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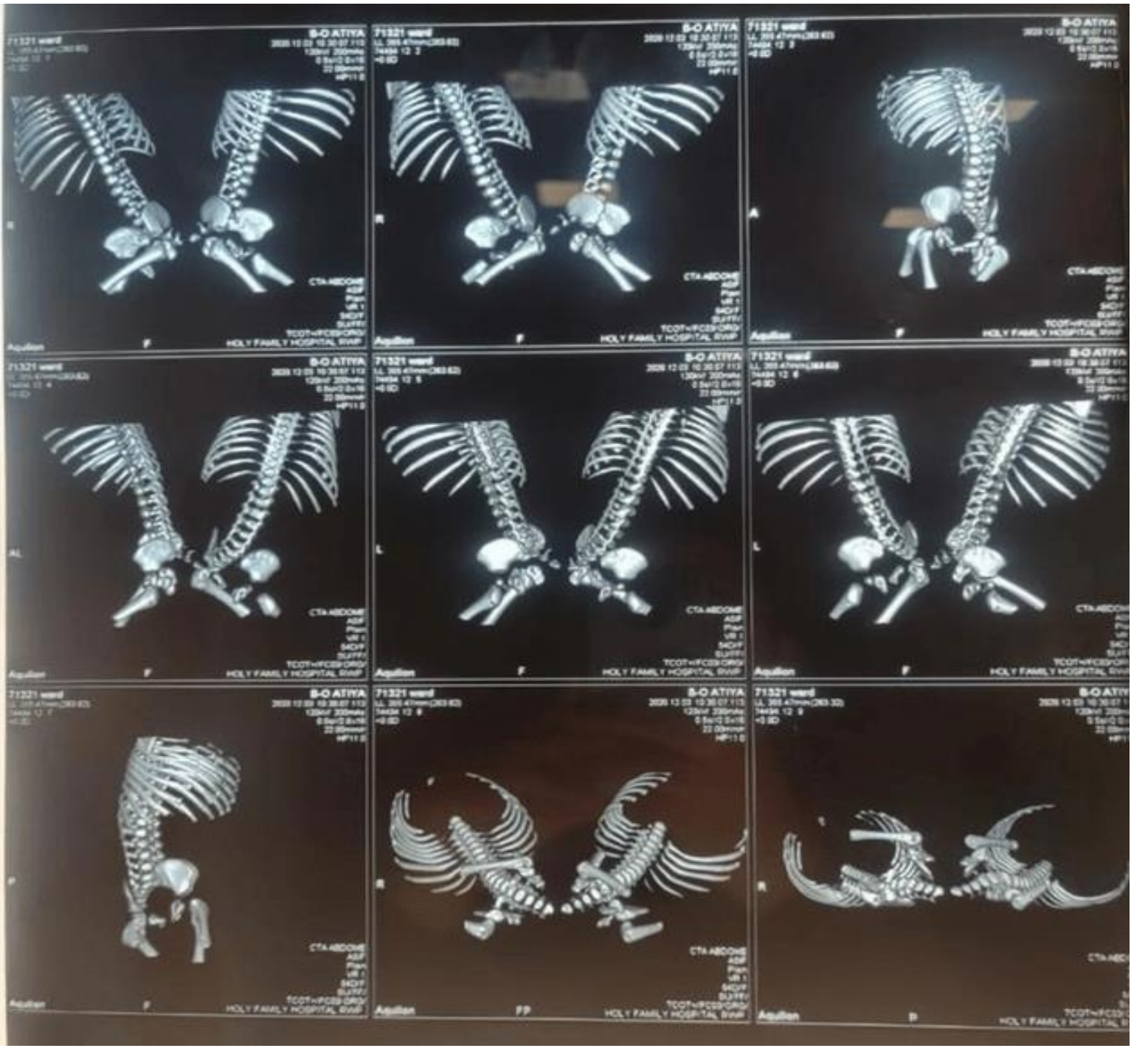

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Novel biochemical prognostic indicators in COVID-19: Can CRP/albumin, urea/albumin, and LDH/albumin ratios be used to predict mortality and length of hospitalization?

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ABSTRACT

Objective: As the coronavirus disease 2019 (COVID-19) pandemic continues, clinical and laboratory predictors of progression to serious and lethal types of the illness are urgently needed. We aimed to investigate how well hematologic, biochemical, and immunologic biomarkers could distinguish between patients with and without severe or fatal COVID-19.

Material and Methods: This retrospective study was performed in the internal medicine departments of two institutions. Data were collected from the hospital database, and the medical files of 280 adult COVID-19 were reviewed. The relationship between hematologic and biochemical parameters which include C-reactive protein (CRP)/albumin, urea/albumin, and lactate dehydrogenase (LDH)/albumin ratios and length of hospitalization, disease severity, and survival were investigated.

Results: Our series consisted of 280 adult COVID-19 patients (147 women, 133 men) with an average age of 58.34 ± 18.64 (range: 19 to 93). Most patients (n=171, 61.1%) had mild-moderate disease, while severe disease was diagnosed in 109 cases (38.9%). A total of 36 patients died due to COVID-19 yielding a mortality rate of 12.9%. The average length of hospitalization was 8.98 ± 5.80 days (range: 1-55 days). We noted that (CRP)/albumin, urea/albumin, and lactate dehydrogenase (LDH)/albumin ratios were significantly associated with severity of disease, mortality, and length of hospitalization.

Conclusion: Several biomarkers have been established that could help with risk stratification models for predicting serious and fatal results of COVID-19 infection. We suggest that clinicians closely track CRP/albumin, urea/albumin, and LDH/albumin ratios in hospitalized patients with respiratory distress as indicators for possible critical illness progression.

Key words: COVID-19, CRP/albumin ratio, Urea/albumin ratio, LDH/albumin ratio, Mortality; Day of hospitalization

Research Article

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INTRODUCTION

To fight against the coronavirus disease (COVID-19), which is currently a global pandemic, laboratory and clinical predictors of progression to serious and lethal forms are critical. Such predictors can help stratify risk, guiding interventional trials of patients at a higher risk of developing a severe disease, and optimizing the use of scarce human and financial resources during the pandemic. Recognition of laboratory parameters that can distinguish between serious and mild cases is also essential. Improved clinical situational understanding would be possible for those exposed more or less to mortality (1). Procalcitonin and platelet count have previously been established as possible severity predictors of disease (2, 3). However, the higher amount of COVID-19 reports which are now available has allowed a more detailed review of laboratory results, which the medical and scientific communities desperately need (2, 3). In COVID-19 patients, reports indicated that white blood cell count, neutrophil count, levels of ALT, AST, total bilirubin, creatinine, lactate dehydrogenase (LDH), urea (blood urea nitrogen), creatine kinase-MB, myoglobin, prothrombin time, D-dimer, cardiac troponin I, and CRP levels erythrocyte sedimentation rate were increased.

On the other hand, lymphocyte, platelet, eosinophil counts, and serum albumin levels were decreased (1). Many people infected with COVID-19 indicate mild to moderate symptoms and are cured with adequate medical treatment (4).

However, moderate, or severe COVID-19 develops in 15–32% of cases, with a 1–15% mortality rate (5). COVID-19 patients have infrequently reported haematological defects (6). COVID-19 has different clinical symptoms, from a mild infection like flu to life-threatening types. Clinical presentation and mortality rates differ significantly across countries, and the reasons for these variations are still unknown. Variety of local testing methods, Genetic factors, and epidemiological reporting among countries can all play a role (7). To better understand this problem, it is critical to have a good image of the general characteristics of COVID-19 patients, as well as a meticulous review of clinical presentation and laboratory test results. There is also a lack of information regarding the clinical features of the infected patients admitted to hospitals (7). We aimed to examine the hematologic and biochemical laboratory anomalies in COVID-19 patients to determine which parameters can be useful to distinguish between those who are more likely to develop severe disease, and those less likely to survive. For this purpose, we investigated whether CRP/albumin, urea/albumin, and LDH/albumin ratios may be useful as prognostic indicators for patients infected with COVID-19.

MATERIAL and METHODS

Study design: This retrospective study was performed in the internal medicine departments of two institutions between April 1, 2021 and August 1, 2021. The local institutional review board approved the trial (date/no). Written informed consent was received from patients or their immediate relatives. Data were collected from the hospital database and the medical files of 280 adults diagnosed with COVID-19 were reviewed. The baseline descriptives, clinical variables, laboratory data, and treatment outcomes were recorded.

Patients: On admission for COVID-19 infection, nasal and pharyngeal swabs were tested using a quantitative real-time reverse transcriptase-polymerase chain reaction (qPCR). COVID-19 infection positivity was diagnosed and treated per World Health Organization interim guidelines and the Turkish Ministry of Health's COVID-19 Diagnosis and Treatment Program (8-10). The clinical conditions during admission were classified as severe condition which requires intensive care or an oxygen saturation <90% or mild-to-moderate condition not needing ICU or oxygen saturation >90% (11). Patients were subjected to routine blood tests, chemical and immunological tests, and chest CT scanning to determine the COVID-19 severity. Following that, every other day, a serial chest CT scan was performed to monitor disease progression and treatment effectiveness. In one commercial multi-detector CT scanner, a single inspiratory phase (Optima CT540, GE Healthcare, U.S.A.) chest CT scans were performed with. To reduce motion and artifacts, patients were instructed to hold their breath. Computed tomography images were obtained using the following parameters: effective tube current, 110– 250 mAs; detector collimation, 0.625 mm; slice thickness, 1 mm; slice interval, 1 mm ; and tube voltage, 100–120 kVp;.

To analyse blood samples, standard methods in the laboratory of our hospital were used. Blood samples were analysed using a standard procedure. A routine blood examination was carried out and the Mindray BC-5390 device was used to count blood cells, classify white blood cells, and calculate biochemical parameters and haemoglobin concentrations (Shenzhen, China). Albumin, CRP, LDH, creatinine, blood urea nitrogen, calcium, sodium, potassium, alanine aminotransferase (ALT), aspartate transaminase (AST), D-dimer, and cardiac troponin I were among the biochemical tests which were performed in the VITROS 5600 Integrated Immunodiagnostic System (VITROS 5600, Johnson, New Jersey, USA).

Statistical analysis: Our data were analysed with IBM Statistical Package for Social Sciences (SPSS) Statistics 20 software (SPSS Inc., Chicago, IL, USA). Logistic regression and ROC analysis were performed to assess diagnostic tests and predictive models and to assess and compare the accuracy between tests and predictive models. Spearman's rank correlation coefficient (Spearman’s rho) was employed as a non-parametric measure of rank correlation and statistical dependence in the rankings of two variables. A p-value of below 0.05 was statistically significant.

Outcome parameters: The baseline descriptive and clinical variables under investigation included age, sex, comorbidity, survival status, disease severity, chief complaint at admission, length of hospitalization, disease severity, and quantitative polymerase chain reaction (qPCR) test results. The hematologic and biochemical lab results involved white blood cell count, haemoglobin level, platelet and lymphocyte counts, serum levels of urea (blood urea nitrogen), creatinine, LDH, AST, ALT, CRP, albumin, sodium, potassium, calcium, cardiac troponin I, D-dimer as well as CRP/albumin, urea/albumin, and LDH/albumin ratios. The results of lab tests performed on the first and fifth days after hospitalization were noted and the correlation between the disease severity, survival status, hematologic and biochemical parameters was sought.

RESULTS

Our series consisted of 280 adult COVID-19 patients (147 women, 133 men) with an average age of 58.34 ± 18.64 (range: 19 to 93). An overview of baseline and demographic data is presented in **Table 1**. Most patients (n=171, 61.1%) had mild-moderate disease while sever disease was diagnosed in 109 cases (38.9%). A total of 36 patients died due to COVID-19 yielding a mortality rate of 12.9%. The average length of hospitalization was 8.98 ± 5.80 days (range: 1-55 days). The most frequent comorbidities were hypertension (n=119, 73.9%), diabetes mellitus (n=67, 41.6%), chronic obstructive pulmonary disease (n=28, 17.4%), congestive heart failure (n=23, 14.3%), and coronary atherosclerotic heart disease (n=22, 13.7%). The most common chief complaints at admission were cough (n=132, 47.1%), fever (n=98, 35.0%), dyspnea (n=92, 32.8%), and fatigue (n=80, 28.6%), respectively. Table 2 demonstrates the results of the evaluation of the clinical variables as for the outcome for survival status. White blood cell and lymphocyte counts, serum levels of urea, creatinine, LDH, CRP, albumin levels were higher on the first and fifth days after admission in patients ending up with mortality. Similarly, serum levels of

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AST and cardiac troponin I were increased on the first day after admission in patients who died due to COVID-19. In contrast, sodium levels on the first day and albumin levels on both the first and fifth days were lower in patients who were lost during treatment. Notably, all three parameters under focus, CRP/albumin, urea/albumin, and LDH/albumin ratios, were remarkably higher in the mortality subgroup (**Table 2**).

The results of the evaluation of the clinical variables per disease severity are shown in Table 3. Accordingly, White blood cell count and serum levels of urea, creatinine, LDH, CRP and ratios of CRP/albumin, urea/albumin, and LDH/albumin, were significantly increased on the first and fifth days after admission in patients with severe disease. The hemoglobin and albumin serum levels were significantly decreased on the first and fifth days in patients with severe disease. ALT, sodium, and cardiac troponin I Serum levels were lower on the first day after admission in COVID-19 patients with severe disease (Table 3).

In Table 4, the relationship between clinical variables and length of hospitalization was demonstrated. Serum D-dimer level on the first day, hemoglobin levels, lymphocyte counts, levels of LDH, CRP, potassium as well as CRP/albumin and LDH/albumin ratios on the fifth day and serum albumin levels on both the first and fifth days seem to be associated with length of hospitalization (**Table 4**).

The performances of clinical variables for discrimination of survival status and severity of disease are shown in Table 5. Our data indicated that hemoglobin level, white blood cell count, lymphocyte count, serum levels of urea, creatinine, LDH, CRP, albumin, cardiac troponin I and D-dimer as well as CRP/albumin, urea/albumin, and LDH/albumin levels may have important implications as predictors of disease severity and mortality (**Table 5**).

The performances of CRP/albumin, urea/albumin, and LDH/albumin levels to predict disease severity and survival are shown in **Graphs 1 and 2**.

Table 1. Demographic and clinical characteristics of patients in our series (n=280).

Variable		Statistics n (%)
Age (mean±standard deviation (min-max))		58.34 ± 18.64 (19 – 93)
Sex	**Female**	147 (52.5)
	Male	133 (47.5)
Comorbidity	**No**	99 (38.1)
	Yes	161 (61.9)
Comorbidity types	**HT**	119 (73.9)
	DM	67 (41.6)
	COPD/asthma	28 (17.4)
	CAHD	22 (13.7)
	CHF	23 (14.3)
	CRF	6 (3.7)
	CVO	4 (2.5)
	Dementia	1 (0.6)
	Hypercholesterolemia	7 (4.3)
	Goitre	5 (3.1)
	Other	21 (13.0)
Chief complaint(s) at admission	**Fever**	98 (35.0)
	Angina pectoris	5 (1.8)
	Cough	132 (47.1)
	Diarrhea	8 (2.9)
	Sore throat	33 (11.8)
	Dyspnea	92 (32.8)
	Sputum	9 (3.2)
	Headache	15 (5.4)
	Myalgia	23 (8.2)
	Dysgeusia	2 (0.7)
	Nausea	11 (3.9)
	Fatigue	80 (28.6)
	Ageusia	2 (0.7)
	Back pain	29 (10.4)
	Arthralgia	10 (3.6)
	Pain	10 (3.6)
	Loss of appetite	9 (3.2)
	Other (otalgia, shivering, sweating, etc.)	14 (5.0)
Survival status	**Alive**	244 (87.1)
	Dead	36 (12.9)
Length of hospitalization (mean±standard deviation (min-max))		8.98 ± 5.80 (1 – 55)
Disease severity	**Mild**	171 (61.1)
	Severe	109 (38.9)
qPCR test result	**Negative**	105 (37.5)
	Positive	175 (62.5)

(Abbreviations: HT: hypertension; DM: diabetes mellitus; COPD: chronic obstructive pulmonary disease; CAHD: coronary atherosclerotic heart disease; CHF: congestive heart disease; CRF: chronic renal failure; CVO: cerebrovascular disease; qPCR: quantitative polymerase chain reaction)

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Table 2. Evaluation of the clinical variables per the outcome for survival (*: Difference was calculated as the alteration between fifth and first days).

		Survival status		
		Alive (n=242)	Dead (n=36)	p
WBC (/µl)	**1st day**	5.84 [4.6 – 7.6]	7.25 [5.35 – 9.14]	**0.013**
	5th day	6.10 [4.6 – 7.61]	9.16 [6.0 – 11.85]	**<0.001**
	Difference*	-0.1 [-1.69 ; 1.0]	1.83 [-0.27 ; 2.92]	**0.002**
Hb (g/dl)	**1st day**	13.4 [12.1 – 14.63]	13.15 [11.35 – 14.53]	0.282
	5th day	12.8 [11.45 – 13.9]	12.3 [10.6 – 13.7]	0.109
	Difference*	-0.60 [-1.30 ; -0.1]	-1.1 [-1.6 ; -0.30]	0.178
Plt (/µl)	**1st day**	198 [160.75 - 249]	188 [142.25 – 225.5]	0.151
	5th day	232.5 [168 - 301]	218 [147 - 256]	0.290
	Difference*	28.5 [-9.25 ; 76.25]	26.0 [-15.0 ; 55.0]	0.721
Lymph (/µl)	**1st day**	1.30 [0.9 – 1.71]	0.93 [0.59 – 1.2]	**<0.001**
	5th day	1.40 [1.0 – 2.02]	0.70 [0.5 – 1.2]	**<0.001**
	Difference*	0.10 [-0.20 ; 0.35]	-0.03 [-0.4 ; 0.11]	0.085
Urea (mg/dl)	**1st day**	28.0 [22.0 – 34.0]	44.55 [32.78 – 79.63]	**<0.001**
	5th day	26.0 [20.4 – 34.0]	53.6 [26.0 – 82.8]	**<0.001**
	Difference*	-2.0 [-8.9 ; 3.95]	16.1 [-8.0 ; 25.0]	**0.001**
Creatinine (mg/dl)	**1st day**	0.80 [0.7 – 1.0]	1.10 [0.87 – 1.49]	**<0.001**
	5th day	0.77 [0.68 – 0.89]	0.96 [0.7 – 1.33]	**0.010**
	Difference*	-0.03 [-0.12 ; 0.01]	0.0 [-0.13 ; 0.20]	0.150
LDH (U/l)	**1st day**	248 [200 - 317]	307.5 [220.25 – 438.25]	**0.004**
	5th day	232 [185 - 324]	449.5 [248.25 – 606.5]	**<0.001**
	Difference*	-15.5 [-71.0 ; 56.3]	125 [-56.25 ; 247.5]	**0.009**
CRP (mg/l)	**1st day**	19.5 [5.23 – 73.98]	108.8 [55.63 – 152.3]	**<0.001**
	5th day	20.0 [3.95 – 57.75]	132.2 [72.0 – 171.0]	**<0.001**
	Difference*	0.0 [-19.5 ; 18.0]	21.0 [-32.7 ; 71.1]	0.264
AST (U/l)	**1st day**	24.0 [19 - 34]	31.0 [21.5 – 45.0]	**0.019**
	5th day	25.0 [18 - 37]	33.0 [18.0 – 48.0]	0.159
	Difference*	-1.0 [-7.0 ; 9.0]	1.0 [-9.0 ; 21.0]	0.468
ALT (U/l)	**1st day**	20.0 [14 - 30]	19.5 [13.25 – 27.75]	0.647
	5th day	25.0 [15 - 36]	21.0 [15.0 – 26.0]	0.239
	Difference*	1.5 [-3.75 ; 9.0]	-1.0 [-6.0 ; 11.0]	0.357
Albumin (g/dl)	**1st day**	43.0 [40 - 46]	39.5 [37.0 – 42.0]	**<0.001**
	5th day	40.0 [36 - 42]	33.0 [28.0 – 36.0]	**<0.001**
	Difference*	-3.5 [-6.0 ; -1.0]	-6.0 [-8.0 ; -3.5]	**0.010**
Sodium (mEq/l)	**1st day**	137 [135 - 139]	135.5 [133 - 138]	**0.031**
	5th day	139 [137 - 140]	138 [134 - 141]	0.406
	Difference*	1.0 [0.0 – 4.0]	2.0 [-2.0 ; 3.0]	0.429
Potassium (mEq/l)	**1st day**	4.1 [3.8 – 4.4]	4.17 [3.60 – 4.43]	0.640
	5th day	4.2 [3.889 – 4.49]	4.1 [3.6 – 4.43]	0.210
	Difference*	0.10 [-0.30 ; 0.40]	-0.10 [-1.0 ; 0.51]	0.131
Calcium (mg/dl)	**1st day**	8.7 [8.3 – 9.0]	8.65 [8.33 – 9.0]	0.875
	5th day	8.5 [8.2 – 8.8]	8.5 [8.24 – 8.98]	0.596
	Difference*	-0.20 [-0.50 ; 0.16]	-0.20 [-0.90 ; 0.10]	0.298
Cardiac troponin I (ng/ml)	**1st day**	0.10 [0.007 – 0.10]	0.1 [0.029 – 0.106]	**0.008**
D-Dimer (ng/ml)	**1st day**	86 [0.79 - 210]	1.63 [0.8 - 417]	0.495
CRP / albumin	**1st day**	0.45 [0.12 – 1.84]	2.83 [1.49 – 4.51]	**<0.001**
	5th day	0.51 [0.10 – 1.58]	3.67 [2.65 – 5.83]	**<0.001**
	Difference*	0.004 [-0.46 ; 0.52]	0.96 [-0.26 ; 2.24]	**0.026**
Urea / albumin	**1st day**	0.65 [0.49 – 1.02]	1.12 [0.81 – 1.95]	**<0.001**
	5th day	0.64 [0.5 – 0.86]	1.52 [0.98 – 2.89]	**<0.001**
	Difference*	0.02 [-0.19 ; 0.14]	0.51 [-0.09 ; 0.95]	**0.001**
LDH / albumin	**1st day**	5.92 [4.57 – 7.83]	8.55 [5.84 – 14.99]	**<0.001**
	5th day	6.31 [4.62 – 8.26]	14.62 [6.52 – 22.63]	**<0.001**
	Difference*	0.11 [-0.17 ; 1.92]	5.62 [-1.57 ; 10.79]	**0.004**

(Abbreviations: WBC: White blood cell count; Hb: hemoglobin level; Plt: platelet count; Lymph: lymphocyte count; LDH: lactate dehydrogenase level; CRP: C-reactive protein level; AST: aspartate transaminase level; ALT: alanine transaminase level)

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Table 3. Evaluation of the clinical variables per the disease severity (* Difference was calculated as the alteration between the fifth and first days).

		Disease severity		
		Mild (n=171)	Severe (n=109)	p
WBC (/µl)	**1st day**	5.77 [4.61 – 7.45]	6.80 [4.83 – 8.98]	**0.035**
	5th day	6.03 [4.6 – 7.36]	7.06 [5.26 – 9.19]	**0.002**
	Difference*	-0.03 [-1.27 ; 1.11]	0.17 [-1.7 ; 2.4]	0.950
Hb (g/dl)	**1st day**	13.6 [12.3 – 14.9]	12.8 [11.6 – 14.2]	**0.007**
	5th day	13.1 [11.8 – 14.3]	11.9 [10.7 – 13.0]	**<0.001**
	Difference*	-0.5 [-1.2 ; 0.1]	-1.1 [-1.6 ; -0.30]	**0.002**
Plt (/µl)	**1st day**	208.5 [170.8 - 251]	181.5 [142.25 – 227.5]	**0.003**
	5th day	238 [175.5 – 300.8]	223 [146 - 311]	0.116
	Difference*	28.0 [-12.0 ; 75.0]	28.5 [-4.5 ; 78.0]	0.873
Lymph (/µl)	**1st day**	1.44 [1.1 – 1.88]	0.97 [0.61 – 1.2]	**<0.001**
	5th day	1.59 [1.19 – 2.19]	0.90 [0.7 – 1.3]	**<0.001**
	Difference*	0.16 [-0.10 ; 0.43]	-0.10 [-0.30 ; 0.11]	**0.001**
Urea (mg/dl)	**1st day**	26.0 [21.0 – 33.8]	41.1 [29.55 – 65.25]	**<0.001**
	5th day	24.0 [20.0 – 30.3]	36.2 [24.03 – 58.5]	**<0.001**
	Difference*	-1.0 [-7.0 ; 3.4]	-2.55 [-14.48 ; 15.58]	0.953
Creatinine (mg/dl)	**1st day**	0.78 [0.68 – 0.90]	1.0 [0.85 – 1.41]	**<0.001**
	5th day	0.73 [0.66 – 0.81]	0.70 [0.9 – 1.22]	**<0.001**
	Difference*	-0.03 [-0.1 ; 0.03]	-0.1 [-0.20 ; 0.00]	**0.008**
LDH (U/l)	**1st day**	233 [197.5 – 303.3]	289 [218 – 374.5]	**<0.001**
	5th day	229 [185.3 – 308.0]	264 [188 – 394]	**0.035**
	Difference*	-11.0 [-62.0 ; 51.0]	1.0 [-7.0 ; 15.0]	0.574
CRP (mg/l)	**1st day**	11.5 [2.58 – 35.68]	96.85 [35.8 – 139.78]	**<0.001**
	5th day	12.5 [2.4 – 35.7]	65.5 [29.1 – 129.6]	**<0.001**
	Difference*	0.0 [-15.33 ; 14.1]	0.35 [-53.2 ; 39.53]	0.747
AST (U/l)	**1st day**	24.0 [19 - 34]	27.0 [20.0 – 38.5]	0.066
	5th day	24.5 [18 - 35]	29.5 [18.0 – 47.75]	0.052
	Difference*	-2.0 [-7.0 ; 6.0]	1.0 [-7.0 ; 15.0]	0.063
ALT (U/l)	**1st day**	21.0 [15 - 30]	18.0 [13.0 – 26.0]	**0.022**
	5th day	25.0 [16 - 35]	21.0 [14.0 – 39.75]	0.251
	Difference*	1.0 [-4.0 ; 10.0]	2.5 [-3.75 ; 13.0]	0.308
Albumin (g/dl)	**1st day**	44.0 [41 - 47]	40.0 [37.0 – 42.0]	**<0.001**
	5th day	41.0 [38 - 43]	35.0 [31.0 – 38.0]	**<0.001**
	Difference*	-3.0 [-5.25 ; -1.0]	-4.0 [-6.5 ; -1.0]	0.112
Sodium (mEq/l)	**1st day**	138 [136 - 139]	136.0 [133 - 138]	**<0.001**
	5th day	139 [137 - 140]	139 [136 - 141]	0.752
	Difference*	1.0 [-1.0 ; 3.0]	3.0 [0.0 ; 5.0]	**0.016**
Potassium (mEq/l)	**1st day**	4.1 [3.82 – 4.37]	4.1 [3.79 – 4.50]	0.689
	5th day	4.2 [3.9 – 4.49]	4.11 [3.8 – 4.42]	0.200
	Difference*	0.13 [-2.0 ; 0.48]	-0.10 [-0.58 ; 0.32]	**0.008**
Calcium (mg/dl)	**1st day**	8.7 [8.4 – 9.0]	8.70 [8.25 – 9.0]	0.685
	5th day	8.57 [8.29 – 8.8]	8.5 [8.1 – 8.87]	0.286
	Difference*	-0.10 [-0.40 ; 0.20]	-0.40 [-0.90 ; 0.01]	**0.002**
Cardiac troponin I (ng/ml)	**1st day**	0.10 [0.018 – 0.10]	0.03 [0.006 – 0.1]	**0.002**
D-Dimer	**1st day**	126 [1.16 – 239.8]	1.21 [0.60 – 165.8]	**<0.001**
CRP / albumin	**1st day**	0.24 [0.06 – 0.79]	2.40 [0.92 – 3.94]	**<0.001**
	5th day	0.31 [0.06 – 0.94]	2.07 [0.81 – 3.71]	**<0.001**
	Difference*	0.04 [-0.27 ; 0.38]	0.16 [-1.29 ; 1.44]	0.735
Urea / albumin	**1st day**	0.57 [0.47 – 0.78]	1.03 [0.73 – 1.63]	**<0.001**
	5th day	0.59 [0.49 – 0.78]	1.04 [0.66 – 1.62]	**<0.001**
	Difference*	0.03 [-0.13 ; 0.14]	-0.04 [-0.33 ; 0.49]	0.774
LDH / albumin	**1st day**	5.42 [4.29 – 6.84]	7.43 [5.74 – 9.79]	**<0.001**
	5th day	5.69 [4.52 – 7.82]	6.89 [5.92 – 12.17]	**<0.001**
	Difference*	0.14 [-88 ; 1.65]	0.46 [-2.17 ; 4.19]	0.649

(Abbreviations: WBC: White blood cell count; Hb: hemoglobin level; Plt: platelet count; Lymph: lymphocyte count; LDH: lactate dehydrogenase level; CRP: C-reactive protein level; AST: aspartate transaminase level; ALT: alanine transaminase level)

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Table 4. The relationship between clinical variables and length of hospitalization.

		Length of hospitalization (n=280)	
		r	p
WBC	**1st day**	-0.053	0.376
	5th day	0.061	0.349
Hb	**1st day**	-0.105	0.079
	5th day	-0.212	**0.001**
Plt	**1st day**	0.031	0.610
	5th day	-0.068	0.302
Lymph	**1st day**	-0.071	0.238
	5th day	-0.327	**<0.001**
Urea	**1st day**	-0.053	0.382
	5th day	0.002	0.979
Creatinine	**1st day**	-0.061	0.311
	5th day	-0.053	0.418
LDH	**1st day**	0.035	0.560
	5th day	0.205	**0.002**
CRP	**1st day**	0.070	0.247
	5th day	0.410	**<0.001**
AST	**1st day**	-0.060	0.315
	5th day	0.067	0.302
ALT	**1st day**	-0.108	0.073
	5th day	-0.058	0.378
Albumin	**1st day**	-0.144	**0.016**
	5th day	-0.353	**<0.001**
Sodium	**1st day**	-0.034	0.570
	5th day	-0.099	0.131
Potassium	**1st day**	-0.033	0.580
	5th day	-0.129	**0.048**
Calcium	**1st day**	0.041	0.491
	5th day	-0.068	0.301
Cardiac troponin I	**1st day**	0.027	0.677
D-Dimer	**1st day**	0.184	**0.002**
CRP / albumin	**1st day**	0.072	0.232
	5th day	0.444	**<0.001**
Urea / albumin	**1st day**	-0.009	0.887
	5th day	0.100	0.127
LDH / albumin	**1st day**	0.107	0.074
	5th day	0.324	**<0.001**

Table 5. The performances of clinical variables for discrimination of survival status and disease severity (*: lesser values are consistent with increased mortality or severe disease).

Variable	Interval	Survival status	Disease severity
WBC	**1st day**	0.646 (0.534 – 0.759)	0.587 (0.506 – 0.669)
	5th day	0.747 (0.635 – 0.859)	0.623 (0.543 – 0.703)
Hb*	**1st day**	-	0.615 (0.537 – 0.693)
	5th day		0.686 (0.616 – 0.756)
Lymph*	**1st day**	0.720 (0.607 – 0.834)	0.735 (0.667 – 0.804)
	5th day	0.785 (0.678 – 0.892)	0.798 (0.739 – 0.858)
Urea	**1st day**	0.725 (0.623 – 0.828)	0.751 (0.682 – 0.819)
	5th day	0.774 (0.651 – 0.897)	0.724 (0.649 – 0.798)
Creatinine	**1st day**	0.695 (0.589 – 0.801)	0.779 (0.711 – 0.846)
	5th day	0.664 (0.526 – 0.802)	0.704 (0.627 – 0.782)
LDH	**1st day**	0.600 (0.451 – 0.749)	0.586 (0.504 – 0.667)
	5th day	0.756 (0.607 – 0.904)	0.585 (0.504 – 0.666)
CRP	**1st day**	0.792 (0.689 – 0.896)	0.795 (0.735 – 0.856)
	5th day	0.801 (0.683 – 0.918)	0.782 (0.721 – 0.843)
Albumin*	**1st day**	0.741 (0.648 – 0.834)	0.794 (0.734 – 0.855)
	5th day	0.825 (0.736 – 0.914)	0.842 (0.785 – 0.898)
Cardiac troponin I	**1st day**	0.64 (0.532 – 0.747)	0.608 (0.531 – 0.686)
D-Dimer	**1st day**	-	0.632 (0.557 – 0.706)
CRP / albumin	**1st day**	0.794 (0.682 – 0.905)	0.804 (0.743 – 0.864)
	5th day	0.822 (0.699 – 0.945)	0.803 (0.744 – 0.863)
Urea / albumin	**1st day**	0.743 (0.641 – 0.845)	0.788 (0.724 – 0.853)
	5th day	0.818 (0.710 – 0.926)	0.774 (0.704 – 0.844)
LDH / albumin	**1st day**	0.705 (0.571 – 0.839)	0.677 (0.601 – 0.752)
	5th day	0.778 (0.627 – 0.928)	0.669 (0.593 – 0.744)

(Abbreviations: WBC: White blood cell count; Hb: hemoglobin level; Lymph: lymphocyte count; LDH: lactate dehydrogenase level; CRP: C-reactive protein level)

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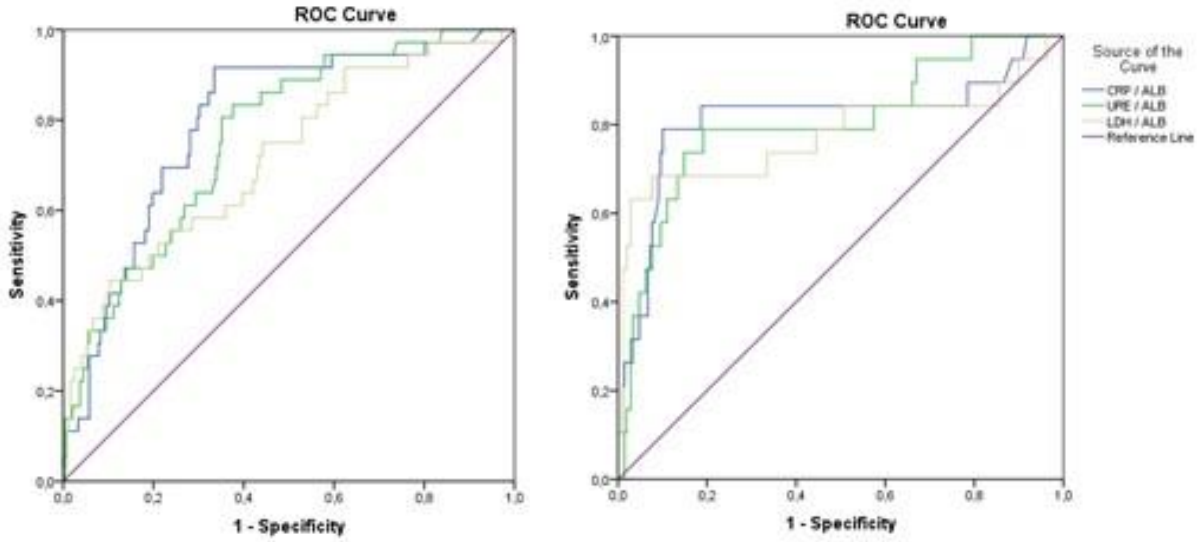
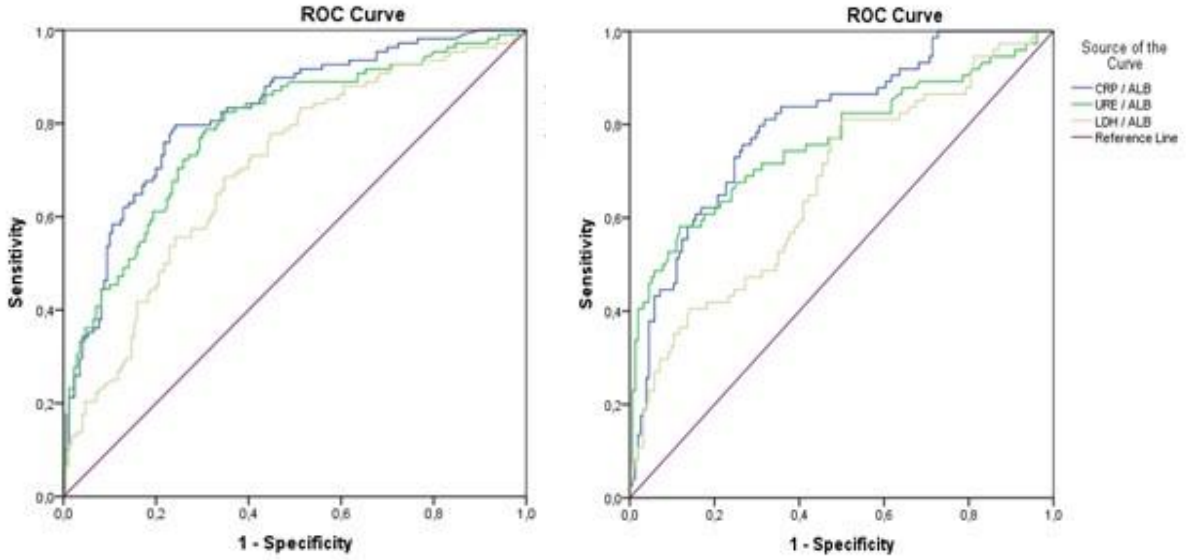


Graph 1. The performances of variables for discrimination of survival. a) Fort he first day, AUC= 0.794 (0.682 – 0.905) for CRP / albumin, AUC= 0.743 (0.641 – 0.845) for urea / albumin, AUC=0.705 (0.571 – 0.839) for LDH / albumin; b) Fort he fifth day, AUC= 0.804 (0.743 – 0.864) for CRP / albumin, AUC= 0.774 (0.704 – 0.844) for urea / albumin, AUC=0.677 (0.601 – 0.974) for LDH / albumin.



Graph 2. The performances of variables for discrimination of disease severity. a) For the first day, AUC= 0.823 (0.773 – 0.873) for CRP / albumin, AUC= 0.788 (0.732 – 0.844) for urea / albumin, AUC=0.704 (0.624 – 0.767) for LDH / albumin; b) For the fifth day, AUC= 0.801 (0.741 – 0.862) for CRP / albumin, AUC= 0.764 (0.691 – 0.836) for urea / albumin, AUC=0.669 (0.594 – 0.745) for LDH / albumin.

DISCUSSION

We assessed whether hematologic and biochemical markers might have significant prognostic value for COVID-19 patients. Our data yielded that LDH/albumin, urea/albumin, and CRP/albumin may have important clinical implications for predicting the prognosis, survival, disease severity, and length of hospitalization in this patient group in our population.

White blood cell count increased just slightly in patients with acute disease, but it increased clinically significantly in patients who died. As a result, a large increase in WBCs in patients with severe disease could indicate clinical deterioration and an increased risk of a poor outcome. Increased neutrophils may drive the rise in WBCs, whereas lymphocytes, monocytes, and eosinophils can decrease. Lymphocytes are thought to be necessary for eliminating virally infected cells in the SARS virus (12), and survival in COVID-19 may depend on the virus's ability to replenish lymphocytes destroyed by the virus (13).

Patients with serious and fatal COVID-19 infection had increasedcardiac and muscle damage biomarkers. At presentation, patients who died had substantially increased cardiac troponin levels, indicating the possibility of cardiac injury from progression to multiple organ failure, viral myocarditis, and secondary cardiac injury from organ-targeted pathologies (e.g. rheumatoid arthritis).

An image of multiple organ failure occurs when large elevations in renal biomarkers (creatinine, blood urea nitrogen), liver enzymes (aspartate aminotransferase and alanine aminotransferase), and coagulation steps were combined (1).

Since growing evidence suggests that ROS are involved in clotting and platelet activation, both compartments may be overactivated in the case of albumin degradation/oxidation. Hypoalbuminemia is linked to thrombosis risk and poor survival because of oxidative stress/inflammation (14). So far, no data on the effect of serum albumin on mortality in patients with COVID-19 have been yet published.

There are several possible explanations for the connection between hypoalbuminemia and decreased survival. First, albumin can protect against the storm of cytokine and the resulting organ failure by acting as an antioxidant and anti-inflammatory protein; in this context, the reverse relationship between troponin levels and serum albumin is of interest. Albumin has anticoagulant properties and prevents clotting and platelet activation caused by oxidative stress. As a result, hypoalbuminemia's detrimental effect on activation of clotting may be another factor contributing to poor survival. Albumin and D-dimer levels have an inverse relationship in COVID-19, with the latter being a known marker of increased mortality and thrombotic risk. Albumin can be inversely correlated with in-hospital mortality as an inverse acute phase reactant precisely because it represents a more impaired clinical presentation of COVID-19. As a result, low levels of albumin should be viewed as an anomaly rather than a predictor of poor prognosis. Since hypoalbuminemia is a symptom of chronic and acute inflammation, and a reverse relationship between albumin levels and CRP was found in this study. Our finding indicates an underlying overactive inflammatory state. Other mechanisms such as higher albuminuria may be involved in lowering albuminemia in the COVID-19 clinical setting. However, hypoalbuminemia reflecting the concomitant acute liver failure can be fairly ruled out by the current and previous studies (14).

Early detection of a severe infection can allow for earlier intervention in therapeutics and supportive measures, resulting in better outcomes [20]. Higher ALT, leukocyte count, and AST, as well as increased LDH and finally increased procalcitonin, were all established as important markers of ICU entry. LDH was the only predictor in our study significantly predicting all three outcomes: ARDS, ICU entry, and mortality. LDH is a metabolic and immune surveillance prognostic biomarker that is released when a cell's cytoplasmic membrane is damaged. The immune response to the viral infection can be weakened by these changes, leading to a more severe disease in patients with high LDH levels. Higher procalcitonin may indicate bacterial coinfection, leading to COVID-19 complications and, as a result, higher ICU admission in these patients (15).

Plasma CRP levels were linked to the severity of COVID-19 pneumonia. Therefore, it may help distinguish mild COVID-19 pneumonia patients from those with moderate to severe COVID-19 pneumonia. These findings may be an early warning sign of severe disease, allowing doctors to better stratify patients for transfer to an intensive care unit (16).

When hepatocytes are stimulated by inflammation, CRP is produced quickly. It binds to a wide range of pathogens, allowing complement activation through the classical pathway, suggesting immune activation, lymphocyte invasion, inflammation outbreak, and immune molecule consumption. Higher CRP levels in COVID-19 patients slowly recovered could be early signs of nosocomial infections, allowing physicians to start empirical antibiotic therapy sooner and avoid a worsening outcome (16).

Pan et al. suggested that the clinical course of patients with severe COVID-19 varied from admission with different outcomes. Lymphocyte count, CRP, platecrit, and LDH dynamic monitoring may help predict the prognosis of severe patients. Furthermore, with the exception of ARDS, the fatal characteristic of COVID-19 is often due to extreme systemic inflammation with induced cardiac dysfunction (17).

Some limitations of the current study must be remembered. Retrospective design, relatively small sample size, and possible impacts of socioeconomical factors may influence our results. However, considering the rapidly emerging pandemic, these findings are clinically important and may aid clinicians in identification and treatment of patients who are under more risk for severe disease. Furthermore, we do not have an external validation cohort. As a result, our findings should be considered exploratory and preliminary. The correlations we note should not be presumed to be cause and effect because subjects who are more likely to die have significant differences in some of these co-variates.

CONCLUSION

As far as we know, this is the first study to provide a detailed insight into the prognostic significances of LDH/albumin, CRP/albumin, and urea/albumin levels in COVID-19 patients. We noted that these 3 novel indicators might possess important clinical implications and their analysis may aid in the early recognition of patients at higher risk for mortality and stratification of patients for intensive care unit transfer. Identification and validation of these novel prognostic indicators necessitate the implementation of further multicentric trials on larger series.

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Conflict of interest: The authors declare no competing interests.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the institutional and/or national research committee's ethical standards and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Clinical investigation of the effects of oral health education in children with Down Syndrome

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ABSTRACT

Objective: The objective of this study is to evaluate the effects of oral health education, which includes parents on children with Down Syndrome (DS).

Material and Methods: The present study included 35 children with DS. Oral hygiene education was given through a program that included parents. The oral hygiene and periodontal health status of all the children were clinically assessed using clinical index measurements such as Bleeding on Probing (BOP), Gingival Index (GI) and Plaque Index (PI) at the baseline, 1st month, 3rd month and 6th month after oral health education programme. Statistical analyses were performed.

Results: All the mean PI, GI and BP measurements showed a statistically significant decrease according to the baseline mean values ($p<0.05$). Moreover, oral hygiene education, in which parents are involved, the fact that the clinical measurements on the posterior teeth are higher than on the anterior teeth. Results clearly indicate the difficulty of brushing on the posterior teeth in children with special needs.

Conclusion: Oral health education has been determined to be rather effective on all clinical measurements. Scientific studies and educational programs in which sufficient information is provided to parents about oral health should be encouraged.

Keywords: Genetics, Pediatric Dentistry, Public Health, Down Syndrome

INTRODUCTION

Poor oral health can cause pain, trouble eating, sleep disturbance, and lack of self-esteem, all of which can significantly impact a person's quality of life (1). Individuals with Down syndrome also have many oral and dental problems, especially periodontal problems that affect their quality of life (2).

Compared with the general population, individuals with DS are more likely to be affected by periodontal diseases and therefore must maintain good oral hygiene habits. (3). Due to the high incidence of active infections and previously treated periodontal diseases, controlling plaque formation becomes critical in individuals with DS. Oral health cannot be achieved and protection against periodontal diseases cannot be ensured unless plaque formation is controlled (4).

Allison et al. (5) showed that 51.4% of DS children under 8 years of age were not supported to brush their teeth daily. It supports the view that hand skills are limited, and this has an effect on oral hygiene (6). Therefore, children need help from their parents when brushing their teeth (7).

Although the importance of dental care in preventing periodontal diseases in individuals with DS is well known, there is limited literature on the effects of brushing on clinical indices through programs in which the parents are also included.

The present study aimed to investigate the effects of education on clinical periodontal indices during a 6-month follow-up period by administering oral hygiene education to children with DS via a program that included their parents.

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MATERIAL and METHODS

The present study included 35 (18 boys, 17 girls) children with DS who were referred to the Department of Paediatric Dentistry at the Faculty of Dentistry."". The Ethics committee approved the study protocol (meeting No. 2016/004). Prior to study initiation, detailed verbal and written information was provided to the parents of all the included children. All the parents read the informed consent forms and provided their written consent for participation in the study. All children's detailed medical histories were obtained from their parents, and the data were recorded on their respective anamnesis forms. Written consultations were requested from the doctors of all children, and treatment was completed as per the recommendations relayed in these consultation reports. Special education centers were visited and interviews were conducted to determine children eligible for inclusion in the study after referral to our clinic. Children with DS, their families, and their instructors at the special education centers they attended—whom we could reach the first visit, were provided with information regarding the prevalence of oral hygiene, periodontal diseases, and susceptibility of children with DS to periodontal diseases. The survey questions prepared for the parents of the children included in the study are presented in **Table 1**.

The The Decayed, Missing, and Filled Teeth (dmf-t/DMF-T) values of all the teeth were calculated and recorded in the index forms prepared for the patient. The oral hygiene and periodontal health status of all the children were clinically assessed using clinical index measurements, such as bleeding on probing (BP), GI (GI, Löe and Silness), and PI (Pi, Löe and Silness), at six points of each tooth in the mouth as distobuccal, midbuccal, mesiobuccal, distopalatinal, midpalatinal, and mesiopalatinal, except for the third molar tooth, using a Williams-type probe (diameter, 0.5 mm).

Oral hygiene education was performed with models and practices with parents. All the measurements were performed at 4 different time points: baseline, 1 month, 3 months, 6 months after education.

Statistical Analysis

The study data were recorded using Microsoft Office Excel 2016. All statistical analyses were performed using SPSS 20.0 (SPSS Inc. Chicago, USA) software. The mean plaque index (PI), gingival index (GI), and bleeding on probing (BP) values at baseline and 1-, 3-, and 6-months post-treatment were separately calculated and analyzed using the Friedman test. The Wilcoxon test was used to compare the effects of the treatment on the clinical measurements of anterior and posterior teeth and to calculate whether there was a difference between the parameters.

RESULTS

Sample of the study consisted of 35 children: 17 (48.5%) girls and 18(52.5%) boys. The patients' general profile and demographic data analysis were analysed (**Table2**). Their mean age was 12.37(±5.81) years. 34% of the children had no systemic diseases, 31% of them had congenital heart disease, 20% of them had hypothyroidism.

The 37% of the sample were brushing their teeth, 74% of them brushing 2-3 times in a week, 20% brushing once a day, 6% of them brushing twice or more in a day. Only 6% of them had received oral hygiene education before. 34% of them had received dental treatment before, 58% of them had dental treatment in the clinic, 42% of them were under general anaesthesia.

The mean value of the DMF-t/DMF-T indices of the 35 children with DS was found as 4.6. Patients' teeth that required treatment were treated in the clinic and under general anaesthesia. Except for the treatment sessions in the clinic or under general anaesthesia, it was determined that no secondary caries occurred through re-examination at 1-, 3-, and 6-month control visits.

All the mean PI, GI and BP measurements showed a decrease compared with the baseline mean values. This decrease was statistically significant ($p < 0.05$) (Table 3).

PI baseline mean values were calculated. This value differed from the mean values obtained in the treatment process's 1st, 3rd and 6th months. According to the initial values 1st month, 3rd month, 6th month values were decreased over time. It was concluded that this decrease was statistically significant ($p<0.05$) (Table 3). According to the data obtained, although there was a significant decrease in the mean PI values in the 1st month, 3rd month, 6th months after the treatment compared to the baseline, the decrease in the 1st month after the onset was significantly higher than the changes in the 3rd and 6th months observed (Figure 1.) ($p<0.05$).

GI baseline value differed from the mean values obtained in the treatment process's 1st, 3rd and 6th months. According to the initial values 1st month, 3rd month, 6th month values were decreased over time. It was concluded that this decrease was statistically significant ($p<0.05$) (**Table 3**). As shown in Figure 1, according to the data obtained, although there was a significant decrease in the mean GI values in the 1st month, 3rd month, 6th months after the treatment compared to the baseline. The decrease in the 1st month after the onset was significantly higher than the changes in the 3rd and 6th months observed ($p<0.05$).

BP baseline mean value differed from the mean values obtained in the 1st, 3rd and 6th months of the treatment process. According to the initial values 1st month, 3rd month, 6th month values were decreased over time. It was concluded that this decrease was statistically significant ($p<0.05$) (**Table3**).

According to the data obtained, although there was a significant decrease in the mean BP values in the 1st month, 3rd month, 6th months after the treatment compared to the baseline, the decrease in the 1st and 3rd month after the onset was significantly higher than the changes in the and 6th months observed (**Figure 1**) ($p<0.05$).

The effect of the oral hygiene education on the clinical measurements of anterior and posterior teeth was examined. Baseline, 1st, 3rd and 6th month's values were higher in posterior teeth than anterior teeth (**Figure 1**)

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Table 1. Questions of the survey administered to the parents of children with DS included in the study

Your child's age	…….	…….	…….
Your educational status	□ Primary School	□ High School	□ University
Your income level	□ 0–5000 ₺	□ 5000–10000 ₺	□ >10000 ₺
Number of siblings	…….	…….	…….
Another child with DS?	□ Yes	□ No	
Is your marriage consanguineous?	□ Yes	□ No	
Does your child have a systemic disease?	□ Yes	□ No	
If yes, please specify	…….	…….	…….
Specify regularly used medicines	…….	…….	…….
Do you have him/her brush his/her teeth?	□ Yes	□ No	
Brushing frequency	□ 2-3 times a week	□ Once a day	□ Twice a day
Have you had him/her receive dental treatment before?	□ Yes	□ No	
If yes, how?	□ Under general anesthesia	□ Under sedation	□ At a Clinic
Has he/she received oral-dental education before?	□ Yes	□ No	

Table 2. General profile and demographic data of the patients

	N	%
Gender		
Female	17	49
Male	18	51
Your educational status		
Primary School	23	66
High School	7	20
University	5	14
Your income level		
0–5000 ₺	21	60
5000–10000 ₺	10	29
>10000 ₺	4	11
Consanguineous marriage		
Yes	2	6
No	33	94
Brushing teeth		
Yes	12	37
No	23	63
Brushing Frequency		
2–3 times a week	26	74
Once a day	7	20
Twice a day	2	6
Previous dental treatment?		
Yes	6	17
No	29	83
If yes, how?		
General Anesthesia	4	66
Sedation	1	17
Clinic	1	17
Previous oral-dental health education?		
Yes	2	6
No	33	94

Table 3. Comparison of the clinical index measurements change in the patients

Clinical indices	Baseline (Mean ± SD)	1st month (Mean ± SD)	3rd month (Mean ± SD)	6th month (Mean ± SD)	*Sig.(p)*[*]
PI	2.01±0.08	1.49±0.07[a]	1.26±0.06[ab]	1.14±0.05[abc]	*0,000*
GI	1.95±0.02	1.58±0.06[a]	1.27±0.05[ab]	1.16±0.04[abc]	*0,000*
BOP	0.95±0.02	0.57±0.06[a]	0.26±0.05[ab]	0.16±0.04[abc]	*0,000*

*a, statistically difference according to initial,
*b, statistically difference according to 1. month follow-up,
*c, statistically difference according to 3. month follow-up.

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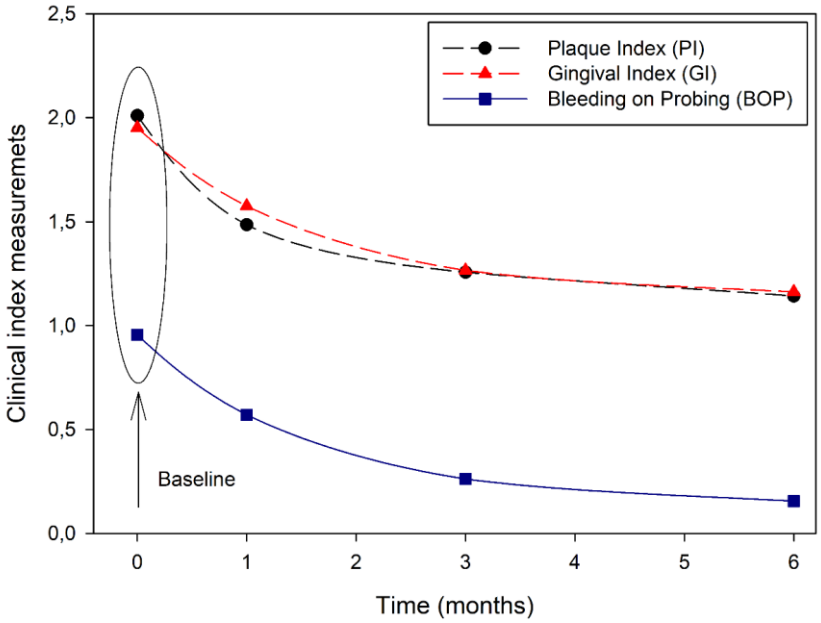

Figure 1. Clinical index measurements

DISCUSSION

When compared with the general population, individuals with DS have a higher prevalence of early-onset periodontitis and edentation. (8). This condition is linked to delays in the development of motor functions and thus the inability to maintain oral hygiene (9). As well as this higher prevalence, plaque control plays a key role in protecting individuals with DS against periodontal disease, and these individuals have poor oral hygiene (10). Hence, prevention of periodontal diseases and protection of natural dentition is considered extremely important in children with DS.

The reason behind the increased incidence of periodontal diseases in individuals with DS is not completely clear. Several studies suggested that multiple factors are involved. For example, the increased prevalence of the disease may related with impaired immune system features, such as decreased neutrophil and monocyte chemotaxis, impaired neutrophil phagocytosis, decreased T lymphocyte count, and immature T lymphocytes (9-12). Furthermore, an association with the oxidative burst capacity of granulocytes and monocytes, suppressed chemotaxis, and impaired oxidative metabolism and immunity relations have been reported (13). Several genetic polymorphisms of IL-1 (IL-1A +4845, IL-1B +3954, and IL-1RN +2018) have also been associated with the loss of periodontal attachment in individuals with DS. also reported an association with reduced CD4+/CD8+ ratio and the resulting changes in immune system regulation and function (14).

Apart from systemic factors, local factors are also reportedly involved. Factors such as macroglossia, malocclusion, tooth morphology, bruxism, and loss of normal chewing function may also have a role. Controlled brushing, good dental care, and protective measures helped improve the periodontal condition (15).

Periodontitis have a diagnostic and therapeutic challenge process. Gingival bleeding on probing is one of more promising diagnostic predictability test (15).

In a study, they reported the rate of bleeding on probing decreased from 85.2% to 69.8% after 6 months educational-therapy, (16), however it is reduced from 95% to 15% after 6 months in the present study.

Some studies reported that periodically provided preventive periodontal care effectively stops disease progression (11,17). Contrarily, some other studies reported that preventive programs cannot prevent the destructive effects of periodontal diseases and that the progression of the disease cannot be suppressed (18-20). The present study, concluded that plaque control can be achieved and that the onset and progression of periodontal diseases can be prevented at an early age through regular control visits to children with DS who cannot effectively maintain their oral hygiene.

In this study, baseline, 1st, 3rd and 6th months clinical index measurements were higher in posterior teeth than anterior teeth. The oral hygiene education, which included parents, was effective in this special healthcare group. However, the results show that posterior teeth are more difficult to clean, and care should be taken by patients.

CONCLUSION

As a result, similar to their general health, the oral health of children with DS, who also have considerable general health problems, is not sufficiently taken care. Thus, long-term participation in follow-up studies is not sufficiently achieved. In our opinion, scientific studies and activities wherein parents are provided sufficient information should be encouraged to gain oral health.

Author Contributions: ŞTK, OG: Concept, Data collection and/or processing, Analysis and/or interpretation, Literature review**, OG:** Writing, Revision**.**

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Conflict of interest: The authors declare no competing interests.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the institutional and/or national research committee's ethical standards and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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The importance of intermediate-dose Valacyclovir in primary CMV prophylaxis after Allogeneic-stem cell transplantation, and the advantages of step-wise pre-emptive treatment in CMV reactivation

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ABSTRACT

Objective: Cytomegalovirus (CMV) reactivation and disease are still one of the most important causes of morbidity and mortality after allogeneic stem cell transplantation (ASCT). Letermovir prophylaxis has been clearly shown to be effective and well-tolerated. Drug interactions and cost are limitations. Alternative regimens such as Valacyclovir 3g-6g a day are of interest. In our study, we investigated the clinical results of intermediate dose (3 gr/d) valacyclovir after ASCT in primary CMV prophylaxis.

Material and Methods: The data of 70 patients who underwent ASCT between 2019-2020 were retrospectively analyzed. Valacyclovir was given at a dose of 3 g/day to all patients for primary CMV prophylaxis after ASCT. If CMV reactivation developed during Valacyclovir prophylaxis, therapeutic oral Valganciclovir or parenteral Ganciclovir was gradually switched according to CMV DNA copy numbers.

Results: The mean age of the patients included in the study was 45.5 years. The D+/R+ seropositivity was 97.2%. CMV reactivation developed in 37/70 (52.8%) patients within the first 100 days after transplantation. While CMV negativity could be achieved with oral VValganciclovir in 17 of the reactive patients (45.9%), hospitalization was required for parenteral ganciclovir use in 20 (28.1%) of them. The median PFS of patients with and without CMV reactivation was 10 months and 18 months, with a one-year PFS were 49.9% and 80.9%, respectively. One-year overall survival rates of patients with and without CMV reactivation were 52.9% and 92.9% respectively.

Conclusion: It has become more important to prevent infections that may develop after ASCT with prophylaxis rather than treating. Post-transplant intermediate-dose Valacyclovir as primary prophylaxis has been shown to reduce CMV reactivation/disease rates at desired levels and reduce hospitalizations.

Keywords: allogeneic stem cell transplantation, ASCT, Cytomegalovirus, CMV, hospitalization, prophylaxis, valaciclovir

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INTRODUCTION

Although allogeneic stem cell transplantation (ASCT) is the only treatment method that can cure many hematological malignancies, complications that increase post-transplant morbidity and mortality continue to cause uneasiness in using this treatment modality. The most threatening post-transplant complications are acute and chronic graft versus host disease (GVHD) and opportunistic infections (bacterial, viral, and fungal).

CMV seropositivity rate in healthy adults is around 70% across the world (1), while in developing countries such as Turkey this rate rises to about 90-99% (2). In immunocompromised patients after ASCT, CMV mostly appears only as reactivation (30-37%), (3) while it is observed as CMV disease at a rate of 1.4-10 % (4, 5). It is not surprising that these rates are high in developing countries. The most common forms of CMV infection in immunosuppressed patients are pneumonia, enteritis, hepatitis, and retinitis (6).

Some non-pharmacological (using CMV negative, leuko-depleted, and filtered blood products) and pharmacological (using prophylaxis or pre-emptive therapy) policies have been adopted to minimize the reactivation and infection risk of CMV (7, 8).

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Although studies are showing the advantages of prophylaxis in terms of reducing CMV reactivation and disease, there is no consensus on the routine use of prophylaxis yet. It is the most valid, objective and constantly updated ECIL (European Conference on Infections in Leukemia) guideline to follow on the complications of CMV after allogeneic transplantation.While pre-emptive treatment was recommended instead of CMV prophylaxis in the first published ECIL guideline, Letermovir prophylaxis was agreed upon in the most recent ECIL-7 guideline ([8], [9]). However, such expensive prophylaxis like Letermovir cannot be used in first-line CMV reactivation due to reimbursement rules of governments in developing countries. So, the treatment approach has been left to the experience and selection priorities of transplant centers (10).

Only high-dose oral Acyclovir, Valacyclovir, Letermovir and parenteral Ganciclovir are proven to be first-line agents that are effective and convenient in CMV prophylaxis (10-12). The use of ganciclovir requires hospitalization due to its parenteral nature and the toxic effects of high dose acyclovir on renal functions have brought Valacyclovir one step ahead in prophylaxis. Based on this awareness, we planned to evaluate the effectiveness of intermediate dose (3 gr/d) valacyclovir in primary CMV prophylaxis after ASCT by comparing our results with recent literature.

MATERIAL and METHODS

The files of 83 patients who underwent ASCT (from related or unrelated donors) due to high-risk hematological malignancy (AML, ALL, MDS, NHL, HL, MM, AA) between January 2019 and December 2020 were retrospectively evaluated within the scope of the study. Based on the inclusion criteria, 13 patients with early mortality in the first 100 days were excluded from the study to rule out the confusion of unknown CMV or transplant-relatedness. The remaining 70 cases were analyzed retrospectively. All protocols, experimental studies, and clinical trials involving human subjects were approved by the ethics committee of the institution before the study began, and that the protocols conformed to the ethical guidelines of the 1975 Helsinki Declaration. Inclusion criteria were given in **Supplementary 1.**

The primary endpoints of our study were CMV reactivation and disease rate, and post-transplant CMV reactivation time. Secondary endpoints were; late CMV reactivation rate, CMV-related hospitalization, and LOS in hospital, and also impact of CMV reactivation on progress-free survival (PFS) and overall survival (OS).

All patients were monitored weekly starting from post-transplant +7 days for CMV DNAemia with an internationally standardized PCR Kit using a whole blood sample with a linear interval of 65-13.000.000 IU/ml (1 IU/ml= 1.2 copies/ml). CMV reactivation is defined as the isolation of the virus or evidence of viral replication ≥1000 copies/ml in the blood or other body fluids in two consecutive measurements in an asymptomatic patient without organ-specific abnormalities.

CMV disease is defined as isolation of the virus from blood or body fluids in patients with symptoms and/or histological evidence of tissue involvement. Late CMV reactivation is defined as the reactivation status of CMV DNA after +100 days from transplantation (7, 8).

An intermediate dose (3 gr/d) of Valacyclovir was started simultaneously with the initiation of the conditioning regimen in all enrolled patients, and the dose was adjusted according to renal function during follow-up. Valacyclovir 3 g/day was planned to be given up to +100 days post-transplant. CMV IgG was studied for CMV serology screening in all recipients and donors before transplantation. D(+)/R(+) and D(+)/R(-) status were admitted as high-risk for CMV reactivation. As a step-wise pre-emptive treatment strategy, asymptomatic patients with CMV reactivation with CMV DNA copies/ml between 1000-5000 were treated with 1800 mg/day oral valganciclovir in an outpatient setting.In patients whose CMV DNA copies/ml ≥ 5000/ml at any time or patients with CMV disease were treated with iv ganciclovir 10 mg/kg/day in an inpatient setting. Pre-emptive treatment was continued until CMV DNA negativity was achieved in two consecutive blood samples. CMV DNA measurements were evaluated at each visit after +100 days depending on the patient's clinical findings and systemic steroid use.

Creatinine (BUN) and creatinine clearance (CrCl) were checked from blood samples at each visit to monitor the most known renal-adverse effects of Valacyclovir. Scoring systems of EBMT (13) for acute GVHD and NIH-consensus 2014 criteria (14) for chronic GVHD were used. All patients received Methotrexate-Cyclosporine A or Post-transplant high-dose Cyclophosphamide combined with Tacrolimus and Mycophenolate Mofetil as GVHD prophylaxis for a minimum of 100 days after transplant. Systemic steroids (1-2 mg/kg/day) were used in acute GVHD grade 3 and above.

Statistical Analysis

IBM SPSS Statistics 25.0 was used for statistical analysis. Descriptive statistics were carried out to assess the central tendency and distribution of study variables (e.g. mean, median, standard deviation, frequencies, minimum/maximum values). Mann-Whitney U test was done to compare the two non-normally distributed variables. Chi-square and Fisher exact tests were used to evaluate the relationship between variables. A binary logistic regression test was performed to ascertain the effects of variables. While OS event was defined as death from any cause, PFS event was defined as relapse or death from any cause. Kaplan-Meier curves were generated for survival analyses, and Log-rank tests were used to assess differences in OS and PFS between study groups. The Cox-regression test was used for the analyses of treatment and prognostic effects of data and assumes a constant hazard ratio. A p-value ≤0.05 was considered statistically significant.

The X-tile model (Version 3.6.1) was used to determine the cutoff values of the CMV DNA copy. Survival curves were plotted using the Kaplan-Meier method, and differences among the individual groups were defined using the log-rank test.

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RESULTS

Patient Characteristics & Outcomes of ASCT

The demographic and Clinical Characteristics of the patients are given in table 1. Patients with high risk for CMV reactivation who underwent ASCT between January 2019 and December 2020 were conducted in our study. Patients characteristics, ASCT characteristics, outcomes of ASCT were summarized in supplementary 1. Median length of stay (LOS) in the hospital for transplantation was 28 days (10-89). When the patients with CMV reactivation were evaluated, secondary graft failure was seen more frequently in patients who were treated with iv ganciclovir (p=0.008).

CMV Reactivation Characteristics

Characteristics of CMV reactivation are given in table 2. No grade 3-4 side effect was seen with valacyclovir prophylaxis. Ganciclovir resistance was not observed in any patient who developed CMV reactivation after valacyclovir prophylaxis. While there was no positive correlation between the use of Fludarabine or ATG use in the conditioning regimen and CMV reactivation (p: 0.157 and p: 0.714, respectively), a statistically significant correlation (p: 0.003) was found between the systemic steroid use (2 mg/kg/d and above) and CMV reactivation. CMV reactivation was observed in all 9 patients using systemic steroids for acute GVHD. In addition, a significant relationship was observed between acute GVHD and CMV reactivation (p: 0.022), independent of steroid use.

Binomial logistic regression was performed to ascertain the effects of acute GVHD, secondary graft failure, donor type on the likelihood that participants have CMV reactivation. The logistic regression model was statistically significant X2(4) = 17.652, p: 0.001. The model explained %29.8 (Nagelkerke R2) of the variance in CMV reactivation and correctly classified %72.9 of cases. Of the four predictor variables, only two were statistically significant: transplantation from a mismatch-unrelated donor (HR: 4.67) and transplantation from a haploidentical donor (HR: 7.97) (table 3).

CMV Reactivation Outcomes

Overall, no statistically significant relationship was found between CMV reactivation and disease progression (p=0.592). Also, when the patients diagnosed with acute leukemia (AML and ALL) were evaluated separately, no relationship was observed between CMV reactivation and disease progression (p: 0.224 and p: 0.635, respectively). However, while CMV reactivation did not affect OS in AML, it was statistically significantly decreased in patients with ALL (p: 0.043). However, this relationship could not be confirmed by cox-regression analysis (p: 0.086). While the mean PFS duration was 10 months in patients with CMV reactivation, it was 18 months in patients who did not develop CMV reactivation. (p=0.093). The 1-year PFS rates were 49.9% and 80.9% in patients with and without CMV reactivation, respectively (figure 1). One year OS of patients with and without CMV reactivation was 52.9% and 92.9% (p: 0.012) respectively; median time for OS for both groups has not been reached (figure 2). Only acute GVHD, CMV reactivation, and disease progression were observed as factors affecting OS in univariate regression analysis. In multivariate regression analysis, both CMV reactivation and disease progression were observed as negative factors for OS with an HR 4.33 and 3.54, respectively (Table 4).

Finding Significant Maximum CMV DNA Copy

There was no association between maximum CMV DNA copy amount and OS (p=0.499). The X-tile model was used to determine the cutoff values of the CMV DNA copy. According to the CMV DNA copy, patients with CMV reactivation were divided into 2 categories: CMV DNA ≤ 7493 copies/ml (n:20), CMV-DNA >7493 copies/ml (n: 17) using the X-tile model. CMV DNA copies/ml >7493 copy were found to have a negative effect on OS with an HR 9.721 (p: 0.004, %95 CI: 2.090 – 45.207) (figure 3A and 3B).

Supplementary 1: Inclusion criteria

1. Being ≥18 years old at the time of transplant
2. Diagnosed with a high-risk hematological malignancy (ALL, AML, etc.)
3. Treated with a myeloablative or reduced-intensity conditioning regimen
4. No previous history of CMV reactivation or disease before transplant
5. Creatinine clearance must be ≥ 50 ml/min
6. Should be no previous solid organ transplantation history
7. Liver enzymes (AST, ALT) must be ≤ 3X higher than normal limits at the transplant

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Table 1. Demographic and Clinical Characteristics of the patients

	CMV Reactivation (+) (n: 37)	CMV Reactivation (-) (n: 33)	Total (n: 70)	*P value*
Age, years (median, range)	45 (18-61)	44 (23-67)	44.5 (18-67)	0.855
Male, sex (n, %)	24 (64.9)	18 (54.5)	42 (60)	0.379
Diagnosis (n, %)				0.884
ALL	11 (29.7)	10 (30.3)	21 (30)	
AML	14 (37.8)	15 (45.5)	29 (41.4)	
MDS	5 (13.5)	3 (9.1)	8 (11.4)	
HL	1 (2.7)	1 (3)	2 (2.8)	
NHL	3 (8.1)	3 (9.1)	6 (8.5)	
AA	1 (2.7)	1 (3)	2 (2.8)	
Conditioning Regimen (n, %)				0.220
MAC	17 (45.9)	20 (60.6)	37 (52.9)	
RIC	20 (54.1)	13 (39.4)	33 (47.1)	
Fludarabine (n, %)	32 (86.5)	30 (90.9)	62 (88.6)	0.714
TBI (n, %)	16 (43.2)	8 (24.2)	24 (34.3)	0.095
ATG (n, %)	7 (18.9)	2 (6.1)	9 (12.9)	0.157
Harvesting (n, %)				1.000
Peripheral	36 (97.3)	32 (97)	68 (97.1)	
Bone marrow	1 (2.7)	1 (3)	2 (2.9)	
Donor (n, %)				**0.005**
Match-related	13 (35.1)	21 (63.6)	34 (48.6)	
Match-unrelated	2 (5.4)	6 (18.2)	8 (11.4)	
Mismatch-unrelated	12 (32.4)	4 (12.1)	16 (22.9)	
Haploidentical	10 (27)	2 (6.1)	12 (17.1)	
Chimerism > %95 (n, %)	35 (94.6)	31 (93.9)	66 (94.3)	1.000
IgG CMV Status D/R (n, %)				0.219
D+/R+	37 (100)	31 (93.9)	68 (97.2)	
D+/R-	-	2 (6.1)	2 (2.8)	
Engraftments (median, range)				
Neutrophil	15 (9-26)	14 (10-26)	15 (9-26)	0.649
Lymphocyte	26 (11-50)	28 (14-62)	27 (11-62)	0.097
Thrombocyte	26 (15-102)	24 (16-66)	25.5 (15-102)	0.762
Engraftment Failure (n, %)				**0.035**
None	21 (56.8)	28 (84.8)	49 (70)	
Primary	5 (13.5)	1 (3)	6 (8.5)	
Secondary	11 (29.7)	4 (12.1)	15 (21.4)	
Acute GVHD (n, %)	13 (35.1)	4 (12.1)	17 (24.2)	**0.025**
Chronic GVHD (n, %)	13 (38.2)	6 (18.2)	19 (27.1)	0.069
CMV Disease (n, %)				
Retinitis	1 (2.7)	-		
Nephritis	2 (5.4)	-		
Colitis	1 (2.7)	-		

CMV: Cytomegalovirus, ALL: Acute Lymphoblastic Leukemia, AML: Acute Myeloblastic Leukemia, MDS: Myelodysplastic Syndrome, HL: Hodgkin Lymphoma, NHL: Non-Hodgkin Lymphoma, AA: Aplastic Anemia, MAC: Myeloablative Conditioning, RIC: Reduced-intensity Conditioning, TBI: Total Body Irradiation, ATG: Anti-thymocyte Globulin, GVHD: Graft versus Host Disease

Table 2: CMV reactivation and CMV disease

Time, day (Median, range)	30 (2-194)
CMV Disease (n, %)	
Retinitis	1 (1.4)
Nephritis	2 (2.9)
Colitis	1 (1.4)
Late CMV Reactivation (n, %)	4 (5.7)
CMV Reactivation Treatment (n, %)	
Ganciclovir	20 (28.5)
Valganciclovir	17 (24.2)
Length of Stay for Ganciclovir, day (median, range)	30.5 (8-74)
CMV DNA, maximum copies/ml (mean + std)	33.941 ± 47.483
CMV clearance, day (median, range)	21 (7-61)

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Table 3: Logistic regression analysis for CMV reactivation

	B	SE	p	HR	95.0% CI for Exp(B)	
Secondary Graft Failure	0,555	0,736	0,451	1,742	0,412	7,366
Mismatch-Unrelated Donor	1,542	0,692	**0,026**	4,674	1,204	18,155
Haploidentical Donor	2,076	0,863	**0,016**	7,972	1,469	43,268
Acute GVHD	1,242	0,692	0,072	3,464	0,893	13,439

Table 4. Univariate and multivariate analysis for overall survival.

Univariate Cox-regression Analysis

	Mean	B	SE	p	HR	95.0% CI for Exp(B)	
CMV Reactivation	0,522	1,466	0,637	**0,021**	4,330	1,242	15,098
Acute Leukemia	0,71	-0,280	0,534	0,959	0,973	0,341	2,771
Conditioning	1,464	0,457	0,487	0,348	1,580	0,608	4,104
Donor Source Match-Related (Reference)				0,233			
Donor Source Match-Unrelated	0,116	-0,270	1,081	0,803	0,763	0,092	6,355
Donor Source Mismatch-Unrelated	0,232	0,594	0,606	0,328	1,811	0,552	5,941
Donor Source Haploidentical	0,174	1,159	0,609	0,057	3,187	0,967	10,509
Graft Failure None (Reference)				0,233			
Primary Graft Failure	0,087	0,632	0,784	0,42	1,882	0,405	8,754
Secondary Graft Failure	0,217	0,879	0,529	0,096	2,410	0,855	6,795
Acute GVHD	0,246	1,016	0,496	**0,04**	2,763	1,046	7,298
Disease Progression	0,159	1,094	0,498	**0,028**	2,986	1,126	7,919

Multivariate Cox-regression Analysis

	Mean	B	SE	p	HR	95.0% CI for Exp(B)	
CMV Reactivation	0,522	1,466	0,679	**0,031**	4,330	1,144	16,386
Acute GVHD	0,246	0,475	0,528	0,368	1,609	0,571	4,530
Disease Progression	0,159	1,265	0,502	**0,012**	3,544	1,326	9,477

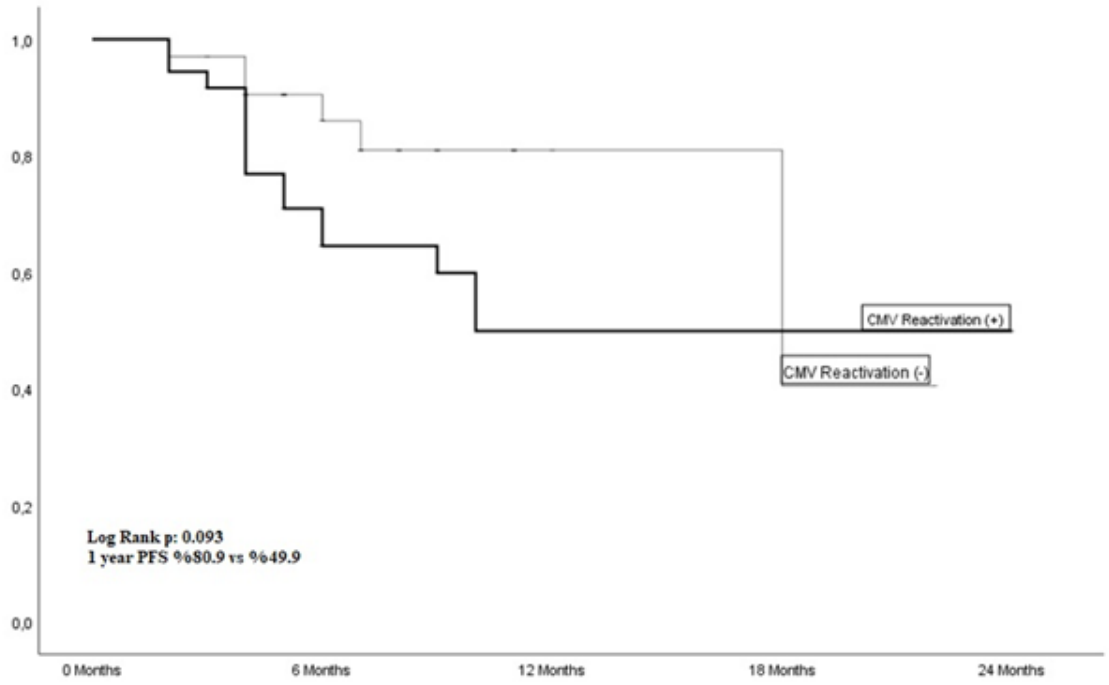

Figure 1. Progression-free survival (PFS) of the patients with and without CMV reactivation

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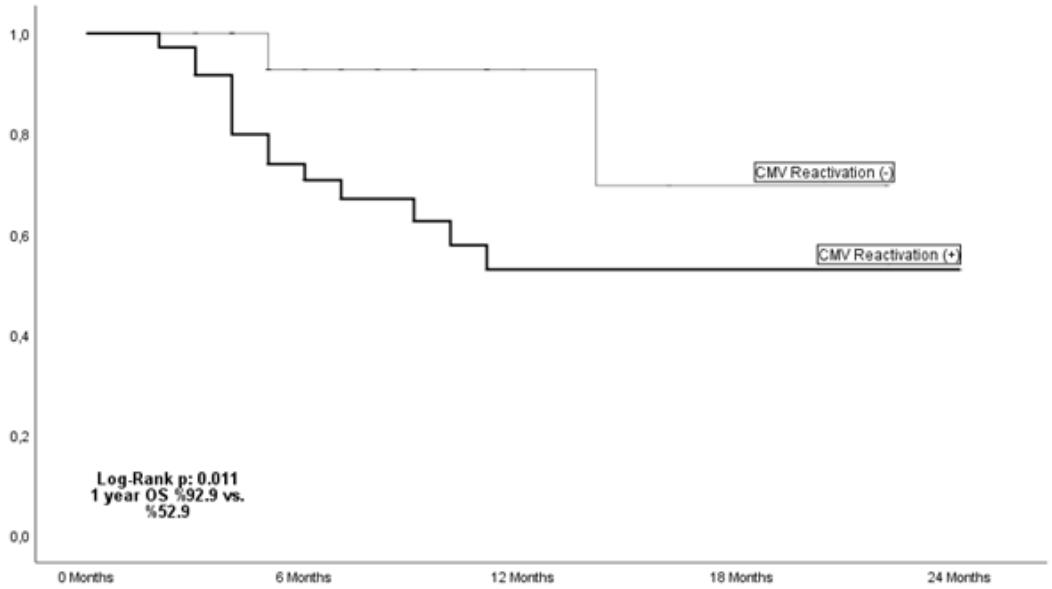

Figure 2. Overall survival (OS) of the patients with and without CMV reactivation

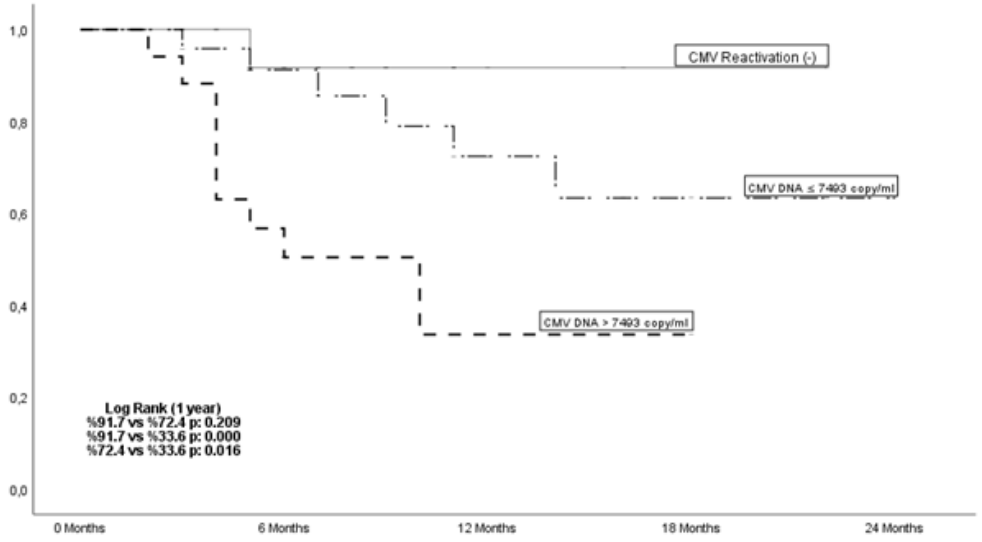

Figure 3A. Overall survival (OS) analysis according to CMV viral load

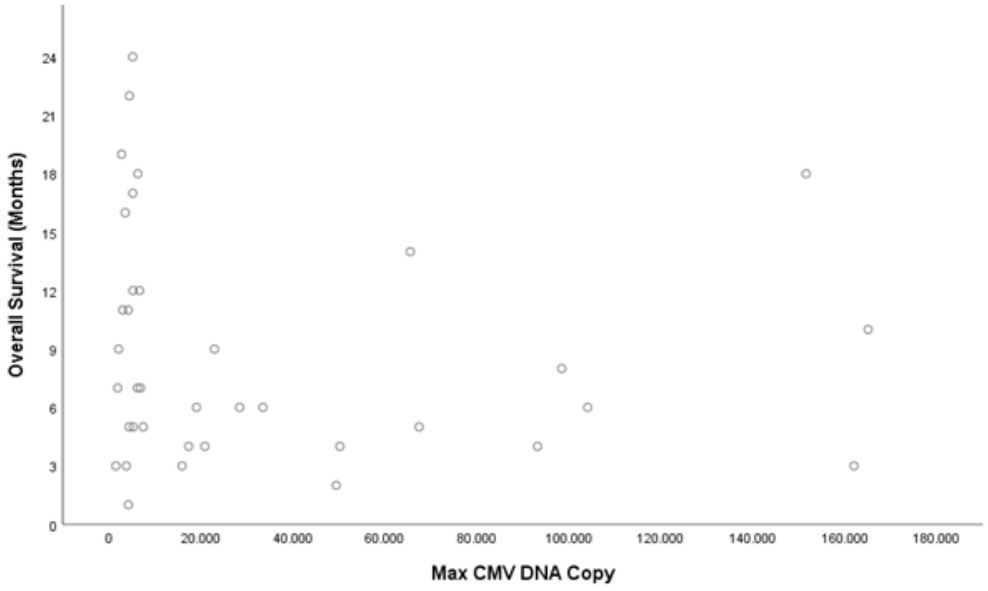

Figure 3B. CMV DNA copy number distribution and overall survival

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DISCUSSION

CMV reactivation and disease occurring after allogeneic stem cell transplantation still pose an important risk on mortality and morbidity (15). Although the approval of Letermovir (16) by the FDA and EMA in 2017 for primary CMV prophylaxis after transplant seems to have marked a breakthrough in OS regard, LTV is not reimbursed in the first-line treatment for CMV prophylaxis by health authorities in many countries. As such, transplant centers continue to work on finding the most suitable conventional antiviral agent for primary CMV prophylaxis, in line with their own experiences.

Considering the CMV seropositivity rates of the population in our country (2) and the recipient/donor CMV serostatus included in the study, our results obtained with the intermediate dose (3 gr/d) Valacyclovir for primary CMV prophylaxis after allogeneic SCT are quite gratifying when compared with both current literature and our historic data. The CMV serostatus of donor and recipient (D+/R+) was 97.2% in the study. Of the 70 patients included in the study, CMV reactivation was observed in 37 patients (52.9%), while CMV disease was detected in only 4 patients (5.7%). According to the step-wise pre-emptive treatment model based on CMV DNA copy number at the time of reactivation, 17 of 37 reactivated patients (24.2%) were successfully treated with oral Valganciclovir, while 20 patients (28.5%) required parenteral ganciclovir and were hospitalized. The average LOS for parenteral ganciclovir was 30.5 days. Ganciclovir resistance was not observed in any patient who developed CMV reactivation after valacyclovir prophylaxis. Late-term CMV reactivation was observed in 4 patients (5.7%). No statistically significant relationship was observed between CMV reactivation and primary disease progression. One-year PFS of patients with and without CMV reactivation were 49.9% and 80.9 respectively (p:0.093). One-year OS of patients with and without CMV reactivation were 52.9% and 92.9% (p: 0.012), respectively; median time for OS for both groups has not been reached.

It was seen that D+/R+ rates of our study (97.2%) are significantly higher than the studies in the literature. In addition, the fact that almost all recipients' seropositivity of CMV puts all patients at high risk for CMV reactivation. While this rate was 77% in the study conducted by Diaz et al. (17) in Latin America, it was found to be 57% in the study conducted by Ljungman et al. (18) from Europe. Post-transplant CMV reactivation rate of 52.8% in this study was not surprising with such high seropositivity of D/R compared to the literature. In a study conducted by Winston et al (19), high-dose Valacyclovir (8 g/d) and ganciclovir were compared after the use of standard Acyclovir in primary prophylaxis, and the CMV reactivation rate was found as low as 14%. The low sensitivity method (urine and blood culture screening) used to detect CMV reactivation in this study, as well as the 54% D/R seropositivity in the study population, may be the main reasons for the differences between these two studies. CMV reactivation developed in 3 (25%) of 12 patients who received primary protection with valacyclovir 3 gr/d in the study performed by Vusirikala et al.20 In this study, pp65 antigenemia searching method, which is much less sensitive than the PCR technique, was used in CMV monitoring. In addition, both low numbers of patients enrolled in the study (12 patients) and low D+/R+ seropositivity (75%) also led to a difference. In a multicenter study conducted by Ljungman et al. (18), high-dose Valacyclovir (8 g/d) and high-dose Acyclovir were compared as primary CMV prophylaxis after using standard parenteral acyclovir treatment in both groups, and the CMV reactivation rate was found to be 33% in the valacyclovir arm. The reasons for the difference between the results can be counted as not using only the PCR technique as a CMV DNA monitoring, low D/R seropositivity (57%) of the study population, dosage of the Valacyclovir used for primary prophylaxis and stem cells source of the patients (most of them from MRD).

With ganciclovir ± low-dose valacyclovir treatment, which we used for primary CMV prophylaxis after ASCT between 2015 and 2018 in our transplant center in a similar patient group (D+/R+ seropositivity 95%), CMV reactivation rate was 61% and our CMV-related disease rate was around 10%. Although it seems that we have achieved very partial success in CMV reactivation and related disease rates with the intermediate dose valacyclovir treatment, we achieved a significant improvement in CMV-related hospitalization rates (from 45.1% to 28.5%) with intermediate-dose valacyclovir prophylaxis with a step-wise preemptive therapy model. This decrease in hospitalization rates had enabled us to cope with both economically and at a time when it was difficult to find a hospital bed like the Covid-19 pandemic.

The rate of CMV-related disease (5.7%) in our study was similar to the literature. In the studies mentioned above (18-20) the rates of CMV disease vary between 2.4-8.3%. There may be two reasons for finding similar CMV disease development rates after Allo-SCT in almost all of the studies in the literature in which different prophylactic anti-viral agents were used; the fact that effected tissue sampling is the only standard method for demonstrating CMV disease and that the frequency of CMV disease development can be reduced at almost similar rates with different anti-viral agents including LTV.

The onset of CMV reactivation was found to be median 30 days after transplant, in line with the literature (19). Late-term (+100 day) reactivation, which is most related to prolonged immunosuppressive use, occurred in only 4 of 37 (5.7%) patients with CMV reactivation. Fludarabine, total body irradiation (TBI), or anti-thymocyte globulin (ATG) used as a part of the conditioning regimen, did not have a negative impact on CMV reactivation. Although a significant relationship was found between Fludarabine and CMV reactivation in a study conducted by Junghanss et al. (21), this correlation was not shown in our study. A significant relationship was observed between systemic steroid use for acute GVHD treatment and CMV reactivation (p: 0.003), consistent with the literature. In patients with CMV reactivation, the mean maximum number of CMV DNA copies/ml was 33.941 + 47.483. Although, we cannot find a correlation between viral load and OS (p: 0.499) in general terms, when we take the CMV DNA copies/ml number as a threshold value for 7493 which was found by X-tile model; it was found that OS was worse in patients with 7493 or more copies (p: 0.004, HR 9.721, %95 CI: 2.090 – 45.207). There are few articles in the literature showing the increment of CMV infections and the decrement of OS time when the CMV DNA copy number exceeds 8200/ml (22).

One of the most important points we want to emphasize in our study is the hospitalization rates and hospital stay processes associated with CMV reactivation/disease. Hospitalization for parenteral ganciclovir treatment was required in 20 of 37 CMV reactivated patients (28.5%). The average hospital stay was 30.5 days (8-74 days). Although there is not much information about CMV-related hospitalization rates and LOS in the literature, in a multicenter study designed by Schelfout et al (23) the first hospitalization period associated with CMV in the first 100 days after transplant was 31.9 days which overlaps with our data. A retrospective study on patients receiving their first ASCT found the incidence of CMV episodes during the first year related to a higher total LOS (average of 26.4 additional days) when compared to those without CMV infection (24). Our hospitalization rates were 45.1% due to CMV-related complications after Ganciclovir±Valacyclovir primary prophylaxis strategy (2015-2018). In times of difficult hospitalization processes, the importance of primary CMV prophylaxis with suitable oral anti-viral agents after ASCT is once again revealed.

The relationship between the prophylactic approach and OS has been pointed in many studies and it has been shown that most of the anti-viral agents except high dose acyclovir and letermovir do not provide an advantage over OS (12, 25-27). Since, we included only the patients transplanted in the last 24 months in our study, the follow-up period was found to be an average of 7 months (1-24 months). In this study 1-year OS rates in patients with and without CMV reactivation were 52.9% and 73.9% ($p = 0.012$), respectively and 1-year PFS was 76.9% and 90.5% (10 months vs 18 months, $p = 0.093$) respectively. In a study conducted by Dwabe et al, (28) 1-year OS and 1-year PFS were found to be 85% and 87%, respectively in the LTV prophylaxis group. Although there are reports that CMV reactivation prevents especially AML recurrence by increasing NK cell activity and triggering the graft versus leukemia effect after transplantation, (29, 30) no effect of CMV reactivation on disease progression was found in any patient group in this study. However, it is too early to say whether there will be a decrease in long-term disease recurrence.

Although there are studies in the literature mentioning grade 3-4 side effects (such as renal dysfunction, mental status changes, persistent nausea, and vomiting) that can cause drug cessation or dose adjustments during high-dose valacyclovir prophylaxis, no such side effects were observed in our study.

Another reason for relatively high CMV reactivation rates after Allo-SCT in our study was the diversity of donor sources used in the transplant setting; HLA full-match relative (MRD) 48.6%, full-match or one-mismatch unrelated (MUD) 34.3%, and HLA haploidentical 17.1%. As is very common in the literature, (31) post-transplant CMV reactivation rates from haploidentical and mismatch unrelated donors were found to be significantly higher in this study ($p=0.05$).

Main limitations of our study were that it was planned in a retrospective design and performed with a limited number of patients. In addition, the absence of another study in the literature conducted with an isolated valacyclovir intermediate dose (3 g/d) made it difficult for us to compare our data fully.

Again, the high CMV IgG rates (90% and above) in our country may negatively affect all study data. Finally, our average follow-up (7 months) period may be considered insufficient.

CONCLUSION

In an environment where CMV reactivation rate can reach up to 80% (32) in patients with ASCT for whom primary CMV prophylaxis is not administered, and where the negative effects of CMV reactivation on mortality and morbidity with/without causing disease are well-known, valacyclovir 3 g/d is effective in primary CMV prophylaxis in case of being unable to use LTV. Another point that should not be forgotten is in cases where hospital occupancy rates are high, it has become more important to prevent infections that may develop after ASCT with prophylaxis rather than treatment. Considering its success in reducing CMV disease and hospitalization periods rather than preventing CMV reactivation, we can say that primary CMV prophylaxis with intermediate-dose Valacyclovir is as successful and cost-effective as Letermovir.

Author Contributions: OK, SA, TE: Concept, Data collection and/or processing, Analysis and/or interpretation, Literature review**, OK:** Writing, Revision**.**

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Conflict of interest: The authors declare no competing interests.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the institutional and/or national research committee's ethical standards and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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The relationship of Growth differentiation factor-15 with renal damage and dyslipidemia in non-albuminuric and albuminuric Type-2 Diabetes Mellitus

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ABSTRACT

Objective: Aim of this study is to investigate the correlation of Growth differentiation factor-15 with renal damage and dyslipidemia in Type-2 Diabetes Mellitus.

Material and Method: The study was conducted prospectively with patients diagnosed with Type-2 Diabetes Mellitus. Two groups were formed as non-albuminuric (n:47) and albuminuric (n:24). Age, gender, Growth differentiation factor-15, glycemic index, lipid panel, glomerular filtration rate, complete blood count, urine albumin/creatin and urine protein/creatin of the groups were compared, and their correlations were examined.

Results: Growth differentiation factor-15, age, and hemoglobin A1c were found to be higher in the albuminuric group, and hemoglobin and hematocrit levels were found to be lower. A positive correlation of Growth differentiation factor-15 with spot urine albumin/creatin and protein/creatin was observed in the albuminuric group. In the non-albuminuric group, positive correlation was observed with Triglyceride and a negative correlation with high-density lipoprotein cholesterol. Negative correlation of Growth differentiation factor-15 glomerular filtration rate was detected in all participants.

Conclusions: Growth differentiation factor-15 has been found positively associated with albuminuria and high triglyceride levels in Type-2 Diabetes Mellitus. It is negatively correlated with glomerular filtration rate and high-density lipoprotein cholesterol. It is strongly associated with renal damage and dyslipidemia.

Keywords: GDF-15, albuminuria, GFR, Triglyceride, HDL-C

INTRODUCTION

Growth differentiation factor-15(GDF-15) is sourced from the transforming growth factor-β(TGF-β) family (1). It is widely expressed in cardiomyocytes, endothelial cells, macrophages, adipocytes, and vascular smooth muscle cells which a critical protein associated with oxidative stress that occurs in hypoxia, organ damage and chronic inflammation (2,3,4).

GDF-15 has been found to have higher levels than healthy controls and obese persons without diabetes in Type-2 Diabetes Mellitus (DM) (5,6). Dyslipidemia is common in Type-2 DM. The most common lipoprotein disorder is increased Triglyceride (TRG) and Low density lipoprotein cholesterol (LDL-C) levels with decreased high-density lipoprotein cholesterol (HDL-C) levels. They have important roles in the formation and progression of atherosclerosis and are associated with increased cardiovascular risk (7,8,9). Also, GDF-15 is an important adipokine (10). Studies on lipid metabolism have generally been carried out with patient groups diagnosed with non-diabetic metabolic syndrome, obesity and prediabetes (11,12).

Studies examining the relationship between dyslipidemia and GDF-15 in type-2 DM are limited and uncertain. Both are important cardiometabolic risk factors, and their correlation needs to be investigated. However, increased GDF-15 levels are a potential marker of diabetic kidney disease. Recent studies suggest that it shows early renal damage independent of albuminuria (13,14).In this context, GDF-15 levels have started to become popular. The aim of our study was to investigate the correlation of GDF-15 with dyslipidemia and renal damage in albuminuric and non-albuminuric patients with a diagnosis of Type-2 DM..

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MATERIAL and METHODS

The study was conducted prospectively between April and December 2021.71 Type-2 DM patients were included. Age, gender, body mass index (BMI), hypertension(HT), hypothyroidism and smoking of all patients were recorded. Venous blood samples of the participants were taken in the morning after at least 8-10 hours of fasting. The blood samples were collected in non-anticoagulant gel tubes and centrifuged for 10 minutes at 2000xg after 30 minutes of coagulation. Approximately 0.5 mL of the obtained serum was taken into microcentrifuge tubes and stored at -80°C until the GDF-15 was studied. In biochemistry tests, serum fasting glucose, glomerular filtration rate (GFR), LDL, HDL, Total Cholesterol (T.COL), TRG, aspartate transaminase (AST), alanine transaminase (ALT), thyroid-stimulating hormone (TSH), thyroxine (T4) were studied. Protein/creatinine (PCR) and albumin/creatinine ratios(ACR) were analyzed from the first spot urine sample taken in the morning. Tests were performed in an autoanalyzer (AU 5840; BeckmanCoulter, Calif., USA) using routine laboratory methods.GFR was calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula(15). Hemoglobin A1c (HbA1c) and complete blood count (CBC) were studied from whole blood samples taken into K2EDTA tubes.HbA1c was measured by HPLC (Premier Hb9210; TrinityBiotech, Co.Wicklow, Ireland). CBC was measured by Sysmex instrument (XN-1000-Products Detail, Japan). Serum GDF-15 concentration (Elabscience, Beijing, China) was measured using the sandwich-ELISA method. The intraassay and interassay coefficient of variation for GDF-15 wereboth<10%. The test was performed strictly according to the kit instructions. Optical density was measured at 450 nm using a microplate reader (SPECTROstarNano, BMG Labtech). Two groups were created in the study. Those with spot urine alb/cr> 30 mg/dl were included in the albuminuria group (n: 24), and those with<30 mg/dl in the non-albuminuria group (n: 47). No classification was made on urine PCR. Demographic data, additional diseases, GDF-15 levels, blood tests, and correlations of the groups were compared.

Inclusion Criteria

- >18 age
- Type-2 DM
- Presence of hypertension and hypothyroidism with type-2 DM
- No known cardiovascular disease

Exclusion Criteria

- <18 age
- Type-1 DM
- Acute or chronic infection
- Chronic obstructive pulmonary disease and asthma
- Malignancy
- Cirrhosis of the liver
- End-stage renal disease receiving replacement therapy (GFR<15 ml/min)
- Atherosclerotic vascular diseases(coronary heart disease, cerebrovascular disease, peripheral artery disease)
- Alcohol and substance addict

Statistics

Statistical analyses were performed using the Statistical Package for the Social Sciences software (SPSS, version 20.0, Chicago, IL, USA). Data were presented as percentages and median (interquartile).

Chi-square test and Mann-Whitney U test were used to evaluate the differences between the groups. The Kolmogorov-Smirnov test was used to determine the normality of the distribution. Correlation analyzes were performed using the Spearman test. Multiple linear regression analysis was used to evaluate parameters associated with urine ACR and urine PCR levels. P-value < 0.05 was considered statistically significant.

RESULTS

The descriptive statistics and group comparisons of the variables and groups that are the subject of the study are given in **Table 1**. These results showed no difference between the groups in terms of gender distribution, BMI ratio, smoking, and comorbidities(P>0.05). However, the mean age(61) was significantly higher in the albuminuria group (P=0.038).

According to laboratory tests; GDF-15 (339 ng/mL)(P=0.007), HbA1C(8g/dL)(P=0.040), urine PCR(250 mg/dL)(P<0.001), urine ACR(81 mg/dL) levels in albuminuria group (P<0.001) was significantly higher. Hemoglobin(Hgb)(13.5 g/dL) and hematocrit (hct) (41.2g/dL) levels were found to be lower(P=0.001).

The correlation of GDF-15 with other variables is shown in **Table-2**. According to this; In the albuminuria group, positive correlations were detected with urine PCR (r=0.448*,P=0.028) and urine ACR (r=0.483* P=0.017). In the non-albuminuria group, a positive correlation was found with TRG (r=0.441** P=0.002) and a negative correlation with HDL-C (r=-0.354* P=0.015).

When the correlation was examined over all participants, urine PCR(r=0.383**,P=0.001), urine ACR (r:0.434**,P<0.001) and TRG(r=0.319**P=0.007) positive, GFR(Negative correlations were observed with r=-0.304*,p=0.010) and HDL-C (r=-0.353**,P=0.003).

These results are statistically significant. In order to evaluate the relationship between urine ACR/PCR and other factors, a linear regression model was applied using the backward elimination method.

The factors affecting the urine ACR level in all participants are shown in **Table-3**. Accordingly, it was determined that GDF-15, age, Hba1C and low hemoglobin were correlated with albuminuria.

The factors affecting the urine PCR level are shown in **Table-4**. Similarly, it was determined that low hemoglobin and increased Hba1C, GDF-15 were correlated with proteinuria. These correlations are statistically strong and significant.

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Table 1. Demographic data and laboratory characteristics of the groups

Variables/Groups	Non-albuminuric (n=47)	Albuminüric (n=24)	P value
Gender (Female%)	59,60%	70,80%	0,352
Age	56(49-62)	61(53-70)	**0,038**
BMI	30,6(28,3-34,1)	32,8(29,6-38,9)	0,056
Smoker (%)	21,3%	29,2%	0,461
Additional disease (HT, hypothyroidism)	66%	66,7%	0,952
GDF-15 (ng/mL)	210(137-297)	339(208-445)	**0,007**
Wbc (10^3/uL)	7,8(6,3-8,9)	8,4(6,4-9,9)	0,198
Hgb (g/dL)	14,4(13,7-16)	13,5(12,3-14,3)	**0,001**
Hct (g/dL)	44(41,4-46,5)	41,2(37,8-42,5)	**0,001**
Mcv (fL)	86,5(83,7-89,1)	85,4(82,2-86,8)	0,102
Mch (pg)	29,2(28-30)	28(26,1-30,15)	0,204
Mpv (fL)	10,2(9,8-10,6)	11(10-11,3)	0,070
Plt (10^3/uL)	259(228-327)	281,5(241-322,5)	0,444
HbA1C (g/dL)	7(6,2-9)	8(7,3-9)	**0,040**
Glucose (mg/dL)	151(125-190)	170(128-232)	0,256
GFR (mL/dk)	95,6(82,4-104)	88,5(69,5-100,6)	0,109
Ast (U/L)	20(17-24)	21(16,5-28,5)	0,715
Alt (U/L)	20(17-29)	20(15,5-29)	0,961
T.Chol (mg/dL)	198(176-255)	215(182-242)	0,405
LDL-C (mg/dL)	119(96-153)	130,5(105-165)	0,422
HDL-C (mg/dL)	51(42-61)	48(45-55)	0,466
TRG (mg/dL)	162(116-226)	211(115-266)	0,282
TSH (uIU/ml)	1,92(1-2,43)	1,74(1,11-2,425)	0,747
T4 (uIU/ml)	0,89(0,83-1,02)	0,98(0,88-1,095)	0,073
Urine ACR (mg/dL)	8,4(5,1-12,9)	81(42,7-179,8)	**<0,001**
Urine PCR (mg/dL)	97(67-124)	250(206-327)	**<0,001**

BMI: Body mass index;HT:Hypertension;GDF-15:Growth differentiation factor-15;WBC:White Blood Cell,Hgb:Hemoglobin;Hct: Hematocrit;Mcv:Main Corpuscüler Volüme;Mch: mean corpuscular hemoglobin;Plt:Platelet;HbA1c: Glycosylated hemoglobin; GFR: Glomerular Filtration Rate; Ast: Aspartate transaminase; Alt:Alaninetransaminase;T.Chol:Total Cholesterol; LDL-C: Low-density lipoprotein Cholesterol;HDL-C: high-density lipoprotein-cholesterol; TRG: Triglyceride;TSH: thyroid stimulating hormone;T4: Thyroxine; ACR:Albumin-creatine ratio;PCR:Protein-creatine ratio.

Table 2. Correlation of GDF-15 with other parameters

Variables/Groups	Total group		Non-albuminüric		Albuminüric	
GDF-15	Correlation coefficient (r)	P value	**Correlation coefficient (r)**	P value	**Correlation coefficient (r)**	P value
Age	0,001	0,991	-0,027	0,856	-0,281	0,184
BMI	0,115	0,340	0,104	0,486	-0,099	0,645
DM duration	-0,127	0,292	-0,084	0,575	-0,164	0,443
WBC	0,098	0,418	0,114	0,445	-0,039	0,856
Hgb	-0,080	0,509	0,154	0,303	-0,131	0,540
Hct	-0,069	0,569	0,170	0,254	-0,129	0,547
Mcv	-0,089	0,461	0,091	0,541	-0,292	0,166
Mch	-0,029	0,810	0,103	0,491	-0,178	0,406
Mpv	0,021	0,861	0,048	0,751	-0,165	0,442
Plt	0,019	0,873	-0,004	0,978	-0,002	0,994
HbA1c	0,104	0,392	0,033	0,828	-0,018	0,936
GFR	-0,304	**0,010**	-0,249	0,091	-0,278	0,188
T.Chol	0,040	0,739	0,063	0,672	-0,159	0,457
LDL-C	0,029	0,810	-0,028	0,852	-0,088	0,682
HDL-C	-0,353	**0,003**	-0,354	**0,015**	-0,252	0,235
TRG	0,319	**0,007**	0,441	**0,002**	-0,040	0,853
Urine ACR	0,434	**<0,001**	0,278	0,058	0,483	**0,017**
Urine PCR	0,383	**<0,001**	0,155	0,300	0,448	**0,028**

Table 3. Multivariate regression analysis of Urine ACR concentration as dependent value.

Variables	β	p value
Age	0,188	0,045
Hgb	-0,413	<0,001
HbA1c*	0,354	<0,001
GDF-15*	0,359	<0,001

* Logarithmic transformation was applied. Hgb:Hemoglobin; HbA1c: Glycosylated hemoglobin; GDF-15:Growth differentiation factor-15

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Table 4. Multivariate regression analysis of Urine PCR concentration as dependent value

Variables	β	p value
Hgb	-0,373	0,001
HbA1c*	0,272	0,011
GDF-15*	0,346	0,001

* Logarithmic transformation was applied. Hgb:Hemoglobin; HbA1c: Glycosylated hemoglobin; GDF-15:Growth differentiation factor-15

DISCUSSION

GDF-15; It is associated with multiple factors in patients with type-2 DM. These may include chronic inflammation, impaired glycemic index, renal damage, and dyslipidemia. These results actually lead to poor prognosis and increased cardiovascular risks. According to our results, primarily GDF-15 levels were found to be significantly higher in the albuminuric group (P=0.007) (Table-1).

Albuminuria is a characteristic feature of diabetic nephropathy (DN) and is a condition associated with chronic inflammation (16). Studies have shown that GDF-15 increases in response to tissue damage in chronic inflammation (4,17). Simons et al. also associated increased GDF-15 levels with renal damage in Type-2 DM(18).Agarwal et al. examined GDF-15 and Galectin-3 levels in healthy, prediabetes, Type-2 DM, and diabetic nephropathy groups. They found that it has the highest rate in DN (19).

This is important in terms of an important prognostic marker that affects progression. Age and Hba1c were significantly higher in the albuminuria group ($p<0.05$) (Table-1). These factors may have contributed to the increase in GDF-15 in the albuminuric group.Because GDF-15 is an important cytokine that increases with age.Oxidative stress, inflammation, and hormonal levels change with age. As a result, an increase in the expression of GDF-15 by the p53 gene is observed (20). Bilson et al. also found a positive correlation with GDF-15 and HbA1C (21). In our study, no correlation was found between GDF-15 and age and Hba1c ($p>0.05$) (Table 2).

This may be due to the low number of patients.However, a strong positive correlation was found between GDF-15 and spot urine ACR/PCR in the correlation made both in the albuminuric group and among all participants ($p<0.001$) (Table 2). Li et al. found a significant correlation of GDF-15 with Mogensen stage in a prospective study involving 80 patients. As albuminuria increased, GDF-15 increased (22). DN is the most common microvascular complication of Type-2 DM (23).

The relationship of GDF-15 with albuminuria/proteinuria can be explained by microvascular damage. Because, as a result of endothelial dysfunction in microvascular damage GDF-15 expression occurs widely (24). Our study showed that there is no correlation was observed between GDF-15 and GFR in albuminuric and non-albuminuric groups ($p>0.05$). However, a negative correlation was detected among all participants. ($p=0.010$)(Table 2). The absence of such a result in the groups may be due to the small number of participants. Chung and Li et al. found a negative correlation between GDF-15 and GFR in their studies.

They suggested that it is a marker of early renal damage independent of albuminuria (14,22). Hbg and hct levels were lower in the albuminuria group than in the non-albuminuric group (Table 1). In a study by Ito et al., the early diagnostic value of anemia in diabetic nephropathy was emphasized (25). In addition, it is known that anemia develops earlier, independent of the stage of chronic kidney disease in diabetic patients (26).

Albuminuria is a pathognomonic finding of renal damage and an important progression marker. It is also associated with increased cardiovascular risk (27). We applied a linear regression model to examine the factors affecting albuminuria and proteinuria. Accordingly, GDF-15 ($p<0.001$), age ($p=0.045$), increased Hba1c ($p<0.001$), and decreased hemoglobin ($p<0.001$) affected albuminuria (Table-3).

It was revealed that other factors, except age, had the same effect on urine PCR (Table 4). Based on these results, we can say that GDF-15 has a strong relationship with renal damage. It can be used as an important indicator. Age is a non-modifiable risk factor. However, lowering Hba1c levels by regulating the glycemic index and new treatment strategies that reduce GDF-15 levels can be used to slow down the progression. One of the important results of our study is the relationship between GDF-15 and dyslipidemia. There was a positive correlation between GDF-15 and TRG, and a negative correlation with HDL-C in the non-albuminuric group.

No relationship was observed in the albuminuric group. This is due to the small number of patients. Because the correlation results made on all participants were observed more strongly in the same direction (Table 2). GDF-15 is an important adipokine that regulates lipid and glucose metabolism. Also known as cardiokines. Ho et al. found a negative correlation of GDF-15 with HDL-C and GFR in a study that included 2991 participants, including DM, HT, smokers, elderly and healthy individuals. They showed that the genome-wide increased GDF-15-associated C allele (rs1054561) was correlated with low HDL-C (30).

Casla et al. also found a correlation between GDF-15, high TRG, and low HDL-C in patients with non-diabetic metabolic syndrome (12). High TRG and low HDL-C levels are important reasons for the development of atherosclerosis(31). The relationship between GDF-15 levels and dyslipidemia is interesting. It has been shown that TRG-rich lipoproteins significantly increase GDF-15 levels in smooth muscle cells of the coronary arteries (32).

However, although the development of GDF-15 in atherosclerosis is not fully understood, it has been shown to regulate inflammatory and angiogenesis pathways (33).

Dyslipidemia may cause the development of atheromatous plaques, resulting in endothelial dysfunction and increased local inflammation, resulting in an increase in GDF-15. Based on these hypotheses, are GDF-15 levels affected by anti-hyperlipidemic therapy? This question comes to mind. Kim et al. applied atorvastatin treatment to patients with hyperlipidemia in Type-2 DM. They observed a decrease in T.COL and LDL levels, but they did not observe any change in GDF-15 levels(34). However, studies have found that GDF-15 is more correlated with high TRG and low HDL than LDL-C (12,30). Therefore, statin therapy may not have caused a change in GDF-15 levels. We suggest conducting studies observing the interaction of anti-triglyceride therapy (fenofibrate, gemfibrozil.) on GDF-15. In our study, The low number of patients in both groups, especially in the albuminuric group, is an important reason for the limitation. We recommend that future studies be conducted with larger participants.

CONCLUSION

Increased GDF15 levels are associated with early renal damage in type-2 DM. It is an important marker in predicting progression. However, there is a significant correlation with dyslipidemia. Both renal damage and dyslipidemia cause increased cardiovascular risk. For this reason, GDF-15 may be use an indicator in the evaluation of predictable risks. Moreover, GDF-15 levels are important for new treatment strategies.

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Prognostic values of LDH and Hematological factors in Patients with Sudden Hearing Loss

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ABSTRACT

Objective: In our study, we aimed to evaluate the relationship between Lactate Dehydrogenase and hematological parameters, neutrophil, Neutrophil lymphocyte ratio, platelet lymphocyte ratio, and mean platelet volume serum levels in patients with Sudden sensorineural hearing loss prognosis.

Methods: 60 patients were hospitalized and treated for Sudden Hearing Loss at Dicle University Hospital Ear Nose Throat Clinic between May 2013 and April 2020, and 60 healthy individuals without any health problems were included in the study. Pretreatment peripheral blood was drawn from all subjects, followed by routine blood cell analysis. The absolute numbers of neutrophils, lymphocytes, and platelets in peripheral blood were obtained, and the Neutrophil lymphocyte ratio, platelet lymphocyte ratio, and mean platelet volume of each case were calculated.

Results: A total of 120 participants, including 60 Sudden sensorineural hearing loss patients case group and 60 healthy individuals in the control group, were included in the study. The median age of the case group was 46.0 (29.0-55.0), and the control group was 48.0 (33.5-58.5), and no statistically significant difference was found between the groups in terms of age ($p = 0.191$). The median Lactate Dehydrogenase, Neutrophil, and Neutrophil lymphocyte ratio of the case group was significantly higher than the control group ($p < 0.05$). There was no significant difference between the groups in terms of thrombocyte, mean platelet volume, lymphocyte, platelet lymphocyte ratio, body mass index, and gender ($p > 0.05$).

Conclusion: We think that the increase in Lactate Dehydrogenase level may be a poor prognostic factor in patients with sudden hearing loss that does not improve. In addition, we believe that the increase in neutrophil count and Neutrophil lymphocyte ratio is quite significant but cannot be considered a poor prognostic factor.

Keywords: Sudden hearing loss, Lactate Dehydrogenase, hematological factors, mean platelet volume, Neutrophil lymphocyte ratio

INTRODUCTION

Sudden sensorineural hearing loss (SSNHL) is characterized by an acute sensorineural hearing loss, almost always unilateral, with a hearing loss of at least 30 decibels (dB) at three consecutive frequencies over 72 hours (1). Although the exact incidence of idiopathic SSNHL is unknown since recovery may be spontaneous, it is thought to range from 11 to 77 per 100,000 people (2). The ratio of men and women is equal. It most commonly affects individuals between 43 and 53 (3).

Although it is caused by various reasons such as neoplastic, infectious, autoimmune, neurological, otological, metabolic diseases, ototoxic drugs, and trauma, most of the SHL cases are idiopathic (4). Various theories have been proposed for idiopathic SSNHL. Viral infections, vascular occlusion, autoimmune, and inflammatory conditions have been associated with sudden hearing loss (5). The total number of white blood cells and their subtypes are inflammatory markers in sudden hearing loss. Increased neutrophil count and decreased lymphocyte count were observed during the inflammatory response (6).

Evaluating these markers as a ratio rather than separately may reveal the inflammatory status more clearly (7). Among these rates, increased neutrophil-lymphocyte ratio and platelet-lymphocyte ratio have been shown in studies conducted with patients with sudden hearing loss (8). Studies say there is a correlation between sudden hearing loss and NLR (9).

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LDH (lactate dehydrogenase) is a tetrameric protein that catalyzes the reversible conversion of pyruvate to lactate. Extracellular LDH itself has no known biological activity and is therefore only considered a biomarker of cellular damage (10).

Neutrophil lymphocyte ratio (NLR), platelet lymphocyte ratio (PLR), and mean platelet volume (MPV) can be determined in the hemogram analysis made from peripheral blood. In recent years, it has been shown that the ratio of neutrophil and platelet count to lymphocyte count (N/L and T/L ratio) can be an indicator of systemic inflammation and is associated with prognosis in many cardiovascular diseases, malignancies, and chronic inflammatory diseases (11-14). Publications are stating that NLR and PLR may be essential parameters for the diagnosis of irritable bowel syndrome (15). A study conducted on type 2 diabetic men suggested that the NLR value and HbA1c value were correlated and that high NLR values could be a marker of poor diabetic control in type 2 diabetic men (16). Another study showed that increased NLR value in patients with Hashimoto's thyroiditis could be a cheap and valuable marker in complicated patients (17). Studies state that high NLR in patients with thyroid nodules may be an indicator of underlying malignant nodular disease in the preoperative period (18).

In one of the studies on PLR, Atak et al. suggested that PLR, an inexpensive and easy-to-use marker, may help predict the development and control levels of type 2 diabetes (19). It was thought that PLR could be beneficial in distinguishing the group with high thyroid uptake from the standard group in thyroid uptake scintigraphy (20). A high PLR value, together with ultrasonography, scintigraphy, and cytology, may be a marker to differentiate malignant thyroid nodules from benign thyroid nodules (21).

A study of MPV and PLR on chronic hepatitis-associated liver fibrosis suggests that high MPV and low PLR values are a feature of chronic hepatitis disease (22). MPV can be a marker for type 2 diabetes mellitus and obese patients to determine the degree of inflammation (23). We can think that platelet ratio with high MPV may be a marker for type 2 diabetes mellitus (24). Publications are stating that low MPV may be associated with rheumatoid arthritis (25). Low MPV may be a marker for nasal polyp formation (26).

As seen in the publications mentioned above, NLR, PLR, and MPV values suggest that they are related to inflammatory diseases. Therefore, we wanted to compare these values in patients with sudden hearing loss. Our study aimed to evaluate the relationship between LDH and hematological parameters, neutrophil, NRL, PLR, and MPV serum levels with prognosis in patients with SSNHL.

MATERIAL and METHODS

Sixty patients were hospitalized and treated for sudden hearing loss at Dicle University Hospital Ear Nose Throat Clinic between May 2013 and April 2020, and 60 healthy individuals without any health problems were included in the study. Before starting steroid medication, blood samples were taken from the patients. All patients were treated with 60 mg prednisone once daily for ten days. Afterward, the treatment was gradually reduced and ended.

Inclusion criteria for the study were as follows:

- SSNHL more than 30 dB appearing at least three consecutive frequencies within three days,
- being over 18 years of age and being hospitalized within three days from the beginning,
- 8th cranial nerve pathology findings,
- neurological disorder,
- head trauma,
- otologic hearing loss due to surgery history,
- drug-induced ototoxicity,
- noise-induced hearing loss, or absence of Meniere's disease were not included in the study.

Exclusion criteria:

- Patients with acute systemic infection,
- malignancy,
- chronic obstructive respiratory disorders,
- asthma were excluded from the study.

Hematological analysis and LDH measurement

LDH measurements were carried out by enzymatic spectrophotometric (UV) method using commercial Olympus kits on Olympus 2700 autoanalyzer. The complete blood counts of the participants were obtained using the Sysmex XE-2100 device (Sysmex Corp, Kobe, Japan), and neutrophil, lymphocyte, and platelet counts were used to determine NLR and PLR. Pretreatment peripheral blood was drawn from all subjects, followed by routine blood cell analysis. The absolute numbers of neutrophils, lymphocytes, and platelets in peripheral blood were obtained, and the NLR, PLR, and MPV of each case were calculated.

Audiological evaluation

A standard pure tone speech audiometry assessment was performed for all SSNHL patients. Pure tone thresholds were obtained for air conduction at 250, 500, 1000, 2000, 4000, and bone at 250, 500, 1000, 2000, and 4000 kHz, respectively. Audiological data were reported using the methods recommended by the American Academy of Otorhinolaryngology and the Head and Neck Surgery Hearing Committee (27). Levels of improvement based on Siegel criteria, a classification was made according to treatment success and average pure tone averages at follow-up after two months (28). Later, the patients were divided into 2 groups as "recovery (Siegel 1 (complete) + Sigel 2 (partial) + Siegel 3 (mild)" and "non-recovery (Siegel 4 (no recovery)).

Statistical analysis: SPSS (Statistical Package for Social Sciences) for Windows 22.0 program was used for statistical analysis. He used Chi-square analysis to compare categorical data. While evaluating the study data, the suitability of the parameters to the normal distribution was assessed with the Kolmogorov-Smirnov test. Descriptive data in the study were shown with n,% values in categorical data, and median, interquartile range (25-75 percentile values) in continuous data. The Kruskal-Wallis test and Mann-Whitney U test were used to compare parameters that did not show the normal distribution in quantitative data. The post Hoc test was used to determine where the significance came from. Value was evaluated at the $p < 0.05$ level.

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RESULTS

A total of 120 participants, including 60 SSNHL patients case group and 60 healthy individuals in the control group, were included in the study. The median age of the case group was 46.0 (29.0-55.0), and the control group was 48.0 (33.5-58.5), and no statistically significant difference was found between the groups in terms of age (p = 0.191). The median value of LDH, Neutrophil, and NLR of the case group was significantly higher than the control group (**Figures 1-3**). No significant difference was found between the groups in terms of platelet, MPV, lymphocyte, PLR, BMI, and gender (p> 0.05) (**Table 1**). When the hearing loss level of the case group included in the study was evaluated, 22 (36.7%) were between 30-70 dB, 25 (41.7%) were between 71-90 DB, and 13 (21.6%) were 91db. And above.

Again, when the healing levels of the patients were evaluated according to Siegel criteria, 37 (61.7%) Siegel 1, 10 (16.7%) Siegel 2, 7 (11.7%) Siegel 3, and 6 (10%) were found as Siegel 4. No significant difference was found between LDH, MPV, NLR, and PLR levels according to the level of hearing loss (p> 0.05) (**Table 2**).

According to recovery levels, a statistically significant difference was found between the groups in terms of LDH (p = 0.028). It has been seen that this difference is due to the difference between Siegel 2 and Siegel 4. In addition, no significant difference was found between MPV, NLR, and PLR levels according to recovery levels (p> 0.05) (**Table 3**).

Table 1: The groups in terms of platelet, MPV, lymphocyte, PLR, BMI, LDH, NLR Neutrophil, and age

Specifications	Control	SSNHL	P-value
Age	48 (33.5-58)	46 (29-55)	0.191
LDH(U\L)	145.5 (129-176.5)	165 (148-215.5)	0.001
Platelet (10^3/U)	252 (198-286)	254 (213-298)	0.457
MPV (fl)	8.36 (7.49-9.42)	8.34 (7.12-9.24)	0.364
Neutrophil (10^3/U)	3.89 (3.25-4.65)	4.4 (4.1-5.78)	0.001
Lymphocyte (10^3/U)	2.20 (1.8-2.32)	2.21 (2.1-2.52)	0.260
NLR	1.99 (1.27-2.33)	2.24 (1.8-3.17)	0.001
PLR	152.7(116.2-237.7)	146.6(109.1-175.1)	0.090
BMI (kg/m^2)	24 (22.3-25.3)	23.4(22.2-24.8)	0.426

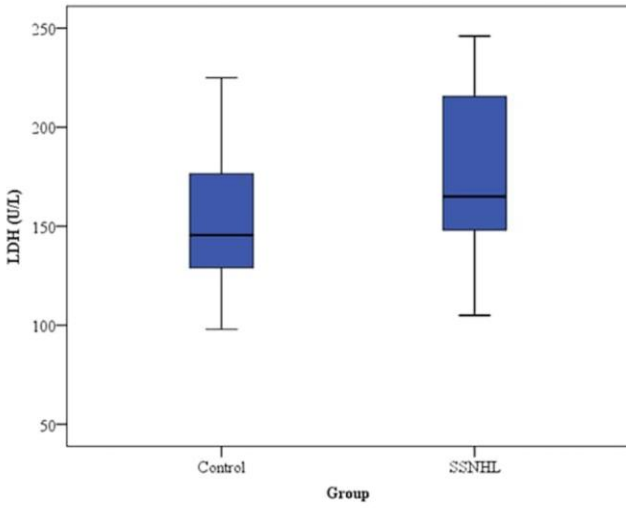
LDH: Lactate dehydrogenases MPV: Mean platelet volüme NLR: Neutrophil lymphocyte ratio PLR: Platelet lymphocyte ratio BMI: Body mass index

Table 2: LDH, MPV, NLR, and PLR levels according to the level of hearing loss

	30-70dB Median (IQR)	71-90 dB Median (IQR)	91- dB Median (IQR)	P-value
LDH(U/L)	181.5 (154-215)	165 (147-184)	216(154-220)	0.089
MPV (fl)	8.28 (7.1-8.6)	8.35 (7.1-8.6)	8.6 (7.5-9.4)	0.590
NLR	2.2 (1.5-3)	2.2 (1.8-2.6)	2.6 (2.2-3.6)	0.114
PLR	150.7 (114-190.3)	133.2(101-158.1)	151(110-175.2)	0.608

Table 3: LDH, MPV, NLR, and PLR levels according to recovery levels

	Siegel 1 Median (IQR)	Siegel 2 Median (IQR)	Siegel 3 Median (IQR)	Siegel 4 Median (IQR)	P value
LDH(U/L)	180(154-209)	155.5(145-165)	154(125-209)	220(216-225)	0.028
MPV (fl)	8.3 (7.1-9.3)	8.5 (8.2-8.7)	8.3 (6.3-8.5)	8.6 (7.5-9.2)	0.782
NLR	2.2 (1.5-3)	2.2 (2.1-2.8)	3.1 (2.6-3.6)	2.3 (2.2-4.3)	0.119
PLR	133.2(110-158.1)	129.9(110-200.1)	160(151-220.2)	131.1(88.5-175.2)	0.334



Figures 1: Median LDH of the case group was found to be significantly higher than the control group.

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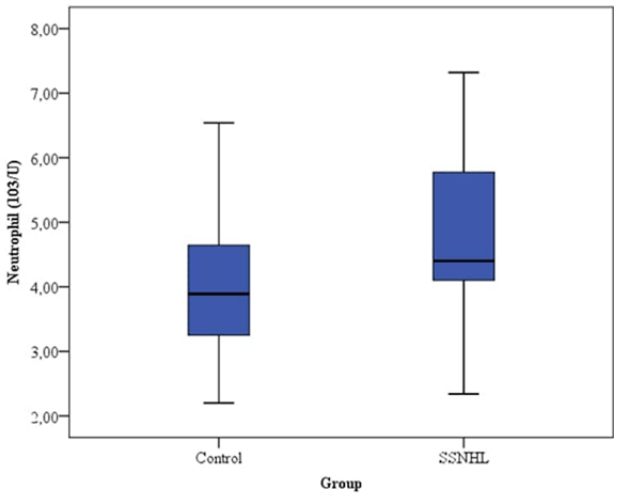
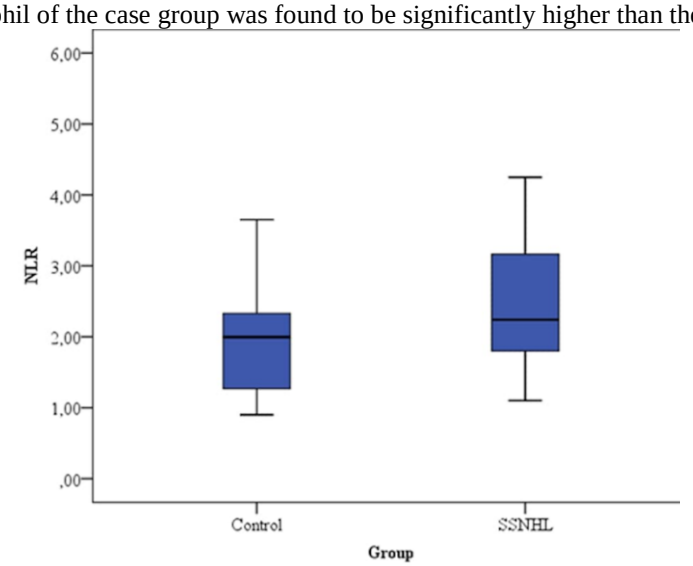


Figures 2: Median of Neutrophil of the case group was found to be significantly higher than the control group



Figures 3: Median NLR of the case group was found to be significantly higher than the control group

DISCUSSION

This study aimed to evaluate whether lactate dehydrogenase and hematological parameters NLR, PLR, and MPV could be prognostic factors in patients with sudden hearing loss. In this study, we observed that although LDH values were average at diagnosis in 60 SSNHL patients, they were higher than the control group. We found a statistically significant difference ($p < 0.01$). We found that the LDH level of patients who did not respond to treatment (nonrecovery) was higher than patients who responded to treatment at various levels (recovery group 1, group 2, group 3). However, we could not detect a difference between the LDH levels of the patients who responded to the treatment. On the other hand, when the patient and control groups were compared, a significant difference was found in terms of neutrophil count and NLR. Although the NLR value in the non-recovery group was higher, there was no statistically significant difference between the recovery group and the non-recovery group. In addition, no significant difference was found between the patient and control groups in terms of PLR and MPV.

Viral infection is considered one of the leading causes of SSNHL. Although acute viral infections can cause this damage, latent infections and reactivation may also explain the lesion. The main latent viruses are part of the herpes virus group. They are found everywhere. They characteristically carry strong neurotropism; they do not always cause symptoms (subclinical infection) and have a complex relationship with SSNHL. A large number of viral agents have been implicated (29). Mechanisms by which viral infection causes SSNHL; first mechanism; although inflammation of neural fibers and ganglia due to viral infection is the most commonly accepted theory, it has not been proven (30). The second mechanism is the reactivation of the latent virus, and the third is the pathological activation of cellular stress pathways within the cochlea (31).

The most important prognostic factor is the degree of hearing loss. The lower the hearing loss at the first presentation, the more likely it is to improve(32). LDH is an intracellular enzyme; therefore, it is not organ-specific. Oxygen-glucose deprivation in auditory cells has been shown to reduce cell viability and increase lactate dehydrogenase (LDH) time-dependent (33).

More increases were detected in these LDH fractions, especially after viral infection. It appears to have been used to indicate various inflammations and diseases. LDH is inflammation biomarker in infectious conditions such as bacterial meningitis, empyema, and arthritis(34). When these data are evaluated together, it is thought that the level of LDH changes due to various reasons affecting the inner ear. The effect of this change on the serum level is unknown. More support for pathologies for which viral pathologies are blamed in patients with sudden hearing loss, for which inflammatory etiology is blamed, suggests that changes in LDH levels may occur. We planned to evaluate the LDH level in SSNHL patients and evaluate its relationship with prognosis in this context. Our study found that the LDH level was significantly higher in patients with sudden hearing loss than in healthy individuals, and we found a statistically significant difference ($p < 0.05$). Clinically this rate was higher in the non-recovery group. We observed that the LDH level triggered by the inflammatory event was higher, especially in the non-recovery group. As the level of damage in the inner ear increases, the level of LDH increases more.

The prognostic effect of hematological indices of complete blood count test on outcomes of SSNHL patients is being investigated. NLR is a widely available inflammation biomarker that can easily measure incomplete blood count tests at no additional cost.

A study involving 348 patients claimed that atherosclerosis-associated NLR and PLR values were significantly higher in SSNHL patients and that endothelial dysfunction may be necessary for SSNHL patients. The mean NLR and PLR values of SSNHL patients were substantially higher than the control group (both <0.001). The NLR value was 5.98 ± 4.22 in the non-recovered group and 3.50 ± 3.38 (<0.001) in the recovered group. However, after adjusting by multivariate analysis, only the NLR level was strongly associated with the recovery of SSNHL. It was then considered an independent risk factor for improving pure tone averages. They stated that the NLR level could be viewed as a new potential marker to predict patients' prognosis for improvement (35).

Again, Cao Z et al., In a meta-analysis of 972 patient groups, showed that NLR, PLR, and neutrophil count are negative prognostic indicators, and lymphocyte level is a favorable prognostic indicator for recovery in SSNHL patients. However, other hematological indices of the hemogram test indicated that it was not associated with the prognosis of SSNHL (36).

IN THEIR EXTENSIVE META-ANALYSIS, Chen L et al. included 1029 SSNHL patients and 1020 healthy individuals to evaluate the relationship between NLR levels and SSNHL. They showed that SSNHL patients had a much higher NLR than healthy populations. They showed that neutrophils and lymphocytes can play essential roles in the pathogenesis of SSNHL and that the level of NLR can be a potential determinant for SSNHL formation. This meta-analysis also compared the NLR levels among SSNHL patients with different degrees of improvement. They found that the NLR of the 'unhealed' patients was significantly higher than that of the 'recovered' patients. This result could be attributed to a higher inflammatory state in 'undiscovered' patients. They stated that the clinician should be afraid of SSNHL patients with higher NLR levels regarding treatment and prognosis (6). We also think that NLR is an essential inflammatory parameter in our study, but it is a weak marker that will affect sudden hearing loss and a poor prognosis.

Durmus et al. stated that PLR, PDW, NLR, PLT, MPV, lymphocyte%, and lymphocyte level could be considered potential markers in predicting the prognosis in recovery (37). In this study, we found that the NLR values (2.24 (1.8-3.17)) in the patient group were significantly higher than the control group (1.99 (1.27-2.33)) ($p < 0.05$). We could not detect a significant difference between the recovery (median 2,2) and non-recovery (median 2,6) groups ($p = 0.114$). Therefore, we think that the NLR value is important because it is an inflammatory marker, but it is not associated with prognosis. We could not find a significant difference between SSNHL patients and the control group.

Ha, et al. showed that NLR could be used in terms of prognosis to study patients with sudden hearing loss in the pediatric age group. Still, there was no significant difference in the comparison of PLR between the control group and the patient group. In our study, we found that there was no difference between PLR and sudden hearing loss, and NLR was a poor prognostic factor (38). Another study reported that NLR and PLR values are high in patients with sudden hearing loss. In our research, it was found that NLR was high and PLR was normal (39).

While vascular occlusion, acute or chronic syndromes, and vasculitis increase MPV levels, infections, autoimmune diseases, and inflammatory conditions reduce it (40). Ulu et al. found that MPV and thrombocyte distribution width was significantly higher in SSNHL in their study with 40 SSNHL patients. SSNHL appeared to be characterized by ischemic or thrombotic events and could contribute to the pathogenesis of SSNHL given increased MPV levels (41). In another study, Sagit M et al. stated that MPV, the determinant of platelet activation in patients with SSNHL, is high. Their findings indirectly support the vascular disorder hypothesis as a pathogenetic factor in sudden sensorineural hearing loss (42).

On the contrary, Karli et al. 46 patients. They observed no significant difference in MPV values between the control and the patient groups receiving SSNHL treatment (43). They stated that this supports the theory that there is no microvascular response in the etiology of SSNHL. In this study, we found no statistically significant difference between the platelet count ($p = 0.457$) and MPV ($p = 0.364$) values in terms of the patient and control group.

CONCLUSION

According to the literature information, this study is the first study evaluating the LDH level of patients with sudden hearing loss. We think that the increase in LDH level may be a poor prognostic factor in patients with sudden hearing loss. In addition, we believe that the increase in neutrophil count and NLR is significant but cannot be considered a poor prognostic factor. We predict that lymphocyte count, platelet count, MPV, and PLR cannot be evaluated as prognostic factors.

Author Contributions: SD, MA, SFT: Concept, Data collection and/or processing, Analysis and/or interpretation, Literature review**, SD:** Writing, Revision**.**

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Ethical approval: All procedures performed in studies involving human participants were in accordance with the institutional and/or national research committee's ethical standards and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Physiological responses during a single rebirthing (Breath work) session

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ABSTRACT

Objective: The present report aimed to look at the physiological responses during a typical single Rebirthing session.

Material and Methods: Ten healthy young women participated in the study. Their mean age, weight, and height were 37±2.7 years, 54.1±6.4 kg, and 161.2±4.9 cm, respectively. The Rebirthing sessions took place at the Israeli Rebirthing Center in Tel-Aviv. The treatments were carried out by a qualified Rebirthing therapist that has experienced thousands of rebirthing sessions during the last 15 years. Sessions were performed in a dark, quiet room and executed in a one-to-one set-up with the same trained therapist. After around 40-50 minutes, the session approached its end. Metabolic, cardiovascular, pulmonary, and gas-exchange variables were measured breath-by-breath using a commercial portable metabolic system. All data were transmitted wirelessly to an adjacent room in the clinic and continuously monitored by the study's chief researcher.

Results: The primary study findings demonstrated that a typical Rebirthing session involving long (45-50 minutes) voluntary hyperventilation generated VO_2, RER, HR, and O_2 pulse matching well with the physiological demands of the procedure (breath work). At the same time, the ventilatory-related responses exhibited, as expected, exaggerated outcomes, illustrated by the high session's peak and average values of the depth (tidal volume), breathing frequency, and minute ventilation. Gas-exchange attributes showed extremely shallow end-tidal CO_2 levels, high end-tidal O_2, high respiratory exchange ratio, and very high levels of O_2 and CO_2 ventilatory equivalents. No significant grievances regarding participants' physical and mental/emotional feelings were reported in the present study.

Conclusions: The present study could not solve the apparent divergence between the observed (acute) physiological responses (mainly severe Hypocapnia) and the subjective participants' pleasant emotional state, and in many cases, spiritually uplifting, at the end of each treatment session.

Keywords: Rebirthing therapy, voluntary hyperventilation, Hypocapnia, body posture

INTRODUCTION

Conscious breathing practices for physical, psychological, emotional, and spiritual healing have a long and extremely varied history. Working with the breath in psychotherapy and other clinical disciplines also enjoys a long and rich history (1, 2), with the assumption that breathwork can resolve psychological pain, soften character armor, release tension in the body and create a sense of embodiment and equanimity (3, 4, 5).

In the early '70s, Leonard Orr created the technique known as Rebirthing (also known as Breastwork, Conscious breathing, Circular breathing, and Connected breathing).

According to numerous reports, predominantly subjective participants who underwent the Rebirthing treatment felt relieved from the stress of daily life and cured of several sicknesses that often stem from weakening our emotional state (e.g., 6, 7, 8).

The primary rationale for such positive effects of the Rebirthing therapy is based on the claims that an intense breathing process can improve focus and sleep quality, battle fatigue and increase energy (6, 9, 10).

Millions of rebirthing advocates worldwide claim that rebirthing treatment helps overcome physical and mental difficulties and improves overall well-being. These subjective reports imply improvement and healing, especially in the following physiological and emotional aspects:

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Mood improvement, lung capacity increase, physical fitness improvement, recovery from various respiratory problems, increase in energy levels throughout the day, recovery from a migraine and headaches, relief and recovery from chronic pain in different body areas, focus enhancement, and better learning ability (6, 7, 8).

The Rebirthing technique is simple and includes deep breathing through an open mouth, pausing between inhaling and exhaling, and vice versa. The process is usually performed lying down on a mattress with eyes shut. Breathing is similar to that during exercise or hyperventilation. However, it is meant to be slower and deeper, and the rhythm changes according to the patient experience before and during treatment.

When performed at upright body position, over-breathing or voluntary hyperventilation causes arterial blood CO2 levels to abnormally drop, blood vessels in the body and brain to contract, the blood supply to the muscles and brain decreases, and their activity is impaired (11, 12). Prolonged hyperventilation (over several minutes) can cause several medical conditions, some of which are risky (13, 14). A partial list of typical physical phenomena seen during acute and extended hyperventilation includes confusion, palpitations, tetanic limb contractions (hands and or feet), paresthesia (primarily hands), feeling of suffocation, and, in severe cases, even fainting (13, 14).

To the best of our knowledge, not a single study has been published that examined the physiological nature, efficiency, effects, and physiological consequences of the Rebirthing treatment technique.

Considering the notable absence of sufficient scientific research and objective quantitative documentation of this challenging issue, and considering the impressive popularity of the Rebirthing therapy worldwide (approximately 20,000,000 participants in the past 40 years) (10), we conducted a controlled scientific investigation to examine both, the physiological responses during a single typical Rebirthing session and the effects a series of Rebirthing sessions on physiological and mental/emotional attributes, in healthy young women.

This manuscript reports the project's first part on the physiological responses observed during a single Rebirthing session.

MATERIAL and METHODS

Participants: Participants were recruited by the Israeli Center for Conscious Breathing from a pool of potential clientele.

Of all potential subjects, eleven healthy young women were selected, all from the central region of Israel. Their mean age, weight, and height were 37±2.7 years, 54.1±6.4 kg, and 161.2±4.9 cm, respectively. All participants underwent a medical examination (including resting and exercise ECG) and were found healthy, and did not take any medications (except birth control pills). Participants were instructed to consume their regular diet, refrain from consuming caffeine or alcohol on the day before the test, and not to engage in intense exercise 24 h before the experiment; they were also instructed to refrain from eating at least two hours before the laboratory visit.

The Helsinki Committee for the Protection of Human Subjects (Institutional Review Board) of the Kaplan Medical Center in Rehovot, Israel, approved all study protocols and procedures. The subjects were fully informed of the procedures, risks, and discomforts in participating in the study, and all gave their signed, informed consent for participation. It was highlighted that participants have the right to withdraw from the study at any time and without any explanation.

Out of the eleven "starters," only one participant withdrew from the study (due to early pregnancy).

Study setting

Preliminary visit: Preliminary measurements for this study were taken at the Human Performance Laboratory at Washington Hill College in Israel.

During the first visit, the participants received a detailed explanation of the study aims and procedures and signed an informed consent form. The participants provided information on their medical history and physical activity habits. Weight and height were measured to the nearest 0.02 kg and 0.1 cm, respectively, using a Shekel model H151-8 scale (Shekel Scales Ltd., Kibbutz Beit Keshet, Israel), while the participants were barefoot and wearing light exercise clothing.

All participants performed the resting Pulmonary Function Test (PFT) (COSMED K4b2, Rome, Italy) to ensure healthy and normal lung function. The tests were carried out according to the ATS guidelines (15).

During this visit, participants were introduced to a short (10 min) demo Rebirthing session using the actual study's apparatus to minimize learning and habituation effects.

The Rebirthing session

Location - The Rebirthing sessions occurred at the Israeli Rebirthing Center in Tel-Aviv. The treatments were carried out by a qualified rebirthing therapist (the same in all sessions) who has experienced thousands of rebirthing sessions during the last 15 years.

Sessions were performed in a dark, quiet room and were executed in a one-to-one set-up (see picture) with the same trained therapist for all.

Rebirthing (Breath-work) Session – Participants were asked to remove their shoes and coat and lie down on a mattress during the Rebirthing session. The therapist then drapes a

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blanket over the patient to help her stay warm and comfortable throughout the session. The therapist then guided the patient to start the connected breathing and build it up to a full, strong, and steady rhythm, pulling high on the inhale and releasing freely on the exhale.

After around 40–50 minutes, the session approached its end. In some participants, the breathing felt difficult, or sleepy, at which time the therapist's job was to recognize such signs and coach the patient through them. At the end of the session, the therapist gently escorted the patient back to the clinic's session.

Measurements taken during the Rebirthing session – Physiological measures were taken continually from the beginning to the end.

Metabolic, cardiovascular, pulmonary, and gas-exchange variables were measured breath-by-breath using a commercial portable metabolic system (COSMED K4b2, Rome, Italy) (see picture above). The metabolic system was calibrated for airflow and gas concentrations before each session, and the system accuracy was checked periodically using a metabolic simulator. A face mask (Hans Rudolph, USA) was used with resistance to breathing up to 10-mm water for air volume up to 200 liters/min.

The heart rate (HR) was recorded electronically (COSMED K4b2, Rome, Italy). The K4b2 gas analyzer has been previously validated and used in studies with varying activities and locomotion (16).

The breath-by-breath data were interpolated to 1s intervals, and the averages of uniform 10-s bins were used for graphical purposes and further calculations.

For safety and quality control purposes, all data were transmitted wirelessly to an adjacent room in the clinic and continuously monitored by the study's chief researcher.

Statistical analysis and calculations –Basic anthropometric characteristics were presented as averages and standard deviations (SD). Physiological data were calculated and presented as averages and standard error of the means (SEM) during the Rebirthing session.

RESULTS

Table 1 presents the group means (±SDs) of the PFT results performed during the preliminary visit. All ten female participants' PFT values (means±SD) were within the normal range, reassuring healthy participants.

Physiological responses during a single Rebirthing session – **Figure 1 (A, B, C, D, and E)** presents the metabolic (VO_2, VCO^2, RER)- and cardiovascular-related responses (HR, O_2 pulse) of the measured parameters.

The kinetic (trending phenomena) of oxygen consumption (VO_2) during the single Rebirthing session was relatively stable at 300–320 ml/min (**see fig. 1A**). An abrupt increase and decrease in VO_2 were observed only during the first and last 6–9 minutes of the session, respectively.

Unlike the relatively stable response of the VO_2, the other two measured metabolic-related parameters, VCO_2 and RER, showed mild elevation during the first 7–8 min of the session, followed by a continuous and linear decrease in values until the session-end (see fig. 1B and 1C).

While VO_2 showed values slightly higher than typical resting VO_2 values for a population similar to the study's participants (young, healthy sedentary women), the level of alveolar CO_2 dropped continually throughout the session (except during the first 5 min), revealing persistently lower than resting pulmonary CO_2 values (**see fig 1-B**).

VO_2 - Oxygen uptake; VCO_2 – Carbon dioxide production/elimination; RER – Respiratory exchange ratio; HR – Heart rate; O_2 pulse – Oxygen pulse;

Consequential to the above VO_2 and VCO_2 responses, the calculated RER values from the 5th minute on demonstrating a continued reduction of the RER values, resulting from the changing proportions between the CO_2 removal (increases) and the O_2 consumption (constant) during the Rebirthing session (**see fig. 1C**).

Figures 1E and 1D present the responses (means±SEM) of the measured cardiovascular-related variables (HR and O2pulse) during the Rebirthing session. Both HR and O_2 pulse showed a relatively stable level throughout the Rebirthing session with the group mean values of 82.3±18.2 b/min and 3.8±0.93 mlO_2/HR/min/kg, respectively. These values fit well with the imposed metabolic demands of the Rebirthing maneuver's energy requirements.

Vt, tidal volume; Bf, breathing frequency; VE, Minute ventilation; Vd/Vt, Estimated physiologic dead space; VE/VO_2 and VE/VCO_2, Ventilatory equivalents for O_2 and CO_2; $PETO_2$, End-tidal O_2; $PETCO_2$, End-tidal CO_2.

Figure 2 presents the response kinetics of the selected ventilatory- (Bf, Vt, VE) and gas-exchange-related variables (VE/VO_2, VE/VCO_2, $PETO_2$, $PETCO_2$, and Vd/Vt) during the Rebirthing session (Means±SEM).

Reviewing figure 2 shows a gradual and continuous increase in breathing frequency (Bf - **fig. 2B**) throughout the treatment, from about 25 breaths/minute at the session starts approximately 65 breaths/min during the final stages of the session (sessions mean 54.8± 21.4 breaths/min). Parallel with the Bf, but in the opposite direction, there was a reduction in the depth of breathing (Vt - **fig. 2A**) from approximately 1.0 liter/breath in the early stage of the treatment to values around 0.5 l/breath during the last few minutes of the treatment (session's mean 0.67±0.36 l/breath). Consequent to the opposing response patterns of the two breathing-related attributes (Bf and Vt), minute ventilation (VE - **fig. 2C**) was relatively stable throughout the session, varying between 25 and 30 l/min (session mean 27.6±8.6 l/min), with an clear swing in VE during the final 10 min of the session (a decrease following by similar increase). Considering the significant increase in estimated physiological dead-space (Vd/Vt - **fig. 2D**) during the session, the observed ventilation pattern suggests a progressive reduction in alveolar ventilation (VA) during the Rebirthing session (17, 18).

The response profile of the ventilatory equivalent for oxygen (VE/VO_2 - **fig. 2E**), signifying the ventilatory efficiency (volume of ventilated air per one liter of oxygen consumed), showed an extremely high and relatively stable response throughout the session, varying between 90 and 100 l/l

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(session mean 95.9±21.1 l/l), with clear short increase and a decrease only at the start and the end of the session, respectively (**fig. 2E**).

A similar trending phenomenon of the respiratory equivalent for CO_2 (VE/VCO_2), representing the ventilation-perfusion ratio (V/Q), was also extremely high from the start to the end of the session (see **fig. 2F**).

In the three additional gas-exchange-related attributes measured in this study ($PETO_2$, $PETCO_2$, Vd/Vt), abnormal responses were noticed. While the $PETO_2$ values, indirectly inferring levels of PaO_2 (Wasserman et al. 2005; Inbar et al. 1994), are relatively high stable throughout the treatment session (~135 mmHg) (typical resting values 90 -120 mmHg) (**see fig. 2G**),

$PETCO_2$ values showed a sharp drop during the first 10 min of the session with relatively stable but exceedingly shallow values thereafter, until the session end (session group means of 14.5±2.6 mmHg) (see **fig. 2H**) (typical resting values 35–45 mmHg). Such low values indicate substantial Hypocapnia and respiratory alkalosis from the start to the end of the Rebirthing session.

All of those mentioned above ventilatory- and gas-exchange-related response dynamics confirm sustained and severe Hypocapnia and respiratory alkalosis, owing to the voluntary hyperventilation (breathwork) during the Rebirthing session.

Table 1: Resting pulmonary function (PFT) (group means ±SD) compared to relevant norms [% of predicted (predicted value)].

Parameter	Mean	SD	% pred.
FVC, Liter	3.65	0.035	110 (3.31)
FEV1, L/sec	3.07	0.26	93 (2.86)
FEV1/FVC, %	84.3	4,67	101 (85.2)
PEF, L/sec	7.05	0.55	94 (6.66)
MEF25-75, L/sec	3.53	0.81	105 (3.71)

FVC, forced vital capacity; FEV1, Force expiratory volume in 1 second; FEV1/FVC, %, The ratio between FEV1 and FVC; PEF, Peak expiratory flow rate; MEF25-75 %, Mid-expiratory flow rate; %pred., percentage of predicted normal (based on ECCS norms (16).

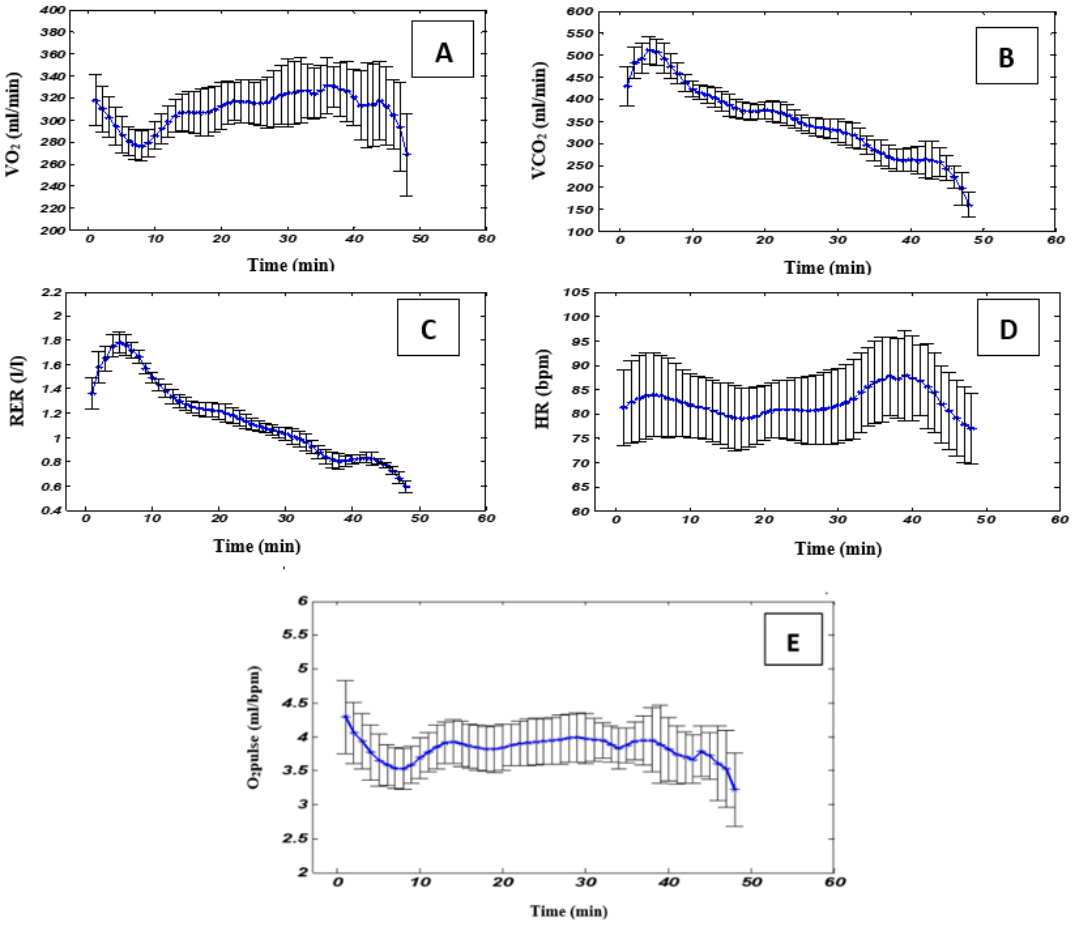

Figure 1: The response pattern of the metabolic- and cardiovascular-related parameters measured during the Rebirthing session (mean± SEM). VO_2 - Oxygen uptake; VCO_2 – Carbon dioxide production/elimination; RER – Respiratory exchange ratio; HR – Heart rate; O2pulse – Oxygen pulse;

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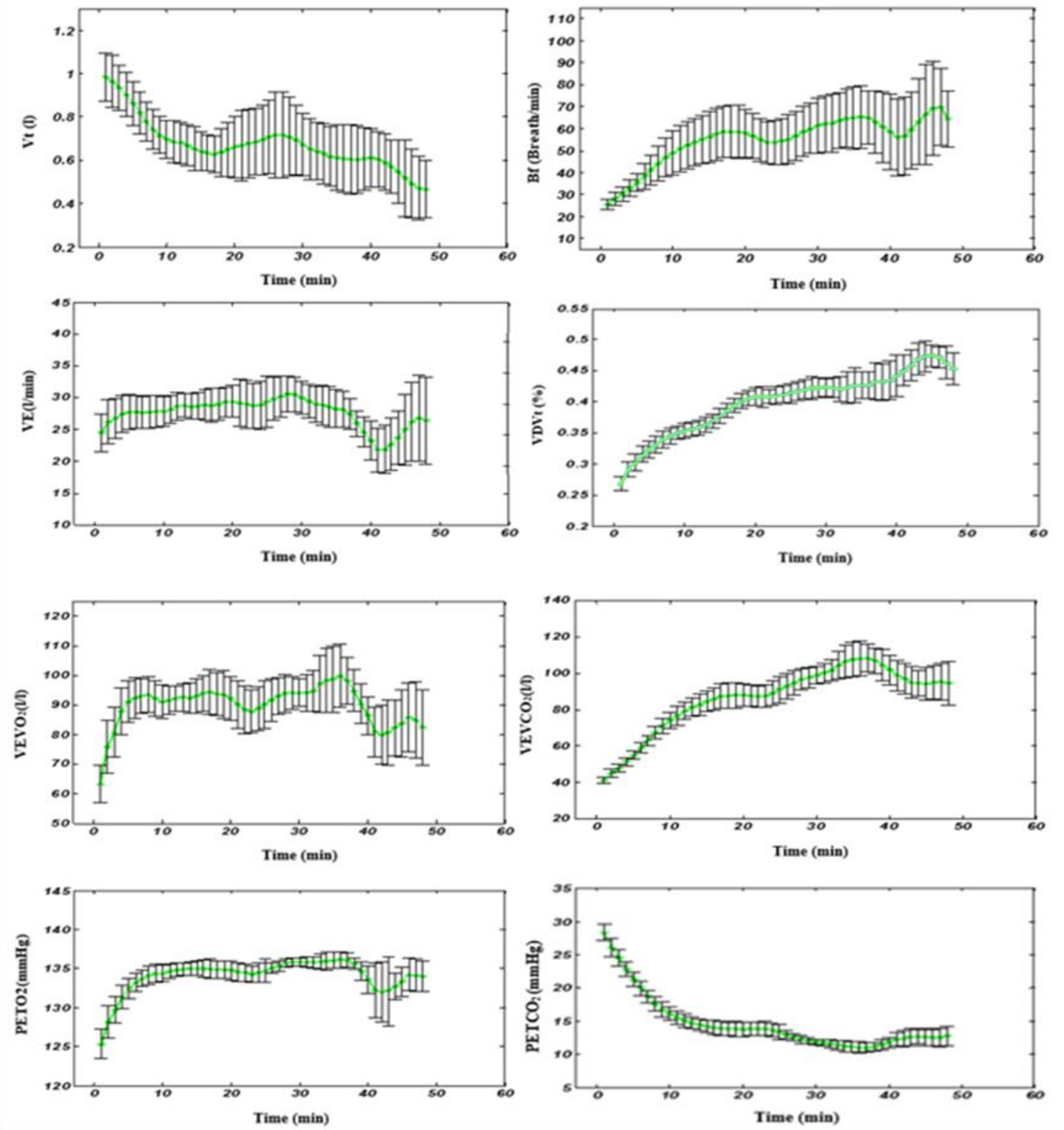

Figure 2: Response patterns of the ventilatory- and gas-exchange-related variables measured during the Rebirthing session (mean± SEM). Vt, tidal volume; Bf, breathing frequency; VE, Minute ventilation; Vd/Vt, Estimated physiologic dead space; VE/VO_2 and VE/VCO_2, Ventilatory equivalents for O_2 and CO_2; $PETO_2$, End-tidal O_2; $PETCO_2$, End-tidal CO_2.

DISCUSSION

Millions of rebirthing advocates worldwide claim that rebirthing therapy helps overcome physical and mental difficulties and improves overall well-being.

This study examined and defined the physiological responses observed during a single Rebirthing session in apparently healthy young women. In general, rebirthing therapy is given in a package, usually of 10–20 sessions.

The main findings of this study corroborate true voluntary hyperventilation (VHV) throughout the Rebirthing session.

True physiologic voluntary hyperventilation (VHV) was implied on the basis of the following measured physiological responses: extremely high ventilatory equivalents (VE/VO_2 and VE/VCO_2) and high and low $PETO_2$ and $PETCO_2$, respectively, throughout a substantial portion of the Rebirthing session (**see figure 2**).

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Furthermore, while the metabolic- and cardiovascular-related responses during the treatment sessions (VO_2, RER, HR, O_2 pulse – see **fig. 1**) match well with the physiological demands of the procedure (breath work), the ventilatory-related responses exhibited exaggerated outcomes, as illustrated by the relatively high session's peak and average values of the depth (Vt), frequency (Bf), and volume (VE) of breathing (**see fig 2**).

In this study, the heightened activation of the respiratory muscles throughout the therapeutic session caused a relatively stable level of oxygen uptake (VO_2) during the session's 40–50 min (session average 320±45 ml/min) (**see fig. 1A**). Since the energy cost of breathing is relatively low (19), it is expected that even with 2 to 3-time resting minute ventilation, the whole-body O_2 consumption will not increase significantly.

As expected from such breathing maneuver, alveolar carbon dioxide levels (VCO_2) showed relatively high values during the first 5 min of the session, followed by a continued and linear drop of the session end (>3-fold drop) (session average 375±53 ml/min) (see fig. 1B). An early increase in expired CO2 was reported previously, suggesting some compensatory mechanisms that help limit the loss of CO_2 from the body's CO2 stores (20, 21), thereby slowing down the alkalotic development.

The exceptionally high VE/VO_2 and VE/VCO_2 values suggest inefficient ventilation and pulmonary gas exchange. Typical (healthy) VE/VO2 values at rest and maximal effort are 30 and 45 l/l, respectively. The respective typical values for VE/VCO_2 are 25 and 40 l/l, respectively (17, 18). Higher than normal resting or exercise values of VE/VO_2 or VE/VCO_2 are typically seen in patients with a gas-exchange abnormality, either at the lungs or at the local (muscle) level, and are often seen in patients being affected by either chronic obstructive lung diseases (COPD) or congestive heart failure (CHF) (17, 21).

Notwithstanding, the heightened ventilatory responses were an obligatory part of the Rebirthing procedure and were voluntarily executed by all participants.

Voluntary hyperventilation is used to expand the boundaries of human adaptation in sports medicine, occupational physiology, aviation, and space medicine (22, 23). The role of hyperventilation in all these conditions is to increase alveolar ventilation and blood oxygen saturation (24). Yet, this is frequently accompanied by several adverse effects, mainly: hypercapnia and alkalosis. However, under conditions of intense physical activity, reduction of partial oxygen pressure in the inhaled air, or the combined effect of these factors, the adverse impact of hyperventilation is largely compensated (17).

Abundant studies have been devoted to studying breathing mechanisms during prolonged voluntary hyperventilation (VHT), mainly during exposure to high-altitude hypoxia or load hypoxia and physical exercise (24, 25). Reported data on this topic (voluntary hyperventilation) is usually limited to only a few minutes of the exposure to hyperventilation (11, 13, 23). However, prolonged hyperventilation in the absence of intense physical activity and under normal O2 partial pressure in the inhaled air in healthy humans remains virtually unexplored [we found only a single published study that matched these criteria (20).

In view of the above, a series of questions arise, the foremost being how could all study participants (and millions of the Rebirthing enthusiasts) "survive" the Rebirthing treatments without reporting any well-known hyperventilation-related adverse effect?

On the one hand, we have overwhelming popularity and positive reports on the effects of rebirthing therapy. However, hyperventilation under "resting" conditions could cause some severe adverse physical and or mental/emotional outcomes.

Considering the notable absence of sufficient scientific research and objective quantitative documentation on the above challenging issue, and in trying to answer this dilemma, the following are some possible tentative considerations/explanations:

One conceivable direction in resolving this puzzling issue (prolong VHV without any adverse effects) could be the body position at which the Rebirthing procedure is being conducted, i.e., recumbent.

It is well known that the loss of consciousness (syncope) results, among other causes, from cerebral hypo-perfusion (26). In an upright position, hypo-perfusion can occur when there is a significant decrease in the amount of blood reaching the heart or the inability of the heart to produce the pressure needed for transporting a sufficient amount of blood to the brain. When lying down, the hydrostatic pressure in the heart and circulation is much lower than during an upright position. Consequently, the ability to eject blood into the brain and other organs requires less energy. Thus, lying supine increases left ventricular filling pressure compared to upright posture (27, 28), causing substantial increases in end-diastolic and stroke volumes in healthy adults (29, 30). Further, Thadani and Parker (28) noted lower heart rates and higher left ventricular filling pressures, stroke indexes, and cardiac indexes in the recumbent position compared with an upright posture.

The above beneficial manifestations of lying prone, similar to those during Rebirthing treatment, may cause, among other changes, an increase in local perfusion pressure, increase in cardiac output, and an increase in cerebral blood flow, despite the partial constrictor effect of the induced hypercapnia on the peripheral and the cerebral vessels, thereby lessening cerebral ischemia/hypoxia (31, 32).

All or some of those mentioned posture-related (lying prone) positive physiological manifestations may, at least, partially explain the unexpected positive outcome of the Rebirthing therapy.

Another rationalization of the unanticipated outcomes of Rebirthing's VHT could be the notion that Hypocapnia is not the solitary cause of cerebral vascular tone. Tercero et al., (32) and Favre et al., (33) showed that cerebral blood flow (CBF) was normal during chronic Hypocapnia, suggesting that CO_2 does not alter the cerebral vascular tone. Kontos et al. (34) probably offered the best evidence that pH rather than CO_2 is the controlling messenger for CO_2-mediated alterations of cerebral vascular tone. By applying artificial cerebrospinal fluid (CSF) topically to the cerebral cortex of

anesthetized cats, they showed that the diameter of cerebral arterioles responded only to changes in pH, regardless of fluid PCO_2.

The above contentions could partially explain defeating the known adverse effects of prolonged voluntary hyperventilation and the positive and pleasant outmoded reported by all study participants and millions of advocates of the rebirthing technique worldwide.

CONCLUSION

As emphasized in the literature, and despite the Rebirthing therapy being practiced and gaining popularity for years, no single published scientific paper directly referring to its physiological aspects, effects, and consequences, was found. Numerous textbooks, guidebooks, and philosophy books have been published about the Rebirthing therapy - all without exception involving solely subjective and prejudiced descriptions and metaphysical explanations about its essence, rationale, physical, mental, emotional, and behavioral effects on healthy and ill participants.

This study looked at the physiological responses during a single Rebirthing session in an objective/scientific manner.

The primary study findings demonstrated that prolonged voluntary hyperventilation (45–50 minutes) causes extremely shallow $PETCO_2$ levels, high $PETO_2$, high RER (relative to resting metabolic requirements), very low VCO_2, and very high levels of ventilatory equivalents (VE/VO_2 and VE/VCO_2). As specified in the discussion, such conditions might have unpleasant and even risky consequences when lasting for a relatively long period (as during a typical Rebirthing session). However, no significant grievances regarding participants' physical and mental/emotional feelings were reported in this study. In the contrast, at the end of all sessions, patients reported calmness, serenity, and, on occasion, some physical lethargy.

This study could not solve the clear divergence between the observed (acute) physiological responses (mainly severe Hypocapnia) and the subjective participants' pleasant emotional state, and in many cases, spiritually uplifting, at the end of each treatment session. Nevertheless, the authors took the liberty to offer some potential mechanisms by which such conflict could be resolved.

Study limitations: As in most studies, there are some limitations to this study. First, the sample size is relatively small, therefore disapproving conclusive inferences.

Second, we measured both $PETO_2$ and $PETCO_2$ as accurate estimates of PaO_2 and $PaCO_2$, which are the actual regulators of CBF. Nevertheless, and since PaO_2 and $PaCO_2$ are not routinely measured (mainly when performed outside of strict medical settings), $PETO_2$ and $PETCO_2$ and the changes in both, at rest, and during exercise, closely approximate the changes in both (17,35).

Third, using a face mask during the Rebirthing sessions may lightly interfere with the usual Rebirthing breathing pattern, affecting the accuracy of physiological responses.

Future directions: Rebirthing's mechanism of action may be explored via psychophysiological measurements concurrent with a clinical trial. Advanced neuroimaging techniques, such as fMRI, may provide a more specific localization of changes in brain activity changes during Rebirthing. To further distinguish between psychological versus direct physiologic effects of Hypocapnia, future studies should examine whether merely prolonged over-breathing, without other aspects of Rebirthing in place, would exert a similar treatment-augmentation impact.

Further, more scientifically oriented studies are needed to determine whether Rebirthing should be a beneficial complementary or alternative treatment for improving well-being and quality of life and common psychiatric disorders.

Using multiple, commonly used outcome measures with well-established psychometric properties and the inclusion of a placebo or wait-list control condition, with random assignment of participants to groups, would significantly enhance the interpretability and validity of findings.

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Conflict of interest: The authors declare no competing interests.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the institutional and/or national research committee's ethical standards and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Body Mass Index as an independent predictor for Mortality and Severe Disease among Patients with COVID-19

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ABSTRACT

Objective: Worldwide studies reported variable death rates and severe disease among patients with COVID-19. The different rate of obesity across countries is one of the main predictors that may explain the diverse rate of COVID outcomes. This study explored the association between body mass index (BMI) and other predictors of COVID-19 severity and mortality.

Methods: We retrospectively reviewed cases with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections. We used univariate and multivariate logistic regression to understand the relationship between patients' characteristics and severe COVID-19 and mortality.

Results: 297 cases (83%) of 354 COVID-19 cases reviewed were symptomatic. 66 (18.6%) were hospitalized, (5.3%) were admitted to the intensive care unit (ICU), and 2.8% (10/354) died. The risk factors associated with mortality were old age (OR 95% CI 1.08[1.0-1.15]; p<0.03) and high BMI (OR 95% CI 9.29[1.92-44.98]; p<0.006). High BMI was also significantly associated with critical disease (OR 95% CI 5.19[2.18-12.38]; P<0.001)

Conclusion: High BMI was the leading independent risk factor associated with symptomatic COVID-19, severe COVID-19, and COVID-19-related mortality. Medical interventions to prevent and treat obesity are urgently needed to reduce covid-19 related mortality.

Keywords: SARS-CoV-2, COVID-19, Coronavirus Disease, 2019-Novel Coronavirus, BMI, Obesity

INTRODUCTION

The novel coronavirus disease 2019 (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus 2 SARS-CoV-2 emerged in Wuhan in China in December 2019. The World Health Organization (WHO) announced the epidemic of COVID-19 as a pandemic in March 2020 (1). The pandemic has killed millions of humans and disturbed global social and financial activities. By the end of May 2022, there were 525 million reported cases and 6 million deaths globally (2). In Saudi Arabia, during the same period, there were 700,000 COVID-19 cases, and the total number of fatalities reached 9000 (3).

Studies have described diverse rates of covid-19 mortality and disease severity across countries, reflecting the role of viral virulence and host chrachteristics (4, 5). Older age, comorbid conditions, and obesity are the main predictors of covid-19 severity and mortality (6.7.8,9). Previous studies have also found the obesity a predictor of severe influenza infection (10,11,12). Data from Saudi Arabia suggest increasing obesity rates (13,14). However, the association of obesity with SARS-CoV-2 infection in Saudi patients is still unclear. This study described the association between obesity and symptomatic SARS-CoV-2 infection. Specifically, we examined the effect of body mass index (BMI) on the development of COVID-19 symptoms, disease severity, and COVID-19-related mortality. Identifying the high-risk group for the severe disease allows for prompt medical intervention and prioritizes medical resources.

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MATERIAL and METHODS

This study was a single-center retrospective observational conducted in National Guard Hospital in Al-Madinah, Saudi Arabia, between April 1 and July 12, 2020. We included Subjects with a positive polymerase chain reaction (PCR) test for SARS-CoV-2Cases and COVID-19 diagnosis, and all data were collected from the hospital's electronic medical records. The institutional review board approved this study on June 1, 2020 (RM20/006/M).

The Abbott Real-time SARS-CoV-2 reverse transcriptase PCR test was used for the qualitative detection of nucleic acid from SARS-CoV-2 from nasopharyngeal (NP) swabs, sputum, or Broncho-alveolar lavage fluid (BAL) nasal swabs. Healthcare workers collected all samples (15).

We defined symptomatic COVID-19 cases as those presented with symptoms of COVID-19 as defined by the Center for Diseases Control (CDC) and WHO and having a positive PCR test for SARS-CoV-2. COVID-19 infection cases were classified as severe if blood oxygen saturation was less than 93% with or without lung infiltrate on chest imaging and critical if one or more of the following were present: acute respiratory distress syndrome (ARDS), sepsis, severe sepsis, septic shock, acute kidney injury (AKI), arrhythmia, myocardial infarction (MI), respiratory failure, disseminated intravascular coagulation and cerebrovascular accident (CVA). We also collected the following variables: gender, sociodemographic, body mass index, smoking, pregnancy (APACHE II), Charlson's comorbidity index, heart failure (HF), Diabetes mellitus (DM), coronary artery disease (CAD), Cerebrovascular accident (CVA), Asthma, Chronic Kidney Disease (CKD), treatment provided during an infection such as antivirals and antimicrobials and home medication were collected the through chart review. Outcomes measured included duration of hospitalization, ICU admission, need for mechanical ventilation, and survival.

We calculated the mean, median, and standard deviation (SD) for continuous variables and the proportions for categorical variables. We used Fisher exact test to compare categorical variables, The student t-test to compare normal-distributed continuous data, and Wilcoxon Rank-Sum to compare continuous non-normal-distributed variables. We included the variables with a significance level of P-value <0.25 from the unadjusted analysis in the multivariable analysis. We used backward stepwise multivariable logistic regression analysis to identify risk factors for severe diseases and mortality. All p values were two-tailed, and values of less than 0.05 were considered statistically significant. Data analysis was performed using STATA version 13 (STATA cooperation, Texas, USA).

RESULTS

From April 1, 2020, to July 12, 2020, 354 COVID-19 cases had a positive PCR test for SARS-CoV-2; we included these cases in this analysis. Of the total cases, 297 (83%) cases presented with COVID-19 symptoms. The most common initial symptoms were fever (67%), cough (55%), sore throat (43%), and fatigue (28%). Of the symptomatic cases, 66 (18.6%) were hospitalized, and of those (5.3%) were subsequently admitted to the intensive care unit (ICU). The median length of stay for the hospitalized cases was 11(5-15) days.

Table 1 shows the baseline demographic and clinical characteristics of symptomatic cases. The median age was 42 (31-58) years, and females represented (52%) of the total cases. The most common chronic comorbidities for the patients were DM (24%), asthma (8%), and CAD (7%). Among hospitalized cases, the most frequent complications observed were septic shock 31(13%), AKI 17 (7%), and respiratory failure 13 (4%). While (4%) cases were treated with Hydroxychloroquine, the most common antivirals used were the combination of Ribavirin plus lopinavir-ritonavir plus interferonbeta1b (8%). In addition, 20% of the cases were treated with antimicrobials for concomitant bacterial pneumonia, and 3% received both convalescent plasma and tocilizumab.

Of the total symptomatic cases, 81 (27%) had severe COVID-19 disease. In unadjusted analysis, the risk factors associated with severe disease included older age (p<0.011), high BMI (BMI over 30 kilogram/meter square) (p<0.001), high Charlson's comorbidity score (p<0.01), and heart failure (p<0.002) (Table 1). In the multivariable analysis, the only factor significantly associated with severe disease was older age (OR 95% CI 2.2[1.2-4.1]; p<0.010).

Of the total cases, 19(6.4%) developed critical diseases (**Table 2**). In unadjusted analysis, the risk factors associated with the critical illness were older age (p<0.001), high BMI (p<0.001), high Charlson's comorbidity score (p<0.001), and high APACHE II score (p<0.001). In multivariable logistic regression analysis, the factors significantly associated with the critical disease were older age (OR 95% CI 1.05[1.00-1.10]; p<0.018) and high BMI (OR 95% CI 5.59[2.32-13.46]; p<0.001). In an additional model which included Charlson's comorbidity index to adjust for chronic comorbidity, the only variable which remained significantly with the critical disease was high BMI (OR 95% CI 5.19[2.18-12.38]; p<0.001)

In this cohort of COVID-19 cases, the median BMI was 29(27-30) kg/m2, and 112(46%) of the subjects had a BMI >30 Kg/m2. Compared to cases with a high BMI (< 30 Kg/m2), subjects with a low BMI (>30 Kg/m2) were older (p<0.029); had a higher mean Charlson's comorbidity index (p<0.032); and were more likely to have CAD (p<0.001), CHF (p<0.001), asthma (p<0.001) and COPD (p<0.027) (**Table 3**). In multivariable logistic regression analysis, the high BMI cases were more likely to have symptomatic COVID-19, critical illness, and higher mortality than those with a low BMI. These findings remained the same after we adjusted for the comorbidities in the model (**Table 4**).

The overall mortality rate was 2.82% (10/354). Most deaths occurred among patients aged 60 years or older, and no death occurred in patients younger than 45 years. All deaths occurred within 60 days of hospitalization; of those (80%) occurred within 30 days of admission. In multivariable logistic regression analysis, risk factors associated with mortality included older age (OR 95% CI 1.08[1.0-1.15]; p<0.03) and high BMI (OR 95% CI 9.29[1.92-44.98]; (p<0.006). Again, when we included Charlson's comorbidity index in the model, the only variable significantly associated with mortality was high BMI (OR 95% CI (8.12[1.6-2 40.54]; p<0.011) (**Table 4**).

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Table 1. Baseline Clinical Characteristics of Severe and Non-Severe COVID-19 Cases from April 1 to July 30, 2020

Characteristics	Total N: 297	Non-severe COVID-19 n / (N %) 216(72.73)	Severe COVID-19 n/ (N %) 81(27.27)	P-value
Age (years)				
Mean	45(16.83)	43(16)	49(17)	0.0111
Median	42(31-58)	41(31-56)	51(33-64)	
Age groups (years)				
> 20	8(3)	3(4)	0	
20-39	122(41)	92(43)	30(37)	0.029
40-59	95(32)	72(33)	23(28)	
>60	71(24)	43(20)	28(35)	
Male	124(48)	98(46)	44(54)	
Female	152(52)	115(54)	37(56)	0.240
Body mass index (kg/m^2)				
Mean	29(3)	27(2)	32(3)	
Median	29(27-30)	28(27-29)	32(31-32)	0.0001
Body mass index <30	161(54)	161(84)	0(0)	0.0001
Body mass index >30	112(46)	31(16)	81(100)	
Smoking	14(5)	10(5)	4(5)	0.999
Pregnancy	6(2)	6(3)	0(0)	0.194
APACHE II score				
Mean	13.7(13)	6.9(5.2)	15.4(14)	0.1193
Median	9.5(5-20)	6.5(2-12)	10(5-23)	
Charlson's comorbidity index				
Mean	1.5(2.4)	1.3(2.1)	2.1(2.8)	0.01
Median	0(0-2)	0(0-2)	1(0-3)	
Heart failure	18(6)	7(3)	11(13)	0.002
Diabetes mellitus	72(24)	56(26)	16(20)	0.290
Coronary artery disease	23(7)	17(8)	6(7)	0.999
Cerebrovascular accident	7(2)	5(2)	2(2)	0.999
Asthma	22(8)	16(8)	6(7)	0.999
Chronic obstructive pulmonary disease	4(1)	2(1)	2(2)	0.303
Chronic Kidney Disease	9(3)	4(2)	5(6)	0.068
Home medication				
Aspirin	47(16)	34(16)	13(16)	0.859
NSAID	7(2)	7(3)	0(0)	0.195
ACEI/ARBs	37(13)	28(13)	9(12)	0.843

Data are presented as mean (SD) or median (IQR), as appropriate, unless otherwise indicated. Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blockers; ARDS, NSAID, nonsteroidal anti-inflammatory drug; BMI, body mass index; Kg/m2, kilogram/meter square; COVID-19, coronavirus disease 2019; P-value significant if <0.05

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Table 2. Baseline Clinical Characteristics of Critical and Non-Critical COVID-19 Cases from April 1 to July 30, 2020

Characteristics	All 296	Non-critical n / (N %) 277(93.58)	Critical n / (N %) 19(6.42)	P-value
Age (years)				
Mean	45(16.8)	44(16)	63(13)	0.0000
Median	42(31-58)	41(31-57)	65(56-74)	
Age groups (years)				
< 20	8(3)	8(3)	0	
20-39	122(41)	121(44)	1(5)	0.000
40-59	95(32)	91(33)	4(21)	
>60	71(24)	57(20)	14(74)	
Male	124(42)	145(53)	12(63)	
Female	152(52)	130(47)	7(37	0.236
Body mass index (kg/m^2)				
Mean	29(3)	29(3)	34(6)	
Median	29(27-30)	29(27-30)	32(32-33)	0.0000
Body mass index <30	160(59)	160(63)	0(0)	0.0000
Body mass index >30	112 (41)	93(37)	19(100)	
Smoking	14(5)	13(5)	1(5)	0.609
Charlson's comorbidity index				
Mean	1.5(2.4)	1.3(2.1	4(3.3)	0.000
Median	0(0-2)	0(0-2)	4(2-8)	
SOFA score (mean)	4.6(5)	2.2(3.1)	9.6(4.3)	0.000
SOFA score (median)	3(0-8)	1(0-4)	9(7.5-12.5)	
APACHE II score				
Mean	13.8(13))	6(4.1)	28.3(212)	0.000
Median	9.5(5-20)	6(2-9)	27(19-33)	
Heart failure	19(6)	0(0)	19(100)	0.000
Diabetes mellitus	72(24)	67(24)	5(26)	0.788
Coronary artery disease	26(8)	7(3)	19(100)	0.000
Cerebrovascular accident	7(2)	5(2)	2(10)	0.068
Asthma	26(9)	7(3)	19(100)	0.000
Chronic Kidney Disease	9(3)	4(2)	5(56)	0.000
Home medication				
Aspirin	47(16)	40(15)	7(37)	0.859
NSAID	7(2)	7(3)	0(0)	0.999
ACEI/ARBs	37(13)	32(11)	5(26)	0.052

Data are presented as mean (SD) or median (IQR), as appropriate, unless otherwise indicated. Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blockers; ARDS, NSAID, nonsteroidal anti-inflammatory drug; BMI, body mass index; COVID-19, coronavirus disease 2019; Kg/m2, kilogram/meter square; Sofa, Sequential Organ Failure Assessment; APACHE II score, acute Physiologic Assessment and Chronic Health Evaluation score; P-value significant if <0.05

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Table 3. Baseline Clinical Characteristics of Obese and non-Obese COVID-19 Cases from April 1 to July 30, 2020

Characteristics	ALL 272	Non-Obese n/ (N %) 160(59)	Obese n/ (N %) 112(41)	P-value
Age (years)				
Mean	29(3)	44(16)	49(18)	
Median	29(27-31)	41(31-55)	50(33-63)	0.029
Age groups				
< 20	6(2)	5(3)	1(1)	0.076
20-39	108(40)	67(42)	41(37)	
40-59	89(33)	56(35)	33(29)	
>60	69(25)	32(20)	37(33)	
Male	130(48)	74(47)	56(50)	0.623
Female	140(52)	84(53)	56(50)	
Smoking	12(4.5)	7(4.43)	5(4.63)	0.581
Comorbidity				
Heart failure	19(7)	0	19(16)	0.001
Coronary Artery Disease	26(10)	0	26(23)	0.001
Chronic Kidney Disease	9(3)	4(3)	5(4)	0.495
Asthma	26(10)	0	26(23)	0.000
COPD	4(1)	0	4(4)	0.027
Diabetes mellitus	68(25)	42(26)	26(23)	0.572
Cerebrovascular accident	7(3)	5(3)	2(2)	0.703
Charlson's comorbidity index				
Mean (DS)	1.5(2.4)	1.43(2.3)	1.9(2.6)	0.032
Median (IQR)	0(0-2)	0(0-2)	1(0-3)	
APACHE II score (Median, IQR)	9.5(5-20)	7(3-12)	10(5-23)	0.235
Outcomes				
Mortality	10(3.6)	0	10(9)	0.001
Critical COVID-19	19(7)	0	19(17)	
Severe COVID-19	81(30)	0	81(72)	0.001
Home medication				
Aspirin	45(17)	29(18)	16(15)	0.617
ACEI/ARBs	36(13)	21(13)	15(14)	0.855

Data are presented as mean (SD) or median (IQR), as appropriate unless otherwise indicated; n of all patients (%) or a proportion of subset, n/N (%); ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blockers; BMI, body mass index; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019.Obese BMI >30 Kg/m2, kilogram/meter square; Non-Obese BMI <30 Kg/m2; P-value significant if <0.05

Table 4. Multivariable Analysis of the Risk Factors for Mortality, Critical Illness, and Symptomatic COVID-19, with and without Adjustment for Charlson's Comorbidity Score

Outcomes	Risk factors	AOR	95% CI	P-value	AOR	95% CI	P-value
COVID-19 symptoms	**BMI**	1.23	1.10-1.37	0.001	1.23	1.10-1.37	0.001
	Age	0.02	1.00-1.05	0.012	1.02	0.99-1.05	0.115
	Charlson's comorbidity	-	-	-	1.02	0.99-1.05	0.522
COVID-19 critical illness	**BMI**	5.59	2.32-13.46	0.001	5.19	2.18-12.38	0.001
	Age	1.05	1.00-1.101	0.018	1.01	0.96-1.08	0.583
	Charlson's comorbidity	-	-	-	1.31	0.92- 1.86	0.129
COVID-19 mortality	**BMI**	9.29	1.92-44.98	0.006	8.12	1.62-40.54	0.011
	Age	1.08	1.00-1.15	0.03	1.05	0.96-1.15	0.251
	Charlson's comorbidity	-	-	-	1.18	0.96-1.16	0.439

BMI, body mass index; Kg/m^2, kilogram/meter square AOR, adjusted odds ratio; CI, confidence interval; COVID-19, coronavirus disease 2019; P value significant if <0.05, Age in Years

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DISCUSSION

Here we described the clinical characteristics and risk factors associated with COVID-19 severity and mortality, and we examined the effect of obesity on COVID-19 outcomes. Of all cases reviewed (83%) were symptomatic, (18%) were hospitalized, (14%) had severe diseases, and (5%) were admitted to the ICU. The above rates are comparable to the rates of symptomatic and severe COVID-19 reported from China during the early periods of this pandemic.4 The reported rates of COVID-19 severity and mortality were variable between countries. These differences have been attributed to the demographic characteristics and prevalence of chronic comorbidities in these countries (16).

In this study, about one-third of the symptomatic cases developed severe COVID-19. Older age and obesity were the main predictors for severe and critical COVID-19 disease. Besides, our data suggest a trend toward an association between severe COVID-19 and pre-existing heart failure and CKD. Many studies demonstrated a similar association between age and obesity with COVID-19 severity (17,18,19,20). Older age and obesity have also been shown to be associated with severe influenza infection (21).

This study's mortality rate was 2.8%, comparable to the mortality rates reported in most other countries. Here, we found the main risk factors associated with mortality were older age and high BMI. Several studies also found an association between mortality and these risk factors (18). Many mechanisms may explain the differential risk of old age in COVID-19 mortality, including age-dependent defects in the immune response to viral infections and the presence of comorbidities. We found no association between age and mortality after adjusting for comorbidities. This suggests that the effect of age on mortality could be due to comorbidities. Many studies that have found an association between older age and mortality also reported higher rates of comorbidities among elderly patients (22).

Our data suggest that obesity is a significant risk for symptomatic COVID-19, severe COVID-19 disease, and mortality. Many recent studies reported similar findings (23,24). Alarmingly, 46% of the COVID-19 cases in our cohort cases were obese. Our high rate of obesity in our institution aligns with the prevalence of Obesity in the country (25) In this study, high BMI cases were older and had more comorbidities than cases with low BMI. However, our data suggest that high BMI was an independent predictor of COVID-19 severity and mortality. The association between high BMI and poor clinical outcomes in the H1N1 influenza infection supports our findings (26). A few mechanisms may explain the association between obesity and COVID-19 severity. Obesity causes a state of chronic inflammation and produces cytokines that increase cytokine storm risk in severe COVID-19 cases (27,28). Nonetheless, the exact mechanism of this association remains unclear as many studies showed that obesity increases the risk of death from any critical illness (29,30).

Our study findings have many implications. We described the number of COVID-19 cases that required hospital and ICU admissions, which projects hospital resources needed in this pandemic. Risk stratification of severe COVID-19 patients using age and BMI may facilitate early medical intervention to improve outcomes, prioritizing the use of the medical resource and early decisions for care goals. High BMI is a modifiable risk, and weight reduction should be encouraged to improve baseline population health.

Our study has several limitations. Due to the relatively small sample size and low rate of deaths in our cohort cases, we could not identify cardiovascular diseases, DM, and CKD as risk factors for COVID-19 mortality, which other studies have shown. However, we found a trend toward this association in the univariate analysis. Since this is a retrospective study, we could not obtain all baseline clinical characteristics and complete follow-up outcomes. Nevertheless, most of our patients seek medical care in the same healthcare system linked to electronic medical records.

CONCLUSION

This is the first study to describe the clinical characteristics, severity, and mortality of COVID-19 cases in Medina in Saudi Arabia. High BMI was an independent risk factor associated with symptomatic COVID-19, severe COVID-19, and death. Therefore, interventions to prevent and treat Obesity may reduce COVID-19 severity and related mortality.

Author Contributions: Authors testify that all persons designated as authors qualify for authorship and have checked the article for plagiarism. In addition, all authors have reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

Z.G: Designed the study, organized, analyzed, and interpreted data, wrote the initial and final draft of the article, and provided logistic support.

JA: Designed the study, collected the data, wrote the initial and final draft of the article

A.A: Collected and organized the data, wrote the initial and final draft of the article

A.O: Collected and organized the data, wrote the initial and final draft of the article

B.A: Organized the data, wrote the paper's initial and final draft, and provided logistic support.

M.A. Organized the data, wrote the initial and final draft of the article, and provided logistic support.

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Conflict of interest: The authors declare no competing interests.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the institutional and/or national research committee's ethical standards and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was approved by King Abdullah International Research Center on June 1, 2020, with the registration number (RM20/006/M).

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The Value of the C-Reactive Protein/Albumin and Fibrinogen/Albumin Ratios in Predicting Disease Severity and Mortality in Elderly COVID-19 Patients

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ABSTRACT

Objective: Due to the high mortality levels associated with the novel coronavirus, reliable predictors for determining disease mortality and severity are needed to permit the careful allocation of health services and for earlier clinical intervention and follow-up. The purpose of this study was to determine the predictive value of the C-reactive protein (CRP)/albumin ratio (CAR) and the fibrinogen/albumin ratio (FAR) in determining mortality and evaluate correlations between these values and thoracic computed tomography (CT) findings.

Material and Methods: COVID-19 patients aged over 65 presenting to the emergency department of a tertiary training and research hospital between Oct 15, 2021, and Jan 15, 2022, were examined in this single-center, retrospective study. The study population was established based on inclusion and exclusion criteria. The patients' mortality status and pulmonary involvement percentages were compared with their laboratory parameters.

Results: The relationships between patients' CAR and FAR values and mortality and disease severity were investigated. Cut-off points of 3.0 for CAR (AUC 0.767, sensitivity 76.5% and specificity 70.1%) and 14.4 for FAR (AUC 0.731, sensitivity 75.0% and specificity 69.0%) were determined for the prediction of mortality. In terms of prediction of disease severity, cut-off points were 4.2 for CAR (AUC 0.786, sensitivity 73.7%, and specificity 75.2%) and 15.2 for FAR (AUC 0.789, sensitivity 84.2%, and specificity 69.6%).

Conclusion: Based on our study findings, CAR and FAR values may be useful in the early differentiation of mortality and pulmonary parenchymal involvement in elderly COVID-19 patients.

Key words: COVID-19, C-reactive protein/albumin ratio, Fibrinogen/albumin ratio

INTRODUCTION

With the rapid global spread of the COVID-19 pandemic declared by the World Health Organization (WHO) on Mar 11, 2020, countries began adopting strict precautionary measures in their health systems (1,2). COVID-19 exhibits a wide clinical manifestation, from asymptomatic infection to mild or severe viral pneumonia or fatal respiratory failure (3). It is assumed that the course of the disease may be more severe in elderly patients or those with previously known chronic diseases (4). Due to its high mortality rates, early diagnosis and prompt treatment are essential in terms of preventing the progression of the disease. It is, therefore, necessary to identify rapid, easily accessible, low-cost, and reliable parameters for predicting disease mortality. Several studies have recommended various laboratory parameters as predictive markers of the severity of COVID-19 (3-6). Also, chest CT imaging is a valuable instrument for diagnosing COVID-19 infection (7).

Studies have suggested that high levels of such inflammatory markers as C-reactive protein (CRP) are associated with disease severity in patients with COVID-19. Albumin, a negative acute phase reactant, has long been used as a marker of nutritional status. However, recent studies have shown that low albumin levels are associated with severe COVID-19 disease (6,8). The CRP/albumin ratio (CAR) has the potential to reflect the patient's inflammatory status. It has recently been described as a prognostic biomarker in several inflammatory conditions and disorders (6,9). Fibrinogen is a positive acute-phase reactant released from the liver that plays an active role in coagulation cascade and inflammation. Recent studies have shown that the fibrinogen-albumin ratio (FAR) is associated with poor clinical outcomes in diseases characterized by inflammation and thrombosis. Moreover, studies have also touched on the importance of FAR in predicting the severity of disease in COVID-19 (10,11).

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We think that CAR and FAR calculated from albumin, CRP, and fibrinogen levels measured among routine laboratory parameters may constitute predictive markers of disease severity in patients with COVID-19. Based on that hypothesis, this study aimed to determine the prognostic value of CAR and FAR in assessing mortality in elderly COVID-19 patients and evaluate the correlations between these values and thoracic computed tomography (CT) findings.

MATERIAL and METHODS

Study Design: COVID-19 patients aged over 65 presenting to the emergency department of a tertiary training and research hospital between Oct 15, 2021, and Jan 15, 2022, were examined in this single-center, retrospective study. Approval was obtained before commencement from the local ethical committee (decision no. 2022/79).

Diagnosis of COVID-19 was confirmed with a positive PCR result from nasopharyngeal swab specimens. All patients aged over 65 with established COVID-19 disease and not meeting the exclusion criteria were enrolled in the study. Patients transferred to another institution, rejecting diagnosis and treatment, with deficient laboratory and tomographic images at the time of presentation to the emergency department, trauma patients, patients with known connective tissue disease, hematological disease, liver function disorder, or thyroid disease, terminal stage cancer patients, and patients who had received albumin transfusion prior to treatment were excluded.

Study Protocol: The demographic characteristics of the patients included in the study and laboratory data (CRP [mg/ll], fibrinogen [mg/dl], and albumin [g/dl]) at the time of presentation to the emergency department and in the subsequent first 24 hours were retrieved from the hospital's digital archive. Thoracic CT findings recorded at the initial presentation were evaluated. Thoracic CT examinations were based on involvement percentages, with cases with pulmonary parenchymal involvement of 0-50% being regarded as mild-moderate and those with involvement exceeding 50% as severe. Patients' lengths of hospital stay, transfer to the intensive care unit requirements, mortality, and discharges were also subjected to analysis.

Whether CAR and FAR obtained within 24 hours after presentation to the emergency department can determine mortality and the severity of lung parenchymal involvement in elderly patients diagnosed with COVID-19 were adopted as the study endpoints.

Statistical Analysis: All analyses were performed on Jamovi v.1.6 statistical software (The Jamovi Project (2021) Computer Software, version 1.6. Sydney, Australia). Categorical data were expressed in frequency (n) and percentage. Normally distributed continuous variable data were described as mean plus standard deviation (SD), and non-normally distributed data as median and interquartile range (IQR). Normality of distribution was evaluated using the Shapiro-Wilk test. Two-group comparisons were performed using Student's t-test or the Mann-Whitney U test, depending on whether or not the data were normally distributed. P values <0.05 were regarded as statistically significant. A receiver operating characteristic (ROC) curve was drawn to determine cut-off points for mortality for CAR and FAR. Finally, sensitivity, specificity, likelihood ratios (+LR and -LR), positive predictive values, and negative predictive values were also calculated for CAR and FAR.

RESULTS

Two hundred fifty-two patients were included in the study, of whom 107 (42.5%) were men and 145 (57.5%) were women. The patients' median age was 77 (IQR 70-83). The mortality rate was 27.0% (n=68). Investigation of thoracic CT findings revealed severe pulmonary involvement in 15.1% of patients (n=38). Patients' demographic data and lengths of hospital stay under the study protocol is shown in **Table 1**.

Patients' laboratory values were calculated using the values at the time of initial presentation (**Table 2**). Examination of the laboratory parameters revealed that neutrophil (p=0.014), lymphocyte (p=0.001), and platelet (p=0.003) counts, CRP (p=0.001), Troponin T (p=0.001), D-dimer (p=0.002), fibrinogen (p=0.001), ferritin (p=0.001), CAR (p=0.001) and FAR (p=0.001) were statistically significant in differentiating patients with and without mortality. Comparison of laboratory findings and thoracic CT involvement identified WBC (p=0.005), neutrophil count (p=0.001), CRP (p=0.001), Troponin T (p=0.001), D-dimer (p=0.001), fibrinogen (p=0.001), ferritin (0.001), CAR (P=0.001), and FAR (P=0.001) values as significant in differentiating patients with severe involvement from those with mild-moderate involvement.

The relationship between CAR and FAR values and patients with and without mortality was also investigated. The relationship between CAR and FAR and patients with and without severe tomographic involvement was also assessed. The prognostic value of CAR and FAR is shown in Table 2. ROC analysis was performed, and the results are shown in **Figures 1A, 1B, 2A, and 2B**

CAR and FAR values were higher in the group with mortality than in the non-fatal group. CAR values in the groups with and without mortality were 4.6 (IQR 3.1-6.2) and 1.8 (IQR 0.5-3.7), respectively (p =0.001). FAR values in the groups with and without mortality were 16.6 (IQR 14.4-21.7) and 12.8 (IQR 10.3-15.4), respectively (p=0.001). Area under the curve (AUC) analysis for the prediction of mortality revealed a value of 0.767 for CAR (95% confidence interval: 0.705-0.829, p =0.001). The cut-off value for mortality of CAR was 3.0, exhibiting 76.5% sensitivity and 70.1% specificity. The AUC value for FAR was 0.731 (95% confidence interval; 0.659-0.802, p=0.001) and the cut-off value for FAR was 14.4, exhibiting 75.0% sensitivity and 69.0% specificity.

Patients' CAR and FAR values were also higher in the group with severe tomographic involvement than in the group without (p=0.001 for both). AUC analysis for predicting severe tomographic involvement revealed an AUC value for CAR of 0.786 (95% confidence interval; 0.714-0.859, p=0.001), and of 0.789 for FAR (95% confidence interval; 0.716-0.862, p=0.001). The detection of patients with severe tomographic involvement with cut-off values of 4.2 for CAR (73.7% sensitivity and 75.2% specificity) and 15.2 for FAR (84.2% sensitivity and 69.6% specificity) confirms these as a powerful predictive factor (**Table 3**).

Table 1 - The Patients' Demographic Data and Lengths of Hospital Stay

		According to Mortality		According to Tomographic Involvement	
		No Mortality (n=184)	Mortality (n=68)	Mild-Moderate Involvement (n=214)	Severe Involvement (n=38)
Gender					
Male	107 (42.5%)	69 (27.4%)	38 (15.1%)	89 (35.4%)	18 (7.1%)
Female	145 (57.5%)	115 (45.6%)	30 (11.9%)	125 (49.6%)	20 (7.9%)
Age (Year	77 (IQR 70-83)	76 (IQR 69.8-82.3)	78.5 (IQR 71.0-83.3)	76 (IQR 70-83)	79 (IQR 73.5-82.8)
Length of Hospitalization (Days)	6 (IQR 0-11)	4.5 (IQR 0-11)	8 (IQR 3-14)	6 (IQR 0-11)	8.5 (IQR 3-18.8)

IQR: *Interquartile Range,* ***Note:*** *Normally distributed data are expressed as Mean ± SD (Min.-Max.), Abnormally distributed data as Median (IQR 25-75)*

Table 2 - Patients' Laboratory Indices

		According to Mortality			According to Tomographic Involvement		
		No Mortality (n=184)	Mortality (n=68)	P Value	Mild-Moderate Involvement (n=214)	Severe Involvement (n=38)	P Value
WBC (10^3/ul)	7.0 (IQR 5.4-9.0)	6.9 (IQR 5.5-8.6)	7.8 (IQR 4.6-11.3)	0.103	6.9 (IQR 5.3-8.7)	8.6 (IQR 6.6-11.0)	**0.005**
Neutrophil (10^3/ul)	5 (IQR 3.6-7.3)	4.9 (IQR 3.6-6.4)	5.9 (IQR 3.6-9.6)	**0.014**	4.9 (IQR 3.4-6.4)	6.6 (IQR 5.0-9.2)	**0.001**
Lymphocyte (10^3/ul)	1.1 (IQR 0.8-1.7)	1.2 (IQR 0.8-1.8)	0.8 (IQR 0.6-1.3)	**0.001**	1.1 (IQR 0.8-1.7)	0.9 (IQR 0.5-1.3)	0.094
Platelet (10^3/ul)	186 (IQR 142-245)	196 (IQR 152-255)	165 (IQR 118-212)	**0.003**	187 (IQR 143-247)	184 (138-227)	0.388
CRP (mg/l)	93.0 (IQR 24.8-157)	64 (IQR 18-122)	151 (IQR 96.9-195)	**0.001**	74 (IQR 20.9-129)	167 (IQR 117-210)	**0.001**
Troponin T (ng/l)	14.0 (IQR 5.9-35.7)	10 (IQR4.6-21.3)	35.3 (IQR 17.9-111)	**0.001**	12.9 (IQR 5.2-29.5)	34.5 (IQR 19.0-95.3)	**0.001**
D-Dimer (µg/ml)	0.6 (IQR 0.3-1.1)	0.5 (IQR 0.3-0.9)	0.9 (IQR 0.5-2.0)	**0.002**	0.5 (IQR 0.3-0.9)	1.1 (IQR 0.5-3.2)	**0.001**
Fibrinogen (mg/dl)	490 (IQR 398-596)	478 (IQR 385-554)	560 (IQR 453-660)	**0.001**	478 (IQR 388-569)	577 (IQR 503-692)	**0.001**
Ferritin (ng/ml)	481 (IQR 270-1088)	390 (IQR 213-834)	928 (IQR 394-1570)	**0.001**	437 (IQR 256-927)	1026 (IQR 458-1560)	**0.001**
CAR	2.5 (IQR 0.7-4.7)	1.8 (IQR 0.5-3.7)	4.6 (IQR 3.1-6.2)	**0.001**	2.0 (IQR 0.5-4.1)	5.1 (IQR 3.7-7.1)	**0.001**
FAR	13.5 (IQR 11-17.6)	12.8 (IQR 10.3-15.4)	16.6 (IQR 14.4-21.7)	**0.001**	12.9 (IQR 10.5-16.3)	18.3 (IQR 15.6-22.1)	**0.001**

WBC: *White Blood Cell,* ***CRP****: C-reactive protein,* ***IQR:*** *Interquartile Range,* **CAR**: C-reactive Protein Albumin Ratio, **FAR**: Fibrinogen Albumin Ratio
Note: *Normally distributed data are expressed as Mean ± SD (Min.-Max.), Abnormally distributed data as Median (IQR 25-75)*
Note 2: *Student t-test was used for normally distributed data, Mann Whitney U Testi was used for abnormally distributed data.*

Table 3 - The Cutoff Value of CAR and FAR for Mortality and Severe Tomography Involvement

	CAR for Mortality	CAR for Severe Tomography Involvement	FAR for Mortality	FAR for Severe Tomography Involvement
AUC ± SD	0.767 ± 0.03	0.786 ± 0.04	0.731 ± 0.04	0.789 ± 0.04
95% CI	0.705-0.829	0.714-0.859	0.659-0.802	0.716-0.862
Cut-off	3.0	4.2	14.4	15.2
Sensitivity (%)	76.5 (64.6-85.9)	73.7 (56.8-86.6)	75.0 (63.0-84.7)	84.2 (68.8-94.0)
Specificity (%)	70.1 (62.9-76.6)	75.2 (68.9-80.9)	69.0 (61.8-75.6)	69.6 (63.0-75.7)
+ LR	2.56	2.98	2.42	2.77
- LR	0.34	0.35	0.36	0.23
PPV (%)	48.6 (42.2-55.0)	34.6 (28.1-41.7)	47.2 (40.9-53.6)	33.0 (27.8-38.6)
NPV (%)	89.0 (83.9-92.6)	94.2 (90.4-96.5)	88.2 (83.0-91.9)	96.1 (92.2-98.1)
Accuracy (%)	71.83 (83.9-92.6)	75.0 (69.2-80.2)	70.6 (64.6-76.2)	71.8 (65.8-77.3)
P Value	0.001	0.001	0.001	0.001

CAR: C-reactive Protein Albumin Ratio, **FAR**: Fibrinogen Albumin Ratio**,** ***AUC:*** *Area Under the Curve,* ***SD:*** *Standard Deviation,* ***LR:*** *Likelihood Ratio,*
PPV: *Positive Predictive Value,* ***NPV:*** *Negative Predictive Value,* ***CI:*** *Confidence Interval*

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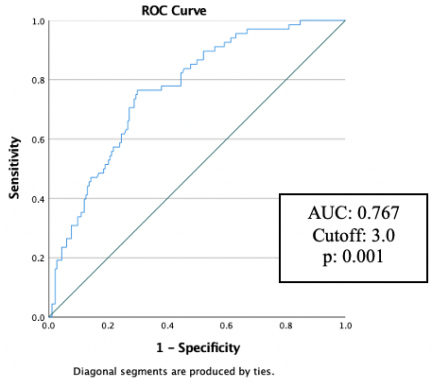
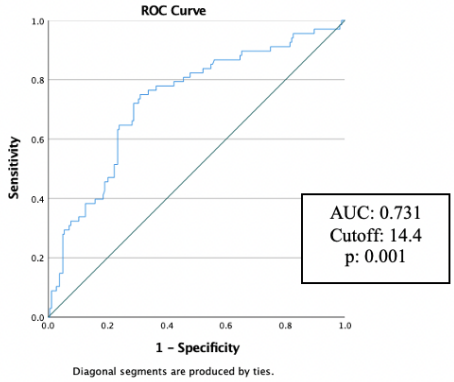
Figure 1A, 1B, 2A, 2B - ROC Curve and Cutoff Value of CAR and FAR at Mortality and Severe Tomography Involvement

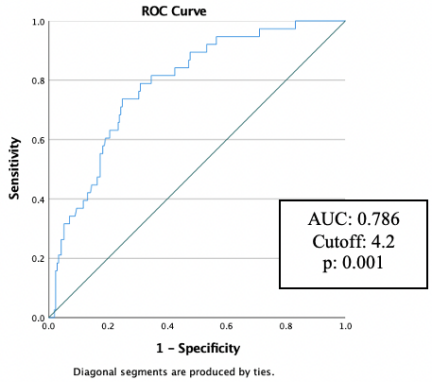
1A- CRP Albumin Ratio for Mortality



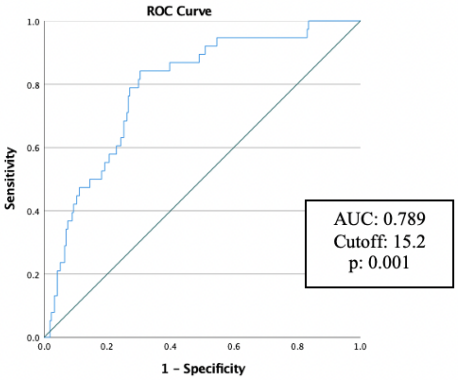
1B- Fibrinogen Albumin Ratio for Mortality



2A- CRP Albumin Ratio for Severe Tomography Involvement



2B- Fibrinogen Albumin Ratio for Severe Tomography Involvement



DISCUSSION

COVID-19 disease remains a significant public health problem with high in-hospital mortality (4). High levels of resources are set aside for combatting this disease in the provision of inpatient health services. Prudent use of healthcare resources, early intervention, and reliable markers for predicting disease severity and mortality and determining clinical outcomes are therefore needed (5).

The present study investigated the relationship between CAR and FAR and severe tomographic pulmonary involvement and mortality in patients with COVID-19. The results showed that CAR and FAR at laboratory examinations in the first 24 hours after presentation to the emergency department could be used as markers of disease severity (CAR p=0.001, FAR p=0.001). ROC analysis was applied to evaluate CAR and FAR in the prediction of disease severity. We determined AUC values of 0.786 for CAR and 0.789 for FAR. Our cut-off point for CAR at 73.7% sensitivity and 75.2% specificity was 4.2, and the cut-off point for FAR at 84.2% sensitivity and 69.6% specificity was 15.2. ROC analysis was also applied to determine the prognostic value of CAR and FAR in terms of mortality in COVID-19 infection. We decided on AUC values of 0.767 for CAR and 0.731 for FAR.

Our cut-off point for CAR, with 76.5% sensitivity and 70.1% specificity, was 3.0, and the cut-off point for FAR, with 75.0% sensitivity and 69.0% specificity, was 14.4. Kalyon et al. investigated the association between the neutrophil-lymphocyte ratio (NLR) and CAR and length of hospital stay in mortality in elderly patients with COVID-19. They reported that NLR and CAR calculated at the initial presentation were helpful in predicting the length of hospital stay and the risk of mortality in those patients (12). Li et al. investigated whether or not CAR values are helpful in terms of risk classification in COVID-19 patients and found that CAR may represent an excellent prognostic biomarker for risk classification in severe COVID-19 cases (13).

Several studies have mentioned the prognostic importance of CAR and FAR in terms of mortality and disease severity in patients with COVID-19. Kalabin et al. investigated the prognostic value of CAR in terms of disease severity in COVID-19 and found that it can be employed as a prognostic factor for disease severity (6). Torun et al. investigated the prognostic value of CAR, FAR, and NLR in determining the severity of disease in COVID-19 and found that these were capable of use for predicting severity. They calculated AUC values of 0.841 for CAR, 0.737 for FAR, and 0.802 for NLR at ROC analysis (14).

Ayrancı et al. investigated the potential for the use of NLR and CAR as markers of mortality in elderly COVID-19 patients and found that both were capable if used as prognostic factors for mortality (15). In another study, Küçükceran et al. investigated whether the D-Dimer-albumin ratio (DAR) and FAR constituted a mortality marker in COVID-19 and found that both were capable of use as a prognostic marker of mortality in COVID-19. Those authors calculated AUC values of 0.773 for DAR and 0.703 for FAR at ROC analysis (3). Various positive and negative biomarkers change with the resulting inflammatory response in infectious diseases. Markers that exhibit a positive change in the inflammatory response are known as positive acute-phase proteins and those that show a negative change as negative acute-phase proteins (16). Examples of positive acute-phase proteins include CRP and fibrinogen, while albumin is a negative acute-phase protein (17,18). Since, apart from the acute phase response, these markers may also increase and decrease due to secondary causes, we hypothesized that their combined use would yield more accurate results than using them alone.

Limitations: There are a number of limitations to this study. In particular, the research was small in scope and single-center, and retrospective in nature. Further studies with more significant numbers of patients and more centers are therefore needed. In addition, similarly to other retrospective studies, there was concern over the possibility of selection bias. However, in order to eliminate that concern, we included all elderly patients presenting to our emergency department during the study period and diagnosed with COVID-19. Further prospective studies are needed to confirm our findings.

CONCLUSION

Based on our findings, we think that CAR and FAR values may be helpful in the early differentiation of mortality and severity of pulmonary parenchymal involvement in elderly COVID-19 patients. We, therefore, think that high values for CAR and FAR, rapid, inexpensive, reproducible inflammatory biomarkers, will attract greater attention in early treatment plans.

Author Contributions: Initials of the contributing authors were listed in brackets after the relevant parts of the research: Literature search (GA, MMY, EN), study design (GA, MMY, AA), legislative applications (GA, MMY), data collection (MMY, EN), supervision and quality control (GA, AA), statistical data analysis (MMY, AA), data interpretation (GA, MMY, EN), drafting the manuscript (MMY, GA). All authors were involved in the writing and critical revision of the manuscript and approved the final version. GA and MMY take the whole responsibility for the paper.

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Conflict of interest: The authors declare no competing interests.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the institutional and/or national research committee's ethical standards and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Treatment of Chorioamnionitis with Piperacillin/Tazobactam and Clindamycin

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ABSTRACT

Objective: Chorioamnionitis is a condition that bacteria infects the chorion and amnion and the amniotic fluid which can affect pregnant women. Infections may affect both the mother and fetus. Cervical insufficiency and chorioamnionitis cause preterm birth and perinatal morbidities.

Case: A 37-year-old patient was referred to the clinic with the diagnosis of preterm labor and cervical insufficiency at 26 weeks + 4 days of pregnancy. Cervix was 3 cm dilated, effaced 70%, the amnion membrane intact, and there was a cerclage thread on the cervix. There was a single, viable fetus in the uterine cavity with fetal measurements compatible with 27-28 weeks on ultrasonography. Ampicillin and azithromycin treatments were started. On the seventh day of observation, leukocytes and C reactive protein values had increased, and there was an onset of serohemorrhagic vaginal discharge. Thereupon, intravenous treatment of piperacillin/tazobactam and clindamycin started. Signs of chorioamnionitis were resolved. The patient was delivered on day 38 of admission due to vaginal bleeding. The newborn and patient outcomes were good. Further studies are needed to evaluate the new treatment regime's efficacy in chorioamnionitis treatment.

Keywords: Cervical Insufficiency, Chorioamnionitis, Clindamycin, Piperacillin/Tazobactam, Preterm delivery

INTRODUCTION

Cervical insufficiency is defined as spontaneous softening, shortening, and opening of the uterine cervix without reason, causing miscarriage or preterm labor in the second trimester (1, 2). Occurrence is about 1% in pregnant women and is more prevalent in pregnancies with recurrent second-trimester pregnancy loss (1, 2).

Cervical mucus has an anatomical and preventive function against the development of ascending infection during pregnancy (3). Preterm premature rupture of membranes (PPROM) or cervical insufficiency results in loss of the cervical mucus plug and allows microorganisms to reach the uterus and fetus. That leads to the development of chorioamnionitis, currently termed inflammation or infection or both or Triple I. When cervical insufficiency or PPROM develops, antibiotic therapy is routinely administered to prevent the development of chorioamnionitis. When chorioamnionitis is evident, birth is necessitated.

In the presented case, routine antibiotic treatment was administered in patients with cervical dilatation due to cervical insufficiency. After one week of routine initial antibiotic therapy, chorioamnionitis findings, such as leukocytes and C reactive protein (CRP) values, had increased. These increases were attributed to an early sign of chorioamnionitis. Thereupon, two different antibiotics were administered together. Chorioamnionitis findings regressed during the antibiotic treatment. Birth took place one month after the new antibiotic treatment. This case report aims to present a new antibiotic administration in patients with chorioamnionitis and to pioneer new studies on this antibiotic regime.

CASE

A 37 year-old patient was referred to my clinic with a diagnosis of preterm labor and cervical insufficiency at 26 weeks and 4 days of pregnancy. The previous day, the patient presented at the hospital due to pelvic pain. She was admitted with a diagnosis of preterm delivery and cervical insufficiency. A tocolytic and steroid therapy was administered. One day later, she was referred to my clinic. Upon speculum examination, a cervical cerclage thread was observed on the cervix.

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The amnion membrane was intact, not prolapsed into the vagina. Bimanual examination revealed that cervical dilatation was 3 cm with effacement (70%). The cervical cerclage thread was not removed to prevent sudden cervical dilatation. Upon obstetric ultrasonography evaluation, there was a single, viable fetus in the uterine cavity with fetal measurements compatible with 27-28 weeks. The amniotic fluid volume was in the normal range. There was no uterine contraction as evaluated by a nonstress test (NST). Magnesium sulfate was administered for its neuroprotective effects. The standard clinical routine treatment of ampicillin (4x1 g IV over seven days) and azithromycin (1000 g oral single dose) was started to prevent the development of chorioamnionitis. The leukocytes count of patient was 16.7 x 103/mm3 on admission.

The pregnancy history of the patient was as follows: her first pregnancy occurred after ovulation induction. Labor started in the 25th week of pregnancy. The patient gave a premature birth with a breech presentation. The baby, 650 g weight, died during labor. The second pregnancy occurred by intracytoplasmic sperm injection (ICSI). The patient gave premature birth in the 26th week of pregnancy with breech presentation. The baby, 850 g weight, died during labor. The patient's third pregnancy occurred by ICSI treatment. Preterm labor started in the 26th week of this pregnancy. The patient gave birth to a 950 g baby with cephalic presentation. The baby died after staying 20 days in the neonatal intensive care unit (NICU). The patient's fourth pregnancy, as described herein, occurred spontaneously. A cervical cerclage was performed in the 14th week of pregnancy with the diagnosis of cervical insufficiency.

During inpatient observation, uterine contraction was not observed. Laboratory evaluations on the following day were; leucocyte: 16.8 x 10^3/mm^3 and C reactive protein (CRP): 18 mg/L. Thromboprophylaxis treatment, progesterone, and ampicillin treatment continued during follow-up. Two days later, the leucocyte count was 12.2 x 10^3 / mm3 and CRP level was 6 mg/L. The patient was continued to observation. Leukocyte count was 14.6 x 103/mm3 and CRP level was 52 mg/L on the 7th day of admission. The values of leukocyte and CRP increased. The patient reported fever, but it was not detected by thermometer.

Vaginal serohemorrhagic discharge started in the patient's 7th day. These findings and symptoms were attributed to the early stage of chorioamnionitis. Thereupon, intravenous treatment of piperacillin/tazobactam (2.25 g four times) and clindamycin (900 mg three times per day) were started. A single dose of steroid (6 mg betamethasone) was administered intramuscularly to decrease inflammation and induce fetal lung maturation. An indomethacin suppository (100 mg) was administered to decrease inflammation severity and prevent uterine contractions' development. During treatment, the patient's vaginal discharge ceased, and preterm labor did not develop. During antibiotic treatment, the leukocytes and CRP values decreased slowly. On the 14th day of treatment, the leukocyte value was 9.5 x 10^3/mm^3 and the CRP value was 9 mg/L. The double antibiotic treatment was stopped on the 15th day. The patient continued to be observed in the hospital. Vaginal bleeding of the patient started on the 38th day of admission.

Uterine contraction and fetal bradycardia were not observed by NST. Since there was no empty ventilator in the NICU of my hospital, the pregnant woman was transferred to a tertiary hospital.

A male baby, 2185 g, was delivered by cesarean section in the transferred hospital. It was observed that the placenta was partially detached (approximately 20%). There was no complication for the mother after birth. The baby was given continuous positive pressure ventilation for two days in the NICU, and then oxygen therapy was given for three days. The baby was discharged on the 21st day of birth at 2575 g weight.

DISCUSSION

According to our knowledge, this is the first time this treatment regimen has been administered to treat chorioamnionitis. This treatment regime prolonged pregnancy duration by about thirty days. Due to United Stated Food and Drug Administration pregnancy category for piperacillin/tazobactam and clindamycin is group B. When vaginal polymicrobial microorganisms reach the uterus and adnexa, they cause pelvic inflammatory disease. Broad-spectrum antibiotics with anaerobic coverage are used to treat pelvic inflammatory disease. The existence of uterine cervical dilatation, PPROM, or both lead to loss of the cervical mucus plug and supply a way for the same vaginal polymicrobial microorganism to reach the uterine cavity and cause intrauterine chorioamnionitis (4). In light of knowledges, to administration of broad-spectrum coverage antibiotics, these two antibiotics were chosen to treat chorioamnionitis.

When pregnant women with cervical dilatation are detected, routine antibiotic treatment is started to prevent the development of chorioamnionitis (5). Despite antibiotic treatment, chorioamnionitis develops in most patients (5). The initiation of antibiotic treatment in the early period of chorioamnionitis, especially in the early stage, is more effective than starting treatment when the disease becomes severe (6). It has been reported that CRP increases as an inflammatory marker in subclinical chorioamnionitis and may have diagnostic value (7).

The diagnosis of chorioamnionitis in the presented case was established by increased leukocyte and CPR values and the development of serohemorrhagic vaginal discharge. Then a new antibiotic treatment regime was administered. During treatment, leukocyte and CRP values decreased, and serohemorrhagic discharge ceased.

It is shown that 76% of pregnant with cervical insufficiency with microorganisms in the amniotic fluid gave birth within the first 48 hours of their hospitalization (8). Mönckeberg et al. investigated the presence of chorioamnionitis by performing amniocentesis to 70 pregnant women with cervical insufficiency. They found that 19% (13/70) of pregnant women had chorioamnionitis. They found that cervical dilatation and leukocyte and CRP values were higher in pregnancy with chorioamnionitis than pregnancy without chorioamnionitis (8).

They observed that the median time of pregnant women with chorioamnionitis between admission to the hospital and the delivery was 1 d (1-10 days). Of these patients, 69% gave birth within the first 48 hours (8).

It is believed that pregnant women with cervical insufficiency and chorioamnionitis cannot be treated successfully (9). Recently Oh et al. reported that chorioamnionitis could be treated with antibiotic treatment, and the duration of pregnancy could be extended in some of the pregnant women with cervical insufficiency and chorioamnionitis (9).

Gravett et al. reported that antibiotic treatment with immunomodulators (dexamethasone and indomethacin) could threat chorioamnionitis, prevent intra-amnionitic infection, placental inflammation, and prolong pregnancy in non-human primate models (10).

In the presented case, the development of subclinical chorioamnionitis in a patient with cervical insufficiency was observed despite routine antibiotic treatment. The findings of chorioamnionitis regressed using the new antibiotic treatment, and the gestation period was prolonged for about one month. More studies need to evaluate the effectiveness of this new treatment modality in patients with chorioamnionitis.

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A case of acute renal failure with COVID-19 under Molnupiravir treatment

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ABSTRACT

Objective: Diarrhea, nausea, and vomiting are the most commonly reported mild side effects of Molnupiravir. However, this case shows that it may indirectly cause acute renal failure.

Case: 67-Year-old male patient diagnosed with hypertension and chronic obstructive pulmonary disease developed severe nausea, vomiting, and diarrhea with the use of drugs. In the patient's examinations, severe deterioration in kidney functions occurred. The patient who developed acute tubular necrosis was taken to hemodialysis twice. Intravenous hydration and supportive treatments were started. In the follow-up, clinical and renal functions improved. In patients over 65 years of age and with comorbidities, the adverse effects of Molnupiravir should be considered and followed closely.

Keywords: COVID-19, SARS-COV-2, Molnupiravir, Diarrhea, Renal Failure

INTRODUCTION

Positive developments have been achieved with vaccination and anti-viral treatments against Coronavirus disease 2019 (COVID-19). Since the RNA-Dependent RNA-Polymerase (RdRp) enzyme plays an important role in the replication of the Severe acute Respiratory Syndrome Virus (SARS-COV-2), it is important for antiviral treatment targets (1, 2).

Favipravir and Remdesivir are RdRp inhibitors and have been approved for treatment. Both drugs have been shown to reduce clinical symptoms and slow down progression (3,4).

However, an alternative treatment has been sought because there are only intravenous (IV) forms of Remdesevir and its weak pharmacokinetic efficacy in Favipravir. Recently, orally available Molnupiravir has been promising due to its tolerability and positive efficacy profile. It suppressed the replication of SARS-COV-2 through inhibition of RdRp and attracted great attention by providing a rapid and effective reduction in viral load. It was developed by scientists at Emory University (USA) (5,6).

Phase-1 and 2 studies have been completed(7,8). However, Phase-3 studies are still in progress, and the efficacy, tolerability, and safety profile remain unclear(9). This case report aims to present a clinical case infected with SARS-Cov-2, who developed severe nausea, vomiting, and diarrhea with Molnupiravir treatment and progressed to severe acute renal failure (ARF) during follow-up.

CASE

A 67-year-old male patient with hypertension and chronic obstructive pulmonary disease diagnosed for 10 years, regularly uses Perindopril/Indamapid (10/2,5mg/day) tablet (TB), metoprolol (50 mg/day) tb, formoterol-budenoside 400 µg/day inhaler (inh), tiotroprium bromide (inh) drugs. The patient, who had been Synovac vaccinated 3 times before, applied to the Kırşehir Training and Research Hospital pandemic emergency on 17.02.2022 with complaints of fever, weakness, fatigue, sweating, and myalgia. There were no complaints of nausea, vomiting, or diarrhea at admission.

Due to patient's clinical status was good, laboratory examination and direct X-ray/thorax CT were not taken from the patient. Infection positivity has been checked with Polymerase Chain Reaction (PCR) test. SARS-COV-2-Omicron variant positivity has been detected. On 18.02.2022, Molnupiravir 200 mg tablet (TB) was started as 2x800 mg outpatient treatment by the healthcare teams.

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Molnupiravir was used regularly for 5 days. He continued to his own previous drugs as well. During this time, he received only paracetamol TB as an analgesic. He did not use non-steroidal anti-inflammatory. However, the patient had severe nausea, vomiting, and diarrhea 15-20 times a day with the use of Molnupiravir. Oral intake is impaired. He could not provide fluid hydration. Diarrhea disappeared after the medication was discontinued, but complaints of nausea, vomiting, and loss of appetite persisted. He applied to the emergency department on 07.03.2022 with these complaints. Fever: 36.1 (C°) Pulse: 106/minute, Blood Pressure: 90/60 mmHg, Respiratory rate: 18/minute, oxygen saturation (SpO2): % 96. His general condition was poor; he had a sluggish and tired appearance. He was re-evaluated for SARS-COV-2 infection. PCR test was negative. Pneumonia or any focus of infection was not detected. In the examinations of the patient at the time of admission to the emergency department, urea: 190mg/dl, Blood urea nitrogen (BUN):89 mg/dl Creatinine (CRE): 8,9 mg/dl, GFR: 5 ml/min, Sodium (Na): 136 mmol/l, Potassium (K): 4.8 mmol/l It came as Calcium (Ca):9 mmol/l, phosphorus(P): 5.5 mmol/l (**Table-1**).GFR levels were calculated with Chronic Kidney Disease Epidemiology Cooperation (CKD-EPI) formula (10). There was severe metabolic acidosis in venous blood gas. Ph: 7.17, PCO_2:39 mmHg, PO_2: 15.2 mmHg, HCO_3-std:13.1 meq/l, Lactate(mmol/l):0.7 detected (**Table-2**).

Urinary catheter inserted. Residual urine output of 10-20 cc was observed. Proteinuria, 1 (+) erythrocyte, and leukocytes were detected in spot urine (**Table-3**). According to clinical and laboratory findings, an emergency hemodialysis (HD) indication was made in the patient. He was placed on hemodialysis for 2 hours with a temporary HD catheter. Ultrafiltration (UF) was not performed because there were no signs of hypervolemia such as pretibial edema and pulmonary edema. Low molecular weight heparin was used as anti-coagulation. 80 cc/hour saline (0.9%) IV was started.

Nephrotoxic agents avoided. After the first dialysis, he was taken to HD for a second time for another 4 hours, as uremia and acidosis continued at a serious rate in his control examinations (**Tables-1 and 2**). UF is not done again. In terms of aetiology, urinary system USG was performed. The parenchymal echogenicity of both kidneys was observed as grade-1 increased (**Table-3**).

In the follow-ups, the patient's clinic tended to improve. Urine output was about 70-80 cc per hour. He was followed up without dialysis. Anti-hypertensive drugs were not used during the hospitalization. Blood pressures remained stable. Renal functions (Table-1) and proteinuria returned to normal (**Table-3**). Haemodialysis and urinary catheters were removed. He was discharged with recommendations.

Table-1. Changes in the patient's kidney function tests and complete blood count

Variables	History						
	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day
Urea (mg/dL)	190	153	123	103	75	59	32
BUN (mg/dL)	89	71	57	48	35	28	15
CRE (mg/dL)	8,9	7	3,13	1,78	1,42	1,31	1,04
GFR (mL/min)	5	7	20	39	51	56	74
Na (mmol/L)	136	137	137	139	142	141	140
K (mmol/L)	5	4,3	4,2	4,1	3,8	4,3	4
Ca (mmol/L)	9	8,4	8,4	8,3	8,1	7,8	7,9
P (mmol/L)	5,5	5,3	4	3,5	3	3	3,2
WBC (10^3/µL)	10,01	8,89	9,58	10,81	13,05	10,57	8,55
Hgb (g/dL)	14,9	13	11,6	11,6	11,3	11,2	11,1
Hct %	47	39,9	34,7	35,1	34	34,4	32,9
MCV (fL)	92,9	90,7	89	88	89,9	89,4	88,4
MCH	29,4	29,5	29,7	29,1	29,9	29,1	29,8
PLT (10^3 /µL)	281	236	185	190	193	204	222

BUN:Blood Urea Nitrogen;Cre:Creatinin;GFR:Glomerular Filtration Rate; Na:Sodium,K:potassium, Ca:calsium ;P: phosphorus,;WBC:White Blood Cell; Hgb:Hemoglobin;Hct: hematocrit;Plt: Platelet; MCV: Main Corpuscular Volume;MCH: Mean Corpuscular Hemoglobin

Table 2. Changes in the patient's venous blood gas

Venous Blood Gas Variables	History			
	First day	Second day	Third day	Seventh day
pH	7,17	7,19	7,40	7,46
PO_2 (mmHg)	15,2	16,3	43,4	24,6
PCO_2(mmHg)	39,7	40,9	29,2	37,2
HCO_3-std(meq/L)	13,1	13,3	19,9	25,9
Lactate(mmol/L)	0,7	0,5	0,5	0,8
SpO_2(%)	26	38	62	68

pH: potential hydrogen; PO_2: partial oxygen; PCO_2: partial carbon dioxide; HCO_3-std: standard bicarbonate; SpO_2: Oxygen saturation

Table 3. Urinary system USG and spot urine examinations

Urinary system USG	Both kidney sizes and localization are normal. Parenchymal echogenicity is compatible with grade-1 nephropathy. Bilateral PVE was not observed. The bladder is not full enough, there is a slight increase in trabeculation in the wall.	
Spot urine variables	**History**	
	07.03.2022	**14.03.2022**
Spot urine protein	2(+)	Negative
Spot urine Density	1024	1012
Spot urine erythrocyte	1(+)	Negative
Spot urinary leukocytes	1(+)	1(+)
Hyaline eraser	Negative	Negative
Granular roller	Negative	Negative
Bacterium	Negative	Negative
Spot urine prote/CRE(mg/dl)	497	148

USG: Ultrasonography; PVE: Pelvicalyceal Ectasia

DISCUSSION

Nausea, vomiting, and diarrhea developed as side effects of Molnupiravir in the patient. As a result, dehydration occurred. This may be due to the SARS-COV-2 infection itself. However, the patient did not have these symptoms when he was diagnosed with COVID-19. It started with the use of medication, and the patient's diarrhea regressed after the treatment was completed. In a randomized study by Bernal et al. on outpatients using Molnupiravir, the most frequently reported side effects were diarrhea, nausea, and dizziness. Painter et al. also reported that the most common side effect of Molnupiravir is diarrhea in a placebo-controlled study (11,7).

Continuation of anti-hypertensive drugs together with these side effects, caused hypotension and hypovolemia, which leading to severe renal perfusion failure.

The patient applied to the emergency department approximately 10 days later, although the use of the medication ended and his complaints continued. Because of this delay in admission to the hospital, the initial prerenal azotemia progressed further, causing severe reductions in renal perfusion. As a result of these, it is possible that acute renal failure due to ischemic acute tubular necrosis has developed.

Because the causes leading to prerenal azotemia (nausea, vomiting, bleeding, burns, dehydration, fluid sequestration into the third spaces) can lead to ischemic acute tubular necrosis. Both have the same spectrum (12). BUN/CRE: 89/8.9=10 at the time of first presentation of the case. This is a finding in favour of acute tubular necrosis. Because this rate is <20, it shows that it is of renal origin (13). A small amount of residual urine after urinary catheterization indicated that there was no post-renal ARF. Nausea and vomiting during emergency admission are due to uremia. He was taken to haemodialysis only 2 times, on 07-08.03.2022. With adequate hydration and hemodynamic stabilization, his clinic tended to improve. Normal urine output was achieved. In this case, diagnostic renal biopsy was not performed due to rapid improvement with emergency haemodialysis and hydration treatments. In fact, this situation also removed the diagnosis of interstitial nephritis. There was no need for steroid treatment. In the last examination of the patient who was hospitalized for 7 days and treated, Urea: 32 mg/dl, BUN: 15 mg/dl, CRE: 1.04, GFR: 74 ml/min.

CONCLUSION

The decrease in haemoglobin values may have resulted from blood loss during venous catheter insertion, repeated tests, and haemodialysis sessions (**Table-1**). Complaints such as nausea, vomiting, and diarrhea due to the use of Molnipiravir in patients over 65 years of age and those with chronic diseases should be seriously considered. It should be included in the category of significant side effects in phase-3 studies. This situation may cause severe dehydration and ARF in patients. The patient should definitely be informed and communicated. When necessary, the nearest health institution should be consulted. Otherwise, it may lead to increased mortality and morbidity. It may bring an additional financial burden. It should be followed closely in terms of chronic nephrotoxicity.

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MSD

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Two cases of Chronic Neutrophilic Leukemia were successfully treated with Allogeneic Stem Cell Transplantation

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ABSTRACT

Objective: Chronic Neutrophilic Leukemia (CNL) is a rarely seen myeloproliferative neoplasia (MPN) in which the BCR-ABL1 gene mutation is negative, and is characterized by persistent neutrophilic proliferation in the bloodstream and granulocytic hyperplasia in the bone marrow. CNL is usually diagnosed incidentally in asymptomatic individuals with persistent neutrophilic leukocytosis. When genetically examined, BCR-ABL1 fusion gene, JAK-2 V617F, and exon12 mutations, CALR mutations, PDGFRA-B, FGRF1 mutations are all not detected, while CSF3R mutation is observed in most of the cases. The WHO-2016 classification determined the presence of CSF3R T618I and other activating CSF3R mutations as diagnostic criteria. While the prognosis is poor in CNL cases with the CSF3R T618I mutation, it is more moderate in the presence of other CSF3R mutations. The average life expectancy is 21-30 months, and 5-year survival rates are around 28%. Although no treatment modality provides an average survival advantage other than hematopoietic stem cell transplantation (HSCT), there is no accepted 'standard of care' consensus. HSCT procedures performed in CNL cases are limited in the literature.

Case: In this study, we presented two cases of CNL who were successfully treated with allogeneic stem cell transplantation and cured.

Keywords: Neutrophilic Leukemia, Stem cell transplantation

INTRODUCTION

Chronic Neutrophilic Leukemia (CNL), which Tuohy first described in the 1920s, is a very rarely seen myeloproliferative neoplasia (MPN) in which the BCR-ABL1 gene mutation is negative and is characterized by persistent neutrophilic proliferation in the bloodstream and granulocytic hyperplasia in the bone marrow (1). Although it was considered as separate myeloid neoplasia until 2001, it was later included in MPN in the WHO-2001 classification of neoplastic diseases (2, 3). The actual incidence is unknown, the mean age at diagnosis is usually 67 years. The male/female ratio is slightly in favor of males (4).

Clinically, it can be presented in different ways. It is usually diagnosed incidentally in asymptomatic individuals with persistent neutrophilic leukocytosis. Fatigue, bone pain, itching, and easy bleeding/bruising are common constitutional findings. Hepatosplenomegaly is seen in approximately 36% of CNL cases (5). The most important laboratory finding is sustained neutrophilic leukocytosis. While mature neutrophils constitute more than 80% of all leukocytes, blasts and other immature cells are rarely observed in peripheral blood. Although monocytosis and basophilia/eosinophilia are not expected findings, mild anemia and thrombocytopenia may be observed (4).

The leukocyte alkaline phosphatase (LAP) score and LDH levels are increased. Progressive thrombocytopenia and splenomegaly may herald blastic crisis. Bone marrow biopsy usually shows hypercellularity (>80%) and myeloid/erythroid ratio >20:1 in bone marrow aspiration. The absence of dysplastic changes and absence of overt reticulin fibrosis can be considered typical for CNL (4).

When genetically examined, BCR-ABL1 fusion gene, JAK-2 V617F, exon12 mutations, CALR mutations, PDGFRA-B, and FGRF1 mutations are not detected, while CSF3R mutation is observed in most of the cases (6). The WHO-2016 classification determined the presence of CSF3R T618I and other activating CSF3R mutations as diagnostic criteria (6, 7). CNL diagnostic criteria are shown in **Table-1**.

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Table 1.WHO diagnostic criteria of Chronic Neutrophilic Leukemia

Peripheral Blood	Leukocytosis ≥25x10^9
	Segmented plus band forms ≥80% of WBC
	Neutrophil precursors < 10% of WBC
	Myeloblast rarely observed
	Monocyte count < 1x10^9
Bone Marrow	M/E >10
	Normal neutrophil maturation
	Myeloblasts <5% of nucleated cells
Genetic	Presence of CSF3R T618I or other CSF3R mutations
	BCR-ABL1 negativity
	JAK2 V617F negativity
	PDGFRA-B negativity
	FGFR1 negativity

While the prognosis is poor in CNL cases with the CSF3R T618I mutation, it is moderate in the presence of other CSF3R mutations. Overall survival is 21-30 months and 5-year survival rates are around 28% (8, 9). Blastic transformation is observed in 10-21.2% (10, 11) of the cases in the literature, while the meantime for transformation is 21 months (12, 13). Although there is no widely accepted prognostic scoring system, platelets lower than 160x10^9 /L, leukocytes more than 60x109/L, and the presence of ASXL1 mutation are accepted as poor prognostic criteria (1).

Although no treatment modality provides an average survival advantage other than hematopoietic stem cell transplantation (HSCT), there is no accepted 'standard of care' consensus. Hydroxyurea is the most common agent used in the first line to reduce leukocytosis and splenomegaly (4). IFN-alpha, hypomethylating agents, ruxolitinib, thalidomide, cladribine, and imatinib are other treatment alternatives that can be considered in cases where hydroxyurea is not successful. HSCT procedures performed in CNL cases are limited in the literature. For the first time, HSCT was performed on two CNL cases by Hasle et al in 1996 and long-term remission was achieved. While poor results are obtained in the HSCT procedure performed in CNL cases in the blastic phase, satisfactory results can be obtained if it is performed in the chronic phase (4, 13). The 1-year median survival after HSCT has been reported to be 40%. CSF3R can be considered as a minimal residual disease marker, and CSF3R follow-up is recommended after HSCT (14). Considering the very few case reports and studies in the literature, it is recommended that especially high-risk CNL cases be referred to HSCT at the most appropriate period.

CASE 1

A 30-year-old female patient was referred to the hematology outpatient clinic due to leukocytosis, which was noticed during a routine check-up in 2016. The only clinical finding detected in the asymptomatic patient was splenomegaly (longitudinal length 19 cm, passing below the ribs). Laboratory findings of the patient are given in **Table-2**. t(9;22) was not detected by FISH. JAK2 V617F, CALR, and MPL mutations were also not observed. While leukocytosis consisting of mature neutrophils was observed in the peripheral smear, no cells were found in the blast morphology. Bone marrow biopsy revealed hypercellularity (>95% cellularity), M/E ratio of 15:1, myeloblast <5%, and mild reticulin fibrosis. Whole-genome sequencing was performed with next-generation sequencing analysis, pThr618IIe CSF3R gene mutation was detected. The patient was diagnosed with CNL and hydroxyurea was started. No clinical or laboratory improvement was observed in the patient who used hydroxyurea for approximately 14 months. In February 2018, by using a myeloablative conditioning regimen, HSCT was applied from her sister, who was fully compatible with HLA (10/10). The patient, whose chimerism rate on the 100th day after transplantation was 98% compatible with donor lymphocytes, is still being followed in the hematology clinic in a healthy and untreated condition.

CASE 2

A 33-year-old female patient applied to the dentist in January 2020. After the detection of neutrophilic leukocytosis in the examinations, the patient was started on a 2-week course of antibiotics. Despite the treatment, the patient's leukocyte count increased, she was consulted to the hematology department. She had splenomegaly in the physical examination and had a leukoerythroblastic blood smear. Chronic myeloid leukemia (CML) was considered at the forefront, and evaluation of t(9;22) by FISH was planned from peripheral blood, and a bone marrow biopsy was performed. FISH for t(9;22) was negative. The bone marrow biopsy result was compatible with myeloproliferative neoplasia without fibrosis. JAK2 V617F mutation, CALR, MPL, PDGFRA-B, and FGRF1 mutations were found negative, in the myeloid mutation panel examined with NGS, heterozygous mutation in the 13th exon of the ASXL1 rs373221034 gene, and on the 14th and 17th exon of CSF3R gene (c2326 and c1853) were detected. The patient who was diagnosed with CNL did not benefit from the hydroxyurea and the WBC count reached 65x109/L in the follow-up. Since she did not have an HLA-matched sibling donor, HSCT was performed from an unrelated donor who had 9/10 HLA compatibility with a myeloablative conditioning regimen in November 2020. No CSF3R mutation was detected for minimal residual disease screening at the fifth month after HSCT. The patient is followed up in a healthy and untreated way in the first year after allogeneic stem cell transplantation.

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Table 2. Laboratory and clinical findings of the patients

	Patient 1	Patient 2
WBC	$26x10^9$/L	$36x10^9$/L
Absolute neutrophil count	$24x10^9$/L	$31.7x10^9$/L
Hb	14.9 g/dL	11.7 g/dL
Platelet	$122X10^3$/L	$258x10^3$/UL
LDH	365 U/L	1269 U/L
Blast (peripheral blood)	No blast	1 blast
Splenomegaly	18 cm	19 cm
JAK2 V617F	-	-
BCR-ABL1	-	-
CALR	-	-
MPL	-	-
PDGFRA-B	-	-
FGRF1	-	-
CSF3R T618I	+	-
CSF3R other mutations	-	+
ASXL1	-	+
M/E (bone marrow)	15	8
Reticulin fibrosis	0	+1 (mild)

DISCUSSION

CNL is an MPN whose laboratory features have been known for many years, but the treatment algorithm has not been fully clarified. Although the prognosis is heterogeneous, it is known that it has an aggressive course, especially in cases carrying the ASXL1 mutation together with the CSF3R T618I mutation.

Although the ASXL1 mutation was positive in one of the cases we presented, if it was thought to be associated with the CSF3R c2326 mutation and the other case had a CSF3R T618I mutation without the ASXL1 mutation, the cases could be placed in the intermediate-risk class. Since both cases were young and had compatible stem cell donors, they were accepted as candidates for HSCT. Similar to the literature, the patients we presented are followed in a healthy and untreated way after allogeneic stem cell transplantation.

CONCLUSION

In CNL cases, HSCT is the only curative treatment modality that should be considered at the earliest time, especially if the patient is young and has the performance to cope with complications that may develop after allogeneic stem cells.

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