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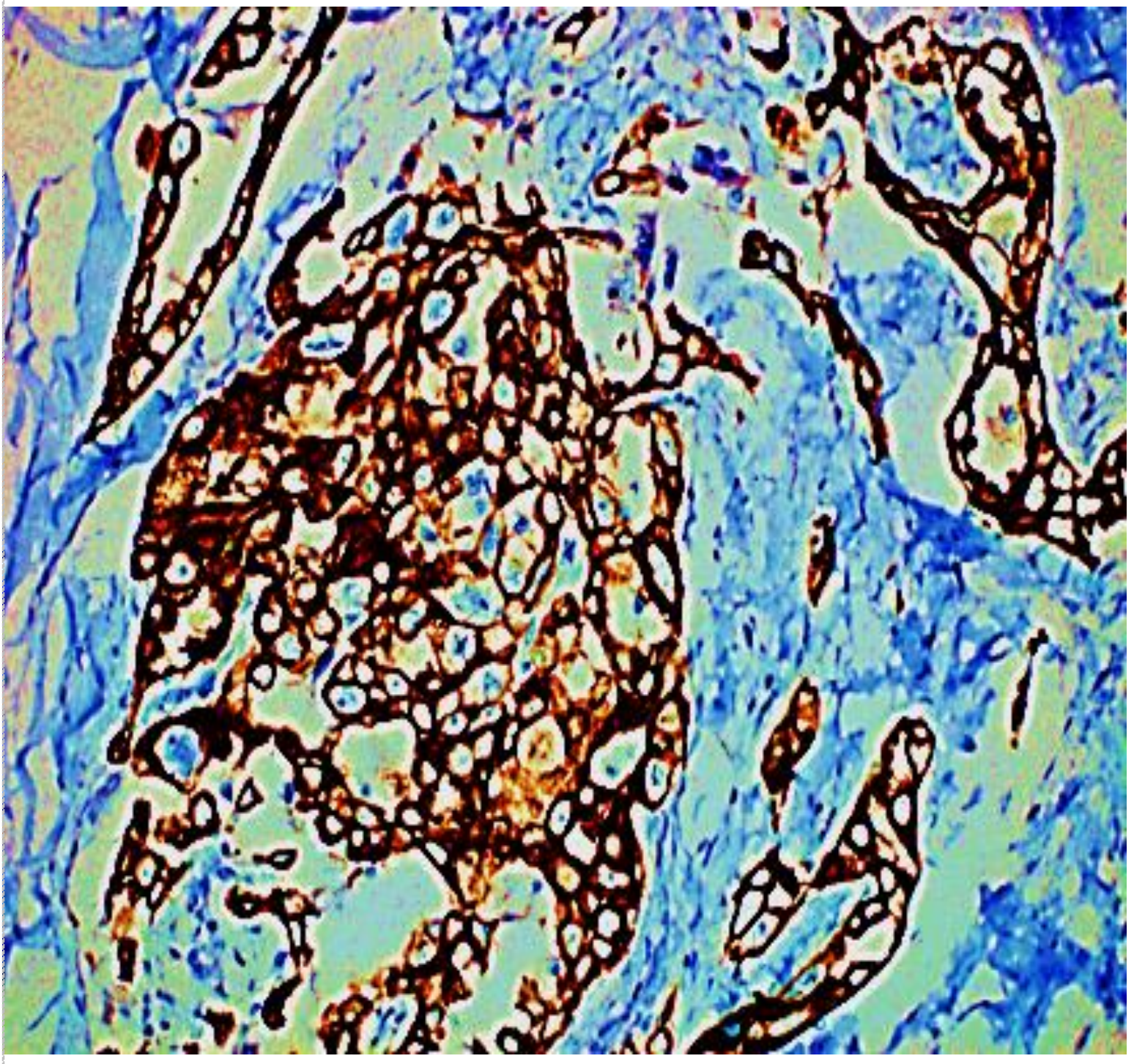

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Speech characteristics of parkinson disease

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ABSTRACT

Objective: Parkinson Disease (PD) is known the second most frequent neurodegenerative age-related disorder after Alzheimer's disease. Although over the six million people worldwide suffer from PD, the main cause of the disease remains are unknown. Speech and language impairments have emerged in most patients with PD during the course of the disease. However, clinical profiles or characteristics that might differentiate individuals with PD who are predisposed to speech and language deficits are generally overlooked. Moreover, factors that expedite language disability have still been remained elusive. It is thought that the awareness of speech and language impairments in PD can significantly help to maintain language abilities as the disease progresses and also may contribute to improving communication skills with patients. For this reason, the present study aims to constitute a comprehensive frame for the speech and language characteristics of individuals with PD.

Keywords: Parkinson disease, language impairments, speech characteristics

INTRODUCTION

PD is one of the most common neurodegenerative chronic disorder characterized by progressive loss of dopaminergic neurons, primarily in the substantia nigra pars compacta (1). The disease is particularly featured by motor symptoms such as bradykinesia, rigidity, resting tremor, postural instability, gait problems, and non-motor symptoms like sleep problems, anxiety, and cognitive impairments (2).

PD diagnosis is generally performed according to the certain criteria which depends on the clinical symptom assessment, but these clinical symptoms do not occur until loss of dopaminergic neurons reaches a level of 60–80% [3,4]. Therefore, in some cases clinical evaluation may be inadequate or lead to misdiagnosis, in particular, at the onset of the disease. On the other hand, there is no exact procedure for a definitive diagnosis of PD. However, in recent studies, it has been shown that speech can be used as an early marker for identification of PD (2).

In the literature, it was determined some important speech manifestations showing the early development of PD which can be summarized as vocal changes, poor articulation, trembling or hoarseness, frequency changes, degraded sound quality, lower tone, decreased rhythm and tonal changes (3-5). Although these manifestations are considered as one of the cardinal symptoms of PD, there is a limited number of studies about speech characteristics of the disease. Therefore, the present study aims to draw a comprehensive frame for the speech characteristics of individuals with PD.

Speech Features in PD

Speech is a complex mechanism, and it arises from sequential or synchronous integration of the central nerve system. The integration of signals sent during the speech is provided with neuromuscular coordination of peripheral level of brain functions. Damage to the motor speech areas of the brain results a number of speech disorders. Diagnostic features of patients with neurological dysfunction are generally seen in the form of difficulty in word finding, impaired speech, prolonged speech time or pronunciation ambiguities. By using these characteristic features, practical research can be done to the patients performing via neurological applications and also it can be investigated the relationship between protatic structure and speech deformation.

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In this manner, coordination anomalies in the neuromuscular system are reflected in the patients' speech in the form of nasal emission, the spread of sound quality to the nasal cavity, the neglect of the voice, the skipping of the voice, the substitution of another voice and vocal disorders (6).

As it is well known speech impairments are quite common in patients with PD. Especially, spontaneous speech is severely reduced. Also, "making verbal communication slower and less accurate, and deficits in verb inflection, verbal fluency, and verb generation" are often seen in this disease (1). As a matter of fact, the prevalence of speech impairments among patients with PD have been reported to be as high as 89% (4). In one of the oldest epidemiology studies made by Atarachi and Uchida (1959), it was found to be speech disturbances in more than two-thirds of individuals suffering from PD (7). Likewise, nowadays as well, it is stated that approximately half of all patients with PD display some kind of speech impairments (8). In particular, dysarthria which is one of the types of speech impairments comes into prominence, because it is accepted as a universal data in PD (3). However, it is stated in the literature more than 90% of patients with PD suffer from dysarthria (5).

In the earliest studies about dysarthria, a number of researchers tried to make a rather wide-ranging neurological classification of this speech impairment. First of all, Zentay (1937) suggested three subdivisions based on neuroanatomical involvement consisting of "fronto-ponto-cerebellar, cortico-striato-pallido-rubro-bulbar-extra-pyramidal and cortico-bulbar areas" (9). Another researcher Foreschel (1943) offered a different classification which comprised of "pyramidal, extrapyramidal, frontopontine and cerebellar distinctions" (10). Also, Brain (1948) made a classification that encompassed "upper motor neurons, corpus striatum, and lower motor neuron lesions, myopathies, and disorders of coordination" (9). Drawing on previous classifications of dysarthrias, in one study which was aimed to define speech impairments in populations with subcortical damage, Peacher (1950) attempted to establish a relationship between features of dysarthria and central and peripheral nervous system lesions. As a result of the study, the author reported that "pure dysarthria", which means misarticulation alone, seldom takes place with central nervous system lesions, but is more often encountered after peripheral nervous system lesions (10).

The acoustics of dysarthric speech reflect the anatomical and physiological changes caused by PD (11). At first, the emerged changes in the respiratory system influence the vocal intensity of the individuals with PD (12). As a second, the emerged changes in the phonatory system mainly affects the vibratory rhythm of the vocal folds, which causes the fundamental frequency increase and prosody alteration (11). Finally, the articulatory system is influenced as well, causing in articulatory inaccuracies and an increase in the number of pauses (13). Although changes in voice quality and intensity are accepted as common early symptoms of dysarthric speech, and this can be detected in the pre-diagnosis process (14), the articulation changes are more likely to emerge in the later stage of the stage (15). On the other hand, a majority number of the patients with PD are unaware that their voice is weak, nor that the voice progressively weakens unless they make a continuous effort during speech (11). In the literature, depending on the location of neurological damage, different types of dysarthria have been classified such as flaccid dysarthria, spastic dysarthria, ataxic dysarthria, hypokinetic dysarthria, hyperkinetic dysarthria, unilateral upper motor neuron dysarthria and mixed dysarthria (3).

Among the types of dysarthria, hypokinetic dysarthria, defined as a slow, weak and monotonous speech, involving motor dysfunctions in respiration, articulation, phonation, resonance, and prosody (11, 16), and characterized by "reduced vocal loudness, monotone, reduced fundamental frequency range, consonant and vowel imprecision, breathiness and irregular pauses" is observed quite often in PD (17, 18). As a matter of fact, it has been reported nearly 90% of patients with PD have hypokinetic dysarthria (19). In one study made by Fabbri et al. (2017) and conducted on 27 patients with late-stage PD, it was found to be 89% of patients with PD have tendency to produce hypokinetic dysarthria as characterized by "imprecise articulation, prosody abnormalities, disturbance of speech rate, and low vocal volume" (20). On the other hand, Whitfield et al. (2017) demonstrated the impaired coordination of speech production in hypokinetic dysarthria is parallel to impaired motor planning in PD such as muscle rigidity, bradykinesia and tremor (21). However, a great number of neuroimaging research have focused on the mechanism of hypokinetic dysarthria in PD. In one fMRI study carried out by Arnold et al. (2014) it was found to be significant correlation between hypokinetic dysarthria and pathomechanism of PD. As a result of the study, authors reported that hypokinetic dysarthria originates from dysfunction in the basal ganglia motor loop which causes deficiencies in the regulation of amplitude, initiation and velocity of movement (22). Likewise, in a PET study Pinto et al. (2004) showed that hypokinetic dysarthria in patients with PD is associated with functional anomalies in the "basal ganglia, orofacial motor cortex, and cerebellum, together with an increased recruitment of premotor and prefrontal cortices during speech production" (23).

Language Production in PD

Language production is formed with a combination of many processes included basic language functions and general cognitive functions, as well. The etiology of language impairments in PD is not definitive; however, a great number of studies have focused on an association with handicapped cognition (1). Besides, while in some studies it was hypothesized that language skills in individuals suffer from PD correlate with cognitive abilities (24, 25), in other studies more specifically it was connected language impairments to working memory and executive function (19). Furthermore, clinical observations have recommended that language performance in patients with PD can be affected from some degree of depression. In one early study conducted by Starkstein et al. (1989), it was reported that PD patients with major depression performed significantly worse than non-depressed PD patients on all parts of neuropsychological function tests, comprising language tasks (26). However, in another study made by Costa et al. (2006), it was not found to be differences in language function among PD patients with minor depression or major depression and the controls (27). As a result, to our knowledge, there is no consensus on how depression affects the language functions. Although language

impairments are seen common in PD, not all patients are affected, even over the long-term progression of the disease (28). It is pointed out in the literature that "clinical profiles or features that might differentiate patients who are predisposed to language impairment have been generally overlooked, and factors that precipitate language disability still remain elusive" (1).

Studies about language production in PD are relatively rare. However, multidisciplineer studies have been carried out and various methodologies are employed in these studies to evaluate the language production skills in PD from different perspectives. In one study conducted by Illes et al (1988) aimed to examine language production in PD in terms of acoustic and linguistic perspective. In the study, speech rate, fluency, syntactic complexity, lexical production, and the relative distribution of content and grammatical phrases were evaluated in 10 patients with PD and 10 age-matched controls while reading the "Grandfather" passage and producing spontaneous speech. As a result of the study, authors stated that the language production of patients with PD differed both acoustically and linguistically from healthy older adults. Also, they demonstrated the speech disfluency in PD group. However, authors reported to be significant correlation between syntactic complexity and PD severity, as evaluated by the Webster scale (29). In another study, Murray and Lenz (2001) investigated the language production in conversational discourse of 10 persons with PD, 9 with Huntington's disease, and 17 controls. As a result of the study authors reported no impairments in syntax among those with PD. However, they remarked the significant positive relationships between degree of dementia, length of utterances and sentence complexity in the PD group (30).

Verbal fluency deficits have also been studied in PD. In one meta-analysis study Henry and Crawford (2004) stated to be significant impairment on measures of both semantic and phonetic fluency in patients with PD. In addition, authors reported the semantic fluency deficit seems to be significantly larger than the phonetic deficit and independent of cognitive speed and effortful retrieval, pointed out that semantic memory is particularly impaired in PD (31). On the other hand, in an interesting study examining spontaneous speech in bilingual individuals with PD conducted by Zanini et al. (2010), it was found that patients with PD exhibited more grammatical errors than the controls, only for their first and not their second language (24).

As a consequence of the study, authors suggested that "first language is more likely to reflect implicit, procedural processing and hence more likely to engage basal ganglia structures, which are impaired with PD. In contrast, a participant's second language is more likely to reflect explicit processing and hence more likely to engage neocortical structures" (24). More recently, in a fMRI study made by Coleman et al. (2009), it was reported a more specific grammatical impairment in PD, a deficit in terms of producing a correct verb form, whereas noun production was not impaired (32).

Prosodic abnormalities are often recognized to be present in the speech of individuals with PD (33). In one study, Pell et al. (2006) pointed out that prosodic disturbances appear early in the disease course of PD and may be present prior to diagnosis in some cases (34). In another study, Kent and Rosenbek (1982) investigated the acoustic speech features of patients with prosodic disturbances due to a variety of central nervous system lesions. As a result of the study, authors summarized the acoustic findings associated with reduced prosody in PD as reflecting an overall "reduction of acoustic contrast or detail" (35). Also, Pell and Leonard (2005) examined the ability of older adults with and without PD to recognize emotions from prosody, facial expressions, and verbal cues. In the study, patients with PD were found to be significantly impaired in their ability to notice emotions based exclusively on prosody (36).

However, authors reported that patients' ability to specify emotions in other modalities (i.e., facial expression, verbal cues) was comparatively preserved, suggesting that prosodic decoding of emotions seems to be differentially influenced in PD. Interestingly, specifying the emotion of disgust from prosody was particularly impaired in individuals with PD (36). Lloyd (1999) also studied prosodic perception features of patients with PD. Findings demonstrated that nondemented PD patients exhibited the impaired ability to accurately identify utterance prosody and displayed deficits for utterance prosody comprehension. As a result of the study, author suggest that verbal and emotional nuances, such as a subtle change in tone of voice or facial expression, may go undetected by patients with PD (37).

Pragmatic abilities, which refers to the social language skills that people use in their daily interactions with others, are impaired in PD. Despite reported evidence, the neuropsychological picture of the pragmatic profile of PD is still unclear (38). Impairment of pragmatic abilities in PD has been for long studied in relation with attentional, short-term memory, and executive deficits (39).

An early report investigating pragmatic production deficits in PD was conducted by McNamara and Durso (2003), in which, patients with PD and control group engaged in brief conversations with a member of the research team. In the study, conversations were coded using the pragmatic checklist. Scheme for classifying social language skills were determined in terms of verbal features such as topic selection, topic maintenance, lexical variation, paralinguistic features such as prosody, vocal quality, and nonverbal features such as gaze, and gestures. As a result of the study, it was reported that patients with PD were impaired on 20.4% of the items relative to the controls. In addtition, both patients with PD and controls did not vary significantly on measures of mental status or verbal fluency. Authors commented these findings that the pragmatic disturbance was not simply reducible to global cognitive deficits or poverty of speech (40). In another study, it was demonstrated the specific pragmatic difficulties in patients with PD, in terms of reduced spontaneous speech production, poor conversational appropriateness, prosody impairment or slowness in processing speed (29). However, there is still lacks a comprehensive description of the pragmatic profile characteristic of PD in the literature. Furthermore, some important aspects that could be associated with pragmatic abilities in PD have been neglected. As a result of this, it can be said, from a clinical perspective, little is known about factors that might help to maintain pragmatic abilities in patients with PD as the disease progresses.

CONCLUSION

Most patients with PD develop speech and language impairments during the course of the disease. In the literature, it was determined some important speech and language manifestations showing the early development of PD. However, clinical profiles or features that might differentiate patients who are predisposed to speech and language deficits are generally overlooked, moreover, factors that precipitate language disability still remain elusive. It is thought that awareness of these speech and language deficits in PD can greatly help to maintain language abilities as the disease progresses and also may contribute to improve communication skills with patients. Therefore, multidisciplineer studies such as collaboration of neurology, software engineering and linguistics are required to diagnosis and treatment of speech and language disorders.

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Investigation of association between ABO blood groups and COVID-19 clinical severity

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ABSTRACT

Objective: COVID-19 has been detected in Turkey since March 11, 2020. Istanbul has become an important center of the pandemic in Turkey. Various risk factors for COVID-19 infection, mortality, and morbidity are under investigation. Recent studies have suggested that certain blood groups are risk factors for the disease. The aim of this study is the evaluation the relationship between blood groups and the risk of contracting COVID-19 disease, clinical severity of the disease, and CT (computed tomography) imaging findings.

Material and Methods: Age, gender, blood group data, clinical severity and CT images of 300 patients who were positive with RT PCR (Reverse transcription-polymerase chain reaction) and were followed up in the clinic were retrospectively scanned and recorded. The clinical severity of the disease and CT imaging findings were scored, and the data were evaluated statistically.

Results: While the incidence of COVID-19 was high in the A blood group, it was low in the 0 blood group. Although there was no significant difference between blood types and clinical severity, the involvement in the B blood group was more severe on CT imaging.

Conclusion: People with A blood group should pay more attention to protection and isolation. Investigating this difference and underlying pathogenic mechanisms can guide science with advanced studies.

Keywords: COVID 19, Blood Group, Clinical Severity, CT imaging

INTRODUCTION

Introduction: COVID-19 emerged in 2019 in Wuhan, China (1). It is an enveloped RNA beta coronavirus. It has phylogenetic similarity to severe Middle East respiratory syndrome (MERS) and acute respiratory syndrome (SARS) coronavirus. While the disease can be asymptomatic, it can cause acute respiratory distress with symptoms such as myalgia, fatigue, dyspnea, cough, fever, and death. CT imaging findings are important in diagnosis and follow-up. In recent studies, it has been suggested that the ABO blood group is associated with virus infection, and people with different blood groups as genetic markers have different susceptibility to the virus and the risk of catching it (2). In our study, the data of the patients who were found to be positive for RT PCR in our hospital were scanned, and the blood group distribution was compared with the normal population. Turkish Red Crescent 5-year data was used (3). Clinical severity with the blood group of the patients; It is aimed to evaluate statistically by comparing blood group and CT imaging findings.

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MATERIAL and METHODS

The files of the patients whose COVID-19 RT PCR test was positive and followed up in the COVID clinic were retrospectively scanned. Three hundred cases were selected. Blood type, age, gender, presence of comorbidity, clinical severity, and CT imaging findings were analysed.

The clinical severity of the patients was scored.

1 = Asymptomatic or outpatient group (Mild)

2 = Inpatient group (moderate)

3= Group of patients requiring intensive care, intubated or deceased (severe)

The image (CT) was scored.

1= Patient group that does not require CT or has mild pneumonia findings,

2= Patient group with typical findings of ground glass appearance in the lung

3= Group of patients with fibrosis and cobblestone appearance, pleural effusion or pericardial effusion in addition to the ground glass appearance of the lung

PCR method: Nasopharyngeal viral samples were collected and evaluated. ORF and N gene regions were studied using primers (Coyote Bioscience test kit, CHINE) and RT PCR method (CFx96 Biorad) device (4).

Statistical analysis; NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used for statistical analysis. While evaluating the study data, Kruskal Wallis test was used to compare the descriptive statistical methods (median, mean, ratio, frequency, maximum, minimum, standard deviation) as well as the groups of three or more that did not show normal distribution in comparison with groups of three or more.

Fisher Freeman Halton test was used to compare quantitative data and qualitative data. Statistically significant results were evaluated at levels of $p < 0.05$.

RESULTS

The distribution of the blood groups of the cases according to the demographic data is given in **Table 1**. In the comparison of the blood groups of the cases and the distribution of the normal population blood groups, A RH (+) blood group (p=0.003) is more common in COVID 19 cases than in the normal population. 0 RH (+) blood group was seen less frequently (p=0.001) (**Table 2, 3**).

There was no significant difference in the blood groups of the cases in terms of age and gender (**Table 4**). The clinical severity of the cases and the presence of comorbidity were not associated with blood type. However, CT imaging findings showed a higher severity of lung involvement in B blood group (**Table 5**).

Table 1: The distribution of descriptive characteristics of the cases

Age (year)	*Min-Max (Median)*	20-97 (61,5)
	Mean±SD	59,45±17,52
Sex	Female	139 (46,3 %)
	Male	161 (53,7%)
ABO blood group	A (Anti B)	156 (52,0%)
	B (Anti A)	48 (16,0%)
	AB (NA)	24 (8,0%)
	O (Anti A+Anti B)	72 (24,0%)
Rh	Rh (-)	35 (11,7%)
	Rh (+)	265 (88,3%)
CT imaging Score	Mild	61 (20,3%)
	Moderate	209 (69,7%)
	Severe	30 (10,0%)
Clinical Score	Mild	42 (14,0%)
	Moderate	169 (56,3%)
	Severe	89 (29,7%)
Comorbidity	Negative	105 (35,0%)
	Positive	195 (65,0%)

Table 2. The ABO blood group distribution in patients with COVID-19 and normal population.

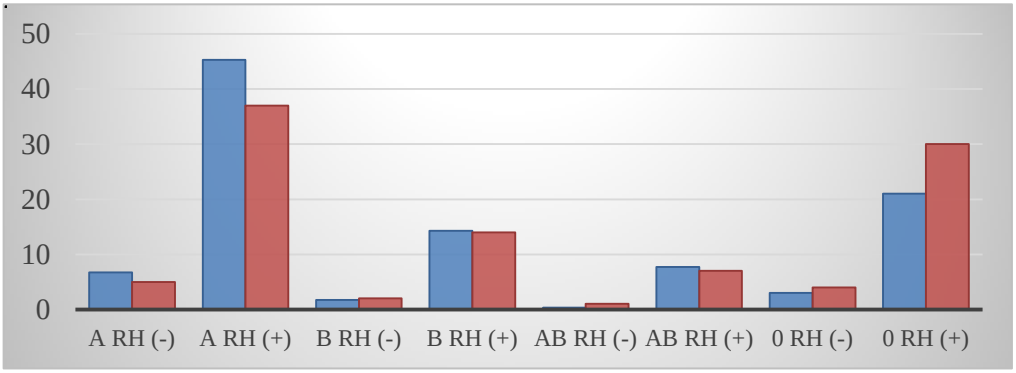

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Table 3. The ABO blood group distribution in patients with COVID-19 and normal population.

	COVID-19 CASES (%)	General in Turkey (%) (5 millon)	P value
A RH (-)	6,7	5,0	*0,185*
A RH (+)	45,3	37,0	*0,003***
B RH (-)	1,7	2,0	*0,680*
B RH (+)	14,3	14,0	*0,868*
AB RH (-)	0,3	1,0	*0,382*
AB RH (+)	7,7	7,0	*0,651*
O RH (-)	3,0	4,0	*0,377*
O RH (+)	21,0	30,0	*0,001***

[b]Pearson Chi-Square Test [c]Fisher Freeman Halton Test **p<0,01

Table 4. Comparison of blood groups with age and gender

		Blood Group A	B	AB	O	Test value P
Age (year)	*Min-Maks (Medyan)*	21-96 (64)	21-97 (63,5)	28-90 (58,5)	20-97 (60)	F:0,390
	Ort±Ss	59,27±19,11	61,67±16,22	59,13±14,10	58,49±15,90	[a]*0,761*
Gender	Female	72 (46,2)	23 (47,9)	12 (50,0)	32 (44,4)	χ^2:0,283
	Male	84 (53,8)	25 (52,1)	12 (50,0)	40 (55,6)	[b]*0,963*

[a]Oneway ANOVA [b]Pearson Chi-Square Test

Table 5. CT imaging score, clinical score and comorbidity comparison according to blood groups

		Blood Group A n (%)	B n (%)	AB n (%)	O n (%)	Test value p
CT imaging Score	Mild	43 (27,6)	5 (10,4)	4 (16,7)	9 (12,5)	χ^2:14,179
	Moderate	103 (66,0)	35 (72,9)	17 (70,8)	54 (75,0)	[c]*0,023**
	Severe	10 (6,4)	8 (16,7)	3 (12,5)	9 (12,5)	
Clinical Score	Mild	26 (16,7)	1 (2,1)	4 (16,7)	11 (15,3)	χ^2:8,470
	Moderate	83 (53,2)	30 (62,5)	12 (50,0)	44 (61,1)	[b]*0,206*
	Severe	47 (30,1)	17 (35,4)	8 (33,3)	17 (23,6)	
Comorbidity	Negative	54 (34,6)	13 (27,1)	10 (41,7)	28 (38,9)	χ^2:2,280
	Positive	102 (65,4)	35 (72,9)	14 (58,3)	44 (61,1)	[b]*0,516*

[c]Fisher Freeman Halton Test

DISCUSSION

In our study, in accordance with other studies, high rate of blood group A and low rate of blood group 0 were detected in cases followed up with COVID-19 (2,5,6). According to a meta-analysis study covering the years 2019 and 2020; The relationship between blood types and COVID 19 varies according to different regions and races. However, it has been found that people with 0 blood group are less susceptible to COVID-19, and people with A blood group are more susceptible. It has been emphasized that people with blood group A should provide better personal protection (2). Zhao et al. reported that case fatality rates are high in the A blood group (5). However, in our study, no relationship was observed between clinical severity and blood group, while those with B blood group in CT imaging scoring had more severe lung involvement. Zietz M. et al. found that Rh positivity was seen at a high rate in blood groups, but it was not associated with comorbidity (6). In our study, Rh positivity is already high in the normal population and no significant relationship was found with the disease.

There are many possible reasons why the risk is higher in blood group A. In cases with type A blood, extra sugar N-acetyl galactosamine is found on the cell surface. This excess sugar is not in group 0. The presence of extra galactosamine is thought to be effective in catching the virus(7). In addition, the relationship of the COVID-19 spike protein with the ACE 2 receptor has been shown (8,9). There is no anti-A in blood group A. Anti A and spike protein are thought to compete in binding to the cell ACE 2 receptor, so those who are Anti A positive have a lower risk of disease. Cross duplication was detected in the 3p21.31 chromosome region. SLC6A20 encodes a known interaction partner with ACE2 at 3p21.31. The coupling signal at 9q34 is located at the ABO blood group locus, and this blood group-specific analysis demonstrated a high risk of developing the disease in A-positive individuals (10). Recent studies with flow cytometry have determined that the predominant isotype of anti-A and anti-B immunoglobulin is IgM in the serum of people with blood group A and B, and IgG is the predominant isotype of anti-A immunoglobulin in people with blood group 0 (11).

In conclusion, the presence of anti-A antibodies and more specifically IgG anti-A in the serum explains why blood group 0 is less risky. In a different study, people with blood type 0 were found to have genetically higher levels of IL 6. IL 6 level has been associated with low ACE 2 receptor and blood pressure (12).

CONCLUSIONS

While blood group and clinical severity were not found to be associated with COVID19-infected patients, it was determined those individuals with A blood group were more likely to develop the disease and individuals with 0 blood group had a low risk of contracting the disease. People with A blood group should pay more attention to protection and isolation. In terms of more severe tomography findings in B blood group and better understanding of these differences, investigation of the underlying pathogenic mechanisms may guide science with further studies.

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Perspectives of physical medicine and rehabilitation specialists and rheumatologists towards fibromyalgia syndrome in Turkey

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ABSTRACT

Objective: Fibromyalgia syndrome (FMS) is a chronic disorder characterized by widespread, unexplained pain in the muscles, including the head, neck, and sides of the hips, and fatigue. We aimed to evaluate the familiarity of physical medicine and rehabilitation and rheumatology physicians with fibromyalgia syndrome (FMS) in Turkey by means of a survey and to determine if these physician groups followed the 1990 FMS diagnostic criteria and 2010 FMS classification criteria for diagnosis.

Material and Methods: The survey questions consisted of two parts; the first part consisted of 10 questions about demographics and professional experience, as well as the number of patients who had been diagnosed, treated, and followed up with in the prior 3 months by physicians. The second part consisted of 15 questions about perspectives on the 1990 FMS diagnostic criteria and 2010 FMS classification criteria.

Results: One hundred and seventy one physicians participated in this survey. The majority of physicians 105 (99.1%) from physical medicine and rehabilitation and 59 (90.8%) rheumatologists could diagnose FMS. The rate of diagnosis and the rate of follow-up for FMS patients were significantly higher with physical medicine and rehabilitation specialists than with rheumatologists (p= 0.013 and p = 0.000; respectively) and were statistically significant.

Conclusion: Differences in the awareness and descriptions of as well as approaches to FMS by physical medicine rehabilitation physicians and rheumatologists were examined in this study.

Key words: Awareness, fibromyalgia, rheumatologists, physical and rehabilitation medicine specialist

INTRODUCTION

Fibromyalgia syndrome (FMS) is a chronic disorder characterized by widespread, unexplained pain in the muscles, including the head, neck, and sides of the hips, and fatigue (1, 2). The prevalence of FMS is reported to be 0.1%-1.0% (3). It occurs in all ethnic groups and across all ages and both genders, but it primarily affects 85%-90% of women aged 40-60 years (3).

FMS is a significant health problem associated with a decreased health-related quality of life. For this reason, in clinical practice, it is important to diagnose FMS. Obtaining an FMS diagnosis can be frustrating for patients and physicians alike because many of its symptoms overlap with those of various chronic conditions. There are considerable differences in the aetiology and lack of reliable treatment methods (4).

The 1990 diagnostic criteria of the American College of Rheumatology (ACR) classify patients as having FMS when there is widespread pain that has lasted for at least three months and tenderness in at least 11 of the 18 points on finger palpation with the application 4 kg of pressure per square inch (2). Fatigue and cognitive impairment were not included in the 1990 ACR diagnostic criteria.

In 2010, the ACR published new criteria that are helpful when it is difficult to obtain a diagnosis owing to the reduced number of identified tender points in patients whose symptom severity has decreased (5).

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Thus, the current study's main objective was to evaluate the familiarity of physical medicine and rehabilitation and rheumatology physicians with FMS in Turkey by means of a survey, to determine whether these physician groups followed the 1990 criteria or 2010 criteria for diagnosis, and to determine the perspectives of these groups on whether FMS is an inflammatory systemic disease or a noninflammatory chronic widespread pain syndrome.

MATERIAL and METHODS

Physicians working in physical medicine rehabilitation and rheumatology clinics in Turkey were contacted via email (using addresses obtained from the relevant specialist associations) to fill a survey comprising 25 questions. The study protocol was approved by Baskent University Medical Faculty Research Council (Date: 04/04/2017, No: KA17/101).

The survey consisted of two parts. The first part consisted of 10 questions about demographic and professional experience and the number of patients who were diagnosed, treated, and followed up in the last three months by physicians. The second part consisted of 15 questions about attitudes toward FMS. The questionnaire sent to physicians in this email survey is included in the appendix.

We divided physicians' age ranges into four groups: 20-29 years, 30-39 years, 40-49 years and age 50 years and older. We also grouped the physicians according to how long they had been working for 1-10 years, 11-19 years, 20-29 years and ⩾30 years.

The number of patients diagnosed with FMS in the last three months was categorized into three groups (1-20), (21-50),and (⩾51). The number of FMS patients who received treatment and follow-up in the last three months were categorized into three groups (1-20), (21-50), and (⩾51).

The questionnaire about attitudes toward the 1990 FMS diagnostic criteria and 2010 FMS classification criteria was based on 15 items (Table 3). These questions were generally answered on a 5-point Likert scale; 1= strongly agree, 2= somewhat agree, 3= neither agree nor disagree, 4= somewhat disagree, 5= strongly disagree. The scale results were spread over a width of 5.00-1.00 = 4.00. By dividing this width into five, the ranges of team leaders' effectiveness of their conflict management styles were determined. According to this; 1.00-1.79 score range is evaluated as "very low," 1.80-2.59 "low," 2.60-3.39 "medium," 3.40-4.19 "high," and 4.20-5.00 "very high." The mean (standard deviation) and variance of all physician responses were calculated for each item in the questionnaire.

Physicians voluntarily completed the survey, for which payment was not made.

Statistical analysis: Statistical analysis was performed using SPSS version 23.0. The Kolmogorov-Smirnov distribution test was used to examine normally distributed data. Because normal distribution was not in evidence, participation levels of the physical medicine and rehabilitation and rheumatology physicians were assessed using the Mann-Whitney U test. Pearson's chi-square and Fisher's exact tests were used to determine the correlation between an FMS diagnosis and treatment provided by the physicians.

The results were evaluated using a 95% confidence interval. Stastistical significance was set at $p \leqslant 0.05$

RESULTS

A total of 171 physical medicine and rehabilitation and rheumatology physicians completed a detailed questionnaire. **Table 1** presents the demographic background of the participating physicians. The survey included responses from 106 physicians from the Physical Medicine and Rehabilitation Department and 65 physicians from the Rheumatology Department. Their mean age was 40.4 ± 8.9 years. The majority of physicians were women 98 (57.3%).

Regarding their medical positions, 29 (17.0%) participants were professors, 16 (9.4%) were associate professors, 8 (4.7%) were assistant professors, 86 (50.3%) were specialists, 15 (8.8%) were research assistant and 17 (9.9%) were minor research assistant. The majority of the physicians (n=63, 36.8%) had worked for 11-19 years.

One hundred and sixty-four (96%) of the physicians had been diagnosed with FMS and 157 (92%) were followed up with patients diagnosed with FMS. The majority of the physician's 105 (99.1%) of the physical medicine and rehabilitation physicians and 59 (90.8 %) of the rheumatologists diagnosed FMS as outlined in **Table 2**. The rate of diagnosis and follow-up of a patient diagnosed with FMS was 98.1% (n=104) for physical medicine and rehabilitation physicians and 81.5% (n=53) for rheumatologists.

The rates of diagnosis and follow-up of FMS patients were significantly higher for physical medicine and rehabilitation specialists than for rheumatologists (p=0.013 and p=0.000 respectively), and the difference was statistically significant.

No statistically significant difference were found between the physical medicine and rehabilitation and rheumatology specialists in terms of the number of patients (categorized as 1 – 20, 21 – 50, and ⩾ 51 patients, respectively) diagnosed (as well as treated and followed-up with) over the previous 3 months (p=0.590 and p=0.172, respectively) (**Table 2**).

These 3-month data were obtained from their career. Since no variables related to patients were used, this study relied on the amount of time physicians could recall their clinical practice, which was the previous three months, based on the number of patients that physicians remembered.

Diagnoses (as well as treatment and follow-up) over the last 3 months were higher in rheumatologists for 1 – 20 patients (46% and 46%), but diagnoses (plus treatment and follow-up) over the previous 3 months for ⩾ 50 patients was higher for physical medicine and rehabilitation physicians (32% and 36%, respectively).

Answers from physical medicine and rehabilitation physicians were compared with those from rheumatology physicians in response to 15 statements about FMS (**Table 3**).

Physical and rehabilitation physicians said more for the following statements, respectively: "FMS is a common pain syndrome" (p=0.060), "This disease is a somatization disorder, according to the 2010 somatic and functional criteria developed by the ACR to establish an FMS diagnosis" (p=0.424), "FMS is over-diagnosed with the use of the 2010 somatic and functional criteria developed by the ACR to

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establish an FMS diagnosis" (p=0.407), "FMS has been identified as an exclusion disease with the use of the 2010 somatic and functional criteria developed by the ACR to establish an FMS diagnosis" (p=0.895), "A new diagnostic criteria set to diagnose FMS is urgently required" (p=0.451), "FMS is a disease that is best diagnosed by clinical experience" (p=0.501), and "A multidisciplinary team, on which there is psychiatric representation, is required to treat an FMS patient" (p=0.282).

However, none of these statements were statistically significant.

In addition, rheumatologists stated more for the following statements, respectively, but a statistically significant difference was not found between these specialists regarding the statements that "FMS is a psychiatric disorder" (p=0.144), "The etiopathogenesis of FMS has been clarified" (p=0.478), and "I use the 1990 diagnostic criteria developed by the ACR as a basis for establishing an FMS diagnosis" (p=0.064).

The difference in correlation between the physicians regarding the statement that "FMS is an inflammatory disease" was found to be statistically significant (p=0.001).

A higher number of physical medicine and rehabilitation physicians agreed with this statement than rheumatologists.

Fewer physical medicine and rehabilitation physicians than rheumatologists made the statement that "An FMS diagnosis is of little importance to these patients. A definition was created simply to describe their pain." The difference in this regard between the groups was statistically significant (p=0.047).

The difference in the extent to which the physicians expressed the view that the "use of the criteria regarding the identification of tender points developed by the 1990 ACR is essential in establishing an FMS diagnosis" was found to be statistically significant (p=0.011). Rheumatologists believed this to be the case more than physical medicine and rehabilitation physicians.

More physical medicine and rehabilitation physicians stated that "I use the 2010 diagnostic criteria developed by the ACR as a basis for establishing an FMS diagnosis" than rheumatologists. The difference in this regard between the groups' mean values was statistically significant (p=0.017).

The difference in the belief that "It is necessary to consider the socio-cultural and psychosocial factors of each country when making an FMS diagnosis" between the two groups of physicians was found to be statistically significant (p=0.018). More physical medicine and rehabilitation physicians made this statement than rheumatologists (**Table 3**).

Table 1. Demographics and professional characteristics of the physicians

		n (%)
Department (*n* = 171)	Physical Medicine and Rehabilitation	106 (62.0)
	Rheumatology	65 (38.0)
Gender (*n* = 171)	Women	98 (57.3)
	Men	73 (42.7)
Age (years) (*n* = 171)	20–29	16 (9.4)
	30–39	72 (42.1)
	40–49	51 (29.8)
	≥ 50	32 (18.7)
Duration of work (years) (*n* = 171)	1–10	50 (29.2)
	11–19	63 (36.8)
	20–29	43 (25.1)
	≥ 30	15 (8.8)
Title (*n* = 171)	Professor Dr.	29 (17.0)
	Associate Professor Dr.	16 (9.4)
	Assistant Professor Dr.	8 (4.7)
	Specialist	86 (50.3)
	Research Assistant/Assistant	15 (8.8)
	Minor Research Assistant/assistant	17 (9.9)
Institution (*n* = 170)	University hospitals	62 (36.5)
	Training and research hospitals	42 (24.7)
	State hospitals	30 (17.6)
	Private hospitals	26 (15.3)
	Clinic	5 (2.9)
	Branch center	5 (2.9)

Data are shown as n (%) unless otherwise stated.

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Table 2. A comparison of the correlation of the number of physicians diagnosing and following-up with fibromyalgia syndrome (FMS) patients and the number of patients diagnosed, treated, and followed-up with in the previous three months

Diagnosis of and follow-up with FMS patients		Departments Physical Medicine and Rehabilitation *n* (%)	Rheumatology *n* (%)	p
Status of diagnosing FMS	Yes No	105 (99.1) 1 (0.9)	59 (90.8) 6 (9.2)	0.013*
Follow-up status of patients with FMS	Yes No	104 (98.1) 2 (1.9)	53 (81.5) 12 (18.5)	0.000*
Number of patients diagnosed with FMS in the previous three months	1–20 21–50 ≥ 51	40 (40.4) 27 (27.3) 32 (32.3)	26 (45.6) 17 (29.8) 14 (24.6)	0.590
Number of patients treated and followed-up with in the previous three months	1–20 21–50 ≥ 51	30 (30.6) 33 (33.7) 35 (35.7)	22 (45.8) 11 (22.9) 15 (31.2)	0.172

Table 3. A comparison of the correlation of agreement between physical medicine and rehabilitation physicians and rheumatology physicians in response to statements about fibromyalgia syndrome (FMS)

Statements made by the participating physicians	Physical Medicine and Rehabilitation physicians		Rheumatology physicians		p
	n	Mean ± SD	*n*	Mean ± SD	
FMS is an inflammatory disease	105	2.5 ± 1.2	64	1.9 ± 0.9	0.001*
FMS is a common pain syndrome	106	4.5 ± 0.8	65	4.3 ± 0.9	0.060
The etiopathogenesis of FMS has been clarified	106	2.0 ± 1.0	64	2.1 ± 1.0	0.478
FMS is a psychiatric disorder	106	2.7 ± 1.2	65	3.0 ± 1.2	0.144
An FMS diagnosis is of little importance to these patients. A definition was created simply to describe their pain	106	2.1 ± 1.2	65	2.4 ± 1.3	0.047*
I use the 1990 diagnostic criteria developed by the ACR as a basis for establishing an FMS diagnosis	105	2.4 ± 1.2	65	2.7 ± 1.1	0.064
Use of the criteria regarding the identification of tender points developed by the 1990 ACR is essential in establishing an FMS diagnosis	106	2.4 ± 1.2	65	2.8 ± 1.1	0.011*
I use the 2010 diagnostic criteria developed by the ACR as a basis for establishing an FMS diagnosis	104	3.7 ± 1.1	65	3.4 ± 1.1	0.017*
This disease is a somatization disorder, according to the 2010 somatic and functional criteria developed by the ACR to establish an FMS diagnosis	106	3.5 ± 1.0	64	3.4 ± 1.1	0.424
FMS is over-diagnosed with the use of the 2010 somatic and functional criteria developed by the ACR to establish an FMS diagnosis	106	3.4 ± 1.0	65	3.3 ± 1.0	0.407
FMS has been identified as an exclusion disease with the use of the 2010 somatic and functional criteria developed by the ACR to establish an FMS diagnosis	106	3.3 ± 1.2	64	3.3 ± 1.0	0.895
A new diagnostic criteria set to diagnose FMS is urgently required	106	3.6 ± 1.1	65	3.5 ±0.9	0.451
It is necessary to consider the socio-cultural and psychosocial factors of each country when making an FMS diagnosis	105	4.2 ± 0.9	65	3.9 ± 0.9	0.018*
FMS is a disease that is best diagnosed by clinical experience	106	3.9 ± 1.0	65	3.9 ± 0.9	0.501
A multidisciplinary team, on which there is psychiatric representation, is required to treat an FMS patient	106	3.9 ± 1.1	65	3.8 ± 1.1	0.282

ACR:American college of rheumatology; FMS: Fibromyalgia syndrome; * Significant difference (p ⩽ 0.05).

DISCUSSION

This study evaluated the perceptions of physical medicine and rehabilitation physicians as well as rheumatologists about FMS and their perspectives on FMS diagnostic criteria.

The rate of FMS is 5% –6% in internal and family medicine settings (6). In one study from Israel, most FMS patients suffering from chronic musculoskeletal pain syndrome were referred to orthopaedic surgeons during the early stages of the disease (7).

There is a high incidence of FMS diagnosis in rheumatology clinics, where 12%–20% of patients presenting for the first time are diagnosed with it (7). On average, it takes 2–3 years from the time of a patient's first consultation with a rheumatologist to be diagnosed with FMS.

One survey from Saudi Arabia received responses from 104 medical practitioners. Rheumatologists reported 28.8% referrals while pain physicians reported 22.1%. This might explain why a high percentage of FMS patients are usually seen by rheumatologists and pain physicians (8). Rheumatologists and pain physicians were more familiar with FMS than were other medical practitioners. This reflects a greater level of expertise in managing such conditions among rheumatologists and pain physicians.

In our survey, it is interesting to note that the rate of diagnosis of FMS in the group of 1–20 patients and the rate of treatment and follow-up in the last 3 months were higher for rheumatologists. On the other hand, the rate of diagnosis, treatment and monitoring of $\geqslant$ 50 patients in the prior 3 months was higher for physical and rehabilitation physicians. Rheumatologist may refer patients to physical and rehabilitation physicians after diagnosis and follow up. In Turkey, physical and rehabilitation physicians are usually the point of first contact for patients with chronic pain.

It is not surprising that physicians are unable to demonstrate FMS as a visible disease (9). Many physicians believe that a true disease is pathologic and changes tissue, whether macroscopically or microscopically; otherwise, if an ailment fails to show these changes, it will be considered a "non-disease" or a "psychological entity." In our survey, the difference in correlation between the physicians regarding the statement that "FMS is an inflammatory disease" was found to be statistically significant (p=0.001). A lower number of rheumatologists agreed with this statement than did physical medicine and rehabilitation physicians. Our findings regarding this statement are compatible with the findings of previous studies. In a survey among only rheumatologists in Scotland (10), most rheumatologists believed that FMS is a distinct clinical but not pathological entity, and another study in France showed that only a quarter (23%) of their rheumatologists considered FMS a disease (11).

In another study, most rheumatologists (92.5%) from Southeast Asia reported FMS is a distinct clinical entity and that this condition is considered an illness rather than a disease (6).

In our study, rheumatologists said more and a statistically significant difference was not found between the physical medicine and rehabilitation and rheumatology specialists regarding the statement that "FMS is a psychiatric disorder" (p=0.14). A study by Arshad et al. (6) showed that 9% of rheumatologists believed that FMS is primarily a psychological illness. Other studies by Merskey and Capen have shown that FMS is a psychological condition rather than a physical disease (12,13).

Despite the fact that FMS patients tend to minimize or deny psychological symptoms, the evidence supports the claim that the burden of psychiatric disease is higher in comparison to controls (14).

At the beginning of this study, it was predicted that rheumatologists who viewed diseases as systemic diseases would use the 2010 ACR classification FMS criteria (5) more in clinical practice whereas rheumatologists used the 1990 diagnostic criteria more (2). On the other hand, we also found that physical medicine and rehabilitation physicians used the 2010 FMS criteria more when we expected them to use the 1990 FMS criteria.

In the current study, the difference in the extent to which physicians expressed the view that the "use of the criteria regarding the identification of tender points developed by the 1990 ACR is essential in establishing an FMS diagnosis" was found to be statistically significant (p=0.011). More rheumatologists believed this to be the case than physical medicine and rehabilitation physicians. In addition to tender point examination, rheumatologists also perform physical examinations and should also identify other inflammatory diseases such as connective tissue diseases (e.g., systemic lupus erythematosus) and vasculitis (e.g., Behçet's disease). Additionally, this group of physicians spent less time using the 1990 diagnostic criteria than the 2010 ACR classification criteria in clinical practice.

In this survey, more physical medicine and rehabilitation physicians stated that "I use the 2010 classification criteria developed by the ACR as a basis for establishing an FMS diagnosis" than rheumatologists. The difference in this regard between the groups' mean values was statistically significant (p=0.017). Questions from the 2010 ACR criteria may be too time consuming for clinical practice.

A study by Blotman et al. reported that the highest proportion of physicians aware of the 1990 diagnostic criteria were rheumatologists and the least familiar were family physicians; this discrepancy was attributed to differences in pain education (11).

The present study determined that rheumatologists preferred using the 1990 ACR criteria to diagnose FMS while physical medicine and rehabilitation physicians favoured the 2010 ACR criteria to diagnose FMS. Only the 1990 ACR criteria were evaluated in the survey by Perrot et al. (15) and it was determined that these criteria were most commonly used by rheumatology physicians (83%), which is similar to the findings of the present study, and used second-most by physical medicine and rehabilitation physicians (77%).

In a survey of Southeast Asian rheumatologists, only 60% used the 1990 ACR criteria to make a diagnosis (6).

In a study in Canada, similar to the current study, 284 physicians were asked about the 1990 diagnostic criteria and the 2010 classification fibromyalgia criteria. In this study, nine physical medicine and rehabilitation specialists (n = 58) used only 1990 criteria (15.5%), 25 (43.1%) used only 2010 criteria; on the other hand, rheumatologists (n = 29), 4 (13.8%) used only 1990 criteria, and 9 (31.03%) used only 2010 criteria. Similar to the present study, 43.1% of physical medicine and rehabilitation specialists were more likely to use the 2010 criteria (16).

In the current study, more physical and rehabilitation physicians than rheumatologists made the following statement, although the difference between the groups was not statistically significant: "FMS is a disease that is best

diagnosed by clinical experience" (p=0.501). One of the questions from the present study was similar to a question asked by Choy et al.; physicians were asked if they were aware of the ACR criteria and had been diagnosed with FMS in patients in the previous two years (n = 725), and 26.0% responded that they did not use the ACR criteria when diagnosing fibromyalgia in their clinical practice (17).

In another study that used only the 1990 ACR FMS criteria, 79 (54.5%) of 145 general practitioners responding to a statement similar to our statement that "FMS is a disease that is best diagnosed by clinical experience" answered that "the diagnosis is clinical and exams are for the differential diagnosis" (18).

The difference in the belief that "it is necessary to consider the socio-cultural and psychosocial factors of each country when making an FMS diagnosis" between the two groups of physicians was found to be statistically significant (p=0.018). More physical medicine and rehabilitation physicians made this statement than rheumatologists (Table 3). This might be because the rate of diagnosing, treating, and monitoring $\geqslant 50$ patients in the previous 3 months was higher for physical and rehabilitation physicians. Additionally, this group of physicians consulted with more patients, and the long implementation period of the 2010 FMS criteria as well as longer communication with the patient resulting from additional questions may have induced physical and rehabilitation physicians to better distinguish socio-cultural and psychosocial levels of patients.

More rheumatologists than physical medicine and rehabilitation physicians made the statement that "An FMS diagnosis is of little importance to these patients. A definition was created simply to describe their pain." The difference in this regard between the groups was statistically significant (p=0.047). In the study by Choy et al., 24% of physicians strongly agreed and 40% somewhat agreed that it is difficult for patients to explain symptoms of fibromyalgia to a physician (17).

In a study by Hayes et al. (19) that evaluated approaches to diagnosing and treating FMS using the 1990 ACR criteria among 189 general practitioners and 139 specialists (anaesthesiologists, neurologists, physiatrists, psychiatrists, and rheumatologists), two-thirds of physicians (63% of general practitioners and 66% of specialists) characterized FMS as diagnosable. Participants (41% of general practitioners and 37% of specialists) reported unclear diagnostic criteria as a barrier in their identification of fibromyalgia in a question similar to our study's statement that "new diagnostic criteria for FMS are urgently required." However, the analysis was performed by evaluating the specialists as a single group.

Perrot et al. compared the approaches used by 1622 physicians. Among them, 206 rheumatologists and 202 pain specialists across eight countries (six European countries, Mexico and South Korea) reported difficulties diagnosing FMS (15) using the 1990 FMS diagnostic criteria. The number of FMS patients seen in a month, the number of patients diagnosed with FMS over the last two years, and the extent of FMS knowledge was greater (with statistical significance) for the 206 rheumatologists than for the 202 pain specialists. In addition, the pain specialists were significantly more likely than the rheumatologists to agree that making an FMS diagnosis was difficult and that it was necessary to give patients more time to identify their symptoms and to effectively distinguish FMS symptoms from others. Unlike the current study, the number of pain specialists and rheumatologists was similar.

Physical medicine and rehabilitation physicians, rheumatology specialists, general practitioners, anaesthesiologists, neurologists, and psychiatrists frequently see FMS patients with primary diseases. However, it has also been reported that depression, insomnia, muscle aches, and muscle weakness are frequently seen in cancer patients and that oncologists may also make an FMS diagnosis. Thus, in this group of oncology patients, it is likely that the use of the 1990 and 2010 FMS criteria may be insufficient to ensure accurate diagnosis (20).

In this regard, the 2010 FMS criteria can be used in clinical practice as an alternative to the 1990 criteria when it is not possible to make a diagnosis based on symptoms because a finger palpation assessment cannot be performed accurately for oncology patients, such as breast cancer patients, or might be carried out incorrectly. Conversely, it may be difficult to evaluate oncology patients according to the 2010 criteria as patients can experience generalized and widespread pain due to malignancy, chemotherapy, and radiotherapy (20).

The current study has several limitations. This was an ambiguous survey using generalized statements that likely relied upon participants' recollections or perceptions. Additionally, this was a de novo questionnaire that reflected perceptions of FMS based on the number of FMS patients that physical medicine and rehabilitation physicians and rheumatologists remembered from clinical practice as well as their perspectives on FMS diagnostic criteria. To overcome this limitation, expanding the surveyed perceptions of and approaches to FMS to include other physicians may be the next research goal.

Another limitation was the exclusion of family physicians, psychiatrists, neurologists, internal medicine specialists, orthopaedic surgeons, and oncologists. The sample size was small. Other limitations might be minimized by adding to the questionnaire statements about the use of treatment modalities, blood tests, or radiologic tools to rule out FMS in undergraduate curricula. Physical medicine and rehabilitation physicians as well as rheumatologists from Turkey were invited to respond; these responses may not reflect global practices. It could also be determined whether physicians' knowledge was impacted by clinical experience. A final limitation was the exclusion of questions regarding the countries in which physicians worked and the countries in which patients lived; if these questions had been included, physicians may have distinguished socio-cultural and psychosocial factors of each country.

CONCLUSIONS

Physical medicine and rehabilitation physicians as well as rheumatologists comprise an important point of early contact between FMS patients and the medical community. It is extremely important to maximize their proficiency and awareness regarding the spectrum of FMS and related symptoms.

Differences in the awareness and descriptions of and approaches to FMS by physical medicine rehabilitation physicians and rheumatologists were examined in this study.

We tried to determine whether physicians from rheumatology or physical medicine and rehabilitation followed the 1990 criteria or the 2010 criteria for diagnosis.

No such study has been performed in this region with these two groups of physicians and with these two sets of fibromyalgia criteria. This questionnaire was determined by physicians from both branches according to the patients they remembered and their comments on the criteria.

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Ethical approval: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by Local Ethical Committee. This study was approved by Baskent University Medical Faculty Research Council (Date: 04/04/2017, No: KA17/101).

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A study on substance abuse among young people (10-24 years) in urban slums of Jorhat, Assam

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ABSTRACT

Objective: To find out the substances abused by the people in urban slums and also find factors contributing to it. Also to recommend suggestions based on the study

Material and Methods: It was a community based cross sectional study and data was collected using Interviewers Performa after getting approved by the Institutional Ethics Committee. A sample size of 174 was calculated . Young People (10 to 24yrs) who were willing to participate were included and whose not ready to take part were opted out.

Results: It was found that males usually 22 to 24yrs used abusive substances than females. Tobacco was the most common substance abused followed by alcohol and majority had been introduced to the substances by their peers between 17 to 24 yrs. Most people consumed the drug multiple times daily followed by weekly and had procured the drug from local retailers. These substances gave them a sense of hallucination and euphoria on consumption. Among the side effects oral problems like ulcers and malignancy topped the list. Respiratory and gastrointestinal issues along with inability to concentrate on work were other side effects.

Conclusions: Based on the results several recommendations were made especially awareness workshops and camps. These programs mainly stressed upon the necessity to create social awareness among the people and their families about the ill effects of substance abuse. All the results were tabulated.

Keywords: substance abuse, Tobacco, alcohol, young people, adolescents, slums, Jorhat,

INTRODUCTION

Every civilization known to us has provided for the use of mind-altering substances. There are medicinal, recreational, religious and social uses for these substances. Each culture develops its own set of parameters norms and practices in order to control the use of these substances and to contain the extent of their abuse. Substance abuse refers to the harmful or hazardous use of psychoactive substances, including alcohol and illicit drugs (1).

It is disturbing to know that, substance abuse has reached an alarming proportion in the recent years particularly among the young population (aged between 10-24 years) (2) as the habit of substance abuse is fast making inroads into their lives (3). The reason of fast growing abuse may be attributed to curiosity and natural tendency to experiment with drugs, disturbed home environment, lack of communication between parents and children, ignorance and its ill after effects, lack of knowledge, early exposure etc. Other important reasons contributing to it may be as a result of escape phenomena from tension and frustrations like unemployment, failure in exams, impact of ‘disco culture’, electronic media, peer pressure or delinquency.

According to ICD-10 (International Classification of Diseases), a medical classification list by WHO recognizes psychoactive substances as those, the self-administration of which produces mental and behavioural disorders (4). This may lead to abuse and subsequently addiction and dependence. The list includes alcohol, opioids, cannabinoids, sedatives and hypnotics, cocaine, caffeine and other stimulating agents, hallucinogens, tobacco and other volatile substances.

The situation of substance abuse among young people is becoming a global health problem and has reached an alarming position in India.

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Research on the abuse of substances has grabbed undivided attention in developing countries like India because of changing trends in the prevalence of substance abuse and the rising magnitude of the menace. Statistically speaking, 2 billion worldwide populations consume alcoholic beverages as documented by WHO, out of which 76.3 million have disorders having suicidal tendencies. Other psychological changes include mood disorders like anxiety, depression, thought disorders, and personality disorders. Tobacco causes more death than all psychoactive substances combined,including 3 million premature deaths (6% of total) and 30% of all cancer deaths (5). Considering our area of study, the socio-cultural, economic, as well as geographical factors has proved to be very conducive for the emergence of drug addiction and alcoholism in this region. The region is close to the 'Golden Triangle', and a major hub of drug transport and the second-largest opium producer, provides an ample push in worsening the scenario. Opium, ganja, bhang, alcohol, khaini, bidi etc. are the most commonly abused substances. Coming back to Indian scenario, the Childline foundation survey in 2008 revealed 63% of patients coming with substance abuse-related disorders were introduced to it in their young age between 10 to 24 years. The cases of drug abuse are fast rising to 12% in age group upto 15 years and 32% in 16-25 years age group (6).

A thorough review of the published papers on this topic can define the causative factors and help to assess planning and suggest further studies in this domain.

In our project, the extent, pattern and trend of drug abuse amongst the young people aged between 10 to 24 years of Jorhat Slum, viz. Pujadubi and .Dhakaipatty.Before commencing the study approval was taken from Institutional Ethics Committee of Jorhat Medical College and Hospital

MATERIAL and METHODS

Young People: The age of young people are taken to be between 10 to 24 years (2).

Substance abuse: In our study the substances considered were Alcohol, Cigarette, Tobacco products, Dendrite and Marijuana (Bhang).

Abuse: A substance was considered to be abused if it was used in an amount harmful to his/her health, either mentally or physically or both.

Scope of study: People of all age groups are seen to be suffering from the harmful effects of substance abuse. The age group included in our project, i.e., between 10 years to 24 years of age, is the major age group involved in such practices, commonly resulting in many physical, mental and social disturbances (7).

Data obtained from our research can be used to conduct educational programmes on substance abuse. We can provide the people information on how to deal with a family member or friend who is struggling with a substance use disorder, make them aware of the probable side effects and also advice them to avoid the use of such substances.

Limitations: Being undergraduates, with minimum resources and limited time, we could not afford a more extensive intervention into the matter. We could only provide our study subjects with on the spot advices and information regarding substance abuse and its effects.

Objectives

1. To find out the substances abused and the factors contributing to it.
2. To suggest recommendations based on our study.

Literature review: According to the study named "Substance abuse among Youths at Guwahati city, ASSAM (India)-major instigators and socio demographic factors" by Himakshi Goswami conducted at Guwahati among 100 substance abused youth, the pattern reveals that 49% of the respondents use alcohol, 22% use heroin, 2% marijuana, 3% ganja, 2% inhalers, 19% polydrug and 3% cocaine . The pattern for the major instigators of substance abuse revealed that 29% used it for fun, 25% by peer influence, 23% reported that their curiosity for the drug acted as propagates and 9% used it to get relief from stress (8).

Research conducted on the extent and patterns of drug abuse by general population surveys in India showed that the prevalence of alcohol abuse varied between 4.2 and 30.7 %, heroin abuse between 0 and 1.3%, and other opiates between 0 and 10.2% in the country. Heroin abuse was frequently reported from Manipur and Kohima and was around 1%, raw opium abuse from Jodhpur and that of cannabis from Uttar Pradesh and Manipur (9).

According to the study "Developing Community Drug Rehabilitation, Rapid Assessment Study of Drug Abuse in Target Communities in India (RAS DATC)" by Mittal and Ch'ien, conducted in nine urban cities namely Bangalore, Chennai, Imphal, Jodhpur, Kolkata, Lucknow, Mumbai, Patna and Pune, it was reported that among a total of 1,271 drug users , commonly used drugs in descending order were: alcohol 43%, heroin 38.2%, opium 9.3%, cannabis 6.1% and other opiates 4.3% (10).

The National Household Survey of Drug Use in the country reported that alcohol was the primary substance used (apart from tobacco) followed by cannabis 3% and opioids 0.7% (11).

According to United Nations office on drug and crime, the states of India bordering Myanmar have experienced very rapid transmission of HIV among drug injecting populations. Zero prevalence among intravenous drug users increased from 0% to 50% within six months in 1989, injecting drug use for non medical purpose increased rapidly during the last decades and was a major contributing factor for various infective diseases like HIV in young generation (12).

In Manipur, Mizoram and Nagaland the anonymous surveys shows that the prevalence of intravenous drug users varies between 1 and 2% of the general population in the states concerned. Thus Manipur accommodates for at least 15,000 intravenous drug users in the entire state, Nagaland 1,500 and Mizoram 2,800 in surveyed urban areas. The intravenous injections are 0.2% in remote areas, 0.9% in areas far away from the highway and 1.3% in areas that are well connected with the highway. Heroin users are much higher in these areas (13).

According to the study, "Substance use among adolescent high school students in India: a survey of knowledge, attitude and opinion" conducted in two high schools in West Bengal, India, among 416 students in classes 8, 9 and 10, the results show that 52 (12.5%) used or abused any of the substance. 26 out of 172 were urban and the others 26 out of 244 were rural students. 73% of respondents expressed their desire to quit and 57.69%had tried to stop (12)

Study design and participants

In our project, a community based cross sectional study was carried out in urban slums of Jorhat (Pujadubi and Dhakaipatty) under the guidance of the Department of Community Medicine, Jorhat Medical College and Hospital, Jorhat. The study sample was selected from among young people (aged between 10 to 24 years of age) to study the substance abused along with the factors responsible for it. The project was completed within a period of four months from MAY to SEPTEMBER, 2017.

Preparatory phase: MAY - JUNE

Data collection: JULY - AUGUST

Report writing: AUGUST - SEPTEMBER

This age group was selected because substance abuse practices were found to be most common among this age group due to various reasons like peer pressure, familial pressure, stress, urge to experimentation, etc.

Sample size:

Based on all the details mentioned above the sample size was calculated using the equation (13).

$n = 4pq/L2$ (15)

Where: p is prevalence

q is non prevalence {1 – p}

L is the absolute error

Taking, p = 12.5%

L = 10%

N = 174

And substituting in the above equation, we get 174 as the sample size.

Inclusion criteria:

All those abusing one substance or the other in the age group of 10 to 24 years.

Those who were willing to participate.

Those using multiple substance of abuse were also included

Exclusion criteria:

Those who did not report any instance of substance abuse till the date of survey.

Those who were not willing to participate.

Those aged below 10 years and above 24 years.

Study area and population

The study was conducted in the urban slums of Pujadubi and Dhakaipatty of Jorhat. It has a population mainly of daily wage workers and their families.

Simple random sampling technique was used for conducting our study. The slums were randomly selected from among the registered slums under Jorhat Development Authority. House to house visit was done in the two slums till 174 young people who reported of substance were reached.

Data collection tools: Informed consent was taken from the participants. They were assured of the confidentiality of the information given. Data was collected by using a pre-designed and pre-tested Performa by the INTERVIEW METHOD.

RESULTS

Table 1: Gender distribution of substance abuse

Gender	Total number	In percentage
Male	153	87.99
Female	21	12.21
Total	174	100

Table 2: Prevalence according to age

Age Group (In Years)	**Total Number**	**In Percentage**
10-13	1	0.6
13-16	13	7.5
16-19	36	20.7
19-22	59	33.9
22-24	63	36.2
TOTAL	174	100

Table 3: Types of drugs abused in percentage

Drugs abused	Total number	In percentage
Dendrite	11	6.32
Marijuana and derivatives	8	4.60
Alcohol	47	27.01
Tobacco and derivatives	92	52.87
Marijuana+Alcohol+Tobacco and derivatives	8	4.6
Alcohol+Tobacco and derivatives	8	4.6
Total	174	100

Table 4: Frequency of drug intake:

Frequency	Total number	In percentage
Daily once	18	10.34
Daily multiple	110	63.21
Weekly	38	21.84
Monthly	8	04.61
Total	174	100

Table 5. Source of drug

Source	Total number	In percentage
Nearby retailer	112	64.36
Drug handler(outside persons)	9	5.17
Friend	11	6.32
Family	1	0.57
Retailer +friend	28	16.1
Retailer +drug handler	2	1.15
Retailer+family	11	6.32
Total	174	100

Table 6. Age at which first introduced:

Age	Total number	In percentage
10-16	60	34.48
17-24	114	65.52
Total	174	100

T**able 7.** The Mental state of the consumer after substance intake

Feelings/effect	Total number	In percentage
Euphoric	26	14.94
Hallucination	13	7.47
Relaxed	45	25.86
Depressed	3	1.72
Addictiveness	17	9.77
Euphoric+relaxed+addictiveness	23	13.21
Relaxed+depressed	4	2.29
Euphoric+hallucination+addictiveness	43	24.71
Total	174	100

Table 8. The health effects of substance use on consumer's work

Effect	Total number	In percentage
Respiratory	26	14.94
G.i	20	11.49
Oral	38	21.83
Cns	6	3.44
Unable to concentrate +loss of work efficacy	34	19.54
Unable to concentrate +absence from work	20	11.49
Loss of work efficacy	21	11.5
No health effects seen	9	5.17
Total	174	100

Table 8. The health effects of substance use on consumer's work:

Effect	Total number	In percentage
Respiratory	26	14.94
G.i	20	11.49
Oral	38	21.83
Cns	6	3.44
Unable to concentrate +loss of work efficacy	34	19.54
Unable to concentrate +absence from work	20	11.49
Loss of work efficacy	21	11.5
No health effects seen	9	5.17
Total	174	100

Table 9. Reasons for taking drugs:

Reasons	Total number	In percentage
Depression	16	9.19
Stress	14	8.04
Personal loss	9	5.17
Peer pressure	83	47.70
Experimentation	33	18.96
Experimentation + peer pressure	6	3.44
Stress + personal loss	1	0.57
Total	174	100

Table 10. Household factors contributing to drug abuse:

Household scenario	Total number	In percentage
Parental violence	33	18.96
Lower socioeconomic condition	44	25.28
Lack of guidance	40	22.98
Lack of bonding	28	16.09
Abused /battered	13	7.47
Neglected	16	9.19
Total	174	100

Table 11. Family history of drug abuse

Family history	Total number	Percentage
Present	76	43.68
Not present	98	56.32

Table 12. Family approach towards the drug abuser:

Attitude and measures	Total number	Percentage
Unaware	88	50.57
Aware but indifferent	50	28.73
Aware and advised	36	20.68

Table 13. Awareness of ill effects

Status	Total number	In percentage
Aware	126	72.41
Not aware	48	27.59
Total	174	100

DISCUSSION

What we have observed at the end of this study is quite remarkable. We have come to a conclusive fact that a very large proportion of the young age group is abusing one kind of drug or another.

We found that 87.99% of males and 12.21% females (**Table1**) were abusing one kind of drug or another in our study while in another study entitled "Drug abuse in slum population" conducted in 2014 in Indore Madhya Pradesh(India) by the department of psychiatry MGM

Medical College Indore 78.2% were males and 28.2% were females. Thus indicating that males have a higher tendency towards vehement consumption of drugs (14).

The majority of drug abusers i.e. 36.2% belonged to age group 22-24 followed by 33.9% in the age group 0f 19-21% (**Table 2**). However, in the study "Prevalence, pattern and familial effects of substance use among the male college students – North Indian Study" by Sorab Gupta the majority i.e. 60% belonged to the age group 19-21 followed by 39.4% in the age group 22-25. Though statistics may vary somewhat in both studies it is seen that young people in the age group 19-25 are most vulnerable (15).

The major drugs abused are tobacco (52.87%) and alcohol (27.01%). The study "Age of substance use initiation among treatment admissions aged 18-30" by Substance Abuse and Mental Health Services Administration (SAMHSA) U.S.A. also revealed that the majorly abused drugs were tobacco (53.8%) and alcohol (19.7%) (18).

73.55% individuals take drugs daily and 21.84% weekly.However the study by Sorab Gupta reveals 49% take drugs daily and 23.8% weekly (18)

The most common source of drug were nearby retailers (64.36%). Also friends in 6.32% cases, drug handlers in 5.17% cases and 23.57% got their drugs from more than 1 source.

The most common reason as concluded was peer pressure (47.5%) by our study. Experimentation stood next at 18.96%. The study"Factors influencing alcohol and tobacco addiction among patients attending de addiction centre of south India" conducted by P. Prabhu and her associates also revealed that the most common reason (41.5%) for getting into drugs was peer influence or peer pressure (17).

Most of the abusers i.e. 65.52% were introduced to drugs in the age group 17-24 years and 34.48% in the age group 10-16 years while in the study "Age of substance use initiation among treatment admissions aged 18-30" by Substance Abuse and Mental Health Services Administration (SAMHSA) U.S.A. majority i.e. 38.6% were introduced to drugs when they were 11 or younger (17) which is understandable as the U.S. is a developed country with easier access to drugs.

It was found that most abusers were into drugs because it provided them with calmness and relaxation feeling (25.86%) followed by euphoria (14.96%). 8.7% of the total seem addicted.

According to our study a number of health effects were seen such as oral problems (21.83%), respiratory problems (14.94%), gastrointestinal troubles (11.49%) and also nervous disorders (3.44%). One of the most significant finding was loss of work efficacy (17.4%); 13.37% of the people also reported absence from school and work. The study "Assessment of causes, prevalence and consequences of alcohol and drug abuse among Mekelle University, CSSL 2nd year students" conducted by Dept. of Psychiatry Mekelle University, Ethiopia revealed that 90% of the subjects had academic consequences, 75% had psychological consequences, 70% had health consequences, 80% had behavioral consequences of drug abuse (19). However the data isn't quite relatable as it is a study conducted in another country with different parameters.

The household factors for drug abuse was found to be financial crisis (25.28%) followed by lack of guidance (22.98%) and household violence (18.96%). However the research by Journal of Pakistan Medical Association shows that family has little or no influence (0.5%) in drug abuse, 8% were due to peer pressure, 6% were due to employment issues, 5% were related to problems with spouse.

43.68% had positive family history of drug use as revealed by our study. The study by Sorab Gupta reveals 57.4% individuals with positive family history of drug use. Thus there seems to be a greater tendency of drug abuse in persons with positive family history.

51.6% of the abusers revealed that their families were unaware of the fact, 22.6% revealed that their families were aware but indifferent, 15.6% had been sent to counselling by family and 10.46% were sent for rehabilitation. The study "Substance use and addiction research in India" by Pratima Murthy, N. Manjunatha and Vivek Benegal throws light upon the results obtained after a 5 year treatment and rehabilitation period of 800 drug addicts at a de addiction centre which is as follows:

- 3% i.e. 504 persons did not use the services provided beyond1 month
- 81% of the remaining i.e. 240 persons of 296 were successful in maintaining abstinence.
- Upon further study it was found that the successful de addiction cases were constantly receiving support from family and friends (20)

6.33% individuals have stopped drug intake while 93.67% continue to abuse drugs, 72.41% are aware of the ill effects of drugs. . However the study by Sorab Gupta reveals 100% of the abusers still are active into drugs, 97.3% are aware of the ill effects.

Summary

On the basis of our project on 'A study on Substance abuse among young people of age group 10-24 years'' in the urban slums of Jorhat Assam' on 174 individuals, it was found that

-87.99% Males and 12.21% Females were abusing one kind of substance or the other.

-Among the 174 respondents, 36.2% belongs to age group 22-24 years, 33.9% of 19-21 years, 20.7 % belongs to 16-18 years,7.56 % belongs to age group 13-15 , 0.58% belongs to age group 10-12 years.

-The substances abused were tobacco by 52.87% abusers, alcohol by 27.01% abusers, and marijuana by 4.60 % abusers, Dendrite by 6.32% while 9.2 % consume combination of two or more drugs.

-Substance abuse is done on a daily basis by 73.55% individuals and on weekly basis by 21.84% individuals and monthly basis by 4.61% individuals.

-The common sources of substanceabuse were nearby retailer in 64.36% individuals, drug handler in 5.17%, friends in

6.32%, family in 0.57% and 23.57 % due to combination of two or more sources

-Out of 174 respondents,34.48% individuals started substance abuse at the age of 10-16 years and 65.52 % started substance abuse at the age of 17-24 years.

-Out of 174 respondents, substance abuse has produce effects like calm and relaxed feeling in 25.86%, euphoria in 14.96%, and hallucination in 7.47% and depression in 1.72 % abusers.

-Out of 174 respondents, health effects were seen like oral problems in 21.83 %, respiratory problems in 14.94%, and gastrointestinal problems in 11.49% and nervous disorders in 3.44 % individuals.

-The common reasons for substance abuse was found to be peer pressure in 47.5%,followed by experimentation in 18.96%,stess in 8.04%,personal loss in 5.17%,depression in 9.19% and in 4.01 % due to two or more of above reasons.

-The household factors responsible for substance abuse are found to be lower socio economic status in 25.28% abusers, lack of guidance in 22.98% and household violence in 18.96% individuals, negligence in 9.19%individuals.

-Out of 174 respondents, 43.68% has family history of substance abuse and 56.32% has no family history of substance abuse.

-Among the substance abusers, 51.6% them revealed that their families were unaware of the fact ,22.6%revealed that their families were aware but indifferent ,15.6% were sent for counselling and 10.46 % were sent for rehabilitation.

- Out of 174 respondents, 72.41% are aware of ill-effects of substance abuse while 27.59% are unaware of ill-effects.

CONCLUSION

As the problem of substance abuse is increasing worldwide, and has in the recent years become a global problem also affecting our country, we tried to find out the trends of substance abuse, i.e. their sources, contributing factors, household environments leading to substance abuse, etc. among 174 young people from Urban slums of Jorhat, Assam. Based on our study, we came to the following conclusions-

Majority of the respondents were males .Among the substance abusers, majority of them belonged to age group of 22-24 years, followed by the age group of 19-21 years. The major drug abused was found to be tobacco, followed by alcohol .For majority of people the source of obtaining drug is from nearby retailers. It was also found that most abusers were into drugs because it provided them with a calm and relaxed feeling followed by euphoria and addiction. A number of health effects were seen among the substance abusers with majority having oral problems and/or respiratory problems. Majority of the abusers were introduced to drugs at the age of 17-24 years that is in school going period. Also, study of the frequency of substance abuse revealed that majority of them take the drugs on a daily basis, while some consume it weekly or monthly. The most common reason for getting into substance abuse was found to be peer influence or peer pressure. It is followed by experimentation. The leading household factor contributing to drug abuse was found to be due to lower socioeconomic status followed by lack of guidance and household violence. A large number of the subjects were aware of the ill effects of substance abuse while the rest were unaware.

Recommendations

- Based on our study it was found out that many people were unware of the ill effects of substance abuse. Under such circumstances, the creating awareness primarily focussing on these unware groups, is utmost required as a viable solution.
- Making people aware of the hamper it does on one's day to day work, leading to a reduction in human efficiency and productivity could make them help in doing away with substance abuse.
- Advices was given to this group of people. There should be counselling calls for moral boosting and it should be explained not to abuse substance to escape problems.
- General advises on good health and adverse effects that substance abuse imparts on one's health should be done by organising health camps, mass gatherings etc.
- Seeking help from rehabilitation centres should be encouraged.
- The dangers of long term ill effects were explained.

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The relationship between first and second trimester biochemical parameters used to screen down syndrome and intrahepatic cholestasis of pregnancy

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ABSTRACT

Objective: To assess the role of first and second-trimester screening biomarkers pregnancy-associated plasma protein-A(PAPP-A), free beta-human chorionic gonadotropin(free ß-hCG), estriol, alpha-fetoprotein and total β-hCG in the early diagnosis of intrahepatic cholestasis of pregnancy (ICP).

Materials and Methods: Patients with ICP admitted to Mersin University Hospital for delivery between 2015 and 2019 and had first and second-trimester aneuploidy screening tests performed in the same facility were retrospectively assessed. Randomly 60 pregnant women without comorbid conditions during the same period were included as controls. Data regarding demographic characteristics, laboratory values including free ß-hCG and PAPP-A in first-trimester screening and total ß-hCG, estriol and alpha- fetoprotein in second-trimester screening were compared.

Results: There were 46 eligible patients with ICP. In first trimester screening, it was found that PAPP-A MoM was significantly lower (0.89±0.55 vs. 1.94±0.73; p=0.035) while free ß-hCG MoM was significantly higher in ICP group when compared to controls (1.84±0.59 vs. 0.99±0.47; p=0.018). In second trimester screening, no significant difference was detected in aneuploidy markers between groups. For prediction of ICP development, first trimester free β-hCG >1.44 MoM was found to have a sensitivity of 50%, a specificity of 80% and positive and negative predictive values of 33% and 88.9% respectively. Similarly first trimester PAPP-A values <1.075 MoM was found to have 80% and 75% sensitivity and specificity with positive and negative predictive values of 75% and 44% respectively.

Conclusion: Low PAPP-A MoM value and elevated free ß-hCG in first trimester seem to be associated with ICP development.

Keywords: Alpha-fetoprotein (AFP), estriol, free beta-human chorionic gonadotropin (free ß-hCG), intrahepatic cholestasis of pregnancy, pregnancy-associated plasma protein-A (PAPP-A)

INTRODUCTION

The intrahepatic cholestasis of pregnancy (ICP) is a common hepatic disorder seen in 0.1% to 15.6% of pregnancies [1]. In ICP, there are elevated hepatic function tests and bile acids levels that result in itching of soles and palms. The itching is more commonly seen at late second trimester and third trimester [2]. The itching shows spontaneous recovery 5 to 6 weeks after delivery [3]. In patients with ICP, poor perinatal outcomes are more common than normal healthy pregnant women because of increased rates of gestational diabetes, pre-eclampsia, meconium in amniotic fluid and preterm delivery [4, 5]. Many physiological alterations occur in organs and the body during pregnancy [6]. The hormonal changes in pregnancy, genetics, and environmental factors affect biliary transport and secretion. Although the etiology of ICP hasn't been fully elucidated, above-mentioned factors have been implied in the etiopathogenesis of the disease [7]. The early diagnosis and treatment are important in the ICP. There are reports suggesting that parameters used in first-trimester screening tests (pregnancy-associated plasma protein A (PAPP-A) and free beta-human chorionic gonadotropin (free ß-hCG) can be used as early markers for ICP [8].

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In our study, we investigated role of first and second trimester screening tests including PAPP-A, free β-hCG, estriol (E3), alpha-fetoprotein (AFP), and total β-hCG in the early diagnosis of ICP.

MATERIAL and METHODS

The study was conducted by reviewing all patients with ICP who were admitted to Obstetrics & Gynecology Department of Mersin University, Medicine School for delivery between 2015 and 2019 and had first and second-trimester aneuploidy screening tests performed in the same facility. Data were extracted from database of the hospital. The following criteria were used as diagnostic criteria for ICP: the presence of general pruritus without skin lesion in late second trimester or third trimester [2]; serum alanine aminotransferase (ALT) level >40 IU [3]; fasting bile acid level >10 mmol/L; and spontaneous recovery of symptoms and laboratory abnormalities after delivery. Patients with multiple pregnancies, those with history of cholecystectomy, those with gestational diabetes mellitus, thyroid disorder or pregnancy-related hypertension, and those with hepatic or biliary diseases were excluded. In addition, we randomly selected 60 pregnant women without the comorbid condition who were admitted to the obstetrics department during the same period as controls.

In all patients, data regarding age, gestational age, hepatic function tests at admission, birth weight and umbilical artery pH values at birth, free ß-hCG and PAPP-A MoM (Multiplies of Median) values in first-trimester screening and total ß-hCG, E3 and AFP MoM values in second trimester, medications and fasting bile acid levels (only for patients with ICP) were extracted from patient files. Demographic data and laboratory results were compared between patients with ICP and controls. In our hospital, first and second-trimester screening tests were studied using Beckman Coulter Dxi 800 analyzer. First-trimester screening is performed between gestational weeks 11 and 14 when CRL (crown-rump length) was measured to be 43-84 mm. In the test, trisomy (trisomy 13, 18 and 21) and age risk is estimated based on free ß-hCG and PAPP-A MoM values calculated according to CRL and NT (nuchal translucency) values. Second trimester screening is performed between gestational weeks 16 and 20 by measuring BPD (biparietal diameter). In the test, risk estimation for trisomy and neural tube defects are estimated based on ß-hCG, E3 and AFP MoM values. In patients diagnosed as ICP, only ursodeoxycholic acid therapy was prescribed based on the recommendation of gastroenterology department.

The delivery decision was made based on worsening in hepatic function tests, intrauterine growth retardation, fetal umbilical Doppler abnormality, severe preeclampsia, and other obstetric indications.

Statistical analysis was performed using SPSS version 22.0 (IBM Corporation, Amork, USA). After testing normal distribution of data, Student's t test was used to compare mean values. ROC curve analysis was performed to determine potential for ICP prediction in parameters found to be significantly higher in patients with ICP. Threshold values, sensitivity, specificity and positive and negative predictive values for ICP development were calculated. A p value <0.05 was considered as statistically significant.

RESULTS

Overall, 46 eligible patients were included. Of the patients, 6 gave birth via vaginal route while 40 gave birth via abdominal route. Of patients with ICP, ursodeoxycholic acid therapy was given to 38 patients. No significant differences were detected between groups regarding mean age, gestational age at admission, fetal weight and umbilical artery pH values at birth (**Table 1**).

When laboratory values were assessed, it was found that ALP, ALT and AST were significantly higher in ICP group while there was no significant difference in GGT level between groups (**Table 1**).

In first trimester screening, it was found that PAPP-A MoM was significantly lower (0.89±0.55 vs. 1.94±0.73; p=0.035) while free ß-hCG MoM was significantly higher in ICP group when compared to controls (1.84±0.59 vs. 0.99±0.47; p=0.018) (**Table 1**).

In second trimester screening, no significant difference was detected in aneuploidy markers between groups (**Table 1**).

The predictive abilities of first and second-trimester PAPP-A and free β-hCG MoM levels for the diagnosis of ICP were depicted in Figure 1 and table 2. Both markers were found to have significant predictive ability for the future development of ICP. First trimester free β-hCG >1.44 MoM was found to have a sensitivity of 50%, a specificity of 80% and positive and negative predictive values of 33% and 88.9% respectively. Similarly first trimester PAPP-A values <1.075 MoM was found to have 80% and 75% sensitivity and specificity respectively with positive and negative predictive values of 75% and 44% respectively (**Table 2**).

Table 1: Characteristics and mean laboratory values in patients with intrahepatic cholestasis of pregnancy and controls

	Patients with intrahepatic cholestasis of pregnancy (n=46)	Controls (n=60)	P
Age (year)	27.1±5.4	29.3±6.3	0.20
Gestational week	32±5	32±7	0.37
Fetal weight (gram)	3035±702	2290±680	0.57
GGT (IU/L)	19±12	27.7±35	0.70
ALP (IU/L)	208±73	137±83	***0.001****
ALT (IU/L)	178±146	52±40	***0.0001****
AST (IU/L)	119±99	44±40	***0.0001****
Bile acid(IU/L)	45±5.6	N/A	N/A
Dose of medication (miligram)	675±38	N/A	N/A
Umbilical Artery Ph	7.32±0.05	7.28±0.09	0.11
PAPP-A (mom)	0.89±0.55	1.94±0.73	***0.035****
Free β-hCG (mom)	1.84±0.59	0.99±0.47	***0.018****
Estriol (mom)	1.12±0.19	0.69±0.21	0.13
β-hCG (mom)	1.1±0.39	0.64±0.21	0.26
AFP(mom)	0.95±0.01	1.19±0.21	0.13

Student T test was employed. * p<0,05, ALT – ALT -serum alanine transaminase, AST– serum aspartat transaminase, GGT – g-glutamyl transpeptidase, ALP – alkaline phosphatase, AFP – α-fetoprotein, hCG – human chorionic gonadotropin, MoM – multiple of the median, PAPP-A – pregnancy-associated plasma protein A.AFP-alfa fetu protein,Free Beta hCG-free human corionic gronadodropin

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Table 2: Likelihood of detecting intrahepatic cholestasis of pregnancy according to fFree β-hCG cut-off value

	AUC	Sensitivity	Specificity	PPV	NPV	P
Free β-hCG (MoM) Cut Off >1,44	0.83	50%	80%	33%	88.9%	0.026*
PAPP-A (MoM) Cut Off <1,075	0.88	80%	75%	44%	93.7%	0.01*

*p<0,05, MoM – multiple of the median, Free Beta hCG-free human corionic gronadodropin AUC – area under the receiver operating curve, MoM – multiple of the median, NPV- negative predictive value, PPV- positive predictive value.

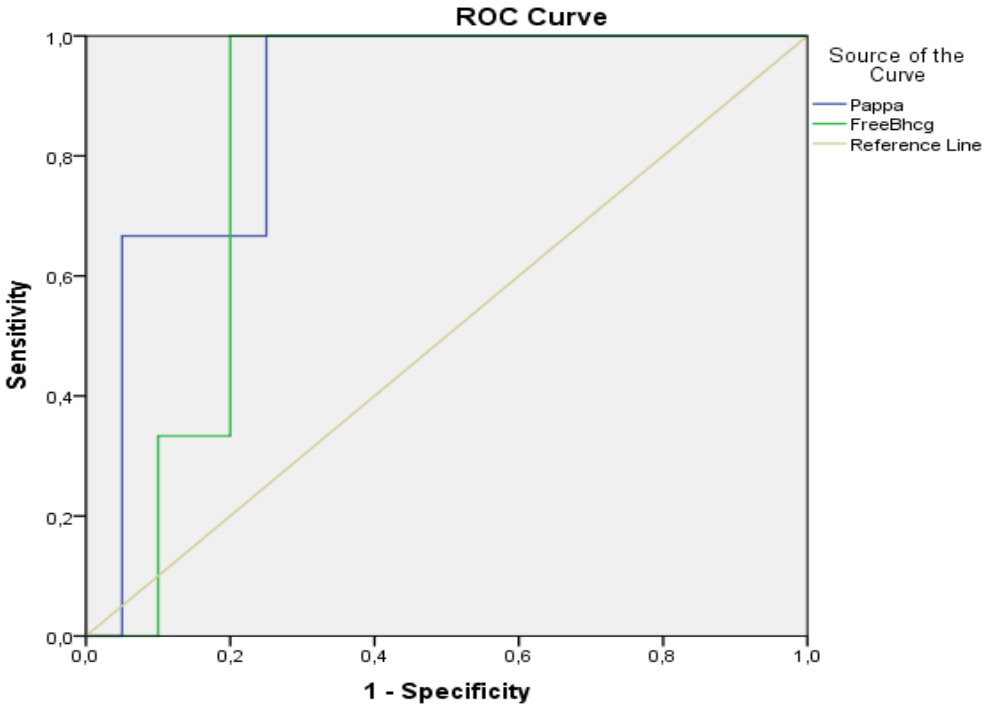

Figure 1: ROC curves for free β-hCG and PAPP-A in intrahepatic cholestasis of pregnancy

DISCUSSION

Intrahepatic cholestasis is the most common liver disease during pregnancy and is associated with adverse maternal and fetal outcomes. It is a diagnosis of exclusion, therefore, it is not always easy to diagnose the disease. Any marker that provide early diagnosis would result in prompt intervention and consequently decrease the adverse outcome risks. In the present study we assessed the roles of first and second trimester aneuploidy markers in the prediction of ICP development and showed that first trimester PAPP-A and free β-hCG MoM levels, but not second trimester markers, may have a comparable predictive potential for the subsequent ICP development throughout the pregnancy. The biochemical markers used for Down syndrome screening can predict poor maternal and fetal outcomes. It was shown that low PAPP-A level is associated with preeclampsia, gestational diabetes mellitus, preterm delivery, intrauterine growth retardation, preterm rupture of membranes and placental ablation [9-12]. A high PAPP-A value was suggested to be associated with a higher rate of meconium-stained amniotic fluid [13] First trimester low hCG levels are reported to be associated with increased risk for intrauterine growth restriction (IUGR), preterm birth, low birth weight (LBW) and low Apgar scores whereas high levels are related with a significantly decreased risk of preterm birth and GDM [14].

In the second trimester both low and high β-hCG levels were found to be associated with increased risk for spontaneous abortion, IUGR and preterm birth [14].

The relationship between these aneuploidy markers and the development of ICP has also been assessed in a few studies. Hancerlioglu et al. reported low PAPP-A and high free β-hCG levels were associated with obstetric cholestasis [8]. They included 35 ICP patients and found the mean PAPP-A and free beta hCG MoM levels as 0.76 ± 0.31 and 1.2 ± 0.79 respectively. Contrary to this study, another study from the same country could not find an association between low first trimester PAPP-A levels and ICP [15]. Destici et al. reported median PAPP-A MoM levels as 0.93 in ICP group. In the present study the median first trimester PAPP-A levels were 0.89 which was significantly lower in ICP patients. In ROC analysis, the area under the curve was found to be 0.88, and values >0.8 is considered to be a strong predictor of clinical sensitivity or specificity (16). In their study, Tayyar et al. found PAPP-A <0.93 MoM as a cut-off value for prediction of ICP with 73.8% sensitivity and 56.3 specificities (95% CI, AUC± SE: 0.663 ±0.042). [17]; however, different than our results, they did not find free hCG to be associated with ICP. They also could not find any relationship between second-trimester markers, including Inhibin A, with subsequent ICP

[17]. In the present study PAPP-A 1.075 MoM was found as a cut-off value with substantial sensitivity, specificity, positive and negative predictive values. Therefore low PAPP-A MoM values seem to have a predictive potential for subsequent ICP.

PAPP-A is a glycoprotein secreted from the extravillous trophoblasts of the placenta [18] and it cleaves insulin-like growth factor binding protein (IGFBP)-4 and -5 [19]. By this way it modulates the activity of insulin-like growth factor (IGF)-1 and -2 [20]. Low levels of PAPP-A results in decreased IGF levels due to decreased cleavage of the binding protein. In addition PAPP-A plays a role in the autocrine and paracrine control of trophoblast invasion of the decidua [21] and any change during this period may have a negative effect continuation of pregnancy.

The failure in invasion (due to IGF effect) increases the risk for intrauterine growth retardation, pregnancy-related hypertension, and intrauterine death in advancing periods of pregnancy. In some studies, it was found that low PAPP-A levels reduced blood IGF level. The decreased IGF levels in pregnancy results in poor pregnancy outcomes such as preterm birth, preeclampsia, gestational diabetes mellitus, and intrauterine fetal death [22, 23].

In a study on choleostatic rats, it was found that IGF therapy increased flow of bile acids and improved clinical presentation [24,25], concluding that there is an association between decreased IGF level and biliary flow. In the study, it was found that IGF is important for bile acid homeostasis and secretion and that endogenous and exogenous IGF decreased blood bile acid. The reduction in PAPP-A levels in early pregnancy can lead decrease in protease effect on IGF-4 and IGF-5; thus, ICP development in advancing weeks of pregnancy as a result of decreased IGF. Contrary to first trimester, high levels of third trimester PAPP-A levels have been observed in ICP patients [9].

Therefore obstetric conditions in which there is inadequate trophoblastic invasion in the first trimester would be associated with a low PAPP-A level, whereas hypoperfusion-related stimulation of the production of placental proteins in the second and third trimesters would be associated with a high PAPP-A level [9].

By initiation of pregnancy, trophoblastic cells begin to secrete glycoprotein ß-hCG. The increased free ß-hCG level in fetal and maternal blood has 80% similarity with luteinizing hormone (LH). The increased free ß-hCG in fetal and maternal blood binds to LH/hCG receptors, inducing steroidogenesis. The induced steroidogenesis results in formation of estrogen and progesterone metabolites. The increased estrogen and progesterone metabolites due to elevated free ß-hCG inhibit bile acid transportation in pregnant women. The increased blood bile acid levels due to failure in excretion lead increase in fetal and/or maternal toxicity and complications [7, 26, 27].

As placental hormone levels are increased by advancing gestational age, ICP is most frequently seen in third trimester. In multiple pregnancies, ICP is more common since placental hormones were higher [28]. In a study by Hancerlioglu et al., a relationship was found between high levels of ß-hCG and ICP [8]. However, no such relationship was found in some studies [29].

In our study, it was found that free ß-hCG level in first trimester was significantly higher in ICP group when compared to controls. In ROC analysis, a relatively higher AUC value was found and a cut-off value of 1.44 MoM was calculated for free ß-hCG. It was also found that the cut-off value can predict ICP by the sensitivity of 80% and specificity of 50%, indicating that first trimester free ß-hCG level can be a potential marker for ICP.

Alpha-fetoprotein (AFP) is a glycoprotein secreted from fetal liver and yolk sac. It is a parameter of second trimester testing used for Down syndrome screening between gestational weeks 16 and 20. There are reports suggesting that low AFP MoM values are associated with gestational DM while high AFP and ß-hCG MoM values were associated with preeclampsia [30, 31].

In previous studies, no relationship was found between the second-trimester screening test and ICP [17, 32, 33]. In a study by Yuan et al., it was concluded that the increased maternal AFP and estriol (E3) levels lead to an increase in risk for ICP in the third trimester [34]. In the study, it was also found that placental anomalies such as placental previa and placental abruption were more common among patients with elevated AFP. The authors concluded that serum AFP level is increased in women with placental dysfunction; as a result, ICP can be seen more commonly in third trimester. In our study, no significant difference was found in AFP between ICP and control groups in agreement with literature.

In previous studies, ICP was more frequently seen in patients with impaired estrogen metabolism [35]. It was found that serum bile acids were increased in non-pregnant women using oral contraceptives with high estrogen and progesterone content [36]. In an in vitro study, it was found that progesterone metabolites, not progesterone itself, impaired bile acid transport [27].

The increased ß-hCG level during pregnancy leads to an elevation in progesterone and its metabolite E3. The elevated E3 levels impair bile acid transport, increasing the risk for ICP. In a study by Raty et al. it was concluded that elevated ß-hCG level is associated with ICP [29]. In two distinct study by Eloranta et al. and Raty et al., no relationship was detected maternal AFP and ß-hCG levels and ICP [29, 33].

However, in the study by Raty et al., ß-hCG levels were increased in the ICP group but did not reach statistical significance. In a study by Leslie et al., no significant difference was found in E3 level between ICP and control groups [37].

In our study, AFP levels in ICP groups were comparable with controls. However, both ß-hCG and E3 levels were higher in ICP group than controls, but the difference did not reach statistical significance. The elevated E3 and ß-hCG levels alone may not result in cholestasis. However, genetic sensitivity or environmental factors can lead ICP in patients with similar E3 and ß-hCG levels.

Although GGT was found to be high in patients with ICP in some previous studies [12, 38] GGT levels were normal in patients with ICP in many studies in agreement with our results [32]. In patients with ICP, the extent of ALT increase was higher than AST [38, 39].

In our study, it was also found that ALT increased more than AST. Since ALP is mainly released from placenta, the use in ICP diagnosis is limited.

Our study is one of the rare studies that evaluated first and second-trimester biochemical markers together in patients with ICP. In our study, the diagnosis and treatment were conducted by the same healthcare providers in a single-center. Major limitations are retrospective design and small sample size. In addition, as it is the case in the studies using biochemical parameters, the values obtained are dependent to study population, laboratory and analyzer used. Thus, it will be appropriate to take these factors into consideration.

CONCLUSION

In conclusion, it was found that low PAPP-A MoM value, one of the Down syndrome screening tests in first trimester, and elevated free ß-hCG were associated with ICP development. Although other biochemical parameters were found to be higher in ICP group, no relationship with ICP was found. The early recognition of ICP is important as perinatal complications are more common in patients with ICP. ICP development can be predicted by low PAPP-A and high free ß-hCG MoM values in the first trimester. If women are already being screened for Down syndrome using the first-trimester aneuploidy test markers, which is routinely performed, the same markers can be employed to screen for ICP, offering a simple and low-cost opportunity to identify groups at high risk of developing this disorder. There is a need for prospective studies with a larger sample size for introduction to routine practice.

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Evaluation of anemia frequency and etiologies in hospitalized patients in a tertiary pediatrics clinic

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ABSTRACT

Objective: Anemia, which is a public health problem on a global scale, continues to maintain its importance in pediatric patients. There are few studies on the prevalence of anemia in hospitalized children. This study was conducted to evaluate the prevalence and etiologies of anemia in hospitalized pediatric patients.

Material and Method: This is a cross-sectional epidemiological study. The study group consists of 1000 patients between the ages of 6 months and 18 years who were hospitalized in the Department of Pediatrics of Prof. Dr. Cemil Tascioglu City Hospital. The data of the patients were reviewed retrospectively. The SPSS 22.0 program was used for statistical analyzes and calculations and $p< 0.05$ was accepted for significance.

Results: Of the patients included in the study, 569 (56.9%) were male, and 431 (43.1%) were female. In the study, the number of patients with anemia was 276 (27.6%), and among those 151 (26.5%) were male and 125 (29.0%) were female. The highest rate of anemia in different age groups was in infancy, with 32.3%.

The number of patients with iron deficiency anemia was 121 (43.8%), anemia of chronic disease was 42 (15.2%), anemia of acute inflammation was 41 (14.9%), and anemia due to B12 deficiency was 31 (11.2%). It was determined that patients with anemia had a longer hospital stay than those without anemia. Moreover, the hospitalization period of patients with anemia of chronic disease was longer than those with iron deficiency anemia.

Conclusion: Anemia is an important problem in hospitalized children as well as in the general population. Iron deficiency is the most common etiology of anemia in hospitalized patients in the pediatric clinic similar to the general population. The hospitalization period was found to be significantly longer in anemic patients than in non-anemic patients. During hospitalization, children should be monitored for anemia and this duration of stay should be regarded as an opportunity to combat anemia or to provide necessary micronutrient or nutritional support to socioeconomically disadvantaged groups.

Keywords: Anemia, iron deficiency, pediatrics, hospital

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INTRODUCTION

Anemia, which is considered a public health problem on a global scale, is defined as a decrease in hemoglobin or hematocrit levels below two standard deviations according to age, race, and gender (1-3). Worldwide, it is estimated that approximately one-quarter of the world's population has anemia, and this prevalence is higher in developing countries. Although anemia is common, the prevalence of anemia may vary according to the development level of countries. The anemia prevalence was reported as 9.1%, 25.7%, and 42.8% in high, middle, and low-income countries, respectively. The 2015 WHO report, derived from the global prevalence of anemia in 2011, showed that the highest frequency (42.6%) was in children compared to other age groups in the world (4). Despite a decline in prevalence globally, total cases of anemia rose from 1.42 billion in 1990 to 1.74 billion in 2019. The greatest burden of anemia was found in west and central sub-Saharan Africa and South Asia, while the highest prevalence of combined anemia (39.7%) was found in children under the age of five (5). When the anemia causes are examined according to etiologies, iron deficiency anemia is the leading one. It is estimated that around 50% of anemia cases are attributed to this micronutrient deficiency, however, this rate likely

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varies considerably between regions and countries. Other causes of anemia include other micronutrient deficiencies such as folic acid and vitamin B12 deficiencies; the presence of infectious diseases and genetic hemoglobin disorders (6, 7).

In epidemiological studies, it was found that anemia is associated with impaired neurocognitive outcomes, including learning difficulties, corrupted memory and processing speed, and emotional instability. Iron, folic acid, and vitamin B12 are essential for brain development and function. In general, a deficiency of these micronutrients results in decreased myelin production by altering gene and protein profiles, which can regulate central nervous system development processes. Impaired synaptogenesis and neural repair may lead to delays in brain development or dysfunction (8-11).

There are different anemia rates among hospitalized children in line with the sociocultural level and developmental state of countries. The presence of anemia augments the treatment process and the risk of hospital stay (4). Our aim in this study is to evaluate our hospitalized patients in terms of the prevalence of anemia and to discuss possible complications of this condition with the precautions that can be taken in this regard.

MATERIAL and METHODS

This study was performed on 1000 children between the ages of 6 months and 18 years who were hospitalized in the Department of Pediatrics of Prof. Dr. Cemil Tascioglu City Hospital between 01.03.2019 and 01.03.2020 and meet the inclusion criteria of the study. The patients with missing data were excluded from the study. This study is a cross-sectional, retrospective epidemiological study. Patients younger than six months and older than 18 years of age, and patients hospitalized in neonatal intensive care, pediatric intensive care, and/or pediatric hematology wards were not included in the study. The patients were divided into three groups according to their ages; those aged 6-24 months were included in the infancy period, those aged 3-9 years were included in the childhood period, and those aged 10 -18 years were included in the adolescence period (12). Demographic characteristics of the patients, diagnosis of hospitalization, duration of hospitalization, presence of anemia, transfusion information, presence of chronic diseases, complete blood count parameters at admission and discharge, ferritin, vitamin B12, foliate, C-reactive protein levels were recorded from the patient files. Anemia was diagnosed based on the reference values (13). This study was carried out in accordance with the Helsinki declaration, after the approval of the clinical research ethics committee of Cemil Tascioglu City Hospital (the date 26/01/2021 and number 26).

Statistical Package for the Social Sciences (SPSS) program was performed to evaluate and analyze the research data. Numerical data were reported as mean (standard deviation) and median (minimum-maximum); while the categorical data were given as numbers and percentages. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to determine the conformity of the data to the normal distribution. Parametric tests were performed if the data met the necessary assumptions, and non-parametric tests were used if they did not. The statistical significance was set at $p<0.05$.

RESULTS

In the study, which was carried out to determine the frequency and the etiology of anemia in hospitalized children, 1000 patients (569 boys and 431 girls) were examined and their demographic characteristics are summarized in Table 1.

While anemia was not detected in 724 (72.4%) of 1000 patients included in the study; anemia was present in 276 (27.6%) patients. Accordingly, the prevalence of anemia was found to be 27.6% in patients hospitalized in our clinic. The prevalence of anemia was found to be 26.5% in boys and 29.0% in girls.

The rate of anemia was 54.7% male and 45.3% female. According to the analysis for the etiology of anemia by gender, 63.6% of patients with iron deficiency, 52.4% of patients with anemia of chronic disease, 51.2% of patients with anemia of acute inflammation, and 43.1% of patients with other etiologies were male and there was a statistically significant difference between the genders regarding the etiology of the anemia (Table 2).

Of 276 patients with anemia, 121 (43.8%) were having iron deficiency anemia, 42 (15.2%) were having anemia of chronic diseases, 41 (14.9%) were having anemia of acute inflammation, and 31 (6.9%) were having B12 deficiency anemia (Table 3).

In addition to being the most common cause of all anemia cases, iron deficiency anemia is the leading cause of anemia with 50.3% in infancy, 23.1% in childhood, and 32.5% in adolescence.

As summarized in Table 4, 849 (84.9%) of the patients did not have a chronic/concomitant disease, while a concomitant chronic disease was detected in 151 (15.1%) of the patients. Among these diseases, epilepsy was the most common with a 24.5% prevalence.

According to the analysis performed to compare the length of stay in the hospital according to the etiology of anemia, the average length of stay (minimum-maximum) of patients diagnosed with iron deficiency was 3 (2-23) days; it was 6 (2-21) days in patients with anemia of chronic disease, 4 (1-25) days in patients with anemia due to acute infection, 4 (2-15) days for anemia due to B12 deficiency, and 4 (2-23) days for other etiologies of anemia.

There was a statistically significant difference between the groups regarding the hospitalization periods ($p<0.001$). When anemia of chronic disease was compared with iron deficiency, a statistically significant difference was found, prolonging the length of hospital stay. No statistically significant difference was found in the pair-wise comparisons of the other groups (figure 1).

As seen in Figure 2, when the duration of hospitalization is compared according to the presence of anemia, the average length of stay in the hospital for patients with anemia (minimum-maximum) was 4 (1-25) days, and the average duration of hospitalization for patients without anemia (minimum-maximum) was 3 (1- 14) days and there was a significant difference between the groups ($p<0.001$).

When the etiologies of anemia were compared according to the erythrocyte suspension transfusion requirements, the highest transfusion requirement was observed in patients with anemia of chronic disease with a rate of 23.8%.

As seen in Table 5, the difference between the groups was statistically significant (p=0.003).

Of the patients included in the study, 384 (38.4%) were in infancy, 451 (45.1%) were in childhood, and 165 (16.5%) were in adolescence. The prevalence of anemia in infancy was 32.3%, it was 24.8% in adolescence, and 24.6% in childhood. According to the analysis performed to compare the presence of anemia by age groups, there was a statistically significant difference between the groups. According to the pair-wise comparisons of the groups, the difference between infancy and childhood was statistically significant in that anemia was more common in infancy. However, the difference between infancy and adolescence and between childhood and adolescence was not statistically significant.

According to the analysis performed to compare the hospitalization and discharge hemoglobin levels of the patients (patients with erythrocyte suspension were not included in the analysis, n=307) the mean (± standard deviation) admission hemoglobin level was 11.71 (±1.63) g/dl and discharge hemoglobin (standard deviation) was 11.50 (±1.54) g/dl, and a statistically significant difference was found between the two groups (p<0.001). Accordingly, the hemoglobin measurement of the patients at discharge was 0.21 g/dl lower than the hemoglobin level at admission. The mean (±standard deviation) hospitalization hemoglobin of the patients in infancy was 10.98 (±1.30) and the mean discharge hemoglobin was 10.70 (±1.25) g/dl, and the difference was statistically significant. The mean (±standard deviation) hospitalization hemoglobin of the adolescent patients was 12.92 (±1.96) and the mean (±standard deviation) discharge hemoglobin was 12.69 (±1.88) g/dl, and the difference was found to be statistically significant (p<0.001).

Table 1. Demographic features of the study participants

Feature	Number (n)	Percentage (%)
Gender		
Male	569	56.9
Female	431	43.1
Age groups		
Infancy period (6 months-2 ages)	384	38.4
Childhood period (3-9 years)	451	45.1
Adolescence period (10-18 years)	165	16.5
Total	1000	100

Table 2. Comparison of the etiology of the anemia between genders

	Gender			
Etiology	Male n (%)	Female n (%)	Total n (%)	p
Iron deficiency	77 (63.6)	44 (36.4)	121 (100.0)	
Chronic diseases	22 (52.4)	20 (47.6)	42 (100.0)	
Acute infections	21 (51.2)	20 (48.8)	41 (100.0)	0.043
Others	31 (43.1)	41 (56.9)	72 (100.0)	
Total	151 (54,7)	125(45,3)	276 (100)	

Table 3. Anemia frequency and etiology

Anemia etiology	Number(n)	Percentage(%)
Iron deficiency anemia	121	43.8
Anemia of chronic diseases	42	15.2
Anemia of acute inflammation	41	14.9
B12 deficiency anemia	31	11.2
Iron and B12 deficiency anemia	14	5.1
Chronic diseases and iron deficiency anemia	5	1.8
Foliate, B12, and iron deficiency	5	1.8
Idiopathic	3	1.1
Anemia due to acute hemorrhage	3	1.1
Foliate deficiency	3	1.1
Thalassemia Intermedia	3	1.1
Sickle cell anemia	2	0.7
Thalassemia Major	2	0.7
Hereditary Spherocytosis	1	0.4
Total	**276**	**100.0**

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Table 4. Chronic/concomitant disease

Feature	Number (n)	Percentage (%)
Concomitant disease		
Present	849	84.9
Absent	151	15.1
Total	**1000**	**100.0**
Disease		
Epilepsy	37	24.5
Frequent asthma or bronchitis attacks	20	13.2
Congenital cardiac disease	16	10.6
Down Syndrome	11	7.3
Cerebral Palsy	9	6.0
Inflammatory bowel disease	9	6.0
Hypothyroidism	8	5.3
Malignity	8	5.3
Celiac disease	7	4.6
Tuberculosis	5	3.3
Nephrotic Syndrome	5	3.3
Autism Spectrum Disorder	5	3.3
Chronic Liver diseases	5	3.3
Factor 8 Deficiency	4	2.6
Others	2	1.3
Total	**151**	**100.0**

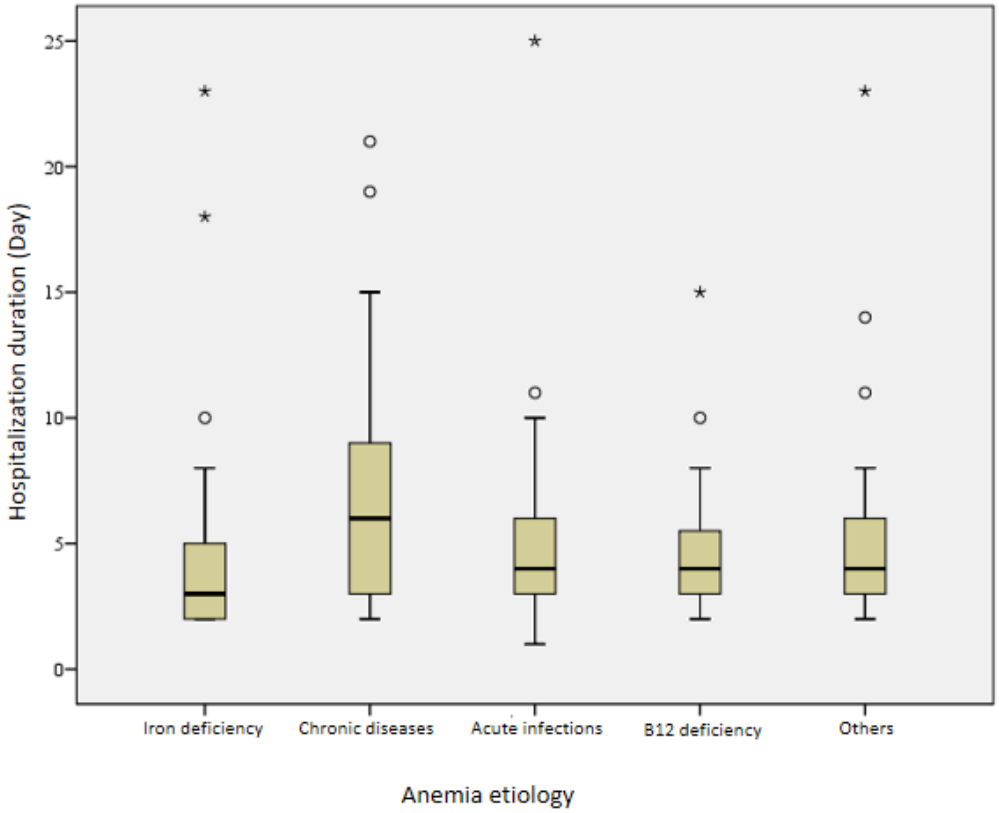

Figure 1. Duration of hospitalization regarding the anemia etiology

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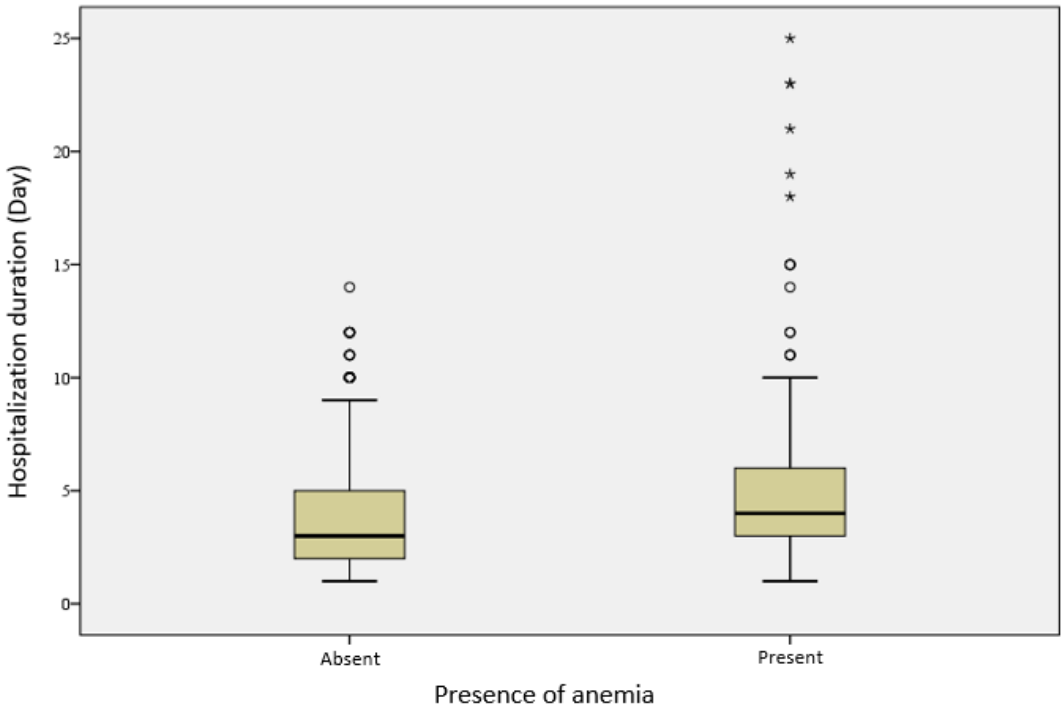

Figure 2. Hospitalization duration in the presence of anemia

Table 5. Comparison of erythrocyte suspension transfusion requirements according to the etiology of anemia

	Erythrocyte suspension requirements			
Etiology	Absent n (%)	Present n (%)	Total n (%)	p
Iron deficiency	115(95.0)	6(5.0)	121(100.0)	0.003
Chronic diseases	32(76.2)	10(23.8)	42(100.0)	
Acute infections	38(92.7)	3(7.3)	41(100.0)	
Others	66(91.7)	6(8.3)	72(100.0)	

DISCUSSION

The general prevalence of anemia in the world is around 42.6%. In our study, the prevalence of anemia was found to be 27.6%. Salami et al. (15) found the prevalence to be 33.2% in a study conducted in hospitalized pediatric patients aged between 1 month and 12 years. The prevalence of anemia was found to be 62.3% in a study conducted on the African continent, (4). In Europe and similarly developed countries, the prevalence of anemia was around 15% which is lower (16). The prevalence of anemia in the world is in a wide range according to age groups and socio-cultural levels. Although the rate we found in our study included hospitalized patients, it is compatible with the general population values of the literature. The decrease in anemia in proportion to the level of development shows that access to micronutrients and other nutrients is a fundamental factor. As a matter of fact, in the study of Salami et al.(15), children with malnutrition encountered anemia 3.4 times more than those without malnutrition.

Anemia in childhood is most common between the ages of 6 months and 2 years (17). The higher prevalence of anemia in infants is mostly attributed to the inability to meet the increasing need proportional to the increasing growth rate (18). In our study, the prevalence of anemia in infancy was found to be 32.3% and higher than that of other age groups, in line with the literature.

Zuffo et al. (19) found the prevalence of anemia in children younger than 24 months to be 39.9%. This rate was higher than other age groups, similar to our study. Sayar et al. (20) found the prevalence of anemia in the neonatal period to be 3.2% in their study conducted with healthy newborns and infants, while they found this rate to be 22.3% in the 6th-month control of the same children. As can be understood from the literature, we can say that this period is important in terms of micronutrient support.

Secondly, the highest frequency of anemia is seen in the adolescent age group (17). In our study, this rate was 24.8%, and it was the period in which anemia was seen with the second frequency. In the study of Dugdale et al., this frequency was found to be 27% in developing countries and 6% in developed countries (21). In a study conducted in our country, this frequency was found to be 12.5% (22). The lower prevalence in our study may be due to the selection of our patient group from hospitalized patients. It can be thought that anemia is more common in this age group, which may be due to the increased muscle strength and growth in boys, and growth and menstruation in girls.

When all age groups were included in our study, 54.7% of the patients diagnosed with anemia were male. Similarly, Enawgaw et al. (4) found that 55.2% of the patients with anemia were male.

This can be explained by the fact that anemia is more common in boys compared to girls due to rapid development in the pre-school period (23). On the other hand, in a study conducted by Salami et al.(15) in which they evaluated hospitalized patients, although anemia was more common in males, they could not find a statistical difference. Some studies are the opposite of this and found anemia more commonly in females (24, 25).

When patients with anemia were examined, the most common etiological factor was iron deficiency anemia with 43.8%. In a study conducted by Çetinkaya et al. in hospitalized patients, they found the prevalence of iron deficiency anemia to be 61.6% (26). In a prevalence study conducted by Balci et al. in adolescent patients, they found the rate of anemia to be 5.6%. Among all anemia, iron deficiency anemia was the most common (24). In the study of Silva et al., they found the prevalence of iron deficiency anemia to be 10.3%, but in the same study, the rate of iron deficiency was found to be 37.4%. In a study conducted on hospitalized children from Turkey, the frequency of iron deficiency anemia was found to be 16.5% (27). As can be seen, studies are reporting different frequencies. Iron is a very important micronutrient, especially in childhood age groups. Its deficiency causes iron deficiency and iron-deficiency anemia. After understanding the importance of this condition, countries started to give iron supplements in certain age groups as a part of preventive health services. As a matter of fact, as a part of this struggle since 2004, prophylactic iron supplementation has been applied to all infants from 4 months of age with the "Turkey as iron" campaign in our country. This treatment is applied free of charge until the age of 1 (28). Although positive results have been obtained with this treatment, unfortunately, we are still far from the target of reducing it to 5% and below recommended by the World Health Organization. More education and nutritional support should be provided to socio-culturally disadvantaged groups. However, patients who do not receive prophylaxis should be checked for anemia with certain follow-up programs. There are follow-up guidelines published for these controls in our country (29).

B12 deficiency anemia is another common nutritional anemia in developing countries, and it has been reported that it is more common especially in children and pregnant women (30). In studies conducted in regions with low socioeconomic status, the frequency of B12 deficiency has been reported to vary between 22-65% (31). Naiboglu et al. (27) found the frequency of B12 deficiency to be 51.5% in their study on children hospitalized due to lower respiratory tract infection. In our study group, the rate of patients with B12 deficiency anemia was found to be 11.2%. When combinations of B12 deficiency with other etiologies are included, B12 deficiency anemia constitutes 18.1% of all anemia cases. B12 deficiency continues to be important in developing countries, especially due to nutritional deficiencies.

In our study, when the relationship between anemia and the length of stay of the patients was examined, a significant relationship was found, and the hospitalization period of patients with anemia was higher than those without anemia. Salami et al (15) did not find any significant relationship between anemia and hospitalization periods. However, it is not easy to explain the hospitalization period only with anemia. It is obvious that this may change depending on the underlying disease and treatment processes. On the other hand, a significant correlation was found between the etiology of anemia and the need for transfusion of erythrocyte suspension during their hospitalization, and the group with the most frequent transfusion requirement was the patients with anemia of chronic disease. These patients may have had higher transfusion needs than non-hospitalized patients with iron deficiency due to their other accompanying health problems. The discharge hemoglobin levels of our patients who were not transfused were found to be lower than the hospitalization hemoglobin levels. Since our hospital is a tertiary center, we think that this situation occurs due to the hospitalization of patients who require more examinations and investigations.

Our study has some limitations. Since our study was retrospective, it was not possible to reach the socioeconomic data of our patient group, the information about whether the patients received iron prophylaxis, their dietary habits, and histories from the file information.

CONCLUSION

In conclusion, this study showed that anemia is an important problem in hospitalized children, iron deficiency anemia is the most common etiological cause in this group, as in the general population, and that the hospitalization period in anemic patients is longer than that of the non-anemic patients. During hospitalization, as in public health monitoring programs, children should be monitored for anemia and hospitalization should be seen as an opportunity to combat anemia or to provide necessary micronutrient or nutritional support to socioeconomically disadvantaged groups.

Author Contributions: IG, ET, HD: Concept, Data collection and/or processing, Analysis and/or interpretation, Literature review, **HD:** Writing**,** Revision

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Use of neutrophil/lymphocyte ratio as a marker in patients with suspicious diaphragmatic attenuation artifact

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ABSTRACT

Objective: This study aimed to evaluate whether neutrophil/lymphocyte (N/L) ratio assists in the diagnosis of coronary artery disease (CAD) in patients with suspected diaphragmatic attenuation artifact (DAA) on myocardial perfusion SPECT (MP-SPECT).

Material and Methods: A total of 255 patients undergoing coronary angiography between 2015-2020 due to unclear DAA of the inferior wall on MP-SPECT were included in this retrospective study. Patients were divided into two groups (CAD and non-CAD) according to angiographic images. Significant CAD was defined as ⩾50% stenosis of coronary arteries feeding the inferior wall. White blood cell count, biochemical parameters, and risk factors for CAD were compared between the two groups.

Results: There was no statistically significant difference between the two groups in terms of age (p = 0.055), gender (p = 0.482), and body mass index (p = 0.305). N/L ratio (OR = 1.397 p = 0.002 95% Cl = 1.128-1.732) and left ventricle ejection fraction (OR = 0.896 p = 0.023 95% Cl = 0.815-0.985) were independent risk factors for CAD in multivariate binary logistic regression analysis. Receiver Operating Characteristic (ROC) curve analysis showed that a cut-off value of ⩾2 for N/L ratio predicted the presence of CAD (sensitivity=63.5%, specificity=60.7%, AUC=0.668, 95% CI=0.596 – 0.740, p<0.001).

Conclusion: N/L ratio is a simple and accessible test and may increase the diagnostic accuracy of MP-SPECT for CAD in patients with suspicious diaphragmatic attenuation on MP-SPECT.

Keywords: neutrophil, lymphocyte, diaphragmatic attenuation, scintigraphy

INTRODUCTION

Coronary artery disease (CAD) is the leading cause of mortality and morbidity in the world (1). Numerous non-invasive methods have been developed to assist in the diagnosis of CAD. Myocardial perfusion single-photon emission tomography (MP-SPECT) is one of the methods with high sensitivity and specificity. However, image artifacts may occur in MP-SPECT due to the patient and technical problems. These artifacts reduce the sensitivity of MP-SPECT. The most common artifact associated with the left ventricular inferior wall in men is the diaphragmatic attenuation artifact (DAA). DAA can cause fixed perfusion defect interfering with a myocardial scar. It was reported that DAA was seen in 25% of MP-SPECT images (2). DAA can frequently occur due to elevation of the left diaphragm and, less often, the effect of the right ventricle. Generally, the elevation of the left diaphragm is seen in obese people and causes DAA. Pulmonary atelectasis and loss of pulmonary parenchyma can also cause diaphragm elevation. There are different methods used for the diagnosis of DAA (3). These methods can be listed as right lateral projection, electrocardiography (ECG) gated images, prone position images, and attenuation correction programs. Generally, prone position and ECG gated images are used (4). However, DAA frequently could not be distinguished despite these methods. Various cells and mediators play a role in the inflammatory process. The most important of these are neutrophils and lymphocytes. Neutrophils damage myocardial cells by releasing proteolytic enzymes such as Leukotriene B4, elastase, and neutrophil chemotactic activity. Many studies showed that these enzymes and low lymphocytes were responsible for acute coronary syndrome (ACS) and stable angina pectoris.

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In recent studies, the neutrophil/lymphocyte (N/L) ratio has been used as an inflammatory marker in atherosclerotic disease. N/L ratio was associated with CAD, mortality, low left ventricle ejection fraction (LVEF), and peripheral artery disease (5, 6).

To our knowledge, the role of the N/L ratio in the diagnosis of suspected DAA and myocardial ischemia differentiation is lacking in the literature. In this study, we evaluated whether the N/L ratio has a discriminative role for patients with suspicious inferior myocardial ischemia on MP-SPECT.

MATERIAL and METHODS

Study Design: This cross-sectional retrospective study was conducted at our institution. Two hundred and twenty-five patients who underwent coronary angiography with suspicious DAA on MP-SPECT between January 2015 and March 2020 were enrolled in this study. The study was designed in accordance with the Declaration of Helsinki and approved by Ethics Committee of Kahramanmaras Sutcu Imam University Faculty of Medicine (30.04.2019 decision no:7).

Participant Population: Data for patients who underwent MP-SPECT imaging between January 2015 and March 2020 were examined. During this time span, 2590 patients who could not be diagnosed by effort test, stress effort test, or who could not tolerate effort tests underwent MP-SPECT imaging. Of these 2590 patients, 269 patients had non-conclusive results due to suspicious DAA in the inferior wall of the left ventricle. Forty-four patients with previously known CAD, ACS, malignancy, suspected infection, hypertrophic cardiomyopathy, severe valve and rheumatologic disease were excluded. The remaining 225 patients were included in this study. Coronary angiographic images for all patients were examined by two physicians. The stenosis percentages for coronary arteries were calculated by the program used in the angiography unit. Stenosis of $\geqslant$50% of the coronary arteries feeding the inferior wall was evaluated as CAD, and <50% stenosis of coronary arteries was assessed as non-CAD according to the American Heart Association classification for coronary artery segments. Age, height, weight, known diseases, and medication use of participants were explored.

Measuring Blood Samples: Blood samples were taken from patients through the antecubital vein at hospital admission. Routine blood tests, including red blood cell count (RBC), white blood cell count (WBC), and platelet count (PLT), were performed using an automated hematology analyzer (Sysmex XN-3100, Kobe, Japan). Biochemical markers, including fasting glucose, triglyceride (TG), high-density lipoprotein (HDL), LDL, total cholesterol (TC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine (CRE) were tested with an automated biochemical analyzer (Roche Cobas 6000, Germany)

Myocardial Perfusion Scintigraphy Design: Routinely, patients were examined with treadmill exercise stress (Bruce or modified Bruce protocol), or pharmacological stress with adenosine (140 mcg/kg) or dipyridamole (0.56 mg/kg). Radiopharmaceutical injection was performed within the 4th minute of maximal effort or pharmacological injection. Tc-99m-MIBI (Methoxyisobutilizonitrile) was used as a radiopharmaceutical. Patients in the supine position underwent 8mCi-22mCi activity for stress and rest protocol, respectively. SPECT was performed from right -45 degrees anterior oblique to 135 degrees according to 90-degree detector positioning with Siemens E. cam dual detector camera. ECG-gated images (8 times per second) were taken simultaneously. Radiopharmaceutical injection was performed approximately 3 hours later, and rest images were taken after 45 minutes after the injection. SPECT was performed in the prone position from -135 degrees to counterclockwise 180 degrees on patients with perfusion defects in the inferior wall. However, patients whose diaphragmatic attenuation wasn't recognized were included in the study. Transverse, vertical, coronal sections and GATED images were examined in all protocols. The SyngoMI system was used for processing images.

Statistical Analysis: Statistical Package for Social Sciences (SPSS) for Windows 21 program was used to analyze the data. Continuous variables are expressed as mean ± standard deviation and categorical variables as a percentage. The participants were divided into two groups as CAD and non-CAD. Kolmogorov-Simirnov test, skewness, and kurtosis were used for the assessment of normal distribution. Independent Student's t-test was used for continuous variables with normal distribution. The Chi-square test was performed on categorical variables. One-Way ANOVA was used in the comparison of more than two groups. Post-Hoc analysis was performed to find which group caused the difference. Multivariate binary logistic regression analysis was used to analyze independent predictors for the presence of CAD. Receiver Operating Characteristic (ROC) curve analysis was used to find the cut-off value for variables that predicted CAD. A 2-sided p-value of <0.05 was considered statistically significant.

RESULTS

One-hundred and ten (48.9%) patients were male, and the mean age of patients was 66.53 ± 9.66 years. Patients were divided into two groups as CAD and non-CAD, according to the presence of significant CAD on coronary angiography. The clinical and demographic characteristics of the patients are shown in **Table 1**. There were no statistically significant differences between the two groups in terms of DM, HT, smoking, age, and lipid profiles. The box plot graphic for the N/L ratio is shown between the two groups (**Figure 1A**). Age, sex, smoking, LVEF, DM, HT, and N/L ratio were entered into multivariate binary logistic regression analysis (**Table 2**). N/L ratio (OR = 1.397 p = 0.002 95% Cl = 1.128-1.732) and LVEF (OR = 0.896 p = 0.023 95% Cl = 0.815-0.985) were independent predictors for the CAD group (**Figure 1B**).

ROC curve analysis showed that a cut-off value of $\geqslant$2 for N/L ratio predicted the presence of CAD (sensitivity=63.5%, specificity=60.7%, AUC=0.668, 95% CI=0.596 – 0.740, p<0.001). Furthermore, the participants were divided into four groups based on the presence of CAD and the cut-off value for N/L ratio. Comparison of these groups according to clinical and demographic characteristics is shown in **Table 3**. Creatinine (p= 0.047) and LVEF (p=0.036) had statistically significant differences between the four groups. Additionally, the diagnostic accuracy of the N/L ratio was 61.33%.

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Table 1. Participant characteristics

	CAD	Non-CAD	p
Age	68.01±7.79	65.65±10.61	0.055
Male, n%	39 (45.9)	71(50.7)	0.482
DM, n%	37 (43.5)	44 (31.4)	0.067
HT, n %	50 (58.8)	70 (50)	0.198
Smoking, n%	33 (38.8)	41 (29.3)	0.140
LVEF, %	57.32±3.22	58.41±2.84	**0.009**
GFR, ml/min/1.73m2	77.72±21.61	81.66±20.01	0.167
BMI	30.01±5.00	29.30±4.99	0.305
HDL, mg/dl	44.72±9.64	44.60±11.13	0.933
LDL, mg/dl	121.38±42.63	123.96±45.13	0.672
TG, mg/dl	189.25±131.09	187.91±113.92	0.935
Glucose, mg/dl	146.52±73.37	129.12±60.07	0.067
Neutrophil count /uL	5558.24±1777.65	4505.14±1599.57	**<0.001**
Lymphocyte count/uL	2300.58±868.16	2445.71±824.92	0.211
N/L ratio	2.86±1.86	2.09±1.25	**0.001**

BMI; body mass index, CAD; coronary artery disease, DM ; diabetes mellitus, GFR; glomerular filtration rate, HDL ; high density lipoprotein cholesterol, HL; hyperlipidemia, HT; hypertension, LDL; low density lipoprotein cholesterol, LVEF; left ventricle ejection fraction, N/L ratio; Neutrophil/ Lymphocyte ratio

Table 2. Multiple binary logistic regression analysis of variables

Variables	B	Odd ratio	p Value	95% Cl
Age	0.024	1.024	0.134	0.993-1.057
Sex	0.379	1.461	0.213	0.805-2.654
Smoking	0.181	1.198	0.567	0.645-2.225
LVEF	-0.110	0.896	**0.023**	0.815-0.985
Hypertension	0.178	1.194	0.553	0.665-2.147
Diabetes Mellitus	0.509	1.663	0.094	0.916-3.020
N/L Ratio	0.335	1.397	**0.002**	1.128-1.732

Abbreviations: Cl; confidence interval, LVEF; left ventricular ejection fraction, N/L ratio; Neutrophil/ Lymphocyte ratio

Table 3. Participant characteristic according to cut off value for N/L ratio

Variables	N_L ratio ≥2 CAD+(n=54)	N_L ratio ≥2 CAD- (n=56)	N_L ratio< 2 CAD+(n=31)	N_L ratio< 2 CAD- (n=84)	P*
Male, n%	28(51,9)	29(51,8)	11(35,5)	42(50)	0,449
Age, year	68,90±7,19	66,41±11,87	66,45±8,63	65,11±9,72	0,169
BMI, kg/m²	29,27±4,76	28,95±5,35	31,29±6	29,54±4,76	0,192
HT, n%	32(59,3)	29(51,8)	18(58,1)	41(48,8)	0,618
DM, n%	24(44.4)	18(32,1)	13(41,9)	26(31)	0,329
Smoking, n%	22(40,7)	19(33,9)	11(35,5)	22(26,2)	0,342
TG, mg/dl	159;107	165;133	134;89	166;149	0,051
LDL, mg/dl	118,12±41,44	112,62±31,47	127,06±44,74	131,52±51,08	0,066
HDL, mg/dl	44,96±8,98	46,39±10,78	44,32±10,83	43,41±11,26	0,435
ALT, u/l	21,64±33,26	19,57±14,39	19±8,82	20,91±14,51	0,919
AST, u/l	20,53±13,47	20,71±9,30	20,77±8,17	21,61±12,43	0,945
Creatinine/dl	0,99±0,41	0.88±0,25	0,79±0,19	0,88±0,33	**0,047**
GFR, ml /min	74,08±22,25	80,28±19,28	84,07±19,17	82,58±20,55	0,073
Glucose, mg/dl	143,90±74,25	127,09±59,82	151,09±72,81	130,48±60,55	0,260
Hemogram, g/dl	13,43±1,55	13,41±1,89	13,10±1,50	13,57±1,66	0,620
LVEF, %	57,48±3,32	58,05±2,88	57,06±3,06	58,65±2,80	**0,036**

ALT; alanine transaminase, AST; Aspartate transaminase, BMI ; body mass index, DM; diabetes mellitus, HT; hypertension, GFR; glomerular filtration rate, HDL; high density lipoprotein, LVEF ; left ventricular ejection fraction, TG; triglyceride

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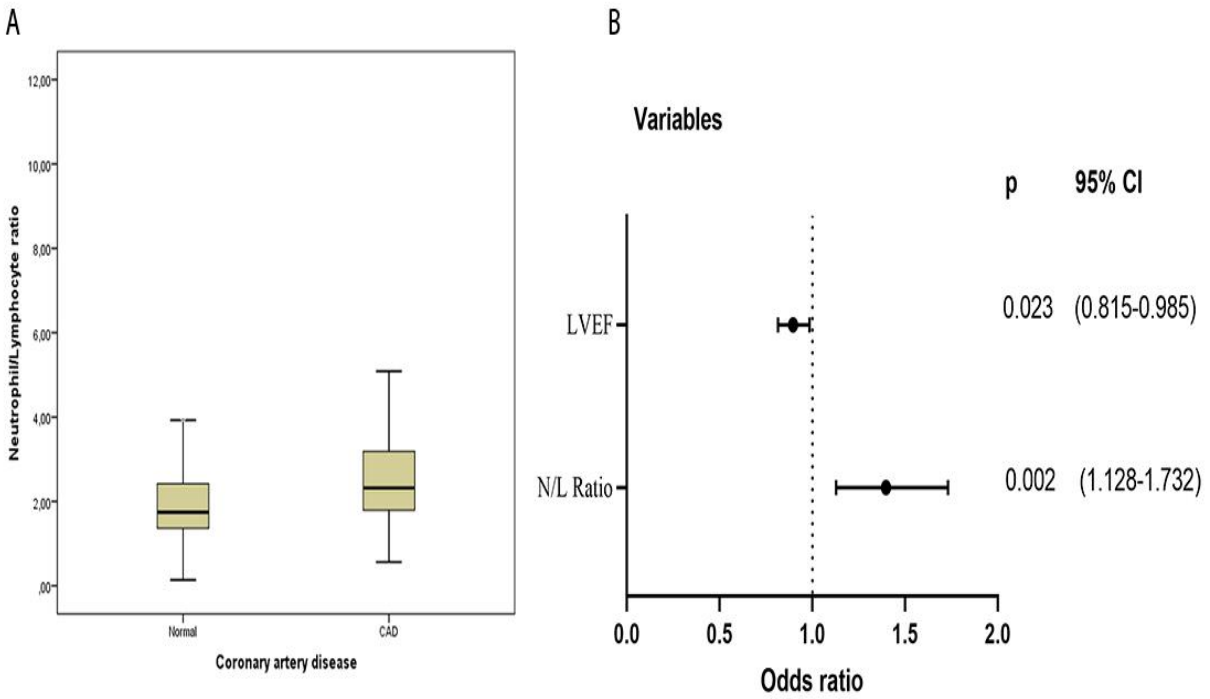

Figure 1. (A) Box plot showing the N/L ratio according to the presence of CAD, (B) Graph showing multivariate logistic regression analysis of statistically significant variables.

DISCUSSION

In this study, the N / L ratio, which is a simple laboratory test, was shown to help the diagnosis of CAD in patients with suspected diaphragmatic attenuation on MP-SPECT.

The purpose of diagnostic tests in clinical cardiology is to identify risky patients for CAD in the early period. MP-SPECT is a test that can provide important prognostic and diagnostic information. MP-SPECT has 90% sensitivity and specificity for the diagnosis of CAD. However, image artifacts reduce the sensitivity of the test. Diaphragmatic attenuation and breast attenuation are the most common image artifacts on SPECT (3-7). Gated ECG, prone images, and CT attenuation correction may be used for the recognition of these attenuations. Attenuation problems were significantly reduced through the use of these techniques. In a meta-analysis involving seventeen studies, the use of attenuation correction images increased the specificity of MP-SPECT (80% -90%) (8). PET and cardiac unique gamma cameras made of solid crystals developed in recent years, such as cadmium zinc telluride (CZT) and cesium iodide, increased sensitivity and specificity (9). In a meta-analysis performed in patients with coronary artery stenosis >50%, the sensitivity and specificity of conventional MP-SPECT studies were 86% and 74%, respectively. Myocardial perfusion images with Rb-82 PET had 92% sensitivity and 85% specificity in a study including 1442 patients (10). However, these methods are expensive and difficult to find. N/L ratio can help us to make the decision for coronary angiography despite artifact reducing methods in patients who were not able to be diagnosed due to DAA. Atherosclerotic heart disease, which is one of the most common causes of death in the world, is associated with multiple genetic and environmental risk factors. It was shown that neutrophils and lymphocytes, which are white blood cells, play a role in every stage of the atherosclerotic process from plaque onset to rupture of the plaque. Naruko et al. reported that the N/L ratio was associated with the progression of atherosclerosis and was an independent predictor of thin cap fibroatheroma (11). It was shown that atherosclerotic plaques in atherectomy specimens including neutrophil infiltration in patients with ACS tended to rupture vessels. Activated neutrophils release numerous proteolytic enzymes, and these enzymes are responsible for endothelial damage. Lymphocytes play a role in the inflammatory response with an inhibitory effect on atherosclerosis. Studies demonstrated that relative and absolute lymphocyte concentrations reduce in patients with cardiac events and a low lymphocyte count might be used as an early marker of physiologic stress and systemic collapse due to myocardial ischemia mediated by cortisol. Increased cortisol levels cause the reduction of lymphocyte counts (12). In our study, increasing neutrophil count was related to CAD. Before ACS occurred, the neutrophil count might increase and guide us for diagnosis of CAD. But the decrease in lymphocyte count was not found to be a factor affecting the N/L ratio. Studies showed that the N/L ratio was an independent risk factor for CAD mortality. When the N/L ratio was integrated into the Framingham risk score, the N/L ratio increased from the intermediate-risk score to the high-risk category.

A meta-analysis demonstrated that the N/L ratio is associated with CAD severity. GENSINI and SYNTAX scores were used to determine CAD severity in this study (13). In light of this information, the N/L ratio can be used for the diagnosis and severity of CAD. However, the cut-off value for the N/L ratio is controversial for CAD. A study found the cut-off the value of the N/L ratio was 2.7 for predicting the severity of CAD. (sensitivity= 72%, specificity= 61%) (14). In another study, including 172 patients, extensive CAD was identified

with a 2.5 cut-off value for N/L ratio (sensitivity = 62%, specificity = 69%) (15). Our study did not determine the severity of CAD. However, increasing the cut-off value for the N/L ratio provided high specificity for CAD.

Increased neutrophil count and N/L ratio were associated with cardiac complications in patients admitted with ACS. In a study of 500 people, including stable coronary disease, the N/L ratio was an independent predictor of MACE (16). Patients who underwent coronary artery bypass grafting operation and percutaneous coronary intervention were also included in this study. In our study, patients without known CAD were included. We showed that the N/L ratio, a simple test, can help us diagnose CAD in patients undergoing MP-SPECT.

There are some limitations to the study. The study is from a single center, and the number of patients is inadequate. Prospective studies including many patients should support these results. N/L ratio may be affected by many factors such as dehydration, overhydration, depression, and anxiety in blood samples taken before coronary angiography. The histories of patients need to be examined rigorously in prospective studies.

CONCLUSION

N/L ratio is a simple and accessible test and may increase the diagnostic accuracy of MP-SPECT for CAD in patients with suspicious diaphragmatic attenuation on MP-SPECT.

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Ethical approval: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by Local Ethical Committee.

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Variants of D9N, G188A, N291S, and 93 T/G Genes in patients with Coronary Artery Diseases

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ABSTRACT

Objective: Our work aimed to study the relationship between LPL variants D9N, G188A, N291S, and 93 T/G genes and CAD in Saudi patients.

Materials and Methods: We recruited 253 CAD patients, who underwent diagnostic coronary angiography, and 207 control subjects. Several biochemical and behavioral markers were obtained, and different genotypes of LPL variants, D9N, G188E, N291S, and 93 T/G, were detected using The PCR-RFLP method.

Results: The current study found D9N genotypes, AA, AG, and GG in 71.14%, 23.72%, and 5.14% in CAD patients, respectively. the AA, AG, and GG control genotypes were found in 81.64%, 16.43%, and 1.93%, respectively. The OR of the D9N AA versus AG genotype with a 95% CI was determined to be 1.65 (1.04–2.65), (p = 0.035). The OR of the D9N AA versus AG + GG genotype with a 95% CI was 1.80 (1.16–2.81), (p = 0.009). A strong relation of the D9N AA was observed with CAD. For the G188E, N291S, 93T/G variants insignificant were observed in both CAD and control groups.

Conclusion: This study revealed the D9N variant has an association with CAD; however, no relation was detected between CAD and G188E, N291S, and 93T/G variants in the Saudi patients.

Keywords: LPL, allele, coronary artery disease, gene variant, lipid.

INTRODUCTION

Lipoprotein lipase (LPL) is an essential glycoprotein enzyme that plays a role in lipid metabolism (1). The LPL activity is an essential step in the clearance of triglyceride-rich lipoproteins. The clearance of lipoprotein is enhanced by binding LPL with chylomicrons and transported to the liver via LDL receptor-related protein (2, 3). The essential function of LPL in adipose tissue, muscle, and macrophages release the free fatty acids by the hydrolysis of triacylglycerol of chylomicrons and very low-density protein. (4, 5). It has been observed that the prevalence of atherosclerosis has increased all over the world associated with the complications of cardiovascular diseases. (6). Cardiovascular diseases (CAD) and their complications are knowing one of the major causes of death worldwide (7). Any disturbance in the level of LPL or its activity leads to diminish the level of high-density lipoprotein cholesterol (HDL-C) and elevated triacylglycerol level, which are risk factors that develop the coronary artery disease (8, 9). In an early atherogenic process, LPL expression in macrophages and other cells in vascular walls is associated with atherosclerosis (10). Genome-wide association studies have identified some loci linked to plasma lipid traits associated with altered LPL gene expression (11). A previous study described that a polymorphism of the LPL gene is linked with plasma lipid concentrations and clinical conditions in different populations (12). Furthermore, many studies have reported that the LPL gene variants are found in coding and non-coding regions (13-15). Moreover, it has been found that 80% of LPL gene variants occur in coding regions, while 20% are found in non-coding regions (16, 17). The length of the LPL gene is 30 Kb on chromosome 8p22 containing 10 exons encoding a 448 amino acid long protein. Several studies reported more than particular 100 mutations and polymorphisms in simple nucleotides of the LPL gene (18, 19). Several genetic LPL mutations and metabolic disturbances that alter the lipid and lipoprotein metabolism have been reported as CAD risk factors. (20).

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In addition to these mutations, four variants identified in the LPL gene impair the catalytic function of LPL, namely Asp9Asn (D9N, rs1801177, G280A) in exon 2, G188E (G188A) in exon 5, and Asn291Ser (N291S, rs268, A1127G) in exon 6 within the gene coding region, and 93 T/G in LPL promoter (21-24). From the transcription start site at position 93 a single nucleotide transition from T to G (93 T/G) was identified; (rs1800590, T93G) (24). The homozygous form of Asn291Ser, Gly188Glu, and Asp9Asn mutations are associated with familial chylomicronemia. In some population groups, the percentage of heterozygous mutations is (3–7%) (19). In the subjects with combined hyperlipidemia, the frequency of Asp9Asn mutations carriers was found to be approximately 4–9.8 %, while it was 3% in healthy subjects (21). In a rare mutation Gly188Glu, the glutamic acid (Glu) substituted to glycine (Gly), at position 188 in the mature enzyme and characterized with high triacylglycerol and low HDL-c plasma levels (25). This substitution represents 22% of the mutant alleles in a cohort of 56 affected subjects that appear to be a common cause of LPL deficiency (26). In hypertriglyceridemia women (16 mmol/L) the Asn291Ser mutation was associated with the high levels of plasma triacylglycerol (27). In CAD Saudi patients, the assessment of four LPL variants (Asp9Asn (D9N, rs1801177, G280A), Gly188Glu (G188A), Asn291Ser (N291S, rs268, A1127G) has not clear. So, this study evaluates the relation between four LPL gene variants and CAD in Saudi patients.

MATERIAL and METHODS

Subjects

Subjects were selected from Cardiology Department, King Khalid University Hospital at King Saud University (KSU) in Riyadh, Kingdom of Saudi Arabia (KSA). They were composed of 253 CAD (137 males and 116 females, mean age 61.73 ± 8.34 years) and 207 participants were healthy control (118 males and 89 females, mean age 58.27 ± 8.46 years). The CAD of the patients was assessed by cardiologists through the review of angiograms. The study was reviewed and approved by the ethics committee, College of Medicine, King Saud University, Riyadh, Saudi Arabia. The subjects who participate in this study Sign their written informed consent.

Blood Sampling and Biochemical Analysis.

Peripheral blood samples were collected after a 10–12 h fast into 2 tubes containing EDTA one of the tubes was centrifuged at 2000 rpm for 10 minutes. The separated plasma was collected in plain polystyrene tubes and used in measuring plasma glucose and lipid profile concentrations by using a Bayer Opera analyzer [Bayer Diagnostics, Munich, Germany]. Glucose, cholesterol, and triacylglycerol kits were purchased from Biotrol, Earth City, USA while HDL-C kit was purchased from Randox Laboratories Ltd., London, UK. The LDL-C levels were calculated (Friedewald formula). The other blood sample tube was used in DNA extraction.

DNA extraction

DNA was isolated from the blood sample using the QIAamp DNA blood Kit from QIAGEN (Germany) according to the manufacturer's instructions. The DNA purity and concentration were determined by a Nanodrop spectrophotometer.

Genotyping and variant analysis

By using the polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) method the D9N (Asp9Asn), G188E (Gly188Glu), N291S (Asn291Ser), and 93 T/G (T93G) variants were determined from genomic DNA. The primers were designated based on previous publications (21, 23, 28). 3 µL (150 ng), of DNA sample, was added to 12.5 µL master mix (2× Promega), 2 µL each of both forward and reverse primers, and 5.5 µL distilled water (final volume of 25 µ).

In the thermal cycler instrument (My Cycler, Bio-Rad) the PCR temperature was adjusted as follows: One cycle of the first denaturation (94 ^{0}C for 5 minutes), 40 cycles of the second denaturation, annealing, and extension (94 ℃ for 20 seconds, 60 ℃ for 30 seconds, and 72 ℃ for 30 seconds, respectively) followed by one cycle of final extension (72 ℃ for 5 min).

For the product analysis, the 10 µL of PCR products were digested with 10 units (1 µL) of appropriate restriction endonuclease enzymes (New England BioLabs) in addition to10× buffer solution (2 µL). The mixture (20 µL final volume) was placed in a water bath at 65 °C for two hours for D9N variant and at 37 °C overnight for G188E, N291S, and 93 T/G variants.

The digested products alongside the 100-bp DNA Ladder (Thermo Fisher Scientific Inc.) were visualized by using UV light after separating on 3% agarose gel. The Oligonucleotide primers for DNA amplification of the studied LPL gene variants, the restriction enzymes, and fragments were described in **Table 1**.

Statistical analysis

The data were statistically analyzed using the SPSS version 24.0 (SPSS, Inc, Chicago, IL, USA). The data were summarized by the mean ± standard deviation (SD) and compared with a t-test. The enumeration data were summarized as the number (%) and compared through the chi-square test ($\chi 2$ test). Following, the allelic and genotypic frequencies calculated from the observed genotypic counts were evaluated, and the Hardy-Weinberg equilibrium expectations were estimated. A similar method was applied to study the associations between the allelic and genotypic frequencies. The relations were determined as odds ratios (ORs) and 95% confidence intervals (CIs). An odds ratio for genotype distributions $\chi 2$ analysis was performed. CAD is the odds of the allelic carriage in the diseased (CAD) group divided by the odds in the control group.

RESULTS

Baseline Characteristics of CAD and Control groups.

Table 2 shows the baseline biochemical features of the study population [253 CAD patients and 207 control subjects]. The CAD and control subjects' mean age was 61.73±8.34 and 58.27±8.46 years, respectively. The plasma level of fasting blood sugar (FBS), total cholesterol (TC), triglycerides (TG), and low-density lipoprotein-cholesterol (LDL-c) were significantly increased in the CAD group compared to the control group (P=0.000 for each). There was no significant change in the high-density lipoprotein cholesterol (HDL-c) level between the two groups (p =0.07).

Clinical risk factors in CAD and Control groups

Table 3 shows some of the clinical risk factors established for CAD such as diabetes, dyslipidemia, hypertension, and smoking. There were significant changes in the previous risk factors percent between CAD and control group. Using the $\chi 2$ test, diabetes mellitus ($p < 0.0001$, OR = 55.16, 95% CI: 31.03- 98.07), dyslipidemia ($p < 0.0001$, OR = 8.97, 95% CI: 5.68 - 14.20), hypertension ($p < 0.0001$, OR = 20.12, 95% CI: 12.31-32.90), and smoking ($p = 0.0001$, OR = 2.58, 95% CI: 1.63-4.08) were determined to be independent risk factors of CAD.

Genotype and allele frequencies for the four SNPs in the LPL variants in CAD and Control groups

The genotype frequencies for D9N (Asp9Asn) (alleles described as A and G), G188E (Gly188Glu) (alleles described as G and A), N291S (Asn291Ser) (alleles described as A and G), and 93 T/G (T93G) (alleles described as T and G) lipoprotein lipase variants in CAD and control groups are represented in **Table 4**. According to the Hardy-Weinberg equilibrium model, the frequencies of the LPL polymorphism genotypes were distributed in the CAD group as the following: the AA D9N genotype in 180 patients (71.14%), 60 (23.72%) represents AG genotype, whereas 13 patients (5.14%) carried GG genotype. In the control group, AA genotype was identified in 169 subjects (81.64%), whereas 34 (16.43%) and 4 subjects (1.93%) carried the AG and GG genotypes, respectively. A significant change in genotype distribution of the D9N variant was detected between the CAD and control groups ($\chi 2 = 7.78$, $p = 0.\ 0.02$). For G188E, the frequency of GG genotype in the control subject was slightly higher than CAD patients, the GG genotype was recognized in 247 CAD groups (97.63%), however, 6 (2.37%) patients carried the GA. In the control group, the GG genotype was recognized in 206 subjects (99.5%), while only 1 (0.5%) carried the GA genotype. The AA genotype was absent in both CAD and control groups. No significant change in genotype distribution of the G188E variant was observed between both CAD and control groups ($\chi 2 = 2.71$, $p = 0.\ 0.09$). For N291S, the frequency of AA genotype in the CAD group was slightly lower in the control group, AA genotype was identified in 250 CAD patients (98.81%), whereas 2 (1.19%) patients carried the AG.

In the control group, the AA genotype was observed in 205 subjects (99.0%), however, 2 persons (1.0%) carried the AG genotype. No significant change in the genotype distribution of N291S variant was identified between CAD and control groups ($\chi 2 = 0.82$, $p = 0.\ 0.051$).

For the 93 T/G genotypes, 228 (90.10 %) CAD patients carried TT genotype, whereas 24 patients (9.50%) carried the TG genotype and 1 patient (0.40%) carried GG genotype. In the control group (n = 207), the TT genotype was identified in179 persons (86.4%) while, 26 healthy subjects (12.6%) carried the TG genotype and 2 subjects (1.0%) carried GG genotype.

For 93 T/G variant, No significant deviations in genotype frequencies between the two groups (X2 = 1.73 and p = 0.42). **Table 5** shows the significant alterations in the A and G alleles distribution of D9N genotype observed between both CAD and control groups (p = 0.003; OR = 1.81 and 95% Cl = 1.22-2.69). No significant changes in the G and A allele distribution of G188E, A and G allele distribution of N291S, and T and G allele distribution of 93 T/G genotype were detected between the CAD and the control groups (= p 0.139, 0.822, and 0.185 respectively).

CAD odds ratio associations with D9N, G188E, N291S, and 93 T/G genotypes

The odds ratios of the D9N (Asp9Asn) genotype AA vs. AG and AA vs. AG + GG genotypes (95% CI) were 1.65 (1.26-4.78), and 1.80 (1.16-2.81). Our results showed a significant association with CAD disease (p = 0.035 and 0.009, respectively). The odds ratios of the G188E (Gly188Glu) genotype GG vs. GA (95% CI) and N291S (Asn291Ser) genotypes AA vs. AG (95% CI) were 5.0 (0.59-41.90) and 1.23 (0.20-7.43), respectively, which shows insignificant association with CAD disease (p =0.137 and 0.821, respectively).

The odds ratio 93 T/G (T93G) genotypes TT vs. TG, TT vs. GG, TG vs. GG, TT vs. TG + GG and TT + TG vs. GG (95% CI) were 0.72 (0.40-1.31), 0.39 (0.04- 4.36), 0.54 (0.05-6.36), 0.70 (0.39-1.24) and 0.41 (0.04-4.52), respectively, represent no significant association between previous genotypes and CAD (p = 0.283, 0.447, 0.626, 0.224, and 0.464, respectively) (**Table 6**).

Table 1: Oligonucleotide primers for PCR amplification of the four studied polymorphisms of the LPL gene, digestion enzymes, and resulting fragments.

Primers	Enzymes	Resulting fragments
D9N (Asp9Asn) polymorphism (21). 5′-CTC CAG TTA ACC TCA TAT CC-3′ 5′-CAC CAC CCC AAT CCA CTC-3′	2U TaqI (New England Biolabs Inc., UK)	A allele: 179 bp and 52 bp G allele: 179 bp and 58 bp
G188E (Gly188Glu) polymorphism) (28). 5′-GAG CAG TGA CAT GCG AAT GT-3′ 5′-CTC CAA GTC CTC TCT CTG CA-3′	2U AvaII (New England Biolabs Inc., UK)	G allele: 131 bp, 88 bp and 86 bp A allele: 219 pb and 86 bp
N291S (Asn291Ser) polymorphism (23). 5′-GCC GAG ATA CAA TCT TGG TG-3′ 5′-CTG CTT CTT TTG GCT CTG ACT GTA-3′	2U RsaI (New England Biolabs Inc., UK)	A allele: not cleaved G allele: 215 bp and 23 bp
93 T/G (T93G) polymorphism (28). 5′-GCT GAT CCA TCT TGC CAA TGT TA-3′ 5′-CCG CGG TTT GGC GCT GAG CAA GT-3′	2U ApaI (New England Biolabs Inc., UK)	T allele: 338 bp and 286 bp G allele: 286 bp, 195 bp and 143 bp

Table 2: Baseline Characteristics of CAD patients and controls.

Characteristic	Subjects		*p*-value
	CAD n=253	Control n=207	
Age, year			
Mean±SD	61.73±8.34	58.27±8.46	0.000
Gender			
Male, %	137 (54.2%)	118 (57.0%)	0.54
Female, %	116 (45.8%)	89 (43.0 %)	
FBS, mmol/L			
Mean±SD	8.90±3.58	4.53±0.70	0.000
TC, mmol/L			
Mean±SD	4.26±1.06	3.83±0.59	0.000
TG, mmol/L			
Mean±SD	1.78±1.03	1.10±0.28	0.000
HDL-C, mmol/L			
Mean±SD	1.16±0.90	1.27±0.39	0.07
LDL-C, mmol/L			
Mean±SD	2.45±0.85	1.66±0.64	0.000

Data represent the mean ± SDs for all quantitative traits. Student's t-test and the X2test were used to compare the values of CAD patients and control subjects. FBS: fasting blood glucose, TC: total cholesterol, TG: triglyceride, HDL-c: high density lipoprotein cholesterol, LDL-c: low-density lipoprotein cholesterol.

Table 3: Clinical risk factors in the CAD patients and control subjects

Parameter	Subjects		OR	95% CI	*p*-value
	CAD n=253	Control n=207			
Diabetes					
Diabetics	218 (86.2%)	21 (10.1%)	55.16	(31.03– 98.07)	< 0.0001
Nondiabetics	35 (13.8%)	186 (89.9%)			
Dyslipidemia					
Positive	155 (61.30%)	31 (15.0%)	8.97	(5.68–14.20)	< 0.0001
Negative	98 (38.70%)	176 (85.0%)			
Hypertension					
Hypertensive	192 (75.90%)	28 (13.5%)	20.12	(12.31–32.90)	< 0.0001
Normotensive	61 (24.10%)	179 (86.5%)			
Smoking					
Smoker	81 (32.0%)	32 (15.5%)	2.58	(1.63–4.08)	0.0001
Nonsmoker	172 (68.0%)	175 (84.5%)			

CAD, coronary artery disease; OR, odds ratio; CI, confidence interval

Table 4: Genotype distributions for the four SNPs in the LPL polymorphisms in CAD Patients and control subjects

Genotype	Subjects		χ^2	*p*-value
	CAD, n (%)	Control, n (%)		
D9N (Asp9Asn)				
AA	180 (71.14%)	169(81.64%)	7.78	0.02
AG	60 (23.72%)	34 (16.43%)		
GG	13 (5.14%)	4 (1.93%)		
G188E (Gly188Glu)				
GG	247 (97.63%)	206 (99.5%)	2.71	0.09
GA	6 (2.37%)	1 (0.5%)		
AA				
N291S (Asn291Ser)				
AA	250 (98.81%)	205 (99.0%)	0.051	0.82
AG	3 (1.19%)	2 (1.0%)		
GG	-	-		
93 T/G (T93G)				
TT	228 (90.10%)	179 (86.4%)	1.73	0.42
TG	24 (9.50%)	26 (12.6%)		
GG	1(0.40%)	2 (1.0%)		

Table 5: LPL allelic frequencies in CAD patients and control subjects

Gene	Allele	CAD, n (%)	Control, n (%)	OR (95% CI)	*p*-value
D9N (Asp9Asn)	A	420 (83.0%)	372 (89.86%)	1.81 (1.22–2.69)	0.003
	G	86 (17.0%)	42 (10.14%)		
G188E (Gly188Glu)	G	500 (98.81%)	413 (99.76%)	4.96 (0.59–41.33)	0.139
	A	6 (1.19%)	1 (0.24%)		
N291S (Asn291Ser)	A	503 (99.41%)	412 (99.52%)	1.23 (0.20–7.38)	0.822
	G	3 (0.59%)	2 (0.48%)		
93 T/G (T93G)	T	480 (94.86%)	384 (92.75%)	0.69 (0.40–1.19)	0.185
	G	26 (5.14%)	30 (7.25%)		

Differences in the allelic frequencies between coronary artery disease (CAD) patients and control subjects were compared using Pearson's X2 test and without adjusting other covariates. Odds ratios with 95% confidence interval (95% CI) are presented

Table 6: CAD odds ratio associations with D9N, G188E, N291S, and 93 T/G genotypes in the LPL polymorphisms in CAD Patients and Control subjects

Genotype	OR	95%CI	*P* value
D9N (Asp9Asn) genotypes			
AA vs. AG	1.65	(1.04–2.65)	0.035*
AA vs. GG	3.05	(0.98–9.54)	0.055
AG vs. GG	1.84	(0.55–6.10)	0.317
AA vs. AG + GG	1.80	(1.16–2.81)	0.009*
AA + AG vs. GG	2.75	(0.88–8.56)	0.081
G188E (Gly188Glu) genotypes			
GG vs. GA	5.00	(0.59–41.90)	0.137
N291S (Asn291Ser) genotypes			
AA vs. AG	1.23	(0.20–7.43)	0.821
93 T/G (T93G) genotypes			
TT vs. TG	0.72	(0.40–1.31)	0.283
TT vs. GG	0.39	(0.04–4.36)	0.447
TG vs. GG	0.54	(0.05–6.36)	0.626
TT vs. TG + GG	0.70	(0.39–1.24)	0.224
TT + TG vs. GG	0.41	(0.04–4.52)	0.464

CI, confidence interval

DISCUSSION

LPL is a vital enzyme play an important role in lipoprotein metabolism that describes the lipid and lipoprotein abnormalities encountered in CAD. Various codon polymorphisms of the LPL gene have been designated, and some of them play a role in the pathogenesis of CAD (29). Therefore, in our study, we have evaluated the effect of LPL gene variations and determined the genetic frequencies in CAD and control groups in the Saudi population. CAD patients had significantly higher concentrations of fasting blood sugar (FBS), and TC, TG, and LDL-C (p = 0.000 for each) in comparison to the control subjects. There was an insignificant change in the HDL-C concentration between the CAD and control groups. Our results were consistent with those of other studies, which reported that CAD male patients had significantly higher concentrations of TC, TG, and LDL-C and low concentrations of HDL-C in comparison to the control subjects (20, 30, 31). The major clinical risk factors listed as (diabetes, dyslipidemia, hypertension, and smoking) in the CAD and control groups. There were significant differences between the CAD and the control groups concerning diabetes mellitus, dyslipidemia, hypertension, and smoking. Using the $\chi 2$ test, diabetes mellitus, dyslipidemia, hypertension, and smoking were found to be independent risk factors of CAD.

A significant effect of smoking and the D9N allele increases the risk of CAD when compared with D9N non-smokers (32). Dyslipidemia is stimulated through the interaction of N291S or D9N mutations with factors, such as pregnancy, obesity, or diabetes (33-35). The patients with CHD had significantly lower HDL-C and higher TG than the control group, whereas no difference was observed in LDL-C in both groups (32). The genotypic frequencies of D9N (Asp9Asn), G188E (Gly188Glu), N291S (Asn291Ser), and 93 T/G (T93G) LPL polymorphisms in CAD patients and control subjects. The distributions of the genotypes were according to the Hardy-Weinberg equilibrium, as expected. The significant differences in the A and G allelic distribution of the D9N genotype were observed between the CAD and the control groups (p = 0.003; OR = 1.81 and 95% Cl = 1.22–2.69). No significant differences in the G and A allele distribution of G188E, A and G allele distribution of N291S, and T and G allele distribution of 93 T/G genotype were observed between the CAD and the control groups. Various studies have reported that the N291S heterozygous carriers had an increase in plasma TG and a decrease in HDL-C (21, 23, 34). However, other studies have been reported in which no association was observed (34-39).

Van Bockxmeer et al. (2001) found a high frequency of the LPL allele in young CHD (38). The T93G, D9N, and 291S have frequencies less than 3%, with the E allele of the G188E variant having a frequency of only 0.03% (40). The higher frequencies of the 291S allele were observed in Chinese Canadians (41), French Canadians (42), and Italian (43). The D9N allele was associated with an odds ratio of 1.9 (1.2–3.0) for CHD in male subjects in Copenhagen city (44). In a meta-analysis, the frequency of D9N allele in CHD and MI Caucasians was found to be two-fold higher than the normal subjects (45). In a previous study, the D9N genotype showed a significant association with CAD, and a similar result was observed for coronary stenosis. The odds ratios of the D9N (Asp9Asn) genotype AA versus AG and AA versus AG + GG genotypes (95% CI) were 1.65 (1.26–4.78), and 1.80 (1.16 p < 2.81). These results demonstrate a significant association with CAD disease (p = 0.035 and 0.009, respectively. There was no significant association with CAD disease. (p = 0.283, 0.447, 0.626, 0.224, and 0.464, respectively) (Table 6). The OR was observed to be 0.89 (95 % CI: 0.81 –0.98) for carriers of the T allele which seems to be protective against CAD. However, the ORs for carriers of -93G, 188E, and 291S were found to be 1.22 (95 % CI: 0.98–1.52), 2.80 (95 % CI: 0.88–8.87) and 1.07 (95 % CI: 0.96–1.20), respectively 40. The D9N alleles are associated with 20% higher triglyceride and 0.08 mmol/L lower HDL cholesterol, which are well-known established risk factors for CAD. The frequencies of LPL N291S and D9N polymorphisms in Australian Caucasians were found to be 1–7% and 17–22%, respectively, similar to those reported from Europe and Scandinavia (45). Many studies have found no difference in the frequency of LPL N291S polymorphism between control and CHD subjects (46, 47). A previous study has concluded that the subjects with LPL N291S polymorphism had no risk of CAD, while subjects with D9N had a significant increase in the risk of CAD (32). The D9N mutation would decrease the activity and concentration of LPL and cause higher plasma triglyceride levels and lower HDL-C levels which would result in the formation of intermediate-density lipoprotein and chylomicrons remnants, and the development of the CAD. Therefore, the D9N mutation could decrease the concentrations of HDL by retarding the LPL activity, accelerating the cholesterol deposition, and promoting the atherosclerosis process, and eventually increased the coronary disease risk (48).

CONCLUSION

In the current study, an association between the D9N variant and CAD was observed, however, no association was observed between the G188E, N291S, 93T/G variants, and CAD. The genetic and environmental features may affect the pathogenesis of CAD, and the LPL variants have a strong role in the development of CAD. Further studies on LPL gene variants are necessary for patients with CAD to explore the effects.

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Evaluation of using distal part of endotracheal tube samples for SARS-COV-2 diagnosis by RT-PCR

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ABSTRACT

Objective: The reverse transcription-polymerase chain reaction (RT-PCR) analyses method is the most important diagnostic method in the diagnosis of SARS-CoV-2 virus infection. In this research, we aimed to investigate the positivity of SARS-CoV-2 by RT-PCR from distal part of the endotracheal tube (DPET) samples, which have not been investigated in any study yet.

Materials and Methods: A total of 48 patients with a diagnosis of COVID-19 hospitalized in the intensive care unit receiving mechanical ventilation and whose conditions resulted in death or extubation were included in the study. The distal 6 cm part of the orotracheal intubation tube was removed from the patient (including the cuff). DPET samples were mixed with viral transport medium and vortexed; then, it was centrifuged at 4500g for 4 minutes. RNA isolation was performed by taking 400 µl from the supernatant and then SARS-CoV-2 RT-PCR was studied.

Results: In 15 patients (31.25 %) the swab samples were PCR positive, 42 patients (87.5 %) had positive computed tomography finding and 48 patients (100 %) had positive clinical findings. Among the patients whose oropharynx (OP)/nasopharynx (NP) combined swab sample was positive for RT-PCR, the rate of RT-PCR positivity detected in DPET samples was 26.7%. While OP/NP combined swab sample was negative, DPET RT-PCR positivity rate was found to be 9.09%.

Conclusions: Patients with positive DPET RT-PCR are detected when the swab is negative. These findings suggest that DPET can be used as a good lower respiratory sample without the risk of particle spread and transmission to healthcare personnel.

Keywords: COVID-19; mechanical ventilation; endotracheal intubation tube; intubation

INTRODUCTION

The new coronavirus disease (COVID-19) caused by the SARS-CoV-2 virus emerged in Wuhan, China, in December 2019 and was identified as a pandemic by the World Health Organization (1). Diagnosis of COVID-19 is based on clinical symptoms, investigation of the viral genome with Reverse-transcription polymerase chain reaction (RT-PCR), chest X-ray or computed tomography (CT) scan, and lastly serological blood tests (2). RT-PCR analysis is the best and most used method for qualitative and quantitative diagnosis of viruses, including coronaviruses (3). Although RT-PCR analysis is usually performed from nasopharynx (NP) swab, oropharynx (OP) swab, combined NF and OF swab, it has been stated that it can be used for RT-PCR from other samples such as sputum, bronchoalveolar lavage, saliva, nasal washing, aspiration fluids and tissue biopsies (4,5). Well taken lower respiratory tract samples are preferred so as to promote the success of the method shown in the article.

It has been shown that approximately 80% of the patients have mild illness, 20% require hospitalization, and also approximately 5% of them need intensive care (6). Patients appear relatively stable at first, but can rapidly deteriorates with severe hypoxia. The basic table noticed in these cases is acute respiratory distress (ARDS) (7).

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When high-flow oxygen therapy and non-invasive mechanical ventilation treatment are insufficient, patients are oxygenated by using invasive mechanical ventilation. As in particular bronchoalveolar lavage or bronchial washing fluid is recommended for COVID-19 patients who are followed up with orotracheal endotracheal intubation in invasive mechanical ventilation. It is generally not preferred because of the spread around and the risk of infection for healthcare personnel (8).

There are many factors affecting the sensitivity of RT-PCR. Analytical factors such as the existence of an inhibitory substance in the sample fluid, the utilization of inaccurate amounts of the components used, errors in the sampling technique, inability to adjust temperature parameters, and finally, mutations affecting the binding site of the primer (9,10) as well as preanalytical factors. Therefore, while clinical and CT findings support COVID-19 in many patients, RT-PCR negative results can be encountered (11,12). Due to the difficulties and the risks in collecting lower respiratory tract samples, these types of samples are used less frequently in diagnosis.

In this study, it is aimed to investigate the positivity of SARS-CoV-2 by RT-PCR from distal part of endotracheal tube (DPET) samples, which have not been used in any study before, in order to indicate the situation in the lower respiratory tract in intubated patients.

MATERIAL and METHODS

Study group

The study was conducted by obtaining a prospective (71522473 / 050.01.04 / 283) approval from Sakarya University Faculty of Medicine ethics committee. A total of 48 patients hospitalized in the intensive care unit of Sakarya University Medical Faculty Training and Research Hospital with the diagnosis of COVID-19 who died as being connected to an invasive mechanical ventilator or who were extubated were involved in the study. The data of the patients were obtained from the hospital information management system used by Sakarya University Medical Faculty Training and Research Hospital (Karmed, Kardelen Software, Mersin, Republic of Turkey). These patients were hospitalized in the intensive care unit, and their clinical findings, radiological findings, or SARS-CoV-2 RT-PCR positivity were evaluated. The data of the patients who accepted to participate in the study (age, hospitalization days, nasopharynx/oropharynx swab SARS-CoV-2 RT-PCR, CT findings, drug therapy for COVID-19 and comorbidities etc.) were recorded regularly. Patients with CT findings consistent with suspected viral pneumoniae and patients with clinical findings such as fever, cough and shortness of breath that could not be explained by another condition were included in the study.

Sample collection

A 6 cm in length sample was taken from the lower end of the orotracheal intubation tube (including the cuff) from patients who were died or extubated during intensive care treatments.

All samples taken were placed in a sterile container and sent to the laboratory in accordance with the cold chain rules with a triple transport system by following the infection prevention and control procedures.

Nucleic acid isolation and RT-PCR for SARS-CoV-2

After the samples were accepted in the microbiology laboratory, the samples were taken to the 3rd level biosecurity negative pressure room. DPET samples were mixed with the viral transport medium and kept for a while, vortexed and then centrifuged at 4500g for 4 minutes. 400 µl of the supernatant was loaded onto the BioRobot EZ1 (Qiagen, Germany) device and 60 µl of elution was taken. Total nucleic acid isolation was performed with EZ1 Virus Mini Kit v2.0 (Qiagen, Germany) in line with the recommendations of the company. For RT-PCR study, a mixture of 10 µl master mix, 2 µl primer, 8 µl template RNA was constituted with the genesig Real-Time PCR COVID-19 (Primer Design, UK) kit and a total reaction volume of 20 µl. PCR temperatures and times were as follows: reverse transcription for 10 minutes at 55° C, enzyme activation for 3 minutes at 95° C, 10 seconds at 95° C,60 seconds at 60° C for a total of 50 cycles of denaturation, binding, and elongation. Curves with a Cycle Threshold (CT) value lower than 45 and observed sigmoidal at the end of the reaction were interpreted as positive for SARS-CoV-2 RNA.

Statistical analysis

Statistical analyses were done by using SPSS for Windows software (ver. 22.0; SPSS Inc., Chicago, IL, USA). Descriptive analysis of the variables was expressed as mean± standard deviation (SD) or as numbers (n) and percent (%). The chi-square test and for small samples the Fisher's exact test was applied to compare qualitative data. A p-value of <0.05 was considered statistically significant.

RESULTS

While 6 (12.5%) of 48 patients who were intubated in the intensive care unit were still alive, 42 (87.5%) of them died. Twenty-one of these patients (43.75%) were female, 27 (56.25%) were male. In 15 patients (31.25%) OP/NP combined swab RT-PCR results were positive, 42 patients (87.5%) had positive CT findings, and 48 patients (100%) had positive clinical findings. The average age of deceased patients was 69.93 ± 14.5, the average age of the survived patients was 58.5 ± 19.70, while the average age of all patients was 68.5 ± 15.5. DPET. The mean day of intubation for all patients was 17.8 days. This number was 6.8 days in DPET positive patients and 13.9 days in negative patients. The demographic data of the patients included in the study are presented in Table 1.

PCR results obtained from swab and DPET samples are presented in Table 2. In terms of SARS-CoV 2 RT-PCR results, no significant difference was found between the OP / NP combined swab and DPET samples of the patients ($p> 0.05$) (Table 2).

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Table 1. Demographic data of the patients included in the study

	n (%)
Gender, n (%)	
Female	21 (43.75)
Male	27 (56.25)
Indication, n (%)	
Swab PCR positive	15 (31.25)
Computed Tomography Findings	42 (87.5)
Clinical Findings	48 (100)
Age average (Mean ± SD)	68.5±15.5
Living patient, n (%)	6 (12.5)
Exitus, n (%)	42 (87.5)
Comorbidity, n (%)	
Hypertension	16 (33.3)
Diabetes mellitus	12 (25)
Heart failure	12 (25)
Chronic obstructive pulmonary disease	10 (20.8)
Coronary Artery Disease	8 (16.7)
Malignancy	5 (10.4)
Cerebrovascular disease	3 (6.25)
Chronic Renal Failure	2 (4.17)

PCR: Polymerase chain reaction, SD: Standart deviation, n: Number of patients, %: Percentiles.

Table 2. SARS-CoV 2 RT-PCR results on swab and DPET samples

Sample type		DPET, n (%)		Total	*p*
		Positive	Negative	n (%)	
Smear, n (%)	Positive	4 (26.7)	11 (73.3)	15 (31. 25)	
	Negative	3 (9.1)	30 (90.9)	33 (68.75)	0.125
	Total	7 (14.6)	41 (85.4)	48 (100)	

DPET: Distal part of entubation tube, n: Number of patients, %: Percentiles.

DISCUSSION

The most commonly used method in the diagnosis of COVID-19 infection is to show the presence of the virus in NF and OF swab samples by RT-PCR (12). However, at the time the samples taken, aerosol is released into the environment and poses a high risk for the healthcare personnel who are in close contact with the patient.

Although the use of lower respiratory tract samples for diagnosis increases the sensitivity, the sampling procedure is riskier and because it is an invasive procedure, much less lower respiratory tract samples are used in diagnosis. In this study, RT-PCR results of DPET samples representing lower respiratory tract samples and OP/NP swab samples were compared in the diagnosis of COVID-19 infection.

It was examined whether or not DPET samples can be used as an effective diagnostic method for the patients receiving mechanical ventilation in intensive care. COVID-19 shows its clinical signs with upper respiratory tract symptoms such as fever, dry cough, and dyspnea (13). Although CT findings may vary according to age, underlying disease, immune status, and stage of the disease, it can be used as a strong recommendation for diagnosis (14). RT-PCR analysis is the most robust and widely used method for qualitative and quantitative diagnosis of viruses, including coronaviruses (3).

Oxygen mask, high flow oxygen therapy, non-invasive mechanical ventilation and invasive mechanical ventilation therapy are generally used in the treatment of patients who need oxygen. Especially after the development of ARDS due to SARS-CoV 2, the need for oxygen increases further, and when non-invasive oxygen therapy is insufficient, oxygenation is exerted to be provided by invasive mechanical ventilation. Orotracheal intubation tube is usually used to connect the patient to a mechanical ventilator. The distal part of the intubation tube (including the cuff) was used to obtain the lower respiratory tract sample, because of the fact that the end of the intubation tube is in the trachea and is in constant contact with the secretions in the lung in the trachea above the carina.

Among the patients whose OP / NP combined swab sample was RT-PCR positive, the rate of RT-PCR positivity detected in DPET samples was 26.7%, while the DPET RT-PCR negative rate was 73.7%. It was thought that the lower rate of DPET RT-PCR was due to the DPET sample taken after the patients were hospitalized for a long time. It was observed that DPET samples were taken after an average of 6 days from the patients who were DPET RT-PCR positive as the swab was RT-PCR positive, and DPET samples were taken after an average of 17.6 days from the patients who were negative for DPET RT-PCR while the swab was RT-PCR positive.

Reliable evidence has shown that the SARS-CoV-2 incubation period is approximately six days (interquartile range, 2-11 days) (15). Similarly, we found that the average time of sampling of DPET RT-PCR positive patients was six days.

While OP/NP combined swab sample was negative, DPET RT-PCR positivity rate was 9.09%. Although combined swab RT-PCRs were negative, DPET RT-PCR was positive in 3 patients, suggesting that DPET could be used as a sample from the lower respiratory tract. Two of the three patients were deceased, and one of them has been extubated with healing. The average number of days of hospitalization for these three patients was eight days.

Although RT-PCR is used as the key standard in diagnosis, finding inaccurate negative and positive results are a substantial problem to be taken into consideration. It has been reported that many suspected cases considered to be typical COVID-19 with clinical and CT findings could not be diagnosed (12). Therefore, RT-PCR is not used as the only criterion to exclude disease. Even though diagnostic errors can always be experienced, it becomes even more important in infectious epidemics such as COVID-19. While false positive or false negative results are a threat to the health of the individual, they can also cause errors and restrictions in the emergency plans and measures created by the national and international authorities to control the epidemic

In particular, reporting a false negative result to a person infected with SARS-CoV 2 causes disruption of isolation and restrictive measures, transmission to the community, and especially insufficient detection of households who are thought to be potentially infected and must be screened. In laboratory studies, not using suitable materials for sampling, inadequate sample collection, insufficient sample volume and quality, inaccurate transportation methods and storage problems come to the forefront in the preanalytic phase.

According to current diagnostic criteria, viral nucleic acid tests play a vital role in determining hospitalization and isolation. However, CT may be a more reliable, practical, and rapid method to diagnose and assess COVID-19 in some cases, especially in the area affected by the epidemic. CT has been a guide in the diagnosis, especially in patients whose clinic is compatible with COVID-19 but whose PCR is negative. In a study conducted in China, it was reported that 59% of 1014 patients had swab RT-PCR positivity, while 88% of them had positive CT findings (11). Recent studies have shown that the sensitivity of chest CT in COVID-19 patients with false-negative PCR results is 98% (16,17). In another study, in 36 patients with COVID-19 pneumonia, the CT sensitivity was 97.2%, while the RT-PCR sensitivity was shown as 83.3%, and it was stated that RT-PCR might initially give false results (18). While different PCR positivity rates were encountered in studies conducted with different methodologies, false negativity was emphasized in all studies. In our study, the positivity rate of CT findings among all patients was 87.5%. While the swab was RT-PCR negative, we found the rate of CT findings being positive to be 84.8%. In all patients, the swab PCR positivity rate was 31.25% (15/48 patients).

In the study conducted by Liu et al., 38.2% of 4880 patient samples were found to have RT-PCR positivity, while the positivity rate was 38.25% in nasal and pharyngeal samples, 49.12% in sputum samples and 80% in bronchoalveolar fluid samples (19). This study shows us that the sample taken at the bronchoalveolar level has a higher RT-PCR positivity rate. It is not preferred in patients with COVID-19 pneumonia because of the high particle release in samples taken under bronchoscopy for bronchoalveolar lavage. In our study, while the OP / NP combined swab RT-PCR positivity rates were similar, the DPET RT-PCR positivity rate, which we used as a lower respiratory tract sample, was 14.6% among all patients (7/48 patients), and 26.7% in patients with swab RT-PCR positive (4/15 patients) required us to look for the differences in sampling time. Tao Ai et al. showed the meantime to become negative is 6.9 days after RT-PCR positivity developed (11). The most important data appeared in the study was that there were 3 patients who were DPET RT-PCR positive while the swab was RT-PCR negative. While the average hospitalization days of these patients were eight days, the average hospitalization days of DPET RT-PCR negative patients was 12.4 days. As a result; in this study, it was investigated whether DPET samples can be used for the first time in the diagnosis of COVID-19. DPET RT-PCR positivity rate was found to be high especially in patients with short hospitalization days. Patients with positive DPET RT-PCR were detected when the swab was negative. These findings suggest that DPET can be used as a good lower respiratory sample without the risk of particle spread and transmission to healthcare personnel. However, more comprehensive studies are needed on this subject.

This study was planned to evaluate DPET as a diagnostic sample. However, our DPET RT-PCR positivity rate remained relatively low due to the long hospitalization period of intensive care patients and the very low rates of cure and extubation of COVID pneumonia. This study was conducted in the early part of the epidemic and at that time, changing the tube of the intubated patient was not part of the protocol in patient management. For this reason, we could not compare OP/NP swab and DPET by taking samples from all patients on the same day. If we were doing this study with our current patient management information, we could evaluate the effectiveness of DPET in diagnosis by taking samples from each patient on the days we determined beforehand.

CONCLUSION

In this study, it is investigated whether DPET samples can be used in the diagnosis of COVID-19 for the first time. DPET RT-PCR positivity rate is found to be high especially in the patients with short hospitalization days. As in our study, we know that there is a group of patients who are clinically compatible with COVID-19 but with negative swab PCR results. We think that DPET can be used to represent the lower respiratory tract, especially in this patient group. These findings suggest that DPET can be used as a good lower respiratory sample without the risk of particle spread and transmission to healthcare personnel. In addition, these results we obtained made us think that DPET could be used as a postmortem diagnostic tool even if it is not in routine diagnosis.

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Ethical approval: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by Local Ethical Committee.

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Effect of some toxic metals in drinking water on male reproductive hormones

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ABSTRACT

Objective: Drinking water contaminated with toxic metals can be a leading cause of infertility in males. The aim of this study was to determine the levels of some toxic metals and trace elements in drinking water and reproductive hormone levels in males who drink water from these sources in Sabongida-Ora , Edo State, Nigeria.

Material and Methods: A total of 90 subjects consisting of 30 subjects who drink borehole water, 30 subjects who drink water from hand-dug well, and 30 subjects who drink table water, aged between 18 and 45 years, were recruited for this study. Serum reproductive hormones were analyzed using ELISA method. Blood lead, cadmium, serum zinc, and copper were analyzed using atomic absorption spectroscopy (AAS). The data obtained were analyzed using SPSS version 23.0.

Results: Blood Cd and Pb levels were significantly higher ($P<0.05$) in hand-dug, borehole and treated water consumers while serum Zn level was significantly lower $P<0.05$) in hand-dug well and borehole water consumers than treated table water consumers. The serum Cu level was not significantly different between the groups. The reproductive hormones were significantly lower among hand-dug well and borehole water consumers than treated table water consumers ($p<0.05$), while PROL($P<0.05$) was significantly higher among hand-dug well, borehole water consumers than treated water consumers. Serum PROL correlated positively with Pb ($r=0.443$; $P<0.05$) and negatively with serum Zn ($r=-0.404$; $P<0.05$) while T correlated positively ($r=0.542$; $P<0.005$) with Zn. Similarly, FSH correlated negatively with Pb ($r=-0.398$; $P<0.05$) and positively with Zn ($r=0.422$;$P<0.05$), while LH correlated inversely with Cd ($r=-0.622$;$P<0.05$) and positively with Zn ($r=0.745$; $P<0.05$). Expectedly, Cd and Pb were higher while Cu and Zn were lower in hand-dug well water consumers than borehole water consumers.

Conclusion: Water consumption from hand-dug wells may have adverse reproductive sequelae among consumers.

Keywords: Toxic metals, Reproductive hormones, water consumers

INTRODUCTION

In Nigeria, access to safe drinking water has become a great problem due to increase in population, most inhabitants have no other option but to engage borehole drillers to sink borehole for them at high cost, and other relies on dugged well water. This has posed serious health challenges, especially infertility which has become social dilemma in Sub-saharan Africa like Nigeria. In most of the cases there are no water supplies and as a result the local population has to depend on shallow aquifers by installing hand pumps or installing electric motors to fetch their water needs. Mostly these new settlements are located around industrial zones. Hence they are at high risk by the effect of heavy metals due to contaminated water consumption. The supply of safe drinking water is crucial to human life, and safe drinking water should not impose a significant risk to humans (1). Although a few metals are essential for human health, an excess amount of these metals can have negative effects (2).

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Toxic metals are not only potential source of oxidants but are also endocrine disruptors. Toxic metals are released into the environment through a natural process and anthropogenic activities (chemical industries and manufacturing processes) and Industrial processes through electroplating and metal smelting. Poorly treated domestic, industrial and agricultural wastewater contains high concentrations of metals, which are often discharged into the environment and water bodies for consumption.

Some toxic metals, such as cadmium, mercury, and lead, may also enter the atmosphere due to traffic pollution and industrial activities, which can be deposited in soils around the reservoir and then enter the water along with the surface runoff (3). The sources of drinking water e.g., surface waters; groundwater and seawater are likely to be polluted by toxic metals (4). Leaching of metals from the water distribution system (WDS) can contaminate drinking water (5).

Small and rural communities and individuals often consume water with a higher level of toxic metals than the guideline values as a result of inadequate supply of treated water to communities (1). To date, the removal of all toxic metals from drinking water with a comprehensive technique has not been reported. Further populations are exposed to drinking water from taps inside the building, where the metal concentrations increase due to stagnation of water in the water distribution system, hot water tanks (HWTs) and plumbing pipes (PP) inside the building.

An adequate supply of pure safe drinking water is one of the major prerequisites for a healthy life, but waterborne disease is still a major cause of death in many parts of the world, particularly in children, and it is also a significant economic constraint in many subsistence economies. Discharges of toxic metals from industrial premises and sewage treatment works are point sources and as such are more readily identifiable and controlled; run off from agricultural land and from hard surfaces, such as roads, are not so obvious, or easily controlled (6).

Such sources can give rise to a significant variation in the contaminant load over time. There is also the possibility of spills of chemicals from industry and agriculture and slurries from intensive farm units that can contain pathogens (7). In some locations, badly sited latrines and septic tanks are a significant source of contaminations. Local industries can also give rise to contamination of water sources, particularly when chemicals are handled and disposed of without proper care. The run-off or leaching of nutrients into slow flowing or still surface waters can result in excessive growth of cyanobacteria or blue-green algae (4).

Many species give rise to nuisance chemicals that can cause taste and odour and interfere with drinking water. However, they frequently produce toxins, which are of concern for health, particularly if there is only limited treatment. Contamination can also take place in consumers' premises from materials used in plumbing, such as lead or copper, or from the back-flow of liquids into the distribution system as a consequence of improper connections. Such contaminants can be either chemical or microbiological. Drinking water treatment as applied to public water supplies can be carried out through coagulation and flocculation, filtration and oxidation. The most common oxidative disinfectant used is chlorine (8).

This provides an effective and robust barrier to pathogens and provides an easily measured residual that can act as a marker to show that disinfection has been carried out, and as a preservative in water distribution. The basis on which drinking water safety is judged is national standards or international guidelines. The most important of these are the WHO Guidelines for Drinking-Water Quality (8).

The Guidelines are now based on Water Safety Plans that encompass a much more proactive approach to safety from source-to-tap (9). A toxic metal refers to a relatively dense metal or metalloid that is recognized for its potential toxicity, especially in environmental contexts (10). The term has particular application to cadmium, mercury, lead, and arsenic, (11) all of which appear in the World Health Organization's list of ten (10) chemicals of major public concern. Other examples include manganese, chromium, cobalt, nickel, copper, zinc, selenium, silver, antimony and thallium.

In some communities where the water distribution network is made of alloys containing toxic metals, those who cannot afford bottled- or mineral water with controlled toxic metal concentrations have no alternative than to consume hand-dug well, borehole or tap water, Therefore, the possible contamination of drinking-water with toxic metals and subsequent accumulation are greatly increased (12). The quality control in drinking-water and detection of its toxic metals is an extremely critical issue for in order to maintain sound human health (13). Toxic metals can bioaccumulate in organisms as they are hard to metabolize (14).

Toxic metals can bind to vital cellular components, such as structural proteins, enzymes, and nucleic acids, and interfere with their functioning(15).

Past studies report toxic metals in drinking water, including their types and quantities, factors affecting metal concentrations, sources, human exposure, risk and removal. Despite significant progress, research is needed to ensure safe drinking water.

Symptoms and effects can vary according to the metal or metal compound, and the dose involved. Broadly, long-term exposure to toxic metals can have carcinogenic, central and peripheral nervous system and circulatory effects. For humans, among others affect reproduction especially toxic metals such as lead.

Studies from rodent models suggest that gonadotropin hormones (both LH and FSH) support the process of spermatogenesis by suppressing the proapoptotic signals and thus promoting spermatogenic cell survival. The Sertoli cells themselves mediate parts of spermatogenesis through hormone production. They are capable of producing the hormones estradiol and inhibin.

The Leydig cells are also capable of producing estradiol in addition to their main product, testosterone. Gonadotropin-releasing hormone (GnRH) is mainly made in the preoptic area of the hypothalamus before traveling to the pituitary gland (16). Circulating levels of FSH, LH, prolactin and testosterone are vital for spermatogenesis and sexual function. Accumulation of toxic metals in the body is harmful for

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sexual function and reproduction. Therefore, the evaluation of some toxic metal levels of drinking water and its possible effect on reproductive hormones is important for public health information.

MATERIAL and METHODS

This is a retrospective case-control study of male participants consuming drinking water from hand dug well, borehole water and treated table water in Sabongida Ora, Edo State. Sabongida Ora is the headquarters of Owan West Local Government Area of Edo State. Sabongida Ora is located at Latitude 6.90N and Longitude5.90E. It has a population of 99,056 according to National Population Commission of Nigeria of population census conducted in 2006.

Participants were gotten from the people living in the local government area. A total of 90 subjects consisting of 30 subjects who drink borehole water, 30 subjects who drink water from hand-dug well and 30 subjects who drink table water, aged between 18 and 45 years were recruited for this study.

Toxic metal concentration of water from 10 hand-dug wells, 10 boreholes, and treated water was determined. The levels of some toxic metal, trace element and reproductive hormones of Individuals who consumed water from these sources were evaluated. Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PROL), estrogen (E2), progesterone (PROG), and testosterone (T) were analyzed using Enzyme linked Immunosorbent assay method. Blood lead (Pb), cadmium (Cd), serum zinc (Zn) and copper (Cu) were analyzed using atomic absorption spectroscopy (AAS).

The study was designed to evaluate the levels of some toxic metals, trace element and reproductive hormones in water consumers (hand-dug well, borehole and table water). The study was carried out within twelve (12) months (July 2018 – June 2019). The sample size was determined using the sample size determination formula for Health studies (17) and the prevalence of hormonal abnormalities in occupational exposure subjects to toxic metal contamination (96.7%) (18). Therefore a minimum of 60 participants and 30 non-occupationally exposed healthy subjects were enrolled for the study

The protocol for the study was reviewed and approved by the ethics committee of the Edo State Ministry of Health. Informed consent form was given by the participants before the commencement of the study.

Healthy men within the reproductive age of 18–45 years, and are drinking water solely from hand dug well , borehole water and treated water were included in the study. Individuals on male contraceptive, or with testicular varicocele, long term medications, known HIV infected, chronic and serious systemic illness, steroid preparations, non-consented and smoking were excluded from the study.

Water Sample was collected from hand-dug wells, borehole and treated water source in clean-glass containers washed by soaking in 20% (v/v) Nitric acid for 24 hrs. Sampling was conducted early in the morning before water abstraction commenced by residents. Two separate samples per water source were collected for analysis. Blood samples were collected aseptically from the antecubital vein of the water consumers in the early hours of the day with sterile disposal syringe and needle and dispensed into anticoagulant bottle (prescreened for cadmium, lead, copper and zinc by vial assay) and plain bottles.

The sample in the plain bottle was allowed to clot at 2-8 ^{o}C, and after clot retraction , it was centrifuged at 3000rpm for 10 minutes . The plasma was separated and stored properly in labeled container at -20 ^{0}C for hormonal assay, and the whole blood was stored at 2-8 ^{0}C for maximum of one week for cadmium, lead, copper and zinc analyses Both qualitative and quantitative data were collected using a semi-structured self-questionnaire. The questionnaire has three (3) sections. Section A (Socio Demographic characteristics), Section B (Exposure/Awareness/Protection) ,Section C. (Medical/family history). The questionnaire was distributed among male participants drinking water from hand-dug well and borehole water as well as treated water.

The reproductive hormones (Luteinizing hormones, Follicle stimulating hormone Prolactin hormones, testosterone, Estradiol and Progesterone hormone) were assayed with ELISA technique (Monobind Inc. Lake Forest, CA 92630, USA. The blood and water cadmium, lead, copper and zinc were analyzed using with Atomic absorption spectroscopy at the wavelength of 217.0 (Pb), 228.8(Cd), 324.8(Cu) and 213.9(Zn) respectively (Buck Scientific Model VGP-210, Germany).

Results obtained are presented as mean ± standard deviation (SD). Data obtained were statistically analyzed using spss version 23.0 software. The comparison of the mean values of measured parameters among hand dug well, borehole and table water consumer was performed using unpaied Student t-test, Chi-Squared and Pearson correlation coefficient was used to test the relationship between toxic metals levels and reproductive hormones. The statistical significance level was set at $p<0.05$

RESULTS

Table 1 shows the socio- demographic variables of the study participants. The age range, sex, educational status, body mass index and ethnicity ($p<0.05$) except marital status ($p>0.05$) were significantly different between the water consumer than the control(treated table water).

Table 2 shows the comparison of reproductive hormones among water consumers. It was observed that levels of reproductive hormones in hand-dug well, borehole were significantly lower than treated table water ($P<0.05$).

Table 3 shows the comparison of toxic metals levels among water consumers. It was observed that the levels of toxic metals in hand-dug well, borehole water were significantly higher than treated table water ($p<0.05$).

Table 4 shows the correlation of reproductive hormones with toxic metals among water consumers.

It was observed that cadmium and lead were negatively correlated with FSH, LH, E2, Progesterone and testosterone except for prolactin but zinc and copper were positively correlated with FSH, LH, E2 and testosterone except in prolactin that showing a negative correlation

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Table 1: Distribution of Demographic Factors of Dug Well and Borehole Water Consumers

	Demographic factors	*Total (n=90)*	*Dug-well and Borehole water Consumers (n=60)*	*Control group (n=30)*	X^2	*p*
Age (years)	***18-25***	56(62.2%)	31(51.6%)	25(83.3%)	71.60	P=0.001
	26-35	20(22.2%)	15(25.0%)	5(16.6%)		
	36-40	8(8.8%)	8(13.3%)	0(0.0%)		
	40-above	6(6.6%)	6(10.0%)	0(0.0%)		
Sex	***Male***	90(100%)	60(66.6%)	30(33.3%)	19.60	P=0.001
Marital status	***Single***	52(57.7%)	32(53.3%)	20(66.6%)	2.18	P=0.140
	Married	38(42.2%)	28(46.6%)	10(33.3%)		
Educational status	***Primary***	5(5.5%)	5(8.3%)	0(0.0%)	62.07	P=0.001
	Secondary	64(71.1%)	44(73.3%)	20(66.6%)		
	Tertiary	21(23.3%)	11(18.3%)	10(33.3%)		
Body mass index (Kg/m^2)	***Underweight***	3(3.3%)	3(5.0%)	0(0.0%)	45.53	P=0.001
	Normal	42(46.6%)	22(36.6%)	20(66.6%)		
	Overweight	32(35.5%)	23(38.6%)	9(30.0%)		
	Obese	13(14.4%)	12(20.0%)	1(3.3%)		
Ethnicity	***Afemai***	81(90.0%)	51(85.0%)	30(100.0%)		
	Yoruba	9(10.0%)	9(15.0%)			

Values in parenthesis are percentage. P<0.05- Significant

Table 2: Comparison of reproductive hormones among different water consumers

Parameters	Dugwell water consumer	Borehole water consumer	Table water consumer	F	P
FSH(mIU/ml)	2.61±0.39	3.12±0.54	5.83±0.39	15.11	P<0.05
LH(mIU/ml)	1.31±0.21	1.63±0.19	6.98±0.34	158.30	P<0.05
E2(pg/ml)	2.00±0.51	2.67±0.43	9.94±2.26	10.38	P<0.05
PROG(ng/ml)	0.66±0.79	1.82±0.16	4.11±1.28	4.02	P<0.05
PROL(ng/ml)	24.51±0.55	19.63±1.02	18.37±0.49	19.91	P<0.05
TESTO(ng/ml)	2.42±0.48	2.84±0.33	6.54±0.27	37.25	P<0.05

Value are expressed in mean±SD, FSH- follicle-stimulating hormone, LH-Luteinizing hormone, E2-Estradiol, Prog-Progesterone, PROL-prolactin, TESTO- testosterone

Table 3: Comparison of metals among different water consumers

Parameters	Dugwell water consumer	Borehole water consumer	Table water consumer	F	P
Cadmium(ug/dl)	7.14±0.75	6.10±0.32	0.92±0.21	46.27	P<0.05
Lead(ug/L)	40.43±2.77	17.35±2.32	4.64±0.48	74.07	P<0.05
Copper(ug/dl)	90.63±1.51	99.43±2.68	97.90±2.23	4.06	P<0.05
Zinc(ug/dl)	38.93±3.33	45.60±2.35	163.30±9.41	139.54	P<0.05

Table 4. Correlation of reproductive hormones with toxic metals among water consumers

Parameters	R- value	P-value
FSH/Cd	-0.275	P>0.05
FSH/Pb	-0.398	P<0.05
FSH/Cu	0.055	P>0.05
FSH/Zn	0.422	P<0.05
LH/Cd	-0.622	P<0.05
LH/Pb	-0.493	P>0.05
LH/Cu	0.033	P>0.05
LH/Zn	0.745	P<0.05
E2/Cd	-0.230	P>0.05
E2/Pb	-0.250	P>0.05
E2/Cu	0.065	P>0.05
E2/Zn	0.412	P<0.05
Prog/Cd	-0.222	P>0.05
Prog/Pb	-0.055	P>0.05
Prog/Cu	-0.087	P>0.05
Prog/Zn	0.229	P>0.05
Prol/Cd	0.282	P>0.05
Prol/Pb	0.443	P<0.05
Prol/Cu	-0.024	P>0.05
Prol/Zn	-0.404	P<0.05
Testo/Cd	-0.426	P>0.05
Testo/Pb	-0.400	P>0.05
Testo/Cu	0.072	P>0.05
Testo/Zn	0.542	P<0.05

FSH- follicle stimulating hormone, LH-Luteinizing hormone, E2-Estradiol, Prog-Progesterone, PROL-prolactin, TESTO- testosterone, Cd-cadmium, Pb-lead, Cu- copper, Zn- zinc, P<0.05- Significant, P>0.05- Non- significant

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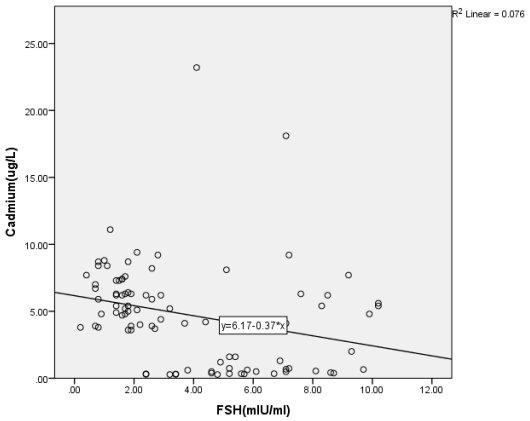

Figure 1: Scatterplot showing negative correlation of cadmium with follicle stimulating hormone (FSH) (r = -0.3 P >0.05)

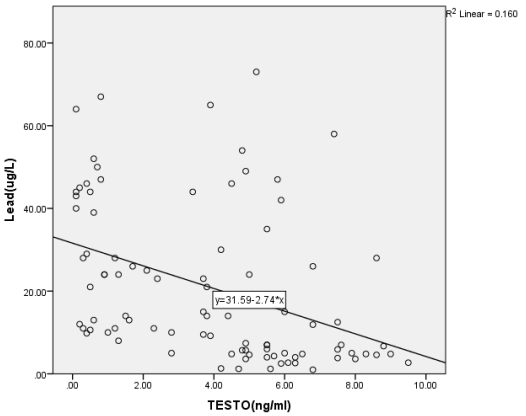

Figure 2: Scatterplot showing negative correlation of lead with luteinizing hormone (LH) (r = -0.4 P<0.05)

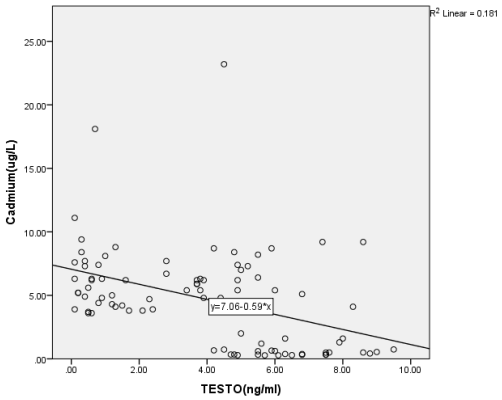

Figure 3: Scatterplot showing negative correlation of cadmium with testosterone (testo) (r = -0.4, P>0.05)

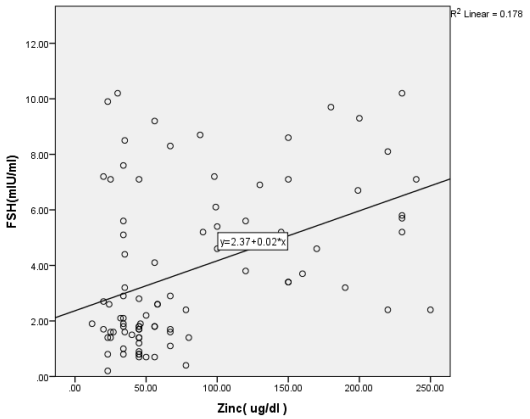

Figure 4: Scatterplot showing positive correlation of zinc on follicle stimulating hormone (FSH) (r = 0.4, P < 0.05)

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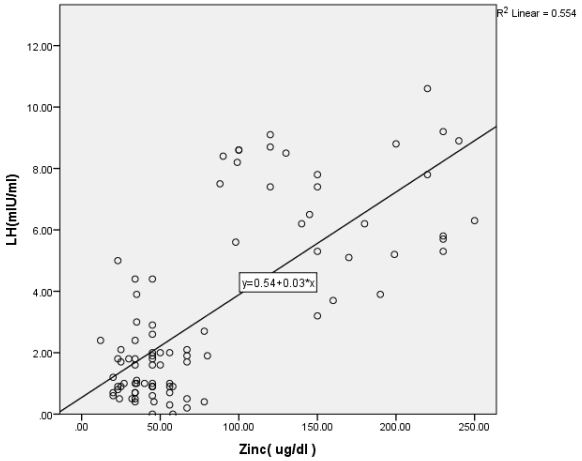

Figure 5: Scatterplot showing positive correlation of zinc on luteinizing hormone (LH) (r =0.7, P<0.05)

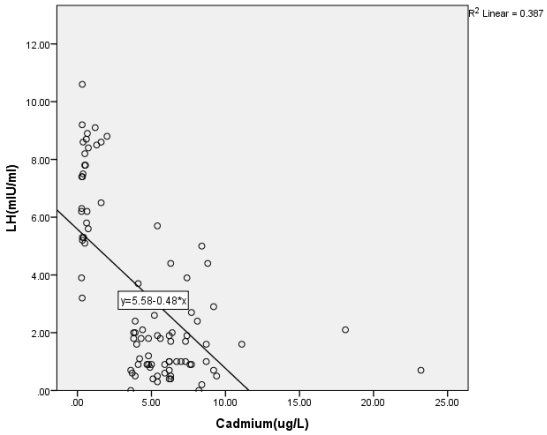

Figure 6: Scatterplot showing negative correlation of cadmium on luteinizing hormone (LH)(r = - 0.6, P<0.05)

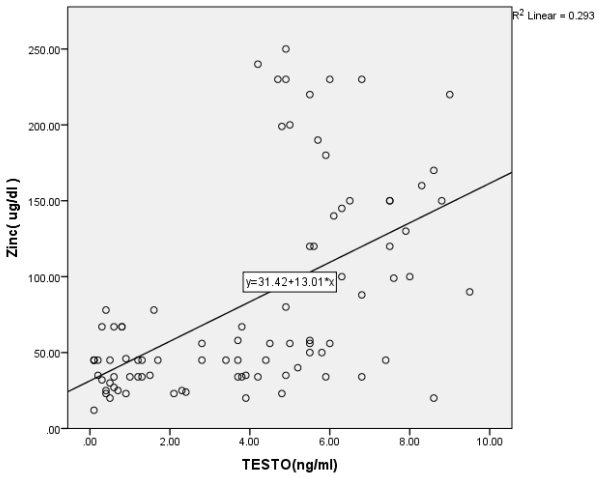

Figure 7: Scatterplot showing positive correlation of zinc on testosterone (testo) (r = 0.5, P<0.05

DISCUSSION

Nigeria belongs to the infertility belt of sub- Saharan Africa, and infertility has assumed a public health challenge. The causes of infertility in both males and female are multifactorial and the contributions of endocrine abnormalities have been reported (19). Toxic metal contamination in drinking water is a potential health risk to humans and has been reported to be the root cause of many chronic health challenges, including cancer, infertility, and organ damage.

The presence of toxic metals cannot be visualized with the naked eyes, but are detected in water through laboratory tests. This study conducted to determine Cd, Pb, Zn and Cu levels in hand-dug well, borehole and treated drinking water and the possible reproductive health effects on the consumers. In order to protect human health, guidelines for the presence of toxic metals in drinking water have been given by International Organizations such as WHO and European Union Commission (20).

These organizations prescribed that toxic metal level in water should not be higher than the maximum permissible level in water as specified. Maximum contaminant level is an enforceable standard in numerical range value to ensure no adverse effect on human health. The upper limit of the highest level of a contaminant is the maximum allowed in a water system for a particular toxic metal.

The toxic metal concentrations in hand-dug well and borehole water showed that Cd and Pb concentrations were higher than WHO recommended permissible limits in drinking water. This is consistent with previous studies in some parts of Nigeria. For example, Momodu and Anyakora (21) observed that 19/30(84.21%) of underground sample water contained Cd concentration higher than the Maximum Contaminant Level (0.003mg/L) with the maximum concentration of 0.098mg/L(9.8µg/dL). The reported concentration is higher than observed in this study (7.14µg/dL). The detected level, which is higher than the Maximum Contaminant Level, is of great concern since Cd has the potential to cause male infertility(22), cancers (23) and long biological half-life (24), leading to chronic organ damage (25). In this study, the concentration of Cd observed in hand-dug well and borehole water is above the recommended concentration permissible in drinking water by the WHO (26).

The concentration of Pb detected in hand-dug well and borehole water in this study was higher than the recommended permissible limit by WHO. The finding is consistent with that of Momodu and Anyakara (21) in Surulere, Lagos. The authors observed that 10 of the well water and 19 borehole water samples contained Pb, and 6 of the well water and 12 of the borehole water samples contained levels above the Maximum Contaminant Level (0.01mg/L) with the maximum concentration detected was 0.024mg/L (2.4µg/dL). These findings are of concern since Pb can bio accumulate and affects the general body metabolism (27) as well as male infertility (28). Lead is also a neurotoxin and may be responsible for the most common type of human metal toxicosis (29). Also, some authors have associated Pb exposures even at low levels with and elevated blood pressure (30) and reduced intelligence quotient in children (31). Lead is a naturally occurring metal present in small quantities in the earth's crust. Although lead occurs naturally in the environment, human activities such as the use of fossil fuel, mining and manufacturing industries contribute to the release of high concentrations. The concentration of Pb observed in hand-dug well and borehole water was higher than in treated water and more than the quantity recommended in drinking water by the WHO. The permissible limit recommended by WHO for drinking water is 0.01mg/L.

From this study, it was observed that the concentration of Pb and Cd in the blood of hand-dug well and borehole water consumers was higher than consumers of treated table water. This finding is consistent with the other studies in Baghdad, Beirut, and Al-Najaf which reported that the blood Pb and other toxic metals were higher among exposed individuals (32,33). Copper and zinc were lower in those that consumed water from hand-dug well, borehole than the treated table water. The higher blood level of toxic metals among the subjects may be traceable to drinking of contaminated water (hand-dugged well and borehole water) (34) since the respondents were occupationally exposed. The respondents reported that they have been consuming water from these sources for several years, being the only source of drinking water as at then of our study. Significant low values of reproductive (sex) hormones of the subjects that consume hand-dug well and borehole water than treated table water was also observed($p<0.05$). From previous studies, it was observed that these toxic metals are potent endocrine disruptors and oxidative stress inducers (35) as well as the hypothalamo-pituitary-gonadal axis inhibitors (36,37,38). These glands have been shown to act with a common function in order to achieve a common goal of regulating reproductive and sex hormone production. The hypothalamic pituitary-gonadal axis plays a critical part in the development and regulation of a number of the body's systems, such as the reproductive and immune systems. Any fluctuations in this axis cause changes in the hormones produced by each gland and have various local and systemic effects on the body. This axis controls development, reproduction, and aging in animals (39). Gonadotropin-releasing hormone (GnRH) is secreted from the hypothalamus by GnRH- expressing neurons. The anterior portion of the pituitary gland produces Luteinizing hormone (LH) and Follicle Stimulating Hormones (FSH), and the gonads produce estrogen and testosterone. The male reproductive system depends upon the activities of these different hormones produced by various body glands and enters systemic circulation. Gonadotropin-releasing hormone is a tropic hormone produced by the hypothalamus and stimulates the anterior part of the pituitary gland to produce the FSH, which stimulates the production of sperm in the testes of men, and LH, which causes the interstitial cells of the testes to produce the hormone testosterone. This concentration in relatively constant testosterone enters the systemic circulation of a healthy, reproductive-age male (40). Testosterone helps to produce and maintain the secondary sexual characteristics of the male and is also responsible for the sex drive as well as work with the FSH to stimulate the production of sperm. If the sperm levels are high, making nutrients for the developing sperm scarce, the testes release inhibin. The inhibin travels through the bloodstream to the brain, where it prevents the secretion of GnRH. In the absence of GnRH, FSH, and LH levels fall, and sperm production slows. This is one of the major mechanisms whereby male hormones are maintained at the relatively constant concentration (16). From the interrelationship of sex hormones and potential confounders, it was observed that lead (Pb) was negatively associated with FSH and Estradiol and positively associated with prolactin which were statistically significant ($p<0.05$). It was observed that cadmium was negatively associated with LH, which was statistically significant. This result is in line with the findings of other studies which negatively affect on LH (41). Zinc was shown to have a positive association with FSH, LH, Estradiol, and testosterone and a negative association with prolactin which were statistically significant ($p<0.05$). Therefore, the observed decrease in the serum levels of these reproductive hormones suggests that the associated increase observed in the serum levels of toxic metals among those that consumed water from hand-dug well and borehole could have caused impairment in the neuron-chemical secretory mechanisms along the hypothalamo-pituitary-gonadal axis responsible for the synthesis and secretion of these hormones. A negative relationship is therefore established between the serum level

of toxic metals and the reproductive hormones of the subjects. This damage of reproductive hormones can result in the damage of the reproductive functions and capacities of affected subjects. The mean blood levels of toxic metals (lead and cadmium) of hand-dug water consumers and borehole water consumer were compared, it was observed that the concentration of toxic metals (Pb and Cd) in the serum of hand-dugged well were higher than the borehole water consumers which was statistically significant ($p<0.05$). This finding is also consistent with the other studies (42,43,44) that groundwater is contaminated as a result of its contact with soil, rocks, and plants and the constituents of these sources. Copper and zinc were higher in the borehole water consumers and statistically significant ($p<0.05$). The increase in the serum level of toxic metals among the hand-dug well water consumers is traceable to Industrial processes; generate wastes, which are mostly discharged into the environment. Industrial activities, especially electroplating, metal smelting and chemical industries and manufacturing processes, are sources of anthropogenic toxic metals in water. Poorly treated domestic, industrial and agricultural wastewater contains high concentrations of toxic metals, which are often discharged into the environment.

CONCLUSION

From this study, it is evident that consumption of contaminated water with toxic metals might pose reproductive health threat to consumers. This is as a result of the water not being treated for toxic metals and other impurities before consumption. This alteration in reproductive hormone levels may cause infertility and other disease conditions in men. In view of the dangers associated with the consumption of water contaminated with toxic metals and other impurities, it becomes necessary to ensure that water from boreholes and especially hand-dug wells are subjected to treatment for purification to make it free from toxic metals and other impurities. Enforcement of the recommended guidelines by WHO regarding the purity of drinking water is suggested.

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Prognostic importance of peripheral blood parameters in HER-2 positive metastatic breast cancer treated by pertuzumab, trastuzumab and docetaxel

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ABSTRACT

Objective: There are some studies about the significance of the peripheral blood parameters in breast cancer. However, there is very few studies about prognostic importance of peripheral blood parameters in human epidermal growth factor-2 receptor (Her-2) positive breast cancer. We aimed to evaluate whether prognostic significance of peripheral blood parameters in patients Her-2 positive metastatic breast cancer with treated Pertuzumab, Trastuzumab, Docetaxel (PTD)

Material and Methods: We included 56 patients with Her-2 positive metastatic breast cancer patients who were treated with PTD. We recorded patients' clinical,demographic features and we obtained peripheral blood parameters such as neutrophil-lymphocyte ratio (NLR), red blood cell distribution (RDW), mean platelet volume (MPV), lymphocyte, neutrophil after the sixth cycle of the treatment and before the treatment. We separated the patients into two groups depending on the progression status. Progression-free survival was analyzed by Kaplan-Meier statistical analysis.

Results: Patients mean age was 50.7. Progression was detected in 34 patients. When we explored and compared hemogram parameters in the groups before the treatment, there wasn't statistically any significant difference between these parameters such as neutrophil, lymphocyte, neutrophil to lymphocyte ratio, mean platelet volume, red blood cell width. In the progressive group;,while pretreatment NLR was 3.83, it was detected 2.72 after six cycle treatment and difference was meaningful (p: 0.043). The pretreamtent MPV was 8.63, and It was 8.15 after six cycle treatment, and difference between these counts was statistically important (p: 0.006). PFS was 18.0 months in the study group.

Conclusion: Peripheral blood parameters were not statistically significant in both group comparisons. In the progression group, the difference between NLR and MPV count was statistically significant after the sixth cycle of the treatment and before the treatment.

Keywords: Breast cancer, Her-2, Pertuzumab, progression

INTRODUCTION

Breast cancer is heterogenous disease, and human epidermal growth factor-2 receptor (Her-2) overexpression approximately occurs in ratio 15-20% in metastatic breast cancer. That survival of patients was longer were detected as a result of development in the treatment of Her-2 positive metastatic breast cancer (1). Pertuzumab and Trastuzumab are anti-Her-2 monoclonal antibody. In the CLEOPATRA study, Pertuzumab,Trastuzumab were applied to patients with Docetaxel, and that this combination treatment was effective was demonstrated in this trial (2). These two drugs prevent Her-2 dimerization with other epidermal growth factor family receptors such as Her-3, Her-4, and these drugs demonstrate activity via hamper the signaling. In addition to, Anti-Her 2 antibodies are thought to mediate tumor regression not only by interrupting oncogenic signaling, but also by inducing antibody-dependent cell-mediated cytotoxicity (ADCC) (3).

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That inflammation is important in cancer progression is known. That many cancer may develop as a result of inflammation and chronic irritation due to infection is known. We may easily obtain some inflammatory markers from the peripheral blood samples. These markers have been shown to have prognostic significance in various solid tumors. (4,5). However, there are insufficient studies about inflammatory markers in patients using dual anti-Her-2 blockage treatment.

The aim of the present study was to investigate whether there was any prognostic significance of hemogram parameters in patients with Her-2 positive metastatic breast cancer receiving Trastuzumab, Pertuzumab and Docetaxel.

MATERIAL and METHODS

Fifty-six patients who got diagnosed Her-2 positive metastatic breast cancer were enrolled to this study. Data of the patients were collected retrospectively. Neutrophil lymphocyte ratio, red blood cell distribution, mean platelet volume, lymphocyte, neutrophil were recorded than the patients blood sample after the sixth cycle of the treatment and before the treatment. The neutrophil lymphocyte ratio was calculated by subdividing neutrophil to lymphocyte. Patient age, tumor location and tumor histology were determined from the patients file. Metastasis sides, SUV max value of metastatic lesion and primary tumor SUV max value in the PET-CT which was used for staging of the disease, were recorded. Moreover, tumor grade, Ki-67 proliferation index and hormon receptor status were obtained. The histopathological diagnosis to patients was made with a biopsy taken from the mass formed in the breast. Her-2 status was confirmed with an immunohistochemical score (IHC) of 3 (positive), and in situ hybridization test was used for those with a score of 2 according to the guidelines of the American Society of Clinical Oncology/College of American Pathologists for Her-2 testing in breast cancer (6). If there were at least 1% positive tumor cell nuclei in the sample that was evaluated by IHC, we accepted hormone receptor-positive. Patients who had kidney and liver disease got treatment for meastatic disease before the study, had a chronic infection or active infection disease, got immunosuppressive therapy and under 18 age were not included to this study. Progression-free survival (PFS) was defined as the time from pertuzumab treatment to either first disease progression or death. All patients were fully informed and approved by the ethics committee of date of 06.05.2020 and 848 decision no from Adana City Training and Research Hospital

Statistical analysis: Continuous variables were summarized in mean and standard deviation, while categorical data were summarized as frequency and percentage. Chi-square test was used to evaluate bivariate associations between categorical variables. Independent samples t test was used to compare two groups while Repeated Measures ANOVA was used to analyze the interaction effect of progression and treatment periods. Continuous variables obtained before and after treatment were compared with Paired samples t test, in each group. Life table and Kaplan-Meier curve were used to evaluate progression free survival. Statistical significance level was considered as 0.05, and statistical analyses were done by SPSS v.20 statistical package.

RESULTS

The mean age of the patients was 50.7. While estrogen receptor status was positive in 35 patients, ıt was negative in 21 patients. Progesterone receptor status was positive in the 26 patients. Her-2 results of 40 patients were detected positive by immunohistochemical method. Her-2 score was 2(+) in 16 patients and that Her-2 positivity was detected by the FISH method. The number of the patients who had grade II and III tumors was equal the each other. While the primary tumor SUV value was 10.2, metastasis SUV value was 9.9. The progression was detected in the 34 patients. The visceral metastasis ratio was 66 %. The most common visceral metastasis sides were lung and liver. There was no progression in the 22 patients. Demographic and clinical features were summarised in the **Table-1**.

We separated the patients into two groups depending on progression status. The mean age was 48.2 in patients having progression, It was 54.8 in patients not having progression (p:0.085). While estrogen receptor positivity was 50 % in the progressive group, It was 63.6% in the non-progressive group. The 18 patients' tumor grade was grade 2 , 16 patients were grade 3 in the progressive group. In the non-progressive group, tumor grade was only grade 2 in 10 patients and grade 3 in 12 patients, respectively. Ki-67 proliferation index was similar in the both group. Primary tumor SUV max value was alike in the both group(SUV max:10.3, in the progressing group, SUV max: 9,6 in non-progressive group, respectively). When we explored and compared hemogram parameters in the groups before the treatment, there wasn't statistically any significant difference between these parameters such as neutrophil, lymphocyte, neutrophil to lymphocyte ratio, mean platelet volume, red blood cell width. These results are summarized in the **Table 2**.

Patients' hemogram parameters before treatment and after six cycle treatment were compared. In the progressive group; while pre-treatment NLR was 3.83, ıt was detected 2.72 after six cycle treatment, and the difference was meaningful (p: 0.043). The pre-treatment MPV was 8.63, and It was 8.15 after six cycle treatment and difference between these count was statistically meaningful (p: 0.006). The pre-treatment RDW was 15.04 , it was determined 17.61 after treatment and the difference was meaningful (p: <0.001). Patients without progression, while pre-treatment NLR was 3.35, ıt was detected 2.71 after six cycle treatment and the difference wasn't meaningful (p: 0.163). The pre-treatment MPV was 8.54, and It was 8.28 after six cycle treatment and the difference between these counts wasn' t meaningful (p: 0.164). The pre-treatment RDW was 15.08, It was determined 17.29 after treatment and the difference was meaningful (p: 0.014). These results were summarized in **Table 3**.

Progression-free survival was detected 18.0 months and it was shown in **Figure-1.**

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Table 1: Demographics and baseline characteristics of patients

Charecteristics	n:56
Age	50.7
Estrogen receptor	
Pozitif	31(.% 55.3)
Negatif	25 (% 44.7)
Progesterone receptor	
Pozitif	26 (% 46.4)
Negatif	30 (%53.6)
Her-2 IHC status	
2(+) with FISH pozitif	16 (%28.5)
3(+)	40 (%71.5)
Grade	
II	28 (% 50)
III	28 (% 50)
Metastasis side	
Visceral	37 (%66)
Non visceral	19 (%34)
Primary SUV	10.2
Metastasis SUV	9.95
Progression	
Present	34 (% 60.7)
Absent	22 (% 39.3)

Table 2: Patients features depending on progression status

	Progression present (n=34)	Progression absent (n=22)	p
Age	48,26±12,87	54,81±14,34	0,085
Estrogen receptor			
Positive	17 (%50,0)	14 (%63,6)	0,316
Negative	17 (%50,0)	8 (%36,4)	
Progesterone receptor			
Positive	16 (%47,1)	10 (%45,5)	0,906
Negative	18 (%52,9)	12 (%54,5)	
Her-2 IHC status			
2(+) with FISH pozitif	9 (%26,5)	7 (%31,8)	0,665
3(+)	25 (%73,5)	15 (%68,2)	
GRADE			
II	18 (%52,9)	10 (%45,5)	0,584
III	16 (%47,1)	12 (%54,5)	
Ki-67 proliferation index	35,12±23,02	34,41±20,89	0,908
Primary SUV	10,34±4,07	9,60±4,87	0,563
Metastasis SUV	10,67±5,22	8,86±5,26	0,212
Pretreatment neutrophil	5638,24±2237,43	5609,09±2805,42	0,966
Pretreatment lymphocyte	1843,53±826,10	1881,82±770,68	0,863
Pretreatment NLR	3,84±2,55	3,35±1,82	0,438
Pretreatment MPV	8,66±1,39	8,54±0,98	0,727
Pretreatment RDW	15,12±3,11	15,08±3,02	0,966

NLR; neutrophil to lymphocyte ratio, MPV; mean platelet volüme, RDW; red blood cell distributio**n**

Table 3: Hemogram parameters

	Progression present (n=34)	p value	Progression absent (n=22)	p value
Pretreatment neutrophil	5557,58±2221,35	0.083	5609,09±2805,42	0.486
[a]Posttreatment neutrophil	4762,12±1942,89		5168,18±1911,22	
Pretreatment lymphocyte	1838,79±838,44	0.234	1881,82±770,68	0.399
Posttreatment lymphocyte	2011,82±662,44		2031,82±523,16	
Pretreatment NLR	3,83±2,59	**0.043**	3,35±1,82	0.163
Posttreatment NLR	2,72±2,03		2,71±1,35	
Pretreatment MPV	8,63±1,40	**0.006**	8,54±0,98	0.164
Posttreatment MPV	8,15±0,95		8,28±0,92	
Pretreatment RDW	15,04±3,10	**<0,001**	15,08±3,02	**0,014**
Posttreatment RDW	17,61±2,70		17,29±3,24	

NLR; neutrophil to lymphocyte ratio, MPV; mean platelet volüme, RDW; red blood cell distribution

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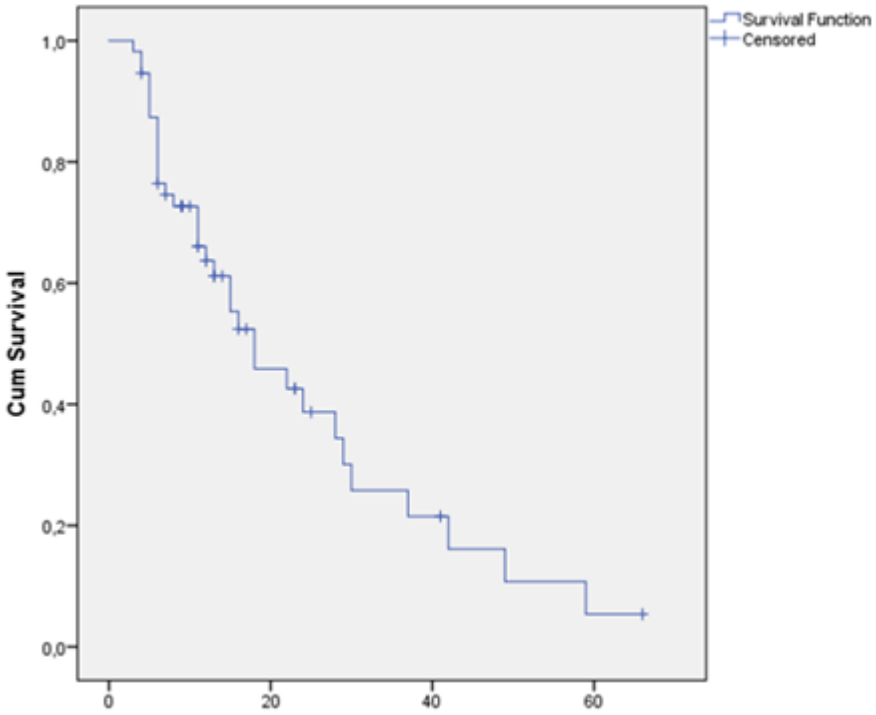

Figure 1: Progression-free survival (18.0 months)

DISCUSSION

Her-2 overexpression approximately occurs 15-20 % in the breast cancer. The breast cancer with overexpression HER-2 has a poor prognosis (7). However, better results were obtained, in study which is called CLEOPATRA, in Her-2 positive metastatic breast cancer via dual blockage with Pertuzumab and Trastuzumab which target the Her-2 (8). Median PFS was detected 18.5 months in the CLEOPATRA study. PFS was 18 months in the our study.

Hormone receptor positivity may accompany in Her-2 positive breast cancer. Hormone receptor positivity was 47 % in patients who were included to CLEOPATRA study (8). In another study which was performed by Esin et al, they published Pertuzumab real real life practice in metastatic patients with Her-2 positivity and they have detected 58.7 % hormon receptor positivity (9). The study was done by Araki et al, hormone receptor positivity was 45 % in Her-2 positive metastatic breast cancer (10). Hormon receptor positivity was ascertained 65.1 % in the study which was performed by Trıpathy et al (11). It was 55.3 % in our study. These results show that these ratios may change from center to center.

Her-2 positive status was confirmed centrally, by means of immunohistochemistry (with 3+ indicating positive status) or fluorescence in situ hybridization (with an amplification ratio ⩾2.0 indicating positive status) (12). Her-2 receptor positivity ratio with immunohistochemistry was 78 % in the study which was performed Araki et al (10). This ratio was detected 87.1 % in the CLEOPATRA study (8). Her-2 receptor positivity ratio with immunohistochemistry was 71.4% in the our study.

The different PFS ratio were seen in distinct studies in patients using Pertuzumab, Trastuzumab because of de novo Her-2 positive metastatic breast cancer.

While the median PFS was 18.5 months in the CLEOPATRA study (8), It was determined 28.5 months in the study which was performed by Esin et al. (9). PFS was 17.7 months in the study which was performed by Trıpathy et al (11). It was 18 months in the our study.

Advanced breast cancer comprises inoperable locally advanced breast cancer, which has not spread to distant organs, and metastatic (stage IV) breast cancer; common sites of spread are bone, the lungs and the liver (13). The most common metastasis sides were lung and liver in our study. Currently, it is a treatable but virtually incurable disease, with metastases being the cause of death in almost all patients, and a median overall survival of 2–3 years. Patients with metastatic breast cancer receive treatments that aim to relieve their symptoms and to prolong quality-adjusted life expectancy . The visceral metastasis more commonly observe in Her-2 positive breast cancer compared to luminal subtypes breast cancer. While Visceral metastasis ratio was 37 % in study that Araki et al were performed (10), It was determined 78.1 % in the CLEOPATRA study (8). Visceral metastasis ratio was 66 % in the our study .

The association between cancer and inflammation are shown distinct studies. Inflammation facilitates tumor development and metastasis (14). That some inflammatory markers are related the poor prognosis was shown in breast cancer (15).There are some studies about peripheral blood parameters for predictive the progression Her-2 positive breast cancer. In the study, including fifty-one patients with Her-2 positive metastatic breast cancer, patients were treated with Pertuzumab, Trastuzumab and systemic chemotherapy. They seperated patients two group according to lymphocyte count.

Patients with high lymphocyte count had longer PFS compared to with low lymphocyte count (10). Another study was performed by Ulas et al in the early stage Her-2 positive breast cancer. This study included 187 patients and median disease free survival was shorter in the high NLR than in the low NLR group, the difference was not statistically significant. In our study, we separated the patients depending on the progression status, and there wasn't a statistically significant difference between two groups peripheral blood parameters such as neutrophil, lymphocyte, neutrophil to lymphocyte ratio, mean platelet volum and red blood cell distribution.

CONCLUSION

Our PFS result was similar with CLEOPATRA study. However, we didn't detect differences in hemogram parameters between groups. Nevertheless, to affirm the prognostic importance of hemogram parameters in these patient populations, large-scale, multi-center, and prospective studies are warranted in the future

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A case of a mixed overdose involving kratom (Mitragyna speciosa) leading to serotonin syndrome

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ABSTRACT

Objective: Drug overdose deaths have risen precipitously over the past two years in the United States. Polysubstance overdose with opiates and amphetamines have been of particular concern. Kratom (Mitragyna speciosa) is an unregulated widely available herb with both stimulant and opiate μ-receptor activity. Studies suggest that its use is quickly increasing.

Case: We describe a patient who presented to a psychiatric hospital with a mixed toxic syndrome due to chronic kratom and prescribed SSRI use compounded by acute intake of methamphetamine. The patient displayed psychosis, tremulousness, myoclonus, and extreme anxiety. Her clinical picture was consistent with both serotonin syndrome and opiate withdrawal.

Conclusion: We call attention to this case because polysubstance overdoses are common, and kratom is widely available. Complex toxic presentations that involve kratom are likely to be increasingly encountered.

Key words: Kratom, Mitragyna, Toxicity, Drug Abuse, Serotonin Syndrome

INTRODUCTION

Deaths from drug overdose are rising precipitously in the US driven particularly by synthetic opioids such as fentanyl (1). At the same time methamphetamine abuse is also on the rise, along with deaths from mixed overdoses involving both methamphetamine and opiates (2). Kratom is an unregulated herbal supplement with opiate μ-agonist characteristics that is widely available and whose use is also increasing (3). We report here a case of a serious but non-fatal mixed overdose that involved kratom, methamphetamine, and chronic SSRI prescription use. Since the prevalence of current antidepressant use in the U.S. approaches 14% of the population, we believe that this case illustrates a serious toxidrome that may be encountered by clinicians more frequently.

Kratom is derived from the leaf of the tree Mitragyna speciosa found in Southeast Asia. Traditionally kratom was brewed into a tea and used by day laborers to increase productivity. In low dosages, it appears to act as a stimulant (1-5 g per day); in higher concentrations, it has opioid and sedative effects (5-15 g) (3, 4, 5). Thus, depending on dosing, kratom can act as a stimulant, anxiolytic, analgesic, or antidepressant. Survey data in the US report that kratom is used for self-management of chronic pain, opioid withdrawal, and mood enhancement (6).

The major alkaloid of kratom is mitragynine, which is an agonist at the opiate μ-receptor. This action has been linked to kratom's analgesic effect but also to its potential to produce physical dependence. An active metabolite of mitragynine, 7-hydroxymitragynine (7-OH), is also responsible for kratom's dose-dependent antinociceptive properties (5, 7). In the native leaf and varying capsule products, 7-OH is found in lesser concentrations ranging from 0.01-0.04%, as compared to 4.7-8.7% mitragynine (8). However, relative amounts in some commercially available preparations appear to vary wildly, as one study found 7-OH in concentrations 109-520% greater than would occur naturally (9). This increased concentration is important because this metabolite has been shown to antagonize the μ-receptor with a potency surpassing that of morphine (10). Kratom also interacts with norepinephrine and serotonin receptors, perhaps adding to its perceived positive effects on mood (3).

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This increased concentration is important because this metabolite has been shown to antagonize the μ-receptor with a potency surpassing that of morphine (10). Kratom also interacts with norepinephrine and serotonin receptors, perhaps adding to its perceived positive effects on mood (3)

Kratom has been considered by some to be a relatively safe alternative to opioids, as it appears less likely to cause respiratory depression. One preclinical study demonstrated that knock-out mice missing the beta-arrestin G protein-coupled receptor regulatory protein showed resistance to the effects of morphine on respiratory depression and constipation, but enhanced and prolonged analgesic responses (11). In vitro studies have demonstrated that kratom is a μ-agonist that does not activate the β-arrestin-2 proteins, potentially explaining its reported lower side effect profile compared to opiate compounds (12). In addition, rodent studies have suggested that mitragynine increases levels of monoamine neurotransmitters including serotonin via hypothalamic-pituitary-adrenal axis interactions and induces Fos expression in the major serotonergic projection center of the brain, the dorsal raphe nucleus (13, 14). Therefore, while kratom is most commonly used as a replacement for opioids, its use as a substitute for antidepressants has also been of interest (5, 15).

Kratom does carry significant risks: One hundred fifty-two overdose deaths in which Kratom was involved were reported over 18 months 2016-2017. In seven cases, it was the only drug found in the blood of the deceased individual (16). In addition, kratom is a strong inhibitor of P450 3A4 and 2D6 and therefore has high potential for drug interactions with opioids, antidepressants, and benzodiazepines (17).

Case Presentation

The patient was a 48-year-old woman with a history significant for PTSD and kratom dependence who was admitted to an inner-city inpatient psychiatry unit for new-onset psychosis following an isolated episode of intranasal methamphetamine use. Notable comorbid conditions included hypertension, COPD, and chronic back pain. She had initiated kratom use four years prior to presentation as a substitute for prescription opiates for back pain. She reported daily use of 50-80 capsules containing 500 milligrams of kratom, an average of 20-40 grams of kratom per day. The patient had an extensive substance use history throughout her lifetime, but several multi-year periods of abstinence from all substances. At the time of presentation, the patient reported using only marijuana and kratom for the previous four years apart from two recent episodes of methamphetamine relapse, one month prior and again six days prior to presentation. Additionally, throughout this time, the patient was prescribed paroxetine 40 mg daily, along with gabapentin, varenicline, and as needed cyclobenzaprine. During the entire 4-year use of kratom and paroxetine prior to relapse on methamphetamine, she had experienced no psychotic symptoms.

Following her use of an unspecified amount of methamphetamine nasally, the patient began to experience psychomotor and psychotic symptoms. She presented to the emergency department voluntarily for complaints of auditory hallucinations and restlessness. Upon admission, approximately 48 hours after last methamphetamine or kratom use, exam was significant for hypertension, sinus tachycardia without fever, auditory and visual hallucinations, ideas of reference, gross disorganization, delusional thoughts, and prominent mood lability. Neurologic exam was significant for diffuse hyperreflexia, shuffling gait, tremulousness and intermittent myoclonus. Urine toxicology was positive for tricyclic compounds (most likely due to cyclobenzaprine) and cannabis. Thyroid function testing was normal; HIV, hepatitis C, and RPR serologies were negative. Routine chemistries were normal. CBC was notable for MCV of 103.7 initially, later measured at 109.7 with normal B12 and folate levels. The patient denied chronic alcohol use.

The patient was administered olanzapine, divalproex, clonidine, and lorazepam. Paroxetine was decreased to 30 mg because of concern about her symptomatology suggesting serotonin syndrome but was not stopped entirely due to concerns about abrupt discontinuation, which can be associated prominent withdrawal agitation. Clonidine was added to address opioid withdrawal, but buprenorphine was withheld due to concern about the degree of the patient's confusion.

Over the next week, the patient's psychotic symptoms gradually resolved; vital signs and neurological examination returned to normal, with resolution of hyperreflexia and myoclonus.

DISCUSSION

To the best of our knowledge, this is the first case study to present a patient with likely serotonin syndrome involving heavy kratom use. We believe that this patient's chronic use of kratom, along with paroxetine, primed the patient for a disorder of serotonin excess. She fulfilled Hunter criteria for serotonin syndrome (18). Preclinical studies have suggested that methamphetamine acts as a serotonin 5-HT2A agonist (19). It is probable that the patient's isolated methamphetamine ingestion acted as the inciting event in the context of kratom and a serotonin reuptake inhibitor.

Interestingly, during the admission the patient was noted to have a significant macrocytosis of unknown origin. As noted above, all standard laboratory investigations related to macrocytosis were normal. One cross-sectional study examining the haematological and clinical-chemistry changes associated with kratom use found no statistically significant change in any hematologic parameter of chronic kratom users; however, the effects of kratom on haematopoiesis remain unexplored (20). Although we cannot be certain that the patient's use of alcohol was not greater than she reported, the possibility that high dose kratom may affect haematopoiesis is open for further investigation. Kratom has been reported to be associated with hypothyroidism, but TSH was normal in this patient (21).

This case may be among the highest doses of daily kratom use reported, although we believe similar presentations are likely to emerge as recognition of kratom use and withdrawal increases. Kratom is regularly advertised and sold as a harmless supplement online, as well as in convenience stores and gas stations. In the case of this patient, she did not report her use of kratom until the second day of admission due to her belief that it was irrelevant to her current symptoms. Kratom cannot be detected by a standard urine drug screen, requiring instead methods such as gas chromatography–mass

spectrometry not in general clinical use (22). Awareness of the dangers of kratom has not kept pace with its increased use. While kratom has potential for future research and clinical application in pain management and in treatment of opioid use disorder, it is more likely to be encountered clinically at this time due to intoxication, abuse, and withdrawal (23). As this case indicates, these presentations can be manifold and can include serotonin syndrome and psychosis when mixed with other common drugs or medications.

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