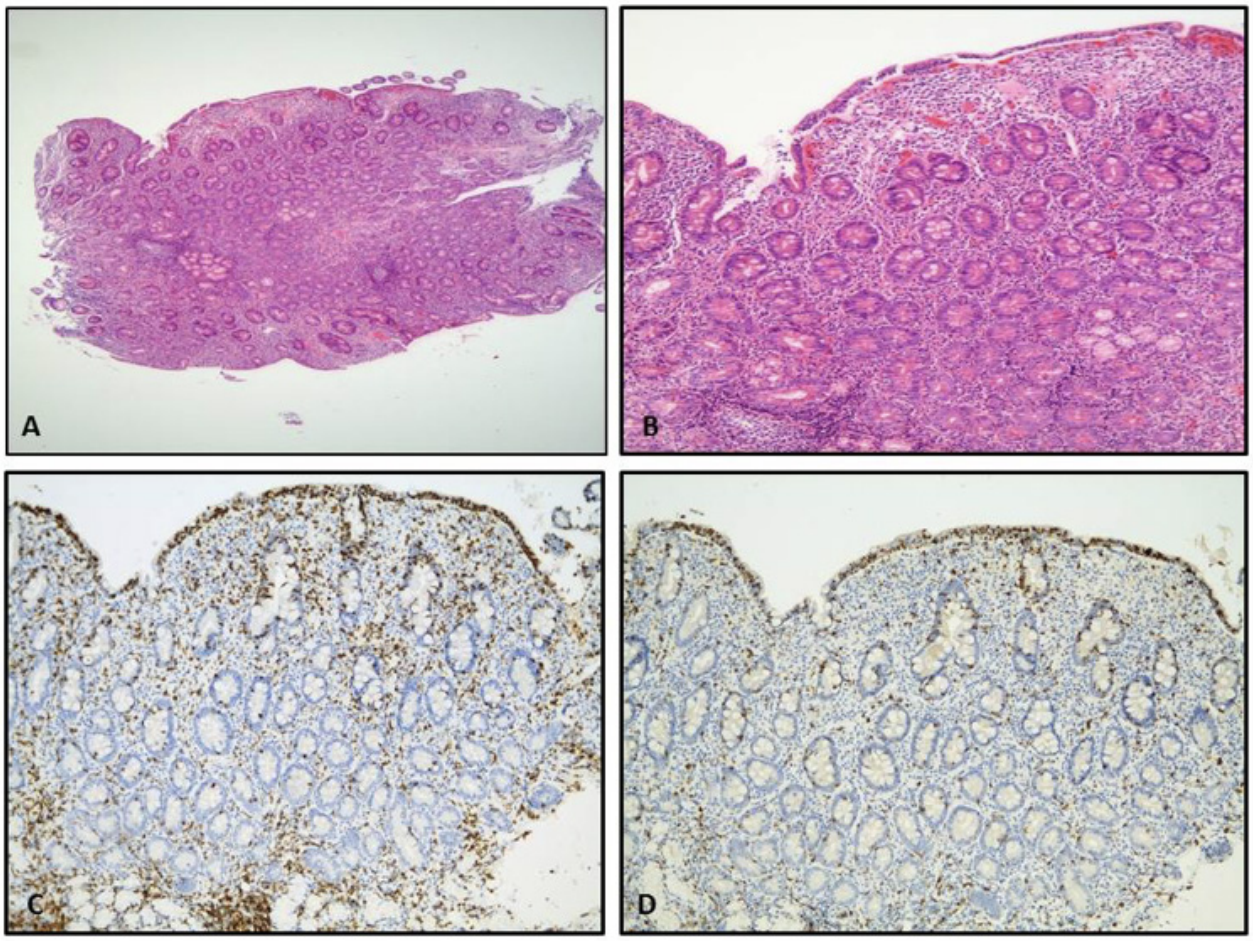

Figure 1A. Duodenal biopsy shows a flat lesion with villous atrophy (hematoxylin-eosin staining, x40). **Figure 1B.** The hematoxylin-eosin staining section shows increased intraepithelial lymphocytes (x100). **Figure 1C**. The immunohistochemical CD3 staining section demonstrates the intraepithelial lymphocytosis in the flat lesion (x100). **Figure 1D.** Increased intraepithelial lymphocytosis is also present in the section of CD8 immunohistochemical staining (x100).

was observed. In contrast, Migliori et al.[10] reported that the search for CD in their 65 subjects with benign pancreatic hyperenzymemia was negative.

The pathophysiological mechanisms explaining PH in patients with CD is not well studied. Several mechanisms underlying PH in CD were reported. Malnutrition-induced low-grade pancreatic inflammation was published as one of the mechanisms.[11,12] Malabsorption of critical nutrients causes reduced production of pancreatic enzyme precursors and protein malnutrition, altering the pancreatic structure. Another mechanism is diseased bowel induced pancreatic dysfunction. Chronic inflammation in the small bowel in CD results in alterations in neuroendocrine cells, and it causes the impaired secretion of the pancreas stimulating hormones. Also, chronic inflammation may cause mechanical obstruction by papillary scaring. Subclinical autoimmune pancreatic damage was speculated to be one of the reasons for hyperenzymemia.[12,13] Many autoimmune disorders such as autoimmune hepatitis, primary biliary cirrhosis, diabetes mellitus, autoimmune thyroid disease, rheumatoid arthritis, psoriasis, pancreatitis accompany CD. Th1-associated cytokines (interferon-gamma and interleukin-18) are increased in both CD and autoimmune pancreatitis. Therefore, autoimmune pancreatic inflammation may be observed in patients with CD. The formation of macroenzymes (macroamylasemia and macrolipasemia) is another mechanism to explain hyperenzymemia in CD.[14,15] Serum amylase and lipase are bound to other macromolecules like immunoglobulins and escape glomerular filtration, resulting in decreased renal clearance and elevated serum levels.

Among asymptomatic celiac patients, pancreatic hyperenzymemia was reported.[16] Since clinical manifestations are limited for these 'silent' patients, serum pancreatic enzyme levels may help to monitor an appropriate gluten-free diet.[16] Considering the underlying possible physiopathological mechanisms, normalization of pancreatic enzyme level with diet might have prognostic value due to the demonstration of intestinal and pancreatic inflammation.

In conclusion, multiple factors can cause isolated hyperlipasemia, and CD should be considered in the differential diagnosis. To understand the relation between CD and pancreatic hyperenzymemia, mainly the frequency and pathophysiological mechanisms, further studies with a high number of patients can be helpful.

Conflict of Interests

Authors declare that there are none.

Authors' Contribution

Study Conception: ABS, MG; Study Design: ABS, MG; Supervision: ABS, MG, NU; Funding: ABS, NU; Materials: ABS, NU, FCH; Data Collection and/or Processing: ABS, NU; Statistical Analysis and/or Data Interpretation: ABS, FCH; Literature Review: ABS, FCH; Manuscript Preparation: ABS, FCH; and Critical Review: MG, NU.

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