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ORIGINAL RESEARCH

Twists and Turns of Peritonsillar Abscess

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Abstract

Objective: *The presence of untreated pus within the peritonsillar space can herald the occurrence of various clinical scenarios that could be difficult to describe. In this article we discuss the "TWISTS AND TURNS OF PERITONSILLAR ABSCESS" we have met in the Northwestern region of Nigeria that have not yet been reported in the literature and to compare the means of symptom duration at presentation among study groups.*

Material and Methods: *It is a retrospective study. A total number of 25 patients formed the study population (N) and were classified into three: Group 1 included patients who presented early with peritonsillar Abscess and had expected clinical course following treatment. Group 2 included patients who presented late and thus had one twist or the other from the usual presentation. Group 3 included patients who died.*

Results: *The mean age = 24 ± 11 years. Sore throat, dysphagia and fever were the most common symptoms. Asymmetrically enlarged tonsil was the most common oropharyngeal finding with occasionally occurring twists and turns such as gangrenous tonsil, auto-tonsillectomy, and severely bleeding tonsil.*

Conclusion: *Peritonsillar abscess (PTA) constitutes a relentless infection that needs urgent, adequate treatment based on acceptable methods, delay in presentation can herald the occurrence of pathologies that could be difficult to interpret.*

Key Words: Peritonsillar Abscess, Auto-tonsillectomy, Gangrenous tonsil.

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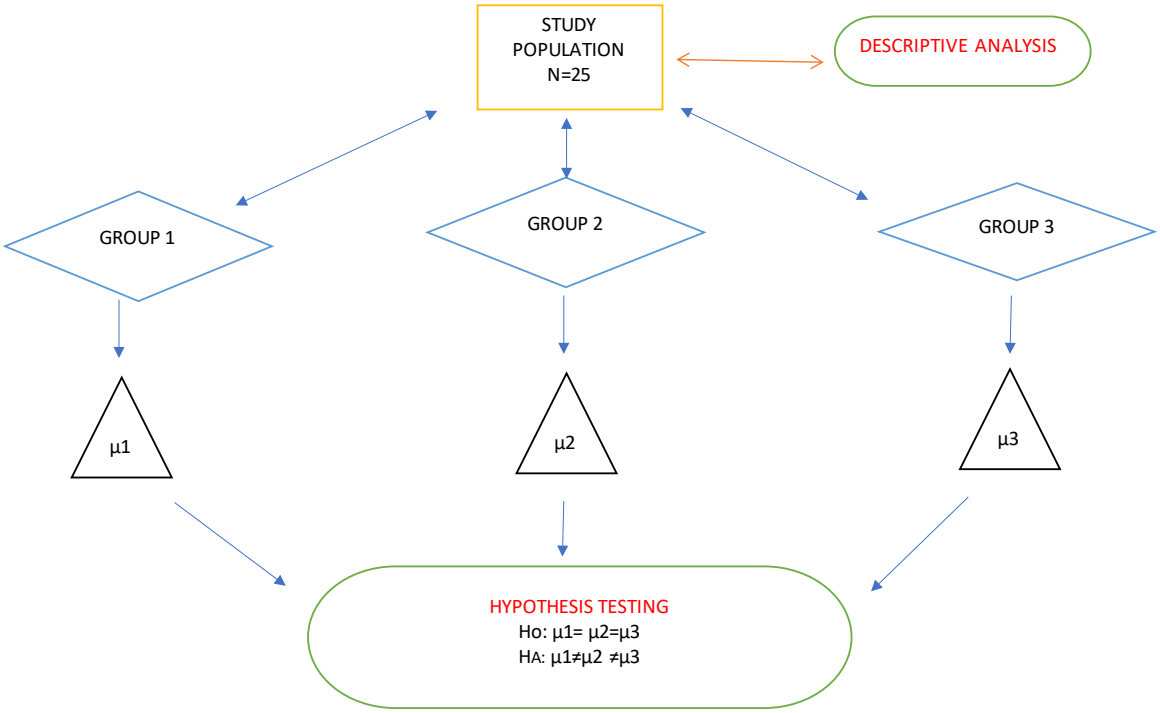
Introduction

Peritonsillar abscess (PTA) first described in the 14th century, is a commonly encountered pathology in ORL practice even in current antibiotic era[1,2]. It is a localized collection of pus within the tonsillar fossa between tonsillar capsule and superior constrictor muscle[3-6]. Presence of untreated pus within the peritonsillar space can herald the occurrence of various clinical scenarios and complications related to wide spread of infection to the heart (carditis), brain (brain abscess) , various neck abscesses, Lemierre's syndrome[7]. In this article we describe the "*TWISTS AND TURNS OF PERITONSILLAR ABSCESS*" we have met in the Northwestern region of Nigeria that have not yet been reported in the literature and to compare the means of symptom duration at presentation among study groups.

Material and Methods

It is a retrospective study done at Federal Medical Centre (FMC), Gusau between November 2020-April 2022. A total number of 25 patients formed the study population (N) and were classified into three groups: Group 1 formed of patients who presented early with peritonsillar Abscess (PTA) and had expected clinical course following treatment (n=14). Patients in Group 2 presented late and had one twist or the other from the usual presentation (n=9). Group 3 were patients who died (n=2). We retrieved clinical records of the patients from the ENT department of the Federal medical Centre, Gusau. The patient's age, gender, occupation, level education, symptoms and duration at presentation, oropharyngeal findings, clinical course of PTA were documented into a Microsoft Excel proforma, and the data was analyzed using SPSS version 25. Microsoft Excel was used for visualization. ANOVA was done to compare the means (μ) of symptom duration between groups 1,2 and 3, other results were presented in descriptive form. Only patients with positive oropharyngeal test aspirate considered to have PTA and those who had unusual twist relating to PTA were included in the study. See the flowchart below for summary.



Results

Age range was 2-40 years with modal age group of 21-30 years and mean age of 24 ± 11 years. Females were 17 (68%) and males 8 (32%) with M:F ratio 1:2.1 There is high illiteracy among the study population, up to (60%) had no formal education with most also not gainfully employed (68%), see table I. The most common symptoms were sore throat, dysphagia and fever noted in 100%, 100% and 60% respectively (Figure 1). Asymmetrically enlarged tonsil was the most common oropharyngeal finding seen in (52%), see figure 2. Group 1 had expected clinical course following treatment (56%), group 2 had occasionally occurring twists and turns such as gangrenous tonsil 4%, Auto-tonsillectomy (8%) and severely bleeding tonsil (4%) and group 3 were patients who died (8%), see figure 3.

A one-way ANOVA to compare μ1, μ2 and μ3 was done and there was a statistical difference. $F(2,22) = 24.3$, ($p=0.000$) at 95% CI, $\alpha \leq 0.005$, a Turkey post-Hoc test revealed statistical difference between μ1 and μ2 ($p=0.000$), between μ1 and μ3 ($p=0.000$) but no statistical difference between μ2 and μ3 ($p=0.078$). Eta- squared, η^2=(SS between groups)/(SS total)=829/1203=0.69, therefore we reject the HO hypothesis. See table II, III and figure 4.

Table 1. Demographics

VARIABLES	FREQUENCY (N)	PERCENTAGE (%)
AGE		
1-10	3	12
11-20	6	24
21-30	9	36
31-40	7	28
TOTAL	**25**	**100%**
GENDER		
MALE	8	32
FEMALE	17	68
TOTAL	**25**	**100%**
EDUCATIONAL STATUS		
PRESCHOOL	1	4
NO FORMAL EDUCATION	15	60
PRIMARY LEVEL	7	28
SECONDARY LEVEL	1	4
TERTIARY LEVEL	1	4
TOTAL	**25**	**100%**
OCCUPATION		
NOT GAINFULLY	17	68
EMPLOYED	1	4
FARMING	2	8
BUSINESS	5	20
CHILD*	**25**	**100%**
TOTAL		

*Not within the working age group.

Figure 1. Symptoms

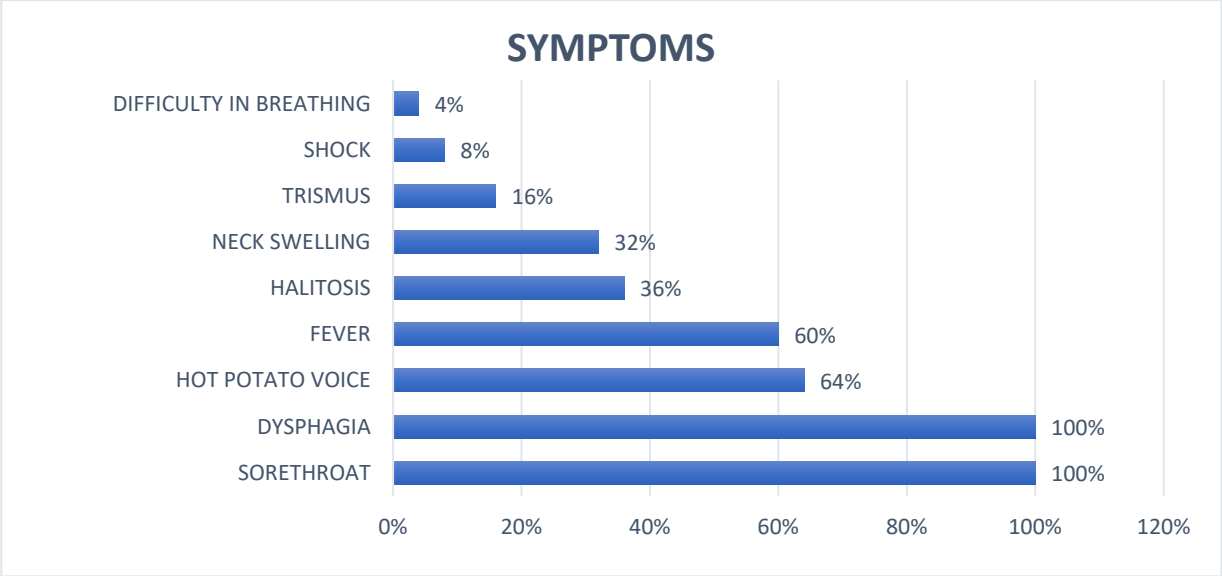


Figure 2: Oropharyngeal Findings

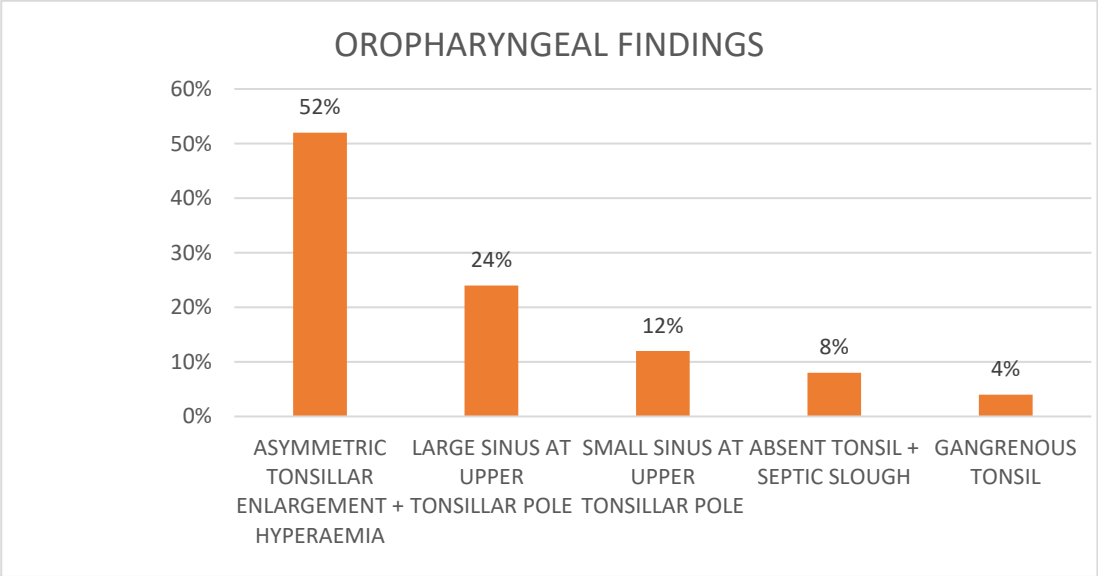


Figure 3: Observed Clinical Course of Pta.

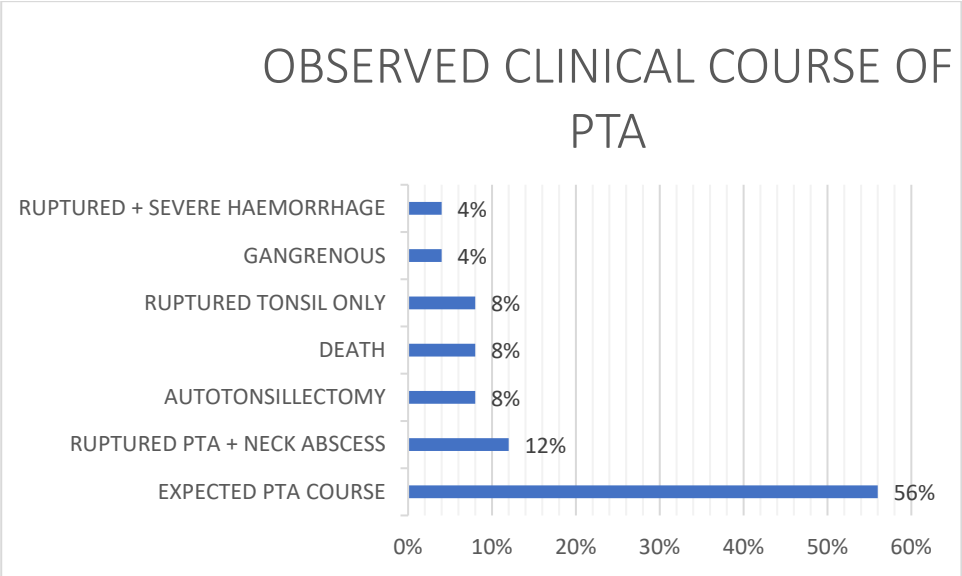


Table 2: Oneway Anova

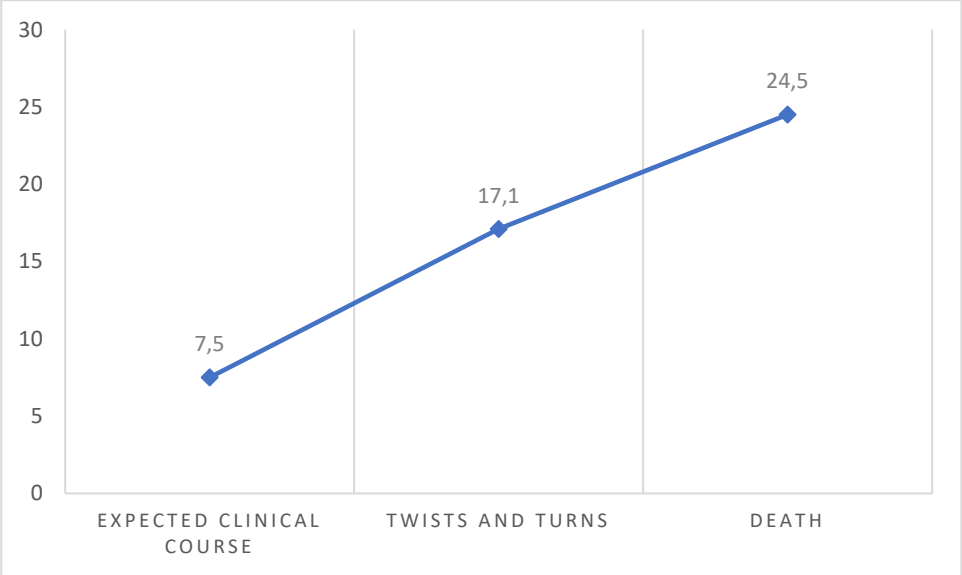
	Sum of squares	***df***	***Mean square***	***F***	***Sig.***
Between Groups	828.551	2	414.276	24.311	0.000
Within Groups	374.889	22	17.040		
TOTAL	1203.440	24			

Table 3. Turkey Post-Hoc, Multiple Comparism

(I) Disease Category	(J) Disease category	Mean Difference (I-J)	Std. Error	Sig
Expected clinical course.	Twists	-9.611*	1.764	.000
	DEATH	-17.000*	3.120	.000
Twists	Expected clinical course.	9.611*	1.764	.000
	DEATH	-7.389	3.227	.078
DEATH	Expected clinical course.	17.000*	3.120	.000
	Twists	7.389	3.227	.078

*. The mean difference is significant at the 0.05 level.

Figure 4: Mean Plot Of Symptom Duration In Days



Discussion

Peritonsillar Abscess (PTA) also called quinsy is a common pathology in otorhinolaryngological practice in our environment as we have treated 25 cases within 17 months compared to 24 cases seen over 12 years at Usmanu Danfodiyo University Teaching hospital Sokoto in earlier study[8], this could be because there is another hospital within the same city rendering ENT services at secondary level so the patients are shared, however similar disparity in frequency of PTA occurrence exist throughout Nigeria as 87% of otorhinolaryngologist practicing in Nigeria who responded to a survey on management policy of PTA reported that it is not a frequent pathology[9]. Looking at other tropical countries such as Brazil a much higher frequency was reported almost similar to that reported in parts of Europe and east Asia[10,11,12]. In this study the mean age of presentation is 24 ±11 years, 12% of our patients were children between 1-10 years and the modal age group is 21-30 years old, this is similar to the study from a neighboring state,Sokoto[8], and this may be due to strong similarities in factors relating to environment, culture and health care seeking behaviors of patients from North-Western Nigeria. There is a slight females' preponderance in this study (M: F= 1:2.1) however equal or males' preponderance have been reported (M: F = 1:2.1)[3,8,11,13] generally speaking tropical countries experience lower frequencies of PTA compared to some European countries even though high temperatures have been reported to have a positive correlation with PTA,[10] therefore other factors relating to lifestyle of the people could play a significant role in the occurrence of PTA. Jochen et al who treated about 680 cases of PTA identified smoking as one of the likely factors[11].

Patients typically present with symptoms like that experienced in tonsillitis, sore throat, dysphagia, hot potato voice and fever were the most common, this is similar in recent or old studies,[4,8,14] others include trismus, halitosis, difficulty in breathing and shock and the presence of which should alert the attending physician to possibility of this pathology called peritonsillar abscess not a straightforward tonsillitis.

A careful physical examination would usually reveal an asymmetrically enlarged tonsil with hyperemia and a displaced uvular, this is expected to hold for patients who present early, however in those who present late for one reason, or the other, additional findings could be seen and sometimes difficult to describe in recognized medical terms, this formed the bedrock of the twists and turns of peritonsillar abscess in this literature. We have seen absent tonsil in 8% of our patients with pus and septic slough on the tonsillar bed resembling post-tonsillectomy patient, in our clinical notes we wrote amputated tonsil initially as the diagnosis but we were not satisfied about the

nomenclature and so we thought of a better term to describe what possibly happened entirely, we therefore coined the term AUTO-TONSILLECTOMY to describe the spontaneous removal or entire separation of the tonsil from its bed due to relentless and untreated peritonsillar abscess with subsequent expulsion or aspiration. Another finding was that of a gangrenous tonsil with pus but still within tonsillar bed and finally ruptured peritonsillar abscess with severe hemorrhage with rapidly expanding neck and chest swellings plus upper airway obstruction and shock, this type of severe spontaneous hemorrhage is ominous signs that have well been documented in the early 20th century with high mortality,[15] our patient survived after tracheostomy, hot tonsillectomy, and blood transfusion. Spontaneous rupture of PTA is well described in other literatures and the resulting sinus vary from small to large seen at the upper tonsillar pole[8,16,17]. (fig 2)

Early presentation and adequate treatment of peritonsillar abscess is the key to preventing these complications, we grouped our patients in to 3 groups depending on the clinical course of PTA. Group 1 comprised of those patients who presented early and had expected clinical course following needle aspiration, antibiotics, steroids and IV fluids, group 2 comprised of patients who presented late and had twists and turns of PTA, group 3 are those who died. Their mean (μ) duration at presentation in days are: (μ1) = 7.5, (μ2) = 17.1 and group 3 (μ3) = 24.5. ANOVA showed statistical difference between the groups (p=0.000), Turkey post Hoc showed a statistical difference between group 1 and group 2 (p=0.000), and also between group 1 and group 3 (p=0.000) but not between group 2 and group 3 (p=0.78) therefore the best mean time for patients to have excellent outcome is 7.5 days any further delay pushes the patient to have either twists and turns of PTA or death. The size effect of symptom duration at presentation calculated using Eta-squared = 0.69 and considered large and further shows that up to 69% of complications of PTA in this study is attributable to symptom duration at presentation.

44% of patients stayed much longer at home compared to some studies in Nigeria,[4,8] Tetsuo et al established that symptom duration was longer in patients with rare bilateral PTA[16]. In our environment some of the reasons why patients present late could be because of reliance on traditional or religious alternative treatments to orthodox medicine, ignorance, poverty, and unwanted quacks input.

Further areas of study should focus on host immune response, bacterial strains among others to see why patients in our environment try to expel tonsil affected by PTA within 4 weeks if not adequately treated. Tetsuo et al reported a much higher range of duration at presentation 0-67 days but have not met spontaneous expulsion of tonsil as seen in this study.

Conclusıon

Peritonsillar abscess (PTA) constitutes a relentless infection that needs urgent, adequate treatment based on acceptable methods, delay in presentation can herald the occurrence of pathologies that could be difficult to interpret.

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ORIGINAL RESEARCH

The effects of renal dopaminergic system on the development of hypertension with high salt diet and L-NNA administration

Received Date: 14.03.2023, Accepted Date: 13.06.2023 **DOI: 10.56484/iamr.1265044**

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Abstract

Objective: *We aimed to investigate the intrarenal dopamine synthesis efficiency, blood pressure changes and the effects of this system on hypertension developed by NOS inhibition and high salt diet.*

Method: *Wistar Albino male rats were administered water containing 50mg/L or 100mg/L concentrations of L-NNA, standard rat feed containing 0.8%salt, or 4%high salt alone or with L-NNA for 7days. Blood pressure measurements were made with the tail-cuff method. 24-hour water intake and urine volume were also measured.*

Results: *Administration of L-NNA or high-salt diet alone for 7days did not cause a change in blood pressure, while their combined administration resulted in a significant increase in blood pressure. Blood pressures were found to be higher in the L-NNA100+HS group compared to the other groups. While the amount of water intake in 24hours did not change, the amount of 24-hour urine was reduced. 24-hour urinary sodium excretion, sodium clearance and GFR was decreased, and 24-hour urine dopamine concentrations were increased.*

Conclusion: *Co-administration of nitric-oxide inhibitor and high-salt diet failed to prevent renal dopaminergic system blood pressure increase. Despite the increase in dopamine synthesis, intrarenal dopamine activity could not be realized by receptor interaction and it is thought that the increase in blood pressure is caused by the development of renal oxidative stress.*

Keywords: Dopamine, Hypertension, L-NNA, Salt.

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Introduction

Essential hypertension may develop because of functional increase and/or decrease in the activities of the systems involved in the regulation of blood pressure at different levels. Factors such as renin angiotensin aldosterone system imbalances, peripheral arterial changes, ion transport changes, endothelial dysfunction, oxidative stress, excessive sodium intake and renal sodium retention are contributors to the development of essential hypertension[1,2]. Progressive insufficiency developing in the activities of endogenous natriuretic and vasodilating agents such as atrial natriuretic peptide (ANP), nitric oxide (NO), prostacyclin (PGI_2) and intrarenal dopaminergic system rather than hyperactivity of endogenous vasoconstrictor and antinatriuretic mechanisms are involved in the development and maintenance of essential hypertension[3,4].

Basal release of nitric oxide (NO) from endothelial cells changes with various stimuli such as blood pressure changes and shear stress and contributes to the regulation of local blood flow. It is known that NO causes strong vasodilation, suppresses platelet adhesion and aggregation and proliferation of vascular smooth muscle cells, and contributes to the regulation of arterial blood pressure[5,6]. In addition, NO, which is synthesized tonically in the kidneys, regulates glomerular filtration rate, total renal and medullary blood flow, pressure natriuresis, epithelial Na^+ transport and the synthesis of vasoactive agents such as renin, and plays an important role in sodium excretion[7]. NO is synthesized from L-arginine by nitric oxide synthase (NOS) enzymes, and in hypertension developed by the inhibition of this enzyme with L-nitro-N-arginine (L-NNA), water and salt retention, increased vascular tone and oxidative stress occur[3,6,8–12]. When the primary role of the kidneys in regulating long-term blood pressure levels was investigated, it was found that renal oxidative stress and NO deficiency mediate the development of hypertension[13–18]. Administration of reactive oxygen species scavengers and antioxidants in experimental hypertension models resulted in improvement in blood pressure levels[19].

Not only does a high salt diet always cause hypertension, but if there is a concomitant deterioration in renal sodium excretion, the harmful effects of high salt occur. Increased sodium intake is a necessary but not sufficient factor alone in the development of hypertension. While a high-salt diet alone can be compensated for in salt-tolerant organisms, it may contribute to the development of hypertension by causing deficiencies in salt-sensitive organisms[2,20]. Approximately 50% of patients with essential hypertension are salt-sensitive individuals[21]. In salt sensitivity, excess salt is not excreted at a sufficient rate and ratio due to the insufficiency of the endogenous natriuretic systems[22]. A high-salt diet is known to cause progressive damage to blood vessels, kidneys, and heart[23]. Treatment of arterial hypertension is necessary to prevent target organ damage

in patients with essential hypertension. Salt restriction is important in terms of preventing target organ damage as well as blood pressure control[22,24].

The intrarenal dopaminergic system, which spontaneously regulates the salt balance of the organism against excessive salt intake, is one of the most important endogenous natriuretic systems[25,26]. L-dopa is converted to dopamine in the kidney by the activity of dopa decarboxylase. The increase in the amount of Na^+ filtered by excessive salt intake increases the amount of L-dopa co-transported with Na^+ in the renal tubule cells. Dopamine synthesized in the tubule cell with increased L-dopa influx stimulates its specific D_1 and D_2-like receptors, inhibiting the Na^+/H^+ exchange pump and Na-K ATPase, thereby reducing Na^+ absorption and removing excess salt. Intrarenal dopamine is the most important system for excretion of excessive salt[27]. In individuals with essential hypertension, abnormal signals were observed in renal dopamine receptors such as D_1 and D_2, and insufficiency in the intrarenal dopaminergic system, and this situation was thought to be related to salt sensitivity and essential hypertension[4,14,25,26,28]. Physiological concentrations of dopamine in the kidney have protective effects against oxidative stress, and dopamine receptors maintain redox balance by inhibiting the production of free oxygen radicals. When any of the dopamine receptor subtypes are missing, an increase in blood pressure is observed, regardless of oxidative stress[29].

The aim of this study is to determine the intrarenal dopamine synthesis ability and efficiency changes that occur because of NOS inhibition and high salt diet, and to investigate the effects of this system on hypertension that develops with these interventions. For this purpose, blood pressure, water and salt balance, glomerular filtration rate, urine flow rate, sodium clearance, fractional sodium excretion, tubular sodium rejection fraction and urinary dopamine levels were measured in subjects who were administered L-NNA and high-salt diet separately and together at doses that would create partial NOS inhibition.

Material and Methods

This study was carried out at Çanakkale Onsekiz Mart University Experimental Research Application and Research Center (ÇOMÜDAM). The experimental protocols were approved by Çanakkale Onsekiz Mart University Animal Experiments Local Ethics Committee (Project No: 2014-125).

Materiel: In this study, a total of 36 8-week-old Wistar Kyoto male rats with an average weight of 205±15gr were used. The rats used in the experiment were obtained from ÇOMÜDAM Animal Laboratory. Rats were housed in standard light (12 hours daylight/12 hours dark, ventilated,

constant temperature) in standard rat cages with an equal number of rats in each cage. The rats were fed with sufficient (ad libitium) water (tap water or water containing L-NNA) and feed (0.8% salt-containing standard rat feed or 4% salt-containing salty feed) for a total of 7 days. On the last day of the experiment, rats were placed in individual metabolic cages.

L-NNA administrations: L-NNA was administered with drinking water and water intake was not restricted. The L-NNA dose taken by the rats was calculated by considering the amount of water they drink[12]. Every day, 50 mg/L and 100 mg/L concentrations of L-NNA were prepared freshly and administered to rats for 7 days.

High salt applications: For 7 days, the rats were fed with high salt diet with 4% salt or one of the standard rat diets with 0.8% salt and their feeding was free of restrictions. Rats were divided into six groups with 6 rats in each group (n=36). Tap water and standard rat feed were given to control group (n=6). High salt (HS) group (n=6): The rats were fed with rat feed containing 4% high salt. L-NNA50 group (n=6): The rats were given 50 mg/L L-NNA in their drinking water. L-NNA100 group (n=6): L-NNA was given to the rats at a concentration of 100 mg/L in their drinking water. L-NNA50+HS group (n=6): The rats were given 50 mg/L L-NNA and rat feed containing 4% high salt. L-NNA100+HS group (n=6): The rats were given 100 mg/L L-NNA in their drinking water and rat feed containing 4% high salt.

Blood pressure measurements: On day 0 and day 7, systolic blood pressure measurements were made using the tail cuff method (MAY BPHR 9610-PC TAIL-CUFF Indirect Blood Pressure Recorder, Ankara, Turkey). Measurements were made in a quiet laboratory environment, after a resting period of approximately 20 minutes, at the time of the regular signal tone obtained. The blood pressure values obtained were recorded on the computer. The mean systolic blood pressure was calculated by taking 3 measurements from each rat.

Collection of urine samples: Rats were placed in metabolic cages on the last day of the experimental protocol. The amount of water they drink and urine output during 24 hours were recorded. 0.1 ml of 6 N HCL was added to the containers for 24-hour urine collection and the urines were collected protected from sunlight. Urine samples were placed in 1.5 ml Eppendorf tubes and stored at -800ºC (SANYO ULTRA LOW TEMPERATURE FREEZER MDF-U4086S) for biochemical measurements.

Na^+, creatinine and urea measurements in serum and urine: Na^+, urea and creatinine levels in serum and urine samples were measured using an autoanalyzer (ROCHE COBAS 6000).

Measurement of urine dopamine levels: For the measurement of urinary excretion of dopamine, 24-hour urine samples were collected, and dopamine levels were measured by Düzen Laboratories group using a high-pressure liquid chromatography device (HPLC, Shimadsu, Japan).

Calculation of water balance, sodium clearance, glomerular filtration rate, fractional sodium excretion: The water balance of the rats (water intake (ml/day)- urine amount(ml/day) = water balance(ml/day)) was calculated by measuring the 24-hour water intake and urine amount of the rats taken into metabolic cages. Using the data obtained from collected urine and blood samples.

Sodium clearance (CNa) (ml/min) = Urine sodium(mEq/L) x Urine flow rate (ml/min) / Plasma sodium(mEq/L)

Glomerular filtration rate (GFR) (creatinine clearance) (ml/min) = (Urine creatinine(mg/dL) / Plasma creatinine(mg/dL)) x Urine flow rate(ml/min)

Fractional sodium excretion (%FENa) = (Plasma creatinine (mg/dL)) x Urine sodium(mEq/L)) / (Plasma sodium (mEq/L) x Urine creatinine(mg/dL))) X 100

Statistical analysis: Data were expressed as mean±standard error of mean (SEM). Statistical differences were calculated with independent student-t tests in independent groups. Paired student-t test was used to evaluate the difference between the values of the same group on days 0 and 7. Student's t test was used to interpret the results obtained, and a P value of <0.05 was considered statistically significant.

Results

Blood Pressures: There was no significant difference between the groups in the baseline blood pressures of the rats (Table 1). In both doses applied in the experimental protocol, L-NNA did not cause a rise in blood pressure by providing partial inhibition of NOS. However, it was observed that the application of both doses of L-NNA together with a high salt diet for 7 days caused a significant increase in the blood pressures of the subjects compared to the baseline blood pressures within the group. In addition, the blood pressures of the L-NNA100+HS group (n=6; 121.2±2.76: 147±3.37) were found to be higher than the L-NNA50+HS group (n=6; 124.3±0.96: 136.9±2.15) (Table 1, $P<0.05$ L-NNA100+HS vs. L-NNA50+HS rats). Partial NOS inhibition increased blood pressure in a dose-dependent manner when co-administered with a high-salt diet.

Table 1. *Baseline (day 0) and final (day 7) blood pressure values of groups*

Groups	Baseline BP (mmHg)	Final BP (mmHg)
Control (n=6)	122,5 ± 1,24	122,7 ± 1,28
HS (n=6)	121,2 ± 1,43	123,1 ± 1,55
L-NNA50 (n=6)	124,2 ± 1,46	127,8 ± 1,89
L-NNA100 (n=6)	122 ± 3,43	128,1 ± 1,89
L-NNA50+HS (n=6)	124,3 ± 0,96	136,9 ± 2,15 *
L-NNA100+HS (n=6)	121,2 ± 2,76	147 ± 3,37 * α

Data were presented as mean±SE. *P<0.05 final BP vs. baseline BP within the groups. α P<0.05 final BP of a group compared to final BP of other groups.

Effects on Water Intake, Urine Volume and Water Balances: L-NNA100 (n=6; 47±1.83) or L-NNA50 (n=6; 50±1.15) applications significantly reduced 24-hour water intake compared to the control group (n=6; 56.1±1.47). While the high salt diet alone did not cause a significant change in the 24-hour water intake of the subjects, it was determined that the co-administration of L-NNA50+HS significantly reduced the 24-hour water intake compared to the control group. L-NNA100 administration alone significantly reduced 24-hour water intake compared to both control and L-NNA100+HS administration (Table 2, P<0.05).

Table 2. *24-hour water intake, urine amount and water balance values*

Groups	24-hour water intake (ml/day)	24-hour urine volume (ml/day)	Water balance (ml/day)
Control (n=6)	56,1 ± 1,47	14,6 ± 0,66	41,1 ± 2,01
HS (n=6)	56,8 ± 2,94	10,8 ± 0,79**	46 ± 2,84
L-NNA50 (n=6)	50 ± 1,15**	10 ± 0,82**	40 ± 1,75
L-NNA100 (n=6)	47 ± 1,83**	11 ± 1,15**	36 ± 1,18**
L-NNA50+HS (n=6)	48,8 ± 2,59**	10,3 ± 1,05**	38,5 ± 2,31
L-NNA100+HS (n=6)	53,1 ± 1,16	6,1 ± 0,91**# β	46,8 ± 1,38** β

Data were presented as mean±SE. ** P<0.05 vs. control. # P<0.05 vs. HS. Ω P<0.05 vs. LNNA50. βP<0.05 vs. LNNA100.

Administration of either dose of L-NNA or high salt alone or in combination significantly reduced 24-hour urine output compared to the control group. In addition, the group administered L-NNA100+HS significantly decreased the 24-hour urine volume compared to the groups treated with both high salt and L-NNA100 and L-NNA50+HS (Table 3, P<0.05).

Table 3. *Serum sodium concentration and sodium concentration in 24-hour urine, calculated sodium clearance (Cl_{Na}), glomerular filtration rate (GFR), fractional sodium excretion (%FeNa)*

Groups	Serum sodium concentration (mEq/l)	Urine sodium concentration (mEq/l)	Cl_{Na}	GFR	%FeNa
Control (n=6)	136 ± 2,4	1,87 ± 0,21	0,095 ± 0,01	17,8 ± 0,13	0,53 ± 0,04
HS (n=6)	138,4 ± 1,4	1,66 ± 0,31	0,083 ± 0,01	13,7 ± 0,12	0,59 ± 0,08
L-NNA50 (n=6)	142,2 ± 0,5**	1,41 ± 0,2	0,069 ± 0,02	15,6 ± 0,14	0,45 ± 0,01
L-NNA100 (n=6)	138,4 ± 1,4	1,53 ± 0,21	0,076 ± 0,01	15,2 ± 0,25	0,52 ± 0,03
L-NNA50+HS (n=6)	143,4 ± 0,9**	1,72 ± 0,33	0,083 ± 0,1	13,3 ± 0,27	0,65 ± 0,01
L-NNA 100 +HS (n=6)	137,6 ± 1,4	0,87 ± 0,16**	0,05 ± 0,1** $^{\beta}$	10,5±0,18**	0,49 ± 0,06

Data were presented as mean±SE.** P<0.05 vs. control. $^{\beta}$ P<0.05 vs. LNNA100.

Administration of L-NNA50 or high salt diet alone or in combination did not affect water balance, while administration of L-NNA100 (n=6; 36±1.18) significantly decreased water balance compared to control group (n=6; 41.5±1.78) (P<0.05). However, co-administration of L-NNA100 with a high salt diet significantly increased the amount of water retained in the body. It was determined that L-NNA100+HS (n=6; 46.8±1.3) application did not change the amount of water consumed in 24 hours, but significantly decreased the amount of 24-hour urine compared to the other groups, thus increasing the water balance compared to the control group. Increased water balance indicates that there may be water-salt retention in the body (Table 2).

Serum sodium and urine sodium levels: L-NNA50 (n=6; 142.6±0.5) and L-NNA50+HS (n=6; 143.4±0.9) applications were found to increase serum sodium values (Table 4, P<0.05).

Table 4. Dopamine levels measured from 24-hour urine samples

Groups	Dopamine (µg/L)
Control (n=6)	9,36 ± 2,75
HS (n=6)	9,24 ± 0,71
L-NNA50 (n=6)	6,72 ± 1,11
L-NNA100 (n=6)	35,88 ± 6,28** $^{\Omega}$
L-NNA50+HS (n=6)	19,74 ± 7,26
L-NNA100+HS (n=6)	20,6 ± 3,33** $^{\#}$

Data were presented as mean±SE.** P<0.05 vs. control. $^{\#}$ P<0.05 vs. HS. $^{\Omega}$ P<0.05 vs. LNNA50.

L-NNA50, L-NNA100 or high salt application alone did not affect 24-hour urinary sodium excretion, while L-NNA100+HS (n=6; 0.87±0.16) administration significantly decreased 24-hour urinary sodium excretion compared to the control group (n=6; 1.87±0.21) (Table 4, P<0.05). Co-administration of a high-salt diet with L-NNA 100 reduced sodium excretion.

Sodium clearance, glomerular filtration rate and fractional sodium excretion calculation: High salt, L-NNA50 or L-NNA100 applications alone did not affect sodium clearance. The sodium clearance of the group administered with L-NNA100 and high salt was found to be significantly lower than the control group and the group administered with L-NNA100. It was determined that L-NNA100+HS application decreased sodium clearance (Table 3, $P<0.05$). The glomerular filtration rate of the L-NNA100+HS applied group was significantly decreased compared to the control group. However, no significant difference was found between the glomerular filtration rates of the other experimental groups (Table 3, $P<0.05$).

Urine dopamine values: Urinary dopamine concentrations were found to be significantly higher in groups where L-NNA100 was administered alone (n=6; 35.88±6.28) or in combination with a high-salt diet (n=6; 20.6±3.33) (Table 4, $P<0.05$).

Discussion and Conclusion

Essential hypertension is a strong risk factor for many diseases such as coronary disease, stroke, peripheral artery disease and heart failure, and the cause is still unknown. The effects of essential hypertension on human health and the fact that it is among the top preventable causes of death explains the fact that many studies have been conducted to elucidate the pathophysiology of the disease[30–32]. In this study, it was aimed to determine the changes in intrarenal dopamine synthesis and activity in blood pressure changes caused by NOS inhibition and high salt diet, and to investigate the effects of this system on developed hypertension.

In this study, 50 mg/L or 100 mg/L concentration of L-NNA or high salt diet (4%) was administered alone or in combination for 7 days. It was observed that L-NNA at both doses applied in the experimental protocol did not cause an increase in blood pressure alone by causing partial inhibition of NOS, but the application of L-NNA in both doses together with a high-salt diet caused a significant increase in the blood pressure of the subjects. In addition, the blood pressures of the L-NNA100+HS group were found to be higher than the L-NNA50+HS group (Table 1, $P<0.05$). Co-administration of partial NOS inhibition with a high-salt diet has been found to increase blood pressure in a dose-dependent manner.

Similar to this study, Tolins et al. concluded that the endogenous NO system directly modulates renal hemodynamics and sodium utilization, and high-salt diet administration also contributes to renal adaptation. Enhanced NO synthesis in response to increased salt intake facilitates sodium excretion, maintaining normal blood pressure. However, it has been shown that with the inhibition of NO synthesis with a high-salt diet, renal hemodynamics change, renal and

systemic vascular resistance increases, and sodium excretion decreases, resulting in increased blood pressure and sodium sensitivity in rats[33,34]. However, it has been reported that high-salt diet alone did not significantly affect water intake, diuresis and natriuresis. Similarly, it had no effect on renal dopamine synthesis, renal parameters and blood pressure[3,35]. Interestingly, another study reported that administration of high 1% salt in drinking water to rats increased natriuresis, diuresis, and urinary dopamine levels. In the related study, it was determined that the water intake, diuresis, and urinary sodium excretion of the control group were not similar to our study, and blood pressure measurement was not performed[36].

Administration of L-NNA alone in both doses resulted in a significant reduction in 24-hour water intake and decreased urine output. This decrease in the amount of urine may be related to the decrease in water intake, but the significant decrease in the amount of urine observed while the 24-hour water intake of the L-NNA100+HS group did not change, suggesting that the amount of water retained in the body increased (Table 2). A significant decrease was found in the 24-hour urine Na excretion of the L-NNA100 and high-salt diet group, while other treatments did not significantly affect sodium excretion. It is known that diuresis and natriuresis occur in the organism due to increased blood pressure[7,37]. However, in this study, natriuresis and diuresis, which were expected to increase blood pressure, did not occur as a result of the co-administration of L-NNA (100mg/L) and high salt. It was determined that in the L-NNA100+HS group, 24-hour urinary sodium excretion and calculated sodium clearance values were significantly reduced compared to the control group. When these data are evaluated, we can think that the pressure-natriuresis relationship was impaired in the L-NNA100+HS group, and that increased Na retention caused an increase in blood pressure. Impairment of pressure-natriuresis due to NOS inhibition is associated with hemodynamics and renal tubules. For example, reduction of NO synthesis directly causes an increase in basal renal vascular resistance or tubular reabsorption, or indirectly leads to a decrease in renal sodium excretion functions in the form of renin angiotensin activation or an increase in renal vascular response to vasoconstrictors[7,38]. In various studies, it has been reported that increased salt retention contributes to hypertension that occurs as a result of long-term low-dose NOS inhibition, and this application causes the development of salt sensitivity[33,34]. In another study, it was reported that long-term low-dose inhibition with L-NAME 16 mg/dL for 8 weeks and high salt for 4 weeks increased blood pressure by increasing the levels of noradrenaline and adrenaline excreted in 24-hour urine from the first week without changing the 24-hour urine amount and sodium excretion. In the development of NOS inhibition-mediated hypertension, the increase in salt sensitivity and sympathetic system activity has been emphasized[8].

In this study, when creatinine and urea levels measured in serum and urine are evaluated, it can be argued that these applications do not cause pathological changes in renal functions (Table 4). However, L-NNA100 and high salt application decreased glomerular filtration rate compared to other groups. In the study of Salazar et al. on dogs, it was shown that although there was no change in blood pressure administered with 50 ng/kg/min L-NAME intravenous infusion for 3 days, there was a decrease in GFR, water and salt retention, renal vasoconstriction, an increase in plasma renin activity and no change in aldosterone levels. It has been reported that NOS inhibition plays a role in the long-term regulation of renal hemodynamics and renal excretion functions as a result of the renal parameters reaching the levels in the control period after the administration is completed[13]. In another study, rats fed a high-salt diet for 2 weeks were infused with 50-125 mg/kg of L-NAME into the renal artery. It was found that only salt loading did not significantly affect glomerular filtration rate, renal perfusion pressure and renal vascular resistance, but adding L-NAME to salt loading dose-dependently decreased glomerular filtration rate, increased renal perfusion pressure and renal vascular resistance[33].

Reabsorption of sodium into the body takes place in the kidneys via Na^+/K^+ ATPase and Na^+/H^+ exchange pumps. The function of this pump is to ensure the reabsorption of Na^+ ions in the lumen into the renal tubules. Dopamine inhibits the activity of this pump in the proximal tubule. In the proximal tubule, this effect is mediated by dopamine receptors and the adenylate cyclase. Dopamine levels measured in 24-hour urine show intrarenal dopamine activity[3,26,27]. In this study, 4% high-salt diet alone for 7 days did not change the dopamine levels measured in 24-hour urine, while its co-administration with L-NNA increased urinary dopamine levels. Alexander et al. showed that increasing the amount of salt consumed for 8 days to 209-259 mEq in normal individuals who take 9 mEq of salt daily increased dopamine levels measured in 24-hour urine in individuals[39]. However, while the 25 times salt diet applied in this study increased the urinary dopamine levels, in our study, the 5 times more salt diet did not affect the urinary dopamine levels. In another study conducted in Dahl salt-sensitive (DS) and salt-resistant (DR) rats, a diet containing 0.4% or 8% salt was applied for 8 weeks, and hypertension developed in salt-sensitive rats, and hypertension did not develop in salt-resistant rats. 24-hour urine sodium values of DS and DR rats treated with high salt were found to be higher than those of DS and DR rats treated with low salt, but no difference was found between 24-hour urine dopamine levels. Changes in the mechanisms controlling renal vascular resistance rather than sodium excretion were thought to be responsible for the development of hypertension in DS rats[40]. To investigate the diuresis and natriuresis efficiency of intrarenal dopamine, normal salt (0.28%) or high salt (4%) was administered to rats under

anaesthesia for 5 days, and it was shown that water, sodium excretion and urinary dopamine concentrations increased in the high salt group[41]. In another study, increasing the amount of salt consumed for 8 days to 209-259 mEq in normal individuals who take 9 mEq of salt daily increased the sodium and dopamine levels measured in 24-hour urine[39].

Under normal physiological conditions, high salt intake increases renal dopamine production, which has been shown to be responsible for maintaining blood pressure with increased sodium excretion, but when oxidative stress is created, high salt intake causes renal dopamine oxidation, renal inflammation and dysfunction, and subsequent development of high blood pressure[4]. In hypertension, the interaction between dopamine receptors is absent or impaired, and natriuresis, diuresis and vasodilation, which are functions of renal dopamine, are impaired[42]. While it is not claimed that intrarenal dopamine is generally involved in the control of sodium excretion or arterial pressure in animals with normal sodium balance, a possible dependence of dopamine's tonic effect on the magnitude of sodium excess in the body has been demonstrated[43]. In this study, applying a high-salt diet alone for 7 days did not cause an increase in blood pressure, nor did it affect 24-hour urine dopamine levels. However, administration of a 4% salt diet with 100mg/L L-NNA increased 24-hour urine dopamine levels, which is an indicator of intrarenal dopamine synthesis. Despite this, while sodium excretion was expected to increase in 24-hour urine, salt excretion decreased and hypertension developed. It suggests that co-administration of L-NNA100 and high salt may cause oxidative stress and may fail to exert the natriuretic activity of intrarenal dopamine, although intrarenal dopamine production is increased as a result of disruption of dopamine G protein dopamine receptor coupling without affecting intrarenal dopamine synthesis ability.

Co-administration of L-NNA and high salt diet decreased 24-hour urinary sodium excretion, sodium clearance and GFR, and increased water salt retention and blood pressure in the body. This application increased 24-hour urine dopamine concentrations. However, the increased intrarenal dopamine natriuresis was insufficient to realize its effectiveness, and the increase in intrarenal dopamine production but its inability to function suggests that there may be a disorder in the G protein coupling of dopamine and D_1 and D_2-like receptor interaction. However, those interactions could not be detected within the scope of the study. Further studies are needed to elucidate the interactions at the dopamine receptor level and the effects of oxidative stress in the pathogenesis of hypertension.

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ORIGINAL RESEARCH

Decreased serum magnesium levels patients with migraine: a case control study

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Abstract

Objective: *Migraine is a common neurological syndrome that affects 15% of the population. The pathogenesis of migraines is not fully understood. In the nervous system; magnesium plays critical roles such as nerve conduction, neuromuscular coordination and protection against excitotoxicity.*

Method: *In this retrospective study, patients were diagnosed with migraine according to the International Classification of Headache Disorders-3 beta criteria were included. Patients were divided into three groups as migraine with aura, migraine without aura and chronic migraine.*

Results: *A total of 168 participant, 71 patients and 97 controls, were included in the study. Serum Mg values were measured as 1.98 mg/dl in the patient group and 2.04 mg/dl in the control group and were found to be significantly lower in the patient group (p=0.021). No significant difference was found in terms of mean serum Mg values in migraine subgroups.*

Conclusion: *We found that serum Mg levels are low in migraine patients and we think that this vital cation may be one of the factors playing a role in the pathogenesis of migraine. Evaluation of serum Mg level in migraine patients may help in predicting migraine attacks and symptoms, as well as in appropriate therapeutic planning for patients.*

Keywords: Migraine, magnesium, headache, measurement, level, pathogenesis.

Introduction

Migraine is a recurrent, usually unilateral common primary headache type characterized by moderate to severe headache attacks. Headache duration varies from hours to days and tends to worsen with routine physical activity. It is often accompanied by nausea, vomiting, photophobia and phonophobia . It is known to affect 15 percent of the general population[1,2]. About 18% of women and 6.5% of men suffer from migraine[3]. The group with 14 or less headaches per month is

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defined as episodic migraine, while chronic migraine (CM) defines patients with migraine who experience headaches 15 or more days per month. Episodic migraine is also divided into two subgroups as migraine with aura (MwA or classic migraine) and migraine without aura (MwoA or common migraine)[4] .

Magnesium (Mg) is a very important dietary macromineral that acts as a cofactor in more than 300 enzymatic reactions in the human body. In the nervous system; plays critical roles such as nerve conduction, neuromuscular coordination and protection against excitotoxicity[5]. Inadequate intake of Mg, due to its wide-ranging functions, may predispose individuals to many health problems, including those related to neurological conditions. In the previous studies, it has been reported that Mg may have a role in the mechanism of diseases such as migraine, chronic pain, epilepsy, Alzheimer's disease, Parkinson's disease, stroke, anxiety and depression[6].

The high incidence and prevalence of migraine and its crippling nature necessitate the elucidation of the underlying mechanism. The pathophysiological mechanisms underlying migraine attacks are not fully understood. Many factors have been blamed for the emergence of attacks. Sex hormones, cortisol, thyroxine, melatonin, hypothalamic axle, autonomic system, ions such as calcium and magnesium are among the factors that play a role in the pathogenesis of migraine headaches[7].

So far, no concrete answer has been found to the question of whether the decrease in Mg serum concentration is a factor that causes migraine headaches. The aim of this study is to compare the serum Mg levels of migraine patients and healthy individuals , to question the role of Mg in the pathogenesis of migraine and to contribute to the diagnosis and treatment planning of the disease.

Materials and Method

In this retrospective, cross-sectional, observational and comparative study, a total of 71 patients between the ages of 18 and 60 who applied to the neurology headache outpatient clinic of a tertiary university hospital and were diagnosed with migraine according to the International Classification of Headache Disorders (ICHD)-3 beta criteria were included. According to these criteria, patients were divided into three groups as MwA, MwoA and CM. Migraine severity evaluated with Migraine Disability assessment (MIDAS) score[8]. Detailed clinical history, sociodemographic characteristics, frequency of migraine attacks, duration of attacks, number of headache days per month, presence of aura were obtained from medical records for each patient.

Those with chronic diseases (such as chronic kidney failure, chronic liver failure, congestive heart failure), gastrointestinal system disease and malnutrition, and those receiving Mg-containing

drug therapy were not included in the study. In addition, patients with a history of drug use such as cyclosporine, cisplatin, aminoglycoside , amphotericin B, furosemide , acetazolamide and thiazide that affect serum levels of Mg were excluded from the study. In addition to the selection criteria used for the patient group, the control group consisted of healthy individuals who did not have a history of any type of headache, and whose blood samples were taken for routine health control.
Using a disposable injector, venous blood sample from the antecubital vein was taken between 8 am and 10 am after an overnight fast, into silicone-coated plastic tubes and centrifuged at 5000 rpm for 10 minutes.

The study was conducted in accordance with the Declaration of Helsinki guidelines and approval was obtained from the local ethics committee.

Statistical analysis

Statistical analyzes were performed with the SPSS version 21.0 program. The conformity of the variables to the normal distribution was tested with kurtosis and skewness analyses. Mean and standard deviation values were used for descriptive analyses. Categorical variables were compared with the Chi-Square Test. The Mann Whitney U Test was used to compare non-normally distributed quantitative variables, and the independent T -test was used to compare normally-distributed quantitative variables. Comparisons between multiple groups was done with one way ANOVA test. The correlation of quantitative data with each other was analyzed with Spearman and Pearson correlation tests. Cases with a P-value below 0.05 were considered as statistically significant results.

Results

A total of 168 participant, 71 patients and 97 controls, were included in the study. Of the migraine patients included in the study, 13 were in the MwA, 34 in the MwoA and 24 in the CM subgroup. The clinical features of migraine patients are shown in Table 1.

Table 1: Clinical features of migraine patients

Frequency of attacks (per month)	5.70
Duration of attacks (hours)	48.22
Headache days (per month)	11.87
MIDAS score	17.78
Episodic migraine (n) Migraine without aura (n) Migraine with aura (n) Chronic migraine (n)	47 34 13 24

While there were 53 (83.1 %) women in the patient group, 81 (83.5 %) of the control group were women. The mean age of the patient group was 35.12, and the mean age of the control group

was 33.74. The patient and control groups are similar in terms of age and gender distribution. Serum Mg values were measured as 1.98 mg/dl in the patient group and 2.04 mg/dl in the control group and were found to be significantly lower in the patient group (p=0.021). The characteristics of age, gender and serum Mg levels of the patient and control groups are shown in Table 2. No significant difference was found in terms of mean serum Mg values in migraine subgroups.

Table 2: Gender distribution and mean ages of the control and migraine groups

		Control		Migraine		p
		n	%	n	%	
Gender	Male	16	(16.5)	12	(16.9)	0.944[1]
	Female	81	(83.5)	59	(83.1)	
Age (year)	mean±Sd	33.74±8.71		35.12±9.87		0.338[2]
Magnesium serum level (mg/dl)	mean±Sd	2.04±0.14		1.98±0.15		0.021[2]

[1]Chi-square test, [2]Independent sample T test

The patients were divided into 4 groups according to their MIDAS scores in terms of migraine severity. The number of patients with Grade 1 was 10, the number of patients with Grade 2 was 22, the number of patients with Grade 3 was 23, and the number of patients with Grade 4 was 16. No significant difference was found in terms of mean serum Mg values in disease severity subgroups.

In the correlation analysis, no significant correlation was found between serum Mg values and frequency of migraine attacks, duration of attacks, number of headache days per month and MIDAS scores.

Discussion and Conclusion

In this study, we found that serum Mg levels were significantly lower in migraine patients compared to the healthy control group. We think that low Mg levels may be one of the physiopathological factors underlying migraine.

Mg plays an important role in the active transport of ions across neuronal membranes. It has been known for many years that Mg is associated with serotonin and N-methyl-D-aspartate (NMDA) receptors and affects nitric oxide synthesis and release[9]. Mg plays a vital role in controlling vascular tonicity and the reaction of vascular membranes to hormones and neuromediators by blocking NMDA channels[10]. It is also necessary for the release of nitric oxide trapped inside the cell. In Mg deficiency, nitric oxide remains in the cell and combines with superoxide to produce a peroxynitrite free radical that induces oxidative stress, lipid peroxidation

and myelin destruction, and neuronal irritability increases[7]. It has been thought that decreased Mg levels may cause cortical spreading depression leading to migraine by opening calcium channels, increasing intracellular calcium with glutamate release, and increasing extracellular potassium[11,12]. In addition, low Mg may be associated with increased platelet aggregation and vasoconstriction, which may be an important underlying etiology for triggering migraine because it affects serotonin receptors[13]. All these mechanisms support the view that Mg is one of the factors playing a role in the pathogenesis of migraine. Low Mg levels in migraine patients, which we found in the results of our study, may have led to migraine attacks by activating mechanisms such as free radical production, neurotoxicity and vasoconstriction.

Conflicting results have been obtained in studies investigating the relationship between migraine and Mg. In a study, it was reported that serum Mg levels were significantly lower in migraine patients compared to controls, and they were similar at the time of attack and between attacks[14]. In a different study , serum Mg levels of women with menstrual migraine were found to be significantly lower than the control group[15]. Our results seem consistent with these studies. On the other hand, there are also studies reporting that there is no significant difference between the serum Mg values of migraineurs and healthy individuals[16,17]. In a different study, it was reported that although Mg serum levels were within physiological limits both during the attack and between attacks, its concentrations during acute migraine attacks were lower than the period between attacks[18]. The inconsistencies found between the studies can be attributed to the presence of modifier factors such as smoking, drug use, intense exercise, nutritional differences, the difference in the number of patients in the study, and the serum Mg measurement method used.

Another entity that supports the role of Mg in the pathogenesis of migraine is the use of this mineral in the treatment of migraine. It is known that agents containing Mg are effective in migraine prophylaxis[19]. It has also been reported that Mg supplementation helps to reduce the dose of valproate in migraine patients treated with valproate[20]. It has been observed that the prophylactic use of oral Mg or the therapeutic administration of intravenous Mg during the attack is a useful approach and reduces migraine attacks. It is thought that this effect occurs as a result of the interaction of Mg with serotonin receptors[21]. Reports on the benefits of Mg use in both exacerbation and prophylactic treatment of migraine support the view that Mg deficiency is one of the factors that play a role in the pathogenesis of migraine.

Our study has some limitations. First of all, our study was designed retrospectively . The results of our study, which included a small patient population from a single center, cannot be generalized. Evaluations were based on a single measurement result and serum analyzes were not

performed during an acute migraine attack. Therefore, dynamic changes in ion levels may have been ignored.

Conclusion

We found that serum Mg levels are low in migraine patients and we think that this vital cation may be one of the factors playing a role in the pathogenesis of migraine. Evaluation of serum Mg level in migraine patients may help in predicting migraine attacks and symptoms, as well as in appropriate therapeutic planning for patients. We think that longitudinal studies with a large patient population are needed to better elucidate its diagnostic and therapeutic role.

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ORIGINAL RESEARCH

Investigation of Sociodemographic and Clinical Characteristics of Forensic Cases Admitted to a Child and Adolescent Psychiatry Outpatient Clinic in Şanlıurfa

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Abstract

Objective: *In this study, we aimed to evaluate the sociodemographic and clinical characteristics of children and adolescents for whom forensic reports were requested by judicial authorities for various reasons.*

Materials-Methods: *The records of 110 cases referred to our hospital's Child and Adolescent Psychiatry Department for forensic psychiatric evaluation between January 2022 and December 2022 were evaluated retrospectively. Sociodemographic data of the cases, clinical diagnoses, contents of forensic events, and forensic report decisions were included in the analyses.*

Results: *The mean age of the 110 patients participating in the study was 14.5 ± 2.9 years and 68.2% (n=75) of the cases were male. The judicial authorities directed the cases most frequently (n=53) for evaluating discrimination under the Turkish Penal Code 31/2, and it was determined that crime against property (41.5%) was committed most frequently. Considering the distribution of perpetrator and victim status by gender, a significant difference was found between the two genders (p<0.001).*

Conclusion: *Determining regional differences regarding individual and familial characteristics of child and adolescent forensic cases may contribute to the determination of factors that may adversely affect children's mental health and to the development of preventive mental interventions.*

Keywords: Child psychiatry; forensic psychiatry; juvenile delinquency

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Introduction

Forensic psychiatry is a branch of science that assists judicial bodies regarding cases that are included in the forensic system and require psychiatric evaluation.1 The number of forensic cases involving children and adolescents is increasing worldwide, and the number of incidents involving children brought to security units in our country increased by 10.8% in 2021 compared to 2020.2 Physicians working in the field of pediatric mental health need increasingly more knowledge and experience about the forensic dimension of cases and practices in clinical practice.

Cases referred to child and adolescent psychiatry clinics for forensic evaluation are frequently evaluated to determine their ability to perceive the legal meaning and consequences of the act they are alleged to have committed and to determine whether their ability to direct their behavior has developed. Additionally, forensic psychiatric evaluation of the minors may also be requested with a court decision for reasons such as marriage permission, custody case, adoption, in order to determine and treat detainees' psychiatric conditions, to understand whether their testimony can be trusted, whether they have suffered psychological damage due to a traumatic event and whether they may defend themselves physically and mentally.3

The definition of a child dragged into crime (CDC) is a young person who is investigated or prosecuted for allegedly committing an act defined as a crime in the Turkish Penal Code (TPC). If the CDC has completed the age of twelve but has not completed the age of fifteen as of the date of the crime, or if the child has completed the age of fifteen but is deaf and mute, it is imperative to conduct an examination to evaluate if the child is ‘sane’ and if he/she can be held responsible for their actions, in other words, the Ability of Realization and Distinction (ARD) of the child.4 While addressing the issue of criminal responsibility of children, the developmental period in which the child is in, the child's medical and psychological condition, and the social environment in which the child grows up should be evaluated holistically. Studies have revealed that one of the most important factors in juvenile delinquency is the negative environmental conditions that the child lives in.5 Therefore, determination of the environmental factors that lead children and adolescents to crime is a critical issue in the rehabilitation of these young people and in the prevention of recurrent juvenile delinquency.

Although there are no precise data on the frequency and prevalence of child abuse in Turkey, it has been shown that the prevalence of sexual abuse and physical abuse is 10.7% and 13.5%, respectively, and children who are victims of sexual or physical abuse are frequently referred by judicial authorities to child and adolescent mental health outpatient clinics for evaluation.6 Although the psychological effects of abuse on children vary depending on many

factors, it is known that exposure to abuse in childhood may lead to various pathologies not only in childhood and adolescence but also in throughout life.7 Therefore, forensic psychiatric evaluations of cases referred to child and adolescent psychiatry clinics due to being victims of abuse are important not only in terms of the legal process but also in terms of detecting possible mental disorders and protecting children from unfavorable mental health outcomes.

Studies conducted in different regions of Turkey report different findings regarding the sociodemographic and clinical characteristics of cases admitted to child and adolescent psychiatry clinics for forensic evaluation.8,9 Determining the forensic case profiles of various regions is necessary in terms of taking appropriate measures, and such studies to be conducted constitute an infrastructure for a rapid and accurate approach to cases. This study aimed to investigate the sociodemographic and clinical characteristics, cognitive capacities, current psychopathologies, and the referral reasons for examination of the children and adolescents who were referred to our hospital for forensic evaluation by judicial authorities in Şanlıurfa.

Materials and Method

Ethical approval of the Harran University Ethics Committee dated 23.01.2023 with decision number 02/02 was obtained. The population of the study consisted of children and adolescents who were referred to our child and adolescent psychiatry outpatient clinic by judicial authorities between January 2022 and December 2022 due to a request for a forensic report for various reasons. The data of the cases were obtained retrospectively from their records after ethics committee approval. The cases were evaluated in terms of age, gender, formal education attendance, reasons for requesting a forensic report, previous forensic report status, and report results. In addition, previous psychiatric admissions and psychiatric diagnoses of the cases, the presence of psychopathologies detected in the cases after forensic evaluation, cognitive levels of the cases, and psychiatric follow-up continuity were examined.

In our study, the Wechsler Intelligence Scale for Children (WISC-R) was performed on the patients by experienced and certified psychologists to determine the cognitive levels of the cases. Standardization, validation, and reliability of the test in the Turkish language have been proven.[10] The psychiatric diagnoses of the cases in the evaluated section were made according to DSM-5 diagnostic criteria as a result of clinical interviews conducted by child and adolescent psychiatry specialists.[11]

Statistics

Data were analyzed with the IBM SPSS Statistics 23 package program. Compliance with normal distribution was evaluated by Kolmogorov-Smirnov test. Mann-Whitney U test was used to compare non-normally distributed data according to paired groups. Chi-square test, Yates correction, and Fisher's Exact test were used to compare categorical variables by groups, and the results were considered statistically significant for $p<0.05$.

Results

A total of 110 cases were evaluated in our study, and information on the sociodemographic characteristics of the cases is shown in Table I.

When the number of forensic evaluations of the cases was examined, 83.6% (n=92) of the total cases (n=110) were forensically evaluated for the first time in our hospital, 7.3% (n=8) were forensically evaluated for a different incident in an external center, and forensic evaluation of 5.5% (n=6) of them had previously been performed in an external center for the same incident, forensic evaluation of 2.7% (n=3) had previously been performed in our center for a different incident, and 0.9% (n=1) had an adult psychiatry evaluation at our center for the same incident.

Table 1: Distribution of the sociodemographic characteristics of the cases

Demographics and family characteristics	
Age (Mean ± SD)	14.52 ± 2.91
Gender, n (%)	
Female	35 (31.8)
Male	75 (68.2)
Number of siblings (Mean ± SD)	5.42 ± 2.27
School attendance, n (%)	
Does not go to school	52 (47.3)
Primary education	24 (21.8)
High school	21 (19.1)
Irregular participation in formal education	4 (3.6)
Dropping out of school after the judicial incident	6 (5.5)
Preschool age group	3 (2.7)
Nationality, n (%)	
Turkish	100 (9.9)
Others	10 (9.1)
Parental coexistence, n (%)	
Together	89 (80.9)
Divorced and separated	12 (10.9)
A parent is dead	9 (8.2)
Mother's education level, n (%)	
Illiterate	39 (35.5)
Literate	33 (30)
Primary school graduate	29 (26.4)
Secondary school graduate	8 (7.3)
High school graduate	1 (0.9)
Father's education level, n (%)	
Illiterate	5 (4.5)
Literate	44 (40)
Primary school graduate	26 (23.6)
Secondary school graduate	20 (18.2)
High school graduate	15 (13.6)

SD: standard deviation, n: number

We found that 87.3% (n=96) of the cases had no previous psychiatric admission and 37.5% (n=36) of the cases with no previous psychiatric admission were found to have psychopathology after forensic evaluation. The most common psychiatric diagnosis in these patients was found to be borderline intellectual functioning (n=10). Information on the clinical characteristics of the patients who underwent psychiatric examinations in our clinic for forensic evaluation is shown in Table II.

Table 2: Distribution of clinical features of cases

	Frequency (n)	Percentage (%)
Intelligence level		
Normal range	90	81.8
Borderline intellectual functioning	12	10.9
Mild intellectual functioning	3	2.7
Evaluation not completed	5	4.6
Diagnosis of psychiatric illness		
None	60	54.5
Borderline intellectual functioning	11	10
Intellectual disability	2	1.8
Attention deficit and hyperactivity disorder	9	8.1
Conduct disorder	2	1.8
Depression	2	1.8
Post-traumatic stress disorder	5	4.5
Substance use disorder	9	8.2
Anxiety disorder	2	1.8
Other	8	7.2
Psychiatric admission history		
Present	96	87.3
Absent	14	12.7
Suggestion to continue psychiatric follow-up		
Present	62	56.4
Absent	48	43.6
Psychiatric treatment initiation status		
Present	8	7.3
Absent	102	92.7
Continuation of psychiatric follow-up		
Present	7	6.4
Absent	55	50
No follow-up was recommended	48	43.6

n: number

Forensic cases, 43.6% (n=48) of them were accompanied by law enforcement officers, and 48.2% (n=53) of the cases were not accompanied by their parents on their first visit. The reasons for requesting a forensic report are presented in Table III.

Table 3: Reasons for requesting forensic evaluation

Reason for Requesting Forensic Evaluation	n (%)
Evaluation ARD within the scope of TPC 31/2	53 (48.1)
Evaluation ARD within the scope of TPC 32	1 (0.9)
Evaluation of whether she/he should be taken under protection under TCC 432	7 (6.3)
Evaluation of whether they can defend themself spiritually within the scope of TPC 102/3	4 (3.6)
Evaluation of psychological effects after the traumatic event	27 (24.5)
Psychological evaluation due to early marriage desire	8 (7.2)
Evaluation of whether their statements can be trusted	3 (2.7)
Evaluation in terms of maturity	2 (1.8)
Evaluation of whether they understand the possible meaning and consequences of the act they are exposed to in terms of TPC 103/1	2 (1.8)
Other reasons	3 (2.7)

n: number, ARD: Ability of Realization and Distinction, TPC: Turkish Penal Code, TCC: Turkish Civil Code

Table IV shows which crime the children who are perpetrators or victims of a crime were evaluated in relation to. Considering the 53 cases who underwent evaluation of ARD status related to the act committed within the scope of TPC 31/2, it was found that 90.6% (n=48) of the patients were male and 49.1% (n=26) of all CDCs did not attend school. While 66% (n=35) of these cases did not have a psychiatric disorder, of those who did, the most common psychiatric disorder in CDCs was borderline intellectual functioning with 15.1% (n=8). It was found that 56.6% (n=30) of the CDCs did not have ARD, 17% (n=9) could not complete their forensic evaluation because they did not attend the necessary interviews, 17% (n=9) had ARD, and 7.5% (n=4) could not be evaluated as to whether they had ARD at the time of the incident considering the time elapsed since the incident. It was suggested that hospitalization in an institution with inpatient services for 1.9% (n=1) of these cases would be appropriate.

Table 4: The nature of the crime and the status of being a perpetrator/victim

Nature of Crime/Judicial Case	Perpetrator n (%)	Victim n (%)
Crime against life	2 (3.8)	0 (0)
Crime against bodily immunity	18 (34)	18 (62)
Torture-torment crime	0 (0)	1 (3.4)
Sexual offense	6 (11.3)	4 (13.7)
Crime against privacy	2 (3.8)	0 (0)
Crime against property	22 (41.5)	2 (6.8)
Against bodily immunity and sexual offense	0 (0)	1 (3.4)
Crime against bodily immunity and against liberty	0 (0)	1 (3.4)
Threat crime	0 (0)	2 (6.8)
Sex crime, threatening crime and crime against privacy	1 (1.9)	0 (0)
The crime of insulting Turkishness, the republic, the institutions and organs of the state	1 (1.9)	0 (0)
The crime of making propaganda for a terrorist organization	1 (1.9)	0 (0)

n: number

When 27 cases who underwent evaluation of psychological effects after a potentially traumatic event were evaluated, 29.6% (n=8) of these cases did not have Post-traumatic Stress Disorder (PTSD), 22% (n=22) had sub-threshold PTSD symptoms and these symptoms impaired functionality, 18.5% (n=5) had PTSD, and 14.8% (n=4) had subthreshold PTSD symptoms but these symptoms did not impair functionality. For 14.8% (n=4) of the cases, forensic evaluation could not be completed because the patient did not attend the necessary interviews. The most common incident was battery (n=12), followed by traffic accidents (n=3). It was determined that 29.6% (n=8) of the cases evaluated in terms of mental trauma did not go to school before the incident, and 11.1% (n=3) left school after the incident. Although 85.2% (n=23) of the cases evaluated in terms of post-traumatic psychiatric effects did not have a psychiatric application before the event, 59.3% of the cases were diagnosed with a psychiatric disorder after forensic evaluation. The most common psychiatric disorder in these patients was PTSD (n=5), followed by conduct disorder (n=2), depression (n=2), and anxiety disorder (n=2).

When the eight cases who applied for early marriage authorization were examined, all of them were female, their mean age was 16.0 ± 0.5 years, none had attended formal education, and none had any psychiatric admission before. Of these cases, 62.5% (n=5) had normal intelligence level, 25% (n=2) had borderline intellectual functioning, and one of the cases had mild intellectual disability; regular psychiatric follow-up was recommended for the patient with mild intellectual disability. For all cases, considering the developmental period they were in, early marriage was considered inappropriate and a forensic report was prepared accordingly.

When the distribution of perpetrator and victimization status by gender was analyzed, there was a significant difference between genders in this respect ($p<0.001$). It was determined that 17.1% (n=6) of the girls and 62.7% (n=47) of the boys were considered as perpetrators. While the most common reason for requesting a forensic evaluation for girls was to investigate whether there was post-traumatic mental effects, the most common reason for requesting a forensic evaluation for boys was ARD evaluation.

When the cohabitation status of the parents of CDCs and victims was compared, no significant difference was found between the two groups ($p=0.44$). There was also no significant difference between the educational status of the mother ($p=0.07$) and father ($p=0.52$) of the CDCs and victims. Our study also examined whether there was a difference between Turkish citizens and foreign nationals in terms of being perpetrators and victims, and no significant difference was found between the two groups in terms of being perpetrators/victims ($p=0.45$).

Discussion

In our study, the sociodemographic and clinical characteristics, intelligence levels, and psychiatric diagnoses of 110 children and adolescents who were referred to our child and adolescent psychiatry outpatient clinic by the judicial authorities for a forensic report were evaluated by retrospectively evaluating the patients' records.

The mean age of the cases was found to be 14.5 ± 2.9 years, and this finding is similar to other studies in the literature.[8,12] In our study, the majority of the cases were males (68.2%), and this result is similar to the results of other studies which revealed that the majority of the cases referred to child and adolescent psychiatry outpatient clinics for forensic evaluation were males.[13,14] However, there are also studies reporting that most of the cases referred to child and adolescent psychiatry outpatient clinics for forensic evaluation were girls.[15,16] It was thought that the different results reported by the studies in this regard might be due to the differences in the rates of perpetrators or victims in the cases referred for forensic evaluation.

When school attendance status was evaluated, we observed that approximately half of the cases (47.3%) did not attend formal education, and this rate is significantly higher compared to the results of similar studies conducted in the western regions of Turkey.[12,16,17] Although the school enrollment rate has gradually increased in the province of Şanlıurfa in recent years, many students in Şanlıurfa cannot effectively continue the education process due to economic inadequacies, and the school attendance rates of children of secondary education age in Şanlıurfa are below the average in Turkey.[18,19] Based on these findings, it was thought that the high rate of cases not attending formal education in our study could be explained by the sociodemographic structure of the city where our hospital is located.

A striking sociodemographic finding in our study was that 10% of the cases referred to the child and adolescent psychiatry clinic for forensic evaluation were foreign nationals. In our country, studies focusing on the inclusion rates of immigrants in forensic systems are extremely rare, and, considering the increasing number of asylum-seekers or children under temporary protection status in our country in recent years, it is thought that more research should be conducted in this field.

In our study, 81.8% of all cases who underwent forensic evaluation were found to have normal intelligence. This rate is higher when compared with the results of a similar study in the literature, but it is thought that this difference between the results of the two studies may be due to the different rates of victimization of the cases referred for forensic evaluation.[16] Different studies predominantly evaluating CDCs reported a similar distribution of intelligence levels to our study.[20,21] In our study, although most of the cases (n=96) had no previous psychiatric admission,

37.5% (n=36) of the cases with no previous psychiatric admission were found to have psychopathology after forensic evaluation. Considering these results, it can be argued that forensic psychiatric evaluations are an important opportunity to provide counseling, diagnosis, and treatment services to children and young people who may not have had access to them before. It was determined that the most common psychiatric disorder was borderline intellectual functioning both in all forensic cases (n=110) and in patients who did not have a previous psychiatric admission but were diagnosed after forensic evaluation (n=36), and this result obtained in our study supports the findings of some studies in the literature.[22,23] Borderline intellectual functioning is used to define the heterogeneous group between cognitive deficiency and normal intelligence, characterized by deficits in daily and social activities, and 1-2 standard deviations below the average intelligence.[24] In many studies, borderline intellectual functioning was not considered to be a separate psychiatric diagnosis and was handled separately when examining the distribution of intelligence levels of the cases. Our study differs from other studies in terms of evaluating borderline intellectual functioning within general psychopathologies. The reason for this is that although it is only included as a descriptive V code in DSM 5, borderline intellectual functioning may lead to negative consequences such as decreasing emotional resilience in individuals, interrupting the processes of overcoming problems, increasing sensitivity to stimuli, and causing insufficient development of the ability to cope with anxiety and impulses.[25] Some researchers suggest that borderline intellectual functioning should be re-included among the diagnostic categories in the next versions of DSM.[26] Considering that presenting descriptive data on children with borderline intellectual functioning who are involved in the forensic system is important in terms of planning and implementing preventive interventions that can protect these children from being involved in forensic processes, this diagnostic group was addressed within psychopathologies in our study.

In our study, it is noteworthy that only 11.2% (n=7) of the patients who were recommended for regular psychiatric follow-up after forensic evaluation continued regular follow-up. In the literature, few studies have addressed the continuation of regular psychiatric follow-up in cases referred to child and adolescent psychiatry clinics for forensic evaluation. In a study conducted in Gaziantep in 2012, it was shown that only 21.4% of the patients who were referred to the child and adolescent psychiatry outpatient clinic for forensic evaluation and who were recommended regular follow-ups after psychiatric evaluation continued follow-ups regularly.[27] In another study evaluating continuity of psychiatric treatment of children and adolescents who were sexually abused, it was found that only 9 of the 35 cases continued regular psychiatric follow-ups.[28] Considering that children and adolescents who are included in forensic evaluation systems for

various reasons are at risk for mental disorders in the later periods of their lives, it is thought that it is important to make some regulations to facilitate the continuation of regular psychiatric follow-up in this patient group.

When the reasons for requesting a forensic report of the cases referred to our clinic were examined, it was found that the most common request was for ARD evaluation within the scope of TPC 31/2. Studies conducted in our country report different results regarding the reasons for requesting forensic psychiatric evaluation of children and adolescents. In two studies conducted in 2018 and 2021, similar to our study, the most common reason for requesting forensic evaluation in children and adolescents was ARD evaluation.[4,12] However, in some studies in the literature, it has been reported that the most common reason for requesting forensic evaluation of cases referred to child and adolescent psychiatry outpatient clinics for forensic evaluation is being a victim of abuse.[16] This difference in the results may be due to the abolition of the concept of deterioration in mental health in Articles 102 and 103 of the TPC regulating sexual inviolability by Law No. 6545, which entered into force on June 28, 2014. In the previous regulation, deterioration in the physical or mental health of the victim was determined as a reason for increasing the penalty, and whether there was deterioration in the physical or mental health of the victims of sexual crimes was evaluated by physical and psychiatric examination in accordance with these articles of law.[29] With the amended article of the law, the need to ask whether the physical and mental health of children who are victims of sexual abuse is impaired has been eliminated, and it is thought that the number of children referred for this reason has decreased.[4]

In our study, most of the CDCs (90.6%) were male, and a significant difference was found between genders in terms of being perpetrator/victim ($p<0.001$). It has been shown in different studies that boys exhibit criminal behavior at a higher rate than girls, and this difference between genders is explained by propositions such as higher rates of exposure to domestic physical violence and higher likelihood of being in social environments that may increase the risk of committing crime, in addition to the biological characteristics of boys.[30,31] In addition, 49.1% (n=26) of the CDCs in our study did not attend school, and this finding supports previous studies that associate the tendency of children and adolescents to commit crime with factors such as school failure, truancy, and low attachment to school.[32] When the crimes allegedly committed by the CDCs evaluated in our study are considered, it was found that these cases were most frequently referred for crimes against property (41.5%) followed by crimes against bodily immunity (34%). In two different studies conducted in Gaziantep and Adıyaman, the two neighboring provinces of Şanlıurfa, it was reported that CDCs were most frequently referred to child and adolescent

psychiatry outpatient clinics for ARD evaluation due to theft and crimes against bodily immunity, respectively.[14,27] The distribution of alleged crimes committed by children and adolescents evaluated in terms of ARD in our study is similar to previous studies conducted in the same region. When the 27 cases who were asked to be evaluated in terms of psychological impact after an event that may have a traumatic effect were considered, it was thought that the reason why only 18.5% (n=5) of the cases were referred because of sexual abuse may be that cases of sexual abuse were not reported to the judicial authorities. It is known that children may not tell someone about sexual abuse for various reasons or may postpone telling.[33] On the other hand, in a study conducted on perpetrators of child sexual abuse, it was shown that the parents of 21.4% of the victims did not report the sexual abuse even though they learned about the sexual abuse and knew the perpetrator.[34] These findings show that not only the victimized children but also the family members who learned about the situation may conceal the sexual abuse experienced by the child.

Our study is a descriptive study conducted by retrospectively evaluating the data in the patient records, and the lack of long-term follow-up of the cases is one of the most important limitations of the study. In order to develop preventive and protective intervention programs for the mental health of children and adolescents involved in the forensic system, to determine nationwide social policies according to the needs of children, and to reintegrate these cases into society as mentally healthy individuals, the sociodemographic and clinical characteristics and needs of forensic child and adolescent cases must first be well understood. Our study is of importance in terms of revealing the sociodemographic and clinical characteristics of children and adolescents referred for forensic evaluation in Şanlıurfa, one of the major cities in the Southeastern Anatolia Region of Turkey. However, it is thought that further nationwide studies are needed to reduce juvenile delinquency and victimization, to make necessary legal regulations, and to make healthier evaluations on the protection of children's mental health.

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REVIEW

Pseudomonas aeruginosa; Virulence Factors and Host Defense Mechanisms

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Abstract

As an opportunistic pathogen, Pseudomonas aeruginosa (P. aeruginosa) can cause both acute and chronic infections. Variable virulence components and antibiotic resistance markers in the bacterium's genome constitute the bacterium's pathogenic profile and provide the bacterium with outstanding metabolic adaptability to many conditions. The interactions of P. aeruginosa with the host are poorly understood, complicating the treatment of its infections and the development of vaccines against them. Despite decades of scientific research focusing specifically on this challenge, vaccines to prevent these dangerous infections still do not exist. The major virulence factors of P. aeruginosa and host immune responses against the bacteria are discussed in this review.

Keywords: *Pseudomonas aeruginosa, virulence factors, host immune response*

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Introduction

Pseudomonas aeruginosa is a Gram-negative non-fermentative bacteria that causes hospital infections and it is resistant to many molecules[1]. In addition to being naturally resistant to many antibiotics, it is a serious problem with an increasing incidence in recent decades, gaining multiple antibiotic resistance owing to its ability to acquire resistance rapidly even during antibiotic use[2–4]. *P. aeruginosa* is on the World Health Organization (WHO) list of "critical" bacterial pathogens demanding new antibiotic development and research[5,6].

There are many incomprehensible parts of *P. aeruginosa*-host interactions. Therefore, the treatment difficulties of infections continue, and preventive vaccines have not yet been developed despite several decades of research[7]. Many extracellular components are concerned with the pathogenesis of *P. aeruginosa.* These systems can increase the movement of bacteria (twitch motility) and enable them to reach nutrients more easily. In addition, they allow bacteria to penetrate tissues more easily or cause more damage to the tissue they colonize through enzymes (elastase, protease, and DNase) that can break down various substances. However, the most important virulence factor for bacteria is biofilm production[8]. The virulence factors of *P. aeruginosa* act on molecules and enzymes in the host cell cytoplasm and activate multimolecular signaling stages in immune cells known as inflammatory cells[9].

Virulence factors of *P. aeruginosa*

P. aeruginosa carries a wide range of virulence factors that contribute to its pathogenicity. Several virulence factors may induce pathogenicity while targeting the extracellular matrix, facilitating adhesion, and/or busting host cell signaling pathways. *P.aeruginosa* can cause a variety of diseases that occupy the host and its immune system, causing infections that are almost impossible to heal[10-12].

The lipopolysaccharide (LPS) is the major component of the outer membrane of *P. aeruginosa*; it involves a distal polysaccharide (O-antigen), a hydrophobic endotoxic element (lipid A), and a single-core oligosaccharide[13]. The LPS activates the host's both innate and adaptive immune responses and ultimately dysregulates inflammation responses that result in increased morbidity and mortality[14].

Flagel, which has a strong immunogenic structure, plays a critical role in the pathogenesis by binding to membrane components of epithelial cells such as AsiolaGM1 and providing adherence[15]. *P. aeruginosa* easily reaches nutrients through flagella, evades the harmful effects of immune system elements and antibacterial agents, translocates into the host cell, and can move freely in the

biofilm[16]. The flagellum stimulates the NFχB-dependent inflammatory response by interacting with TLR5 and TLR2, so leads to the initiation of IL-8 synthesis by activation of the calcium-dependent kinase pathway. It is found in most of the strains isolated from the environment and hospital infections, whereas the 10 strains isolated from patients with cystic fibrosis and chronic infections do not have flagella to evade the host immune system response[17].

Type-IV pili have the ability to adhere to cells, thereby providing tissue tropism and attachment to certain tissues. These adhesions mediate non-opsonic phagocytosis and biofilm formation[18-20].

P. aeruginosa has six secretory systems (Type I to VI) that release hydrolytic enzymes and various toxins to invade the host[21,22]. Type I (T1SS) and Type V (T5SS) secretion systems are simple secretory mechanisms that secrete substances into the extracellular environment. T1SS is a one-step secretory system without the need for a periplasmic intermediate. It consists of an outer membrane protein (OMP), an ATP-binding cassette (ABC) transporter, and an adapter protein binding the two together. Alkaline protease (AprA) and heme acquisition protein (HasAp) are known substrates of T1SS; they are directed into the T1SS secretion tool by a C-terminal secretory signal. The type II secretion system (T2SS) is the most multipurpose secretion system of *P. aeruginosa*[21]. The type III secretion system (T3SS) is the most important secretory system used to disable and destroy the host's immune system[23]. The type VI secretion system (T6SS) is one of the most recently described bacterial secretion systems. T6SS consists of a hemolysin-coregulated protein (Hcp-TssD) tube containing double-spike proteins. It is important for bacterial competition as it produces bacterial toxins (Tse) that destroy the host's microbial flora but also play a minor role in host defense[24]. Unlike the other three systems with a one-step mechanism, T2SS and T5SS use a two-step secretion mechanism that involves the passage of secreted proteins into the periplasm. On the other hand, T3SS and T6SS inject proteins directly into the cytoplasm of the host cell[21,22].

The exotoxin A is secreted through T2SS, which uses a pilus-like tool to secrete proteins into the extracellular environment, including lipase, phospholipase, alkaline phosphatase, and protease. The contribution of these factors to the virulence of bacteria has been demonstrated in animal experiments[25]. Besides, exotoxin A has been demonstrated to be involved in regional tissue invasion and injury [26]. Elastase LasB type metalloproteases also cause tissue damage in the host, especially in burned wound infections and pulmonary infections[27].

Alginate, another substance secreted by *P. aeruginosa*, is an anionic mucoid exopolysaccharide. It consists of repetitive polymers of D-mannuronic acid and L-glucuronic acid and protects bacteria against adverse environmental conditions such as oxidative stress, host defense systems and especially the harmful effects of aminoglycoside antibiotics[28,29].

Quorum Sensing (QS) is a "cell-to-cell" bacterial communication mechanism via diffusible chemical compounds. The quorum is required to produce a sufficient amount of a secreted signal molecule (auto-inducer) to prompt the expression of a large regulon[30]. The most common class of autoinducers are the acyl-homoserine lactones (AHL) used by Gram-negative bacteria. The autoinducer diffuses freely throughout the bacterial membranes. Butanoyl-homoserine lactone and oxohexanoyl-homoserine lactone are the AHL signals of the bacteriae[31]. These signals generated by AHL synthase (LasI/RhlI) are emitted into the microenvironment. The increase in bacterial density during infection also increases the autoinducer concentration. When a certain bacterial population density is reached, AHL molecules accumulated in the medium activate genes that induce biofilm formation and encode virulence factors[32].

A recently discovered QS System (IQS) uses "2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde" as a new signaling molecule. The cognate receptor is not yet known[33]. IQS inhibits host cell growth, impairs host DNA damage repair, and induces apoptosis dose-dependently[34].

Biofilms play an important role in the development of resistant to chronic infections caused by *P. aeruginosa.* Biofilms are microbial communities adhering to a surface and surrounded by an exopolysaccharide (EPS) matrix. The function of the biofilm is to ensure that the microorganisms contained in them are protected from attack by the internal and external environment. Thus, it gains resistance to antibiotics, disinfectants, and host defenses and impairs bacterial clearance[35,36]. Compared to the planktonic form, biofilm formation of *P. aeruginosa* is generally associated with higher drug resistance and leads to evading the host immune response. Microorganisms in the biofilm also exhibit altered phenotypes concerning growth rate and gene transcription[37–42].

Table 1. Virulence factors and pathogenicity mechanisms of *P. aeruginosa*

Virulence factors	Mechanism
Lipopolysaccharide (LPS)	Activates the host's innate and adaptive immune system by Toll-like receptor 4 (TLR4), NOD-like receptor pyrin domain containing 1 (NLRP1), NLRP2, and NLRP3), and dysregulates inflammation responses.
Flagellum	Strongly immunogenic; interacts with TLR2 and TLR5; binds to membrane components of epithelial cells (Asialo GM1). Gives motility to access nutrients, escape from immune system elements and antibacterial agents, migrate in the host cell, and move freely within the biofilm.
Type-IV Pili	Type-IV pili have the ability to adhere to cells, thereby providing tissue tropism and attachment to certain tissues.
T1SS (Type 1 Secretory System)	One-step secretory machinery consists of an outer membrane protein (OMP), an ABC transporter, and an adapter protein. The substrates are alkaline protease (AprA) and Heme acquisition protein (HasAp).
T2SS (Type 2 Secretory System)	This molecular nano-machine consists of three parts: General secretory pathway (Gsp) proteins, a large channel embedded in the OM, and the pseudo-pilus functioning as a piston that secretes exotoxin A.
T3SS (Type 3 Secretory System)	The most important secretory system used to disable and destroy the host's immune system. Supports the transfer of virulence proteins called effectors from the bacterial cytoplasm to the eukaryotic cell in a single step.
T4SS (Type 4 Secretory System)	The most cosmopolitan secretory system. It differs from other secretion systems by having the ability to transfer DNA in addition to proteins.
T5SS (Type 5 Secretory System)	Consists of a two-stage secretory mechanism involving the passage of secreted proteins into the periplasm.
T6SS (Type 6 Secretory System)	An integrated secretory system within the cell membrane. Transfers toxic substrates to eukaryotic and prokaryotic cells. It plays a crucial role in pathogenesis and competition among bacteria.
Exotoxin A	It is secreted via T2SS. It plays a role in local tissue damage and invasion.
Proteases	Several proteases are "elastase LasB-type" metalloproteases. They destroy the host tissue and play a crucial role in both acute lung and burn wound infections.
Alginate	A mucoid anionic exopolysaccharide. Protects the bacteria against adverse environmental conditions, oxidative stress, the host defense system, and especially the harmful effects of aminoglycoside antibiotics.
Quorum Sensing	A bacterial "cell-to-cell" communication mechanism via diffusible chemical compounds. Quorum must produce a sufficient amount of a secreted signaling molecule (auto-inducer) to activate the expression of a large regulon. Acyl-homoserine lactones (AHL) are the most common class of autoinducers used by Gram-negative bacteria.
Biofilm Formation	Biofilms are communities of microorganisms that adhere to a biotic or abiotic surface surrounded by an exopolysaccharide (EPS) matrix. They protect the microorganisms in their contents from the microbial attack of the external or internal environment.

Host immune response against *P. Aeruginosa*

P. aeruginosa is an opportunistic pathogen and causes secondary infections in immunocompromised humans, so the main factor determining the occurrence of infections is the immune status of the host[43,44]. The immune system is suppressed in cases where barrier integrity is impaired as a result of the use of broad-spectrum antibiotics, catheter applications, trauma, wound, ulcer, or burn infections, etc. Bacteria, which find the opportunity for colonization, produce alginate from extracellular virulence factors in such cases, causing tissue damage and spreading in the bloodstream[45].

During *P. aeruginosa* infections, potent agonists of Toll-like receptors (TLR) -TLR2, TLR4, TLR5, and TLR9 - which recognize bacterial lipopolysaccharide (LPS), lipopeptides, unmethylated bacterial CpG DNA, and flagellin, are expressed in the host[46–48]. Of these, the most crucial for infection clearance is the TLR4-dependent inflammatory response to LPS[47]. TLR4 sensing of LPS leads to activation of important inflammatory cytokines such as TNF-α, IL-6, and IL-8[49]. The absence of TLR4 increases sensitivity to two distinct signaling pathways: the primary response pathway of myeloid differentiation 88 (MyD88) and the adaptive pathway involving the beta interferon-inducing Toll/IL-1R domain (TRIF pathway). The MyD88 pathway activates the nuclear factor kappa light chain enhancer of activated B cells (NF-κB), thereby releasing numerous proinflammatory cytokines and chemokines – TNF-α, IL-6, and macrophage inflammatory protein (MIP). The TRIF pathway regulates the transcription of chemokines such as IFN-α and IFN-β, IP-10 (Interferon γ-inducible protein 10) and RANTES (regulated in the activation of normally expressed and secreted T cell)[50]. UT12 - the TLR4/MD2 agonistic monoclonal antibody - was shown to support host defense against chronic *P. aeruginosa* lung infection, increase neutrophil levels and inflammatory MIP-2 concentrations in the lungs, and improve bacterial clearance in mice[51]. Animal experiments have shown that TLR4 and TLR5 are required for the appropriate host immune response[52-55]. It has been shown that lung cells infected with *P. aeruginosa* in mice were unable to induce invasive lung infection when TLR5 was blocked with anti-TLR5 antibodies[56]. Inflammatory responses dependent on TLR2 and TLR9 are shown to be important in bacterial clearance. The deficiencies of TLR-2 and TLR-9 increased bacterial clearance and protected mice against *P. aeruginosa* pulmonary infection[57, 58].

Studies have shown that the MyD88 pathway is essential for the rapid migration of neutrophils to the infection site[59]. Substances released by *P. aeruginosa* target the host cell cytoplasm and activate the assembly of multimolecular signaling stages in inflammatory cells. NOD-like receptors (NLRs) are a group of patern recognition receptors (PRRs) that can recognize

various endogenous and exogenous ligands and thus play a critical role in innate immunity. NLR inflammatories - NLRC4 and NLRP3- are involved in the detection and reaction of *P. aeruginosa* infections[60]. NLRP3 is involved in the recognition of *P. aeruginosa* infection, followed by macrophage secretion of caspase-1 and IL-1β. As a secondary signal, the human cathelicidin LL-37/h-CAP18 promotes the death of epithelial cells infected with *P. aeruginosa* and acts as a "fire alarm" to stimulate inflammatory responses that will counteract uncontrolled infection. Then IL-1β and IL-18 are released from infected epithelial cells in order to promote neutrophil efflux[61].

P. aeruginosa also activates NLRs by the release of outer membrane vesicles (OMV). Thus, TLR-dependent reactions occur in epithelial cells via proteins and LPS. These membrane vesicles also activate NF-κB signaling and mitogen-activated protein kinase (MAPK) within epithelial cells[48,62]. This may suggest the use of NLRs as therapeutic adjuvant targets during *P. aeruginosa* infection, thereby reducing inflammatory responses in bacteria-infected cells[48].

P.aeruginosa and innate immunity

Recognition of *P. aeruginosa* pathogen-associated molecular patterns (PAMPs) is the first step of a robust inflammatory response that facilitates bacterial clearance and the migration of macrophages and neutrophils to the site. A weak immune response results in a poor response of phagocytic cells and failure of bacteria-killing and clearance, while an excessive immune response causes host tissue damage. Therefore, the host response should be optimal[48,63–67]. The chemokine receptors - CXCR1 and CXCR2- are the chemokine receptors on the neutrophils that regulate host defense and neutrophil migration, especially in pulmonary infections caused by *P. aeruginosa*. CXCR1 regulates anti-*Pseudomonas* neutrophil responses by modulating reactive oxygen species and interacting with TLR5[48,67–69]. CXCR1, also binds to IL-8 and to GCP-2 specifically leading to a proinflammatory effect[48,68]. Both CXCR1 and CXCR2 are essential in the response to *P. aeruginosa* because they recruit neutrophils that provide bacterial clearance and have other functions in the immune system[70,71]. While killing *P. aeruginosa*, neutrophils may also contribute to host lung injury due to the synthesis of reactive oxygen compounds (ROS) and proteins released from their acidophilic granules[72]. Therefore, a neutrophil recruitment level that will provide bacterial clearance but not cause excessive tissue damage is crucial during infection control.

There is increasing evidence that *P. aeruginosa* can survive inside mammalian cells. In the study of Garai et al., it was shown that *P. aeruginosa* can be detected first in phagosomal vacuoles and then in the cytoplasm of macrophages. This indicates phagosomal escape of the bacteria.

Among the *P. aeruginosa* virulence factors, T3SS and ExoS play an important role in the intramacrophage survival of bacteria and can induce host macrophage lysis[73].

Complement activation also plays a role in the host response to *P. aeruginosa*. The OprF porin located in the outer membrane of the bacterium acts as a binding acceptor molecule for C3b, enabling the formation of the membrane attack complex (MAC). A study by Mishra et al. showed that C3b binding was significantly reduced in an OprF-deficient *P. aeruginosa* strain[74]. The innate immune system is important in the control of *P. aeruginosa* infections, but further studies are needed on the details of these pathways and how they are integrated in vivo.

P. aeruginosa and adaptive immunity

If proinflammatory pathways are weakened during the acute phase of *P. aeruginosa* infections, the inflammatory response may resolve. T helper cells (Regulatory T cells; Treg) secrete anti-inflammatory cytokines, inhibit the secretion of pro-inflammatory cytokines, and dendritic cells initiate adaptive responses. *P. aeruginosa* infection, which cannot be eradicated during the acute phase, progresses to a chronic infection characterized by mucoid biofilm formation[75]. As neutrophil inflammation prolongs, high expression of IFN-γ, IL-6, IL-1β and IL-17 and a decrease in IL-10 and Treg are observed, followed by an effector T cell response[48]. This response inhibits bacterial antigen presentation and an effective host immune response against *P. aeruginosa*[75]. A Th1-like response may improve lung function by releasing IFN-γ[76-78]. With the induction of IFN-γ by alveolar macrophages, apoptotic neutrophils are removed, progression to necrosis occurs and thus further inflammation is prevented[75]. Increasing the Th1 response reduces IL-8 - a chemoattractant for neutrophils- thus reducing inflammation in the lung due to decreased neutrophil recruitment. A low Th2 response reduces both tissue damage and the formation of immune complexes. Furthermore, decreased IL-13 levels may result in decreased mucus production[79].

Another acquired immune response in *P. aeruginosa* infections is antibody production and subsequent immune complex (IC) formation. Although IgG antibody production in chronic *Pseudomonas* infections is associated with high NF-KB expression, particularly in cystic fibrosis (CF) patients, it should be kept in mind that the response to specific antigens may vary depending on the stage of infection. While some antigens are more expressed in the acute phase, causing an intense immune response, some antigens are recognized in the chronic phase. The presence of specific antibodies against antigenic structures of the mucoid phenotype of *P. aeruginosa* has been associated with a poor prognosis[75].

Immunoglobulin A (IgA), the dominant antibody isotype of the mucosal immune system, is also of great importance in the humoral immune response against respiratory tract infections caused by *P. aeruginosa*[80]. Based on the concentration of secretory IgA against *P. aeruginosa* in nasal secretions and saliva, it can be predicted whether patients are colonized, infected, or chronically infected with *P. aeruginosa*[81,82].

Conclusion

The numerous virulence factors of *P aeruginosa* and their expression at different levels according to the environment and conditions cause infections and treatment approaches to be quite complex. Despite more than 50 years of research to develop a vaccine against *Pseudomonas*, no marketable product has yet been produced. *P. aeruginosa* remains one of the most resistant organisms to antibiotics in the pharmaceutical industry. Researchers are still looking for new drugs or new treatment options that could stop infections caused by this multi-drug-resistant, problematic bacterium.

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