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Phone: +90 (530) 177 30 97 / +90 (539) 307 32 03
E-mail: info@galenos.com.tr • yayin@galenos.com.tr
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Message from the Editor-in-Chief

Message from the Editor-in-Chief,

Dear colleagues who closely follow the developments in medical science,

Preparations for the first issue of 2024 were spent in the fight against viral infections brought by the winter period. However, with the outstanding efforts of all healthcare professionals, it was overcome without a major social health problem arising. With the hopeful energy of the new year and your intense interest, our first issue is ready for publication, as if welcoming spring. An interesting issue awaits you, in which you will be satisfied with current developments with valuable original research and interesting case series covering different areas of medicine. In this respect, GMJ continues its work without compromising its inclusiveness.

On this occasion, we would like to thank our editorial board, our authors and you, our valued readers, for ensuring that this issue is presented to you in the best possible way.

M. Ali Gülçelik, M.D., Prof.
Editor-in-Chief

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Synergistic effects of royal jelly and glycine on the healing of skin wounds in mice

Zahra Pazoki[1], Maryam Eidi[1], Yasaman Ebrahimikia[2], Seyedemaryam Zarei[1], Narges Parhizkari[3]

[1]*Islamic Azad University, Biological Sciences College, Department of Biology, Varamin, Iran*
[2]*Islamic Azad University Tehran Medical Sciences Faculty of Medicine, Anatomical Sciences and Cognitive Neuroscience Department, Tehran, Iran*
[3]*Islamic Azad University Tehran Medical Sciences, Faculty of Advanced Science and Technology, Department of Cellular and Molecular, Tehran, Iran*

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Corresponding Author:
Zahra Pazoki, M.D., Islamic Azad University, Biological Sciences College, Department of Biology, Varamin, Iran
+989192702258
zahrapazoki760@gmail.com

ORCID:
orcid.org/0000-0001-8583-3180

Keywords: Royal jelly, glycine, skin wound, collagen, mice

ABSTRACT

Aims: This study aims to assess how the synergistic effects of royal jelly (RJ) and glycine, which are easily accessible and economically viable substances, can enhance the restoration of skin affected by wounds. This study provides valuable insights into pioneering strategies for addressing the critical concern of effectively treating skin wounds.

Methods: The experimental design of this study involved the formulation of different concentrations of RJ and glycine. A total of 80 male NMARI mice were categorized into two groups of 10, each following specific oral and topical treatment protocols. Additionally, a 5-mm diameter wound was created on the back of the neck. These wounds were then treated orally and topically with varying doses of RJ and glycine, and their combination, over 9 days. Wound measurements were taken and recorded daily throughout the study period. On the 10th day, mice were anesthetized under ethical conditions, and skin tissue samples were collected for subsequent histological examinations and hydroxyproline measurements.

Results: The synergistic effects of combined oral treatment with RJ (50 mg/kg) and glycine (3 and 12 mg/kg) and simultaneous topical treatment of RJ (2.5%) and glycine (0.2 and 1%) caused a significant reduction in skin wound diameter ($p<0.001$) as well as an increase in new blood vessels, fibroblast accumulation, epithelial tissue formation, and collagen synthesis in histopathological sections compared with their single doses.

Conclusions: The results showed that RJ and glycine significantly increased collagen synthesis, epithelial formation, and hydroxyproline levels in wound tissue.

Introduction

The skin acts as a protective barrier for living organisms, covers the entire body surface, and has a high regenerative capacity. However, deep injuries, such as deep burns or extensive cuts, are associated with scar tissue and require quick and effective repair. Achieving healthier skin may require complementary treatments (1).

Wound healing therapies can generally be divided into two categories: traditional and modern, with varying degrees of efficacy, clinical acceptance, and side effects. Contemporary treatments for skin ulcers primarily involve chemical agents and invasive procedures, which can be expensive, time-consuming, and detrimental in the long term (2,3). Bee products are not only utilized in therapy and skincare as cosmetic ingredients. Royal jelly (RJ) serves as a traditional remedy for wound repair; nevertheless, the underlying mechanisms and ingredient profiles remain largely unknown. RJ is a yellow-white viscous substance with a sweet and sour taste and a faint phenol odor and is

secreted by the hypopharyngeal glands of young worker bees (4). RJ has many useful properties, including anti-inflammatory, antioxidant, anti-microbial, anti-tumor, and wound healing properties (5). In recent years, the use of RJ has increased because of its many benefits as a natural honeybee product and its great potential for use in medical and pharmaceutical products (6-8). In particular, in the field of healing skin wounds due to the antioxidant and anti-inflammatory properties of RJ, this substance can be an alternative treatment for many chemical compounds in the future (9). Glycine, the simplest amino acid characterized by its single carbon atom and side chain, accounts for approximately 11.5% of total amino acids and 20% of nitrogen content in body proteins (10). Moreover, it serves as a widely used analyte in clinical applications because of its prominence (11). Glycine functions include cell protection, anti-inflammatory responses, and body growth (12,13). Its involvement in the synthesis of glutathione, a natural antioxidant crucial for diminishing free radicals and thus mitigating risks of diseases and aging, further underscores its significance (14). Proline, glycine, and hydroxyproline are involved in 57% of all amino acids in collagen, and glycine is used as a cost-effective additive in animal diets more than other amino acids (15). Glycine is one of the amino acids found in collagen, and the abundance of skin collagen can be a sensitive indicator of the importance of glycine in various metabolic processes (16). The collagen index can be measured by measuring hydroxyproline because in creature tissues hydroxyproline and hydroxylysine are found as in collagen (17). Hydroxyproline-containing di- and tripeptides in human blood plasma increment in a dose-dependent manner after the expending hydrolyzed collagen (18). Prolyl hydroxyproline and hydroxypropyl glycine are the major food-derived collagen peptides found in human blood plasma (19).

The objective of this study was to investigate the potential of readily available substances such as glycine and RJ in enhancing collagen production in skin injuries, a topic explored comprehensively within this research.

Methods

Study design

The experimental design involved the preparation of different material concentrations for oral and topical treatments, the formation of experimental groups of mice, wound induction, histological and biochemical studies, and subsequent statistical analysis.

Animals

Eighty male NMARI mice (20-30 g) were obtained from Pasteur Institute (Iran). Forty mice were divided into 10 groups for oral and topical treatments (Table 1).

Procedures

RJ was obtained (Roodin Company, Iran) and concentrations of 50, 100, and 200 mg/kg of body weight were prepared for oral treatment with gavage (20) and concentrations of 2.5%, 5%, and 10% were arranged by physiological serum for topical treatment (21).

Glycine was obtained (Pajuhesh Chemistry Company, Iran) in concentrations (3, 12, and 50 mg/kg) for oral treatment with gavage, and concentrations of 0.2%, 1%, and 2% were arranged for topical treatment. The combined concentrations for oral treatment (RJ 50 mg/kg + glycine 3 mg/kg and RJ 50 mg/kg + glycine 12 mg/kg) and the combined concentration for topical treatment (RJ 2.5% + glycine 0.2% and RJ 2.5% + glycine 1%) were arranged.

Table 1. Experimental groups

Experimental groups for oral treatment			Experimental groups for topical treatment		
Group number	**Group name**	**Treatment**	**Group number**	**Group name**	**Treatment**
1	Intact group	No wound, no treatment	1	Intact group	No wound, no treatment
2	Control group	Wounded, no treatment	2	Control group	Wounded and received physiological serum
3	Experimental group	RJ 50 mg/kg	3	Experimental group	RJ 2.5%
4	Experimental group	RJ 100 mg/kg	4	Experimental group	RJ 5%
5	Experimental group	RJ 200 mg/kg	5	Experimental group	RJ 10%
6	Experimental group	Glycine 3 mg/kg	6	Experimental group	Glycine 0.2%
7	Experimental group	Glycine 12 mg/kg	7	Experimental group	Glycine 1%
8	Experimental group	Glycine 50 mg/kg	8	Experimental group	Glycine 2%
9	Experimental group	RJ 50 mg/kg + glycine 3 mg/kg	9	Experimental group	RJ 2.5% + glycine 0.2%
10	Experimental group	RJ 50 mg/kg + glycine 12 mg/kg	10	Experimental group	RJ 2.5% + glycine 1%

Note: Each group consisted of 4 mice for both oral and topical treatments. RJ: Royal jelly

5-mm diameter wound on the back of the neck of mice using a skin punch (14). The size of the wound was measured and recorded daily for 9 days. Approval was obtained from the Ethical Committee on the Use and Care of Laboratory Animals of Islamic Azad University of Varamin Pishva Branch, Iran.

Outcomes

The thin tissue pieces, measuring 5 m thick (22), were sliced with a microtome and stained with hematoxylin and eosin. The changes considered in tissue samples included the arrangement of epithelium, collagen synthesis, formation of new blood vessels and presence of fibroblasts. All the above items were scored (23) and measured using ImageJ software (INH, USA) and compared with the control group.

Tissue samples were analyzed using a hydroxyproline measurement kit (Kiazist Company, Iran). The sample was mixed in the presence of strong acid, and after oxidation, it reacted with chromogen, and its absorbance was measured at a wavelength of 540-560 nm.

Statistical Analysis

The Statistical Package for the Social Sciences Software (version 21, IBM Crop., Armonk, NY, USA) was used in the data analyses. The data are expressed as mean±standard error of the mean (SEM). Before conducting parametric tests, the normality of the data was evaluated using the Shapiro-Wilk test. Variances among the groups were examined using the Student's t-test and one-way analysis of variance (ANOVA), followed by Tukey's test. P<0.05 was considered statistically significant.

Results

Effects of oral and topical treatments on skin wound diameter

Measurements of the progression of skin wounds and the effects of oral and topical treatments were performed over 9 days. Wound size significantly decreased compared with the control group in the group of animals receiving oral glycine at 50 mg/kg on day 7 and RJ at a concentration of 200 mg/kg on day 8 ($p<0.05$). The combination of oral RJ (50 mg/kg) and glycine (3 and 12 mg/kg) resulted in a significant reduction in skin wound diameter compared with isolated doses of each substance ($p<0.001$). Topical wound treatment using 10% RJ solution or glycine solution (1 and 2%) also significantly decreased skin wound diameter on day 7 compared with the control group ($p<0.05$). Additionally, combining RJ (2.5%) with glycine (0.2 and 1%) led to a substantial reduction in skin wound diameter on day 7 compared with the use of each substance alone ($p<0.001$) (Figure 1).

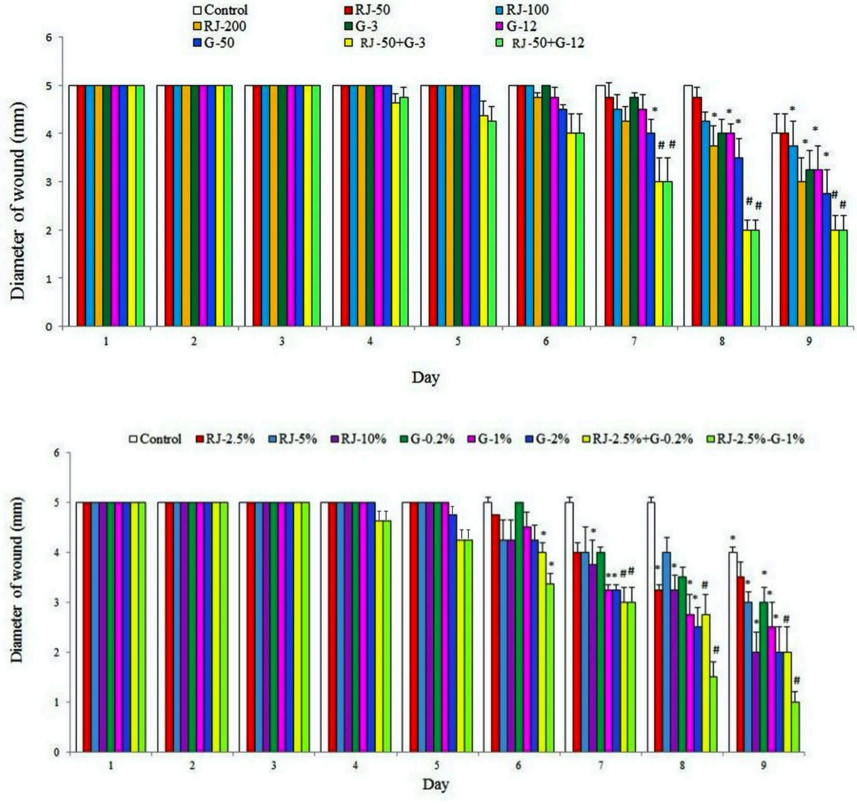

Figure 1. The effects of oral RJ and glycine (marked with G) on skin wound diameter in small laboratory mice for 9 days (top chart). The effects of topical RJ solutions and glycine on skin wound diameter in mice small laboratory for 9 days (down chart). *p<0.05 vs control group. #p<0.001 synergistic treatment vs single dose of RJ and glycine

RJ: Royal jelly

Results of the histological studies

Histological studies revealed that compared with the control group, oral RJ or glycine significantly increased epithelial tissue repair, collagen synthesis, fibroblast accumulation, and new blood vessel formation in a dose-dependent manner for 9 days ($p<0.001$). The combination of these substances also resulted in a significant increase in epithelial tissue repair compared with the individual doses of each ($p<0.01$) (Figure 2). Similarly, topical treatment with RJ or glycine solution in a dose-dependent manner significantly increased epithelial tissue repair, collagen synthesis, fibroblast accumulation, and new blood vessel formation compared with the control group ($p<0.001$). Combining RJ and glycine caused a notable increase in epithelial tissue repair compared with individual doses of each ($p<0.01$) (Figure 3).

Measurement results for hydroxyproline

After a 9-day treatment period, oral administration of RJ or glycine significantly increased hydroxyproline levels in the injured tissue in a dose-dependent manner compared with the control group ($p<0.001$). Moreover, the oral combination of these substances resulted in a significant increase in hydroxyproline concentration compared with individual doses of each ($p<0.05$) (Figure 4a). Similarly, topical treatment with RJ (5 and 10%) or glycine solution (1 and 2%) led to a significant increase in hydroxyproline levels in the wounded tissue compared with the control group ($p<0.001$). Combining RJ and glycine led to a distinctly higher concentration of hydroxyproline than when either substance was individually applied in topical treatment ($p<0.05$) (Figure 4b).

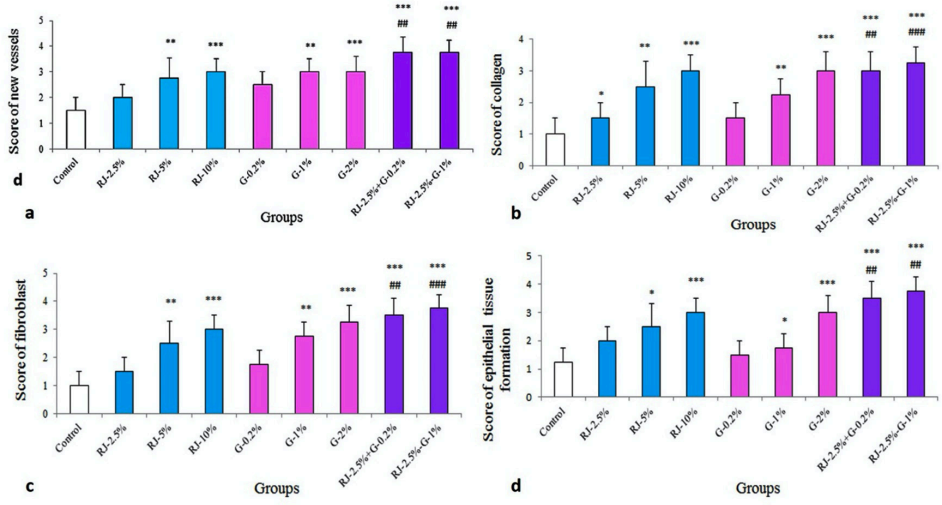

Figure 2. The effects of oral treatment on the number of new blood vessels (a), collagen synthesis (b), presence of fibroblasts (c), and formation of epithelial tissue (d). *p<0.05, ***p<0.001 vs control group. ##p<0.01 synergistic treatment vs single dose of RJ and glycine
RJ: Royal jelly

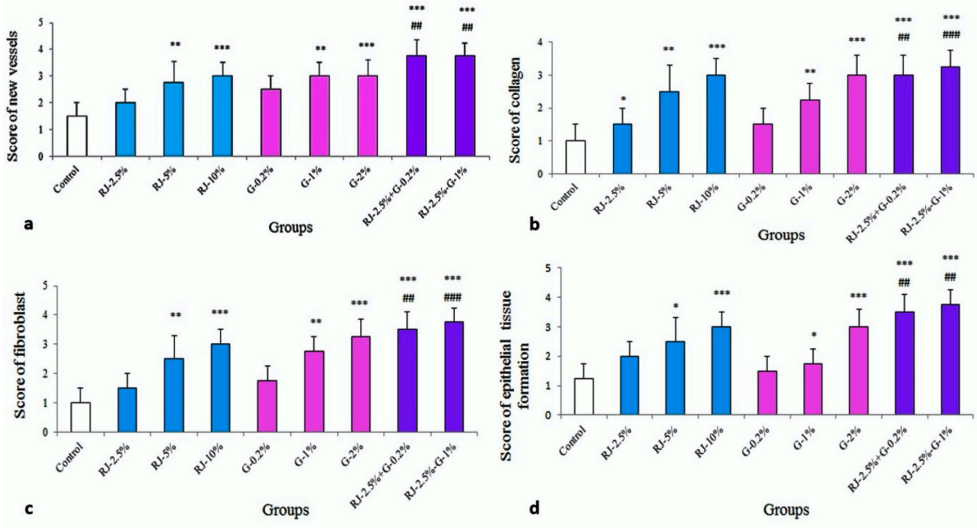

Figure 3. The effects of topical wound treatment on the number of new blood vessels (a), collagen synthesis (b), presence of fibroblasts (c), and formation of epithelial tissue (d). *p<0.05, ***p<0.001 vs control group. ##p<0.01 synergistic treatment vs single dose of RJ and glycine
RJ: Royal jelly

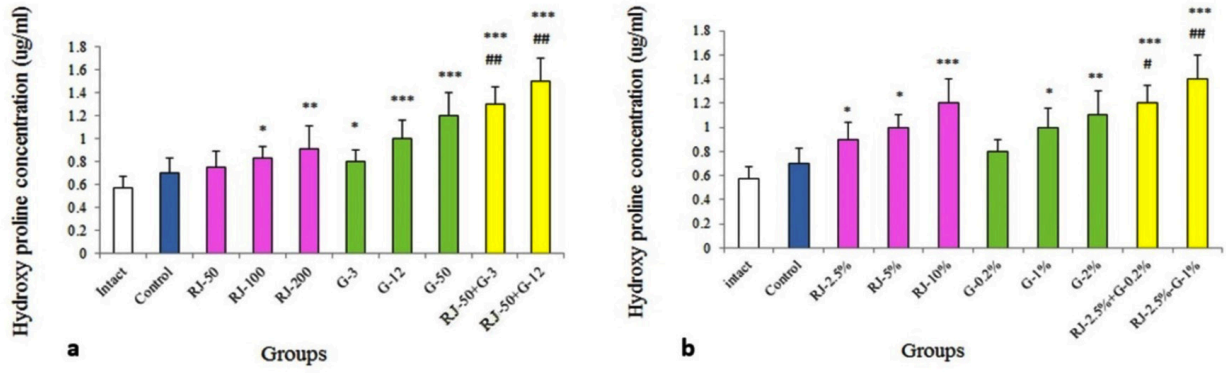

Figure 4. The effects of oral treatments on the amount of hydroxyproline (a). The effects of topical treatments on the amount of hydroxyproline (b), *p<0.05, ***p<0.001 vs control group. #p<0.05, ##p<0.01 synergistic treatment vs single dose of RJ and glycine
RJ: Royal jelly

Discussion

The present study demonstrated that administering higher doses of oral and topical treatments with RJ or glycine yielded more favorable effects than lower doses. Particularly, RJ at a concentration of 200 mg/kg and glycine at a concentration of 50 mg/kg led to significant reductions in the diameter of the skin wound on the 8th and 6th days, relative to the control group in oral treatment. In addition, the topical treatment of RJ with a concentration of 10% and glycine solution of 2% caused a significant decrease in the diameter of the skin wound on the 7th and 6th days, respectively, compared with the control group. Furthermore, oral administration of RJ and glycine yielded greater improvements when compared with topical treatment. This disparity was evident in the daily photographic documentation, where the wounds exhibited a smaller and more closed diameter following the oral regimen of RJ and glycine compared with their topical application. The results obtained from histological examinations and hydroxyproline measurements emphasize the enhanced effectiveness of the synergistic interaction between RJ and glycine in wound healing compared with their isolated applications. Similar results from other studies showed the validity of our results; for example, in past studies, it has been proven that RJ induces the proliferation of fibroblasts that produce collagen, thereby healing the wound (24). RJ is also effective in healing the skin through other mechanisms; RJ promotes wound healing by increasing the activity of keratinocytes (25), increasing nitric acid (26), modulating inflammation (27), increasing transforming growth factor-β secretion and decreasing tumor necrosis factor-alpha (28). RJ increases the migration of human skin fibroblasts and prevents skin aging in mice in an in vivo model by determining the expression levels of procollagen type I protein and matrix metalloproteinase (29).

In this study, glycine increased the number of skin repair cells both orally and topically, and similar to the results of scientists who had already discovered the various functions of glycine (30,31), this study showed the repair properties of this amino acid. The main function of glycine is the synthesis of proteins. Most proteins contain small amounts of glycine; an exception in this regard is collagen, which contains approximately 33% glycine due to the formation of a spiral structure (32). Glycine and proline can increase collagen synthesis in pig, chicken, and fish skin (12). Overall, our study proved that using RJ and glycine to treat wounds helps in effective skin healing. In addition, when RJ and glycine are used in combination, they show more effectiveness in wound healing and collagen synthesis than either treatment alone.

Study Limitations

The study was conducted on male NMARI mice, which may limit the generalizability of the findings to other species. The relatively short study duration (9 days) may not have captured the long-term effects of the combined treatment of RJ and glycine on wound healing. The study was conducted on mice, and the direct applicability of the findings to human subjects remains to be established.

Conclusion

This study showed the effectiveness of RJ and glycine alone and in combination on skin wound healing in mice. The observed synergistic effect of RJ and glycine in wound healing offers a promising direction for potential applications in both traditional and modern skin treatments. Further studies and testing in human populations are necessary to explore their clinical utility in disease conditions.

Ethics

Ethics Committee Approval: Approval was obtained from the Ethical Committee on the Use and Care of Laboratory Animals of Islamic Azad University of Varamin Pishva Branch, Iran.

Informed Consent: Animal experiment.

Authorship Contributions

Surgical and Medical Practices: Z.P., S.Z., N.P., Concept: Z.P., M.E., Design: M.E., S.Z., Data Collection or Processing: Z.P., M.E., N.P., Analysis or Interpretation: Z.P., Y.E., N.P., Literature Search: Y.E., S.Z., Writing: Y.E.

Conflict of Interest: No conflict of interest was declared by the authors.

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Mental health of mothers with children with attention deficit hyperactivity disorder after distance learning measures during the COVID-19 pandemic

Duygu Kaba[1], Hande Ayraler Taner[1], Burcu Akın Sarı[1], Ayşegül Efe[2], Beril Aydın[3]

[1]Başkent University Faculty of Medicine, Department of Child and Adolescent Psychiatry, Ankara, Türkiye
[2]Ankara Etlik City Hospital, Clinic of Child and Adolescent Psychiatry, Ankara, Türkiye
[3]Başkent University Faculty of Medicine, Department of Pediatrics, Ankara, Türkiye

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Corresponding Author:
Duygu Kaba, M.D., Başkent University Faculty of Medicine, Department of Child and Adolescent Psychiatry, Ankara, Türkiye
+90 505 230 34 44
duygukaba72@gmail.com

ORCID:
orcid.org/0000-0002-4261-8509

Keywords: Distance education, ADHD, Coronavirus disease-2019, COVID-19 pandemic, parent child relationship, burnout

ABSTRACT

Aims: This study aimed to evaluate the mental health of mothers of children with attention deficit hyperactivity disorder (ADHD) after distance learning measures during the Coronavirus disease-2019 (COVID-19) pandemic, considering factors that may influence their well-being.

Methods: In a cross-sectional design, the levels of burnout, depression, anxiety, stress, coping strategies, social support, and perceived changes in mental health among the mothers were assessed using the Maslach Burnout Inventory (MBI), Depression Anxiety Stress Scale-21 (DASS-21), Brief Coping Orientation to Problems Experienced Inventory, Multidimensional Scale of Perceived Social Support Scale, and Global Rating of Change (GRC) scale. Furthermore, the mothers completed the Atilla Turgay Scale (T-DSM-IV-S) to evaluate their perceptions of their children's ADHD and behavioral problems.

Results: Of the 72 mothers (mean age: 42.46±6.73 years, mean children age: 11.39±3.12 years), 50%, 44.4%, and 43.1% had depression symptoms, anxiety, and stress, respectively. According to the GRC, most mothers (67%) reported deterioration in mental well-being compared with the pre-pandemic period. The strongest correlations were between the subscales of T-DSM-IV-S and the total MBI score (r=0.51, p<0.001), DASS-21 score (r=0.35, p=0.003), and GRC score (r=-0.40, p=0.004), particularly with the inattention subscale. Child's attention deficit score, maternal education level, maladaptive coping level, and frequency of outdoor activities were identified as predictors of GRC.

Conclusions: This study showed that the distance learning during the COVID-19 pandemic was associated with deteriorated mental health among mothers of children with ADHD. The mother's maladaptive coping strategies, frequency of outdoor activities, education level, and attention deficit level were identified as independent predictors of deteriorated mental health in mothers.

Introduction

Attention deficit hyperactivity disorder (ADHD), a neurodevelopmental disorder characterized by distractibility, mobility, and impulsivity, is prevalent in children and adolescents (1). A limited number of studies conducted during the Coronavirus disease-2019 (COVID-19) pandemic have highlighted more dramatically increased parenting stress (2) and decreased quality of life in parents of children with ADHD than in those of the controls (3,4). This may be due to increased home time spent with children, reduced educational support from teachers, the burden of responsibility on parents, increased family conflicts, and reduced social support. Moreover, studies conducted on extraordinary conditions such as pandemics have reported that parental stress was positively correlated with a child's ADHD

symptom severity (2,3); among ADHD symptoms, hyperactivity-impulsivity symptoms were stated to be a stronger predictor of parenting stress (5-7).

After the COVID-19 outbreak was declared a pandemic, schools in Türkiye switched to online education, and home confinement was applied for individuals under 18 years. Türkiye continued online education for a longer period than European countries and applied long-term home confinement for children. Parents of children with ADHD may be more adversely affected in Türkiye because of prolonged interaction with their children and the increasing challenges faced by children with ADHD during home confinement. However, the mental health of children with ADHD and their parents has not been adequately evaluated during the distance learning process stemming from the pandemic in Türkiye.

While previous studies provide valuable insights, it remains unclear how specific factors contribute to the deterioration of parental mental health in individuals with ADHD, as well as the relationship between parental mental health and the child's ADHD symptoms during distance learning. This study differs from other studies in the literature in that it also addresses various factors that may be related to the mother's mental health, such as the level of social support, coping strategies, and the child's behavioral problems. Taking these factors into consideration, the present cross-sectional study aimed to investigate the following aspects through self-reports from mothers during the home confinement and distance learning imposed by the pandemic: (a) levels of maternal general distress and burnout, (b) increasing problems of children with ADHD, (c) the relationship between maternal mental health and parental coping strategies, perceived social support, and the severity of the child's ADHD symptoms, and (d) risk factors associated with deteriorated maternal mental health. The primary outcomes of the study were focused on the mental health of mothers with children with ADHD, encompassing burnout, depression, anxiety, stress, and perceived changes in mental health associated with the pandemic. The secondary outcomes included exploring various factors that may impact the well-being of these mothers, including coping strategies, perceived social support, the severity of their children's ADHD and behavioral problems, and sociodemographic factors.

Methods

Participants and the procedure

This cross-sectional study included mothers of children with ADHD during the distance learning period. The data were collected between May 15 and July 15, 2021, when 1-year distance learning and home confinement continued for individuals under 18 years of age. The data were registered during follow-up visits to the Child and Adolescents Clinic of the Faculty of Medicine, Başkent University Hospital. A non-probability convenience sampling technique was used in this study. The inclusion criteria for participant selection were as follows: having a child under the age of 18 years diagnosed with ADHD and residing with the child. The exclusion criteria encompassed the presence of intellectual disability, autism spectrum disorder, specific learning disability, psychotic disorder, bipolar mood disorder, or chronic physical diseases (such as cancer, diabetes, epilepsy, etc.) in the child.

The ADHD diagnosis of the children was reconfirmed by the clinician according to the Diagnostic and Statistical Manual of Mental Disorders, 5th ed., Text Revision (8) criteria. The Wechsler Intelligence Scale for Children (fourth edition) was requested in cases suspicious for an intellectual disability (9). During the specified period, 184 mothers with children with ADHD visited the outpatient clinic. Among this group, 119 mothers met the research criteria, and 81 voluntarily agreed to participate. After obtaining verbal and written informed consent from the parents who met the inclusion criteria and voluntarily agreed to participate in the study, the mothers completed the questionnaires. Of the 81 mothers who filled out the research questionnaire, 9 participants with missing data were excluded, and the final analysis included 72 participants.

Assessments

Sociodemographic form: The form created by the authors consists of parent-proxy reports regarding the sociodemographic characteristics and medical history of all children in the family, types and duration of medication used by children, COVID-19 backgrounds of parents, frequency of outdoor activities such as tracking and exercise during the last year, and any changes in the children's behavioral problems.

Maslach Burnout Inventory (MBI): This scale consists of three subscales: emotional exhaustion, depersonalization, and lack of personal accomplishment (10). The reliability and validity of the Turkish version were evaluated (11) and adapted to parents (12). Each of 22 items in this scale is scored from 0 to 4. The analysis involved the general burnout score derived from reversing the scoring of the items in the personal accomplishment subscale. As the total score on the scale increased, burnout also increased. In the current study, the data were recorded and analyzed as continuous variables. The Cronbach's alpha reliability coefficients of the emotional exhaustion, depersonalization, and personal accomplishment subscales were 0.86, 0.69, and 0.72, respectively.

Depression Anxiety Stress Scale-21 (DASS-21): The scale was developed in 1995 (13), and the Turkish version was validated (14). Each item is scored between 0 and 3, and the total score ranges between 0 and 63. The cut-off scores of the Turkish version for depression, anxiety, and stress are 5, 4, and 8, respectively. In a recent study, the DASS-21 total score was

found useful to determine the general distress score (15). As the total score on the scale increased, the level of general distress also increased. In the current study, the data were recorded and analyzed as continuous variables. The Cronbach's alpha was 0.95.

Brief Coping Orientation to Problems Experienced Inventory (Brief COPE): Brief COPE developed by Carver consists of 14 subscales, each containing two items (16). Each item is scored between 1 and 4. Psychometric validation of the Turkish version was performed (17). The score obtained from the scale also reflects the frequency of the use of that coping strategy. There are different categorizations of scale items, among which the most commonly used are adaptive versus maladaptive and problem-focused versus emotion-focused (18). In the current study, the subscales active coping, planning, emotional support, instrumental support, positive reframing, acceptance, religion, and humor were grouped as "adaptive coping strategies", whereas venting, denial, substance use, behavioral disengagement, self-distraction, and self-blame were grouped as "maladaptive coping strategies". The data were recorded and analyzed as continuous variables. The Cronbach's alpha was 0.84.

Multidimensional Scale of Perceived Social Support Scale: The scale consists of 12 items with a total score ranging between 12 and 84 (19). High scores obtained from the scale indicate high perceived support. Psychometric validation of the Turkish version was performed (20). In the current study, the data were recorded and analyzed as continuous variables. The Cronbach's alpha was 0.93.

Global Rating of Change (GRC) Scale: To evaluate the perceived change in the mental health of mothers, a single question was asked: "How would you rate your current mental health compared with your situation before the COVID-19 pandemic?" Responses were as follows: 1 = "much worse", 2 = "worse", 3 = "minimally worse", 4 = "no change", 5 = "minimally improved", 6 = "improved", and 7 = "much improved". This one-question scale was previously shown to be easy to understand by the participants (21). It was also used in another study to assess changes in mental health in the COVID-19 study (22). In the current study, those who scored between 1 and 3 were grouped as "*low GRC*" (for deteriorated mental health), and those who scored between 4 and 7 as *"high GRC"* (for improved mental health/no change).

Atilla Turgay DSM-IV Based Screening and Evaluation Scale for Behavioral Disorders in Children and Adolescents (T-DSM-IV-S): T-DSM-IV-S is a 41-item scale for ADHD, developed by Atilla Turgay based on the DSM-IV criterion, and psychometric validation was performed (23). Each item is scored between 0 = "not at all" and 3 = "very much". It consists of attention deficit (9 items), hyperactivity/impulsivity (9 items), oppositional defiant disorder (ODD) (8 items), and conduct disorder (CD) (15 items) subscales. In the current study, mothers completed this scale, which can be completed by either parents or teachers (23). The data were recorded and analyzed as continuous variables. The Cronbach's alpha was 0.94 in this study.

Ethics

The study was approved by Başkent University, Medical and Health Sciences Research and Ethics Committee (project number: KA21/223, date: 28.04.2021). This study was conducted in accordance with the principles of the Helsinki Declarations revised in 2013.

Statistical Analysis

Power analyses (G power software) indicated that 67 participants would provide 80% statistical power, with an alpha of 0.05, for a medium effect size of r=0.3 (for correlational analyses). Statistical Package for Social Sciences (SPSS) 25.0 [(SPSS) Armonk, New York: IBM Corp.] software was used for the analysis. The distribution of the data was examined with histogram graphics and skewness and kurtosis values. After descriptive statistical analysis, the groups were compared using the independent sample t-test or the Pearson chi-square and Fisher's exact tests. The homogeneity of the variances was interpreted according to the Levene statistical results. Correlations between the scores from the scales were analyzed using Pearson correlation coefficients. Finally, to determine the factors that predicted deteriorated mental health, the variables with $p<0.1$ in the independent sample t-test, Pearson's chi-squared test, or Fisher's exact test were included in the logistic regression model with the 'Enter' method. In all analyses, $p<0.05$ was considered statistically significant.

Results

Descriptive statistics

The mean age of mothers was 42.46±6.73 years (minimum-maximum=28-58 years). The majority (89%) were married and had a high education status (12.5% primary/secondary school, 19.4% high school, 68.1% college and over education). Approximately 50% of mothers were public employees, 29% were retired or housewives, and the remaining 21% were tradesmen. Regarding the psychiatric and medical background, 7% (n=5) of the mothers had a mental illness (4 depression, 1 anxiety disorder); 16.7% of them had at least one chronic disease such as hypertension or diabetes mellitus. The descriptive features of the participants are summarized in Table 1.

The mean age of children with ADHD was 11.39±3.12 years (minimum-maximum=6-17 years), whereas the mean diagnosis age was 7.65±2.19 years. A vast majority of the children (73.6%) were males. In the scope of ADHD type, the most common type

Table 1. Comparison of maternal socio-demographics and clinical features of children with ADHD according to GRC score

	All mothers (n=72)	GRC score ≥4 (n=24)	GRC score <4 (n=48)	p
Age, n (%)				
<43 years	40 (55.6)	15 (37.5)	25 (62.5)	0.402[a]
≥43 years	32 (44.4)	9 (28.1)	23 (71.9)	
Marital status, n (%)				
Married	64 (88.9)	22 (34.4)	42 (65.6)	0.710[b]
Divorced/separated	8 (11.1)	2 (25.0)	6 (75.0)	
Education status, n (%)				
Senior high school or below	23 (31.9)	12 (52.2)	11 (47.8)	**0.020**[a]
College education or above	49 (68.1)	12 (24.5)	37 (75.5)	
Occupation, n (%)				
Healthcare worker	8 (11.1)	2 (25.0)	6 (75.0)	0.823[a]
Other public employees	28 (38.9)	11 (39.3)	17 (60.7)	
Tradesman/private sector	15 (20.8)	5 (33.3)	10 (66.7)	
Retired/housewife	21 (29.2)	6 (28.6)	15 (71.4)	
Income status, n (%)				
Very low (0-2999 TRY)	8 (11.1)	2 (25.0)	6 (75.0)	0.804[a]
Low (3000-5999 TRY)	17 (23.6)	7 (41.2)	10 (58.8)	
Moderate (6000-8999 TRY)	18 (25.0)	5 (27.8)	13 (72.2)	
High (>9000 TRY)	29 (40.3)	10 (34.5)	19 (65.5)	
Working status in pandemic, n (%)				
Not working	37 (51.4)	12 (32.4)	25 (67.6)	0.932
Working	35 (48.6)	12 (34.2)	23 (65.8)	
Psychiatric background, n (%)				
Present	5 (6.9)	2 (40.0)	3 (60.0)	0.544[b]
Absent	67 (93.1)	22 (32.8)	45 (67.2)	
Medical background, n (%)				
Present	12 (16.7)	1 (8.3)	11 (91.7)	0.051[b]
Absent	60 (83.3)	23 (38.3)	37 (61.7)	
Child number under 12-year-old, n (%)				
<2	53 (73.6)	20 (37.7)	33 (62.3)	0.186[a]
≥2	19 (26.4)	4 (21.1)	15 (78.9)	
Psychiatric disorders of other children, n (%)				
Present	4 (5.6)	0 (0.0)	4 (100.0)	0.294[b]
Absent	68 (94.4)	24 (35.3)	44 (64.7)	
Medical disorders in other children, n (%)				
Present	2 (2.8)	0 (0.0)	2 (100.0)	0.549[b]
Absent	70 (97.2)	24 (34.3)	46 (65.7)	
COVID-19 background, n (%)				
Yes	15 (20.8)	7 (46.7)	8 (53.3)	0.218[a]
No	57 (79.2)	17 (29.8)	40 (70.2)	
Quarantine background, n (%)				
Yes	17 (23.6)	6 (35.3)	11 (64.7)	0.844[a]
No	55 (76.4)	18 (32.7)	37 (67.3)	
Loss of loved one due to COVID-19, n (%)				
Yes	5 (6.9)	1 (20.0)	4 (80.0)	0.659[b]
No	67 (93.1)	23 (34.3)	44 (65.7)	
Outdoor activities, n (%)				
Less than 1 per week	50 (69.4)	12 (24.0)	38 (76.0)	**0.011**[a]
At least 1 day a week	22 (30.6)	12 (54.5)	10 (45.5)	

Significant p values are shown in bold. [a]Pearson χ^2 test, [b]Fisher's exact test was used when expected cases were <5 in two-group comparisons.
ADHD: Attention deficit and hyperactivity disorder, GRC: Global Rating of Change Scale, TRY: Turkish Lira, COVID-19: Coronavirus disease-2019

was the mixed type (%65), followed by attention-deficit type (%31) and hyperactivity type (%4), respectively. In addition, 64% of children have at least one comorbid psychiatric disorder. The most common psychiatric comorbidity was ODD (43%), whereas anxiety disorder (14%), conduct disorder (7%), tic disorder (4%), and depression (3%) were less frequent. Data on other characteristics that define children with ADHD are displayed in Table 2.

Comparison of children's symptoms with those in the pre-pandemic period according to mothers' self-reports

The majority of parents (58%) reported a deterioration in their children's mental health compared with the pre-pandemic period, manifested by an increase in symptoms of irritability (53%), distraction (46%), and agitation/impulsivity (44%). There was also a significant increase in screen time outside online lessons (86%). A significant portion of parents/mothers stated that their children's academic success (53%), fulfilling their responsibilities such as doing homework (64%), and total sleep time (40%) decreased, whereas the parent-child arguments (58%) increased compared to the pre-pandemic period. However, few of the mothers reported that their children's mental health was better (11%) than that in the pre-pandemic period and that they adapted well to distance learning (18%).

Associated factors of maternal burnout, general distress levels, and deteriorated mental health

The mean MBI score of the mothers was 23.39±11.80, and the mean DASS-21 score was 15.51±12.18. Based on the cut-off scores for DASS-21, the prevalence of clinically significant depression, anxiety, and stress symptoms in mothers was 50%, 44.4%, and 43.1%, respectively. According to the GRC, most mothers (67%) reported experiencing a deterioration in their mental well-being compared with the pre-pandemic period. Based on the reported reasons, the highest percentage (79%) was attributed to the increasing difficulties faced by children with ADHD, followed by restrictions on social activities (73%), COVID-19 anxiety (65%), and economic hardships (33%). In addition, those with low GRC had higher levels of education (p=0.020) and were less able to go out for social sports activities (p=0.011) than those with high GRC (Table 1). The results of the analyses, which compared individuals with low and high GRC scores and their children in terms of clinical and sociodemographic characteristics, as well as scores on other scales, are presented in Tables 1-3. GRC showed a moderately negative correlation with both MBI and DASS-21 (r=-0.52, p<0.001; r=-0.56, p<0.001, respectively). The strongest correlation was observed

Table 2. Characteristics of children with ADHD and their comparison according to GRC score

	All children (n=72)	GRC score ≥4 (n= 24)	GRC score <4 (n=48)	p
Age, n (%)				
<11 years	30 (41.7)	12 (40.0)	18 (60.0)	0.310[a]
≥11 years	42 (58.3)	12 (28.6)	30 (71.4)	
Sex, n (%)				
Female	19 (26.4)	6 (31.6)	13 (68.4)	0.850[a]
Male	53 (73.6)	18 (34.0)	35 (66.0)	
Education status, n (%)				
Primary school	28 (38.9)	12 (42.9)	16 (57.1)	0.305[a]
Secondary school	26 (36.1)	6 (23.1)	20 (76.9)	
Senior high school	18 (25.0)	6 (33.3)	12 (66.7)	
ADHD diagnoses age, n (%)				
<7 years	24 (33.3)	11 (45.8)	13 (54.2)	0.112[a]
≥7 years	48 (66.7)	13 (27.1)	35 (72.9)	
ADHD type, n (%)				
Attention deficit type	22 (30.5)	7 (31.8)	15 (68.2)	0.994[a]
Mixed type	47 (65.3)	15 (31.9)	32 (68.1)	
Medication for ADHD, n (%)				
Methylphenidate	47 (65.3)	16 (34.0)	31 (66.0)	0.342[b]
Atomoxetine	5 (6.9)	3 (60.0)	2 (40.0)	
Comorbid psychiatric disorders, n (%)				
Present	46 (63.9)	12 (25.5)	34 (74.5)	0.083[b]
Absent	26 (36.1)	12 (48.0)	14 (52.0)	

[a]Pearson χ^2 test, [b]Fisher's exact test was used when expected cases were <5.
ADHD: Attention deficit and hyperactivity disorder, GRC: Global Rating of Change Scale

Table 3. MBI, DASS-21, MSPSS, Coping styles, and T-DSM-IV-S scores based on GRC score

	GRC score ≥4 (n=24)	GRC score <4 (n=48)	p
MBI score, mean±SD	14.79±9.73	27.69±10.36	**<0.001**
DASS-21 score, mean±SD	6.33±6.35	20.10±11.83	**<0.001**
MSPSS score, mean±SD	61.33±18.51	61.72±17.92	0.931
Coping styles			
Adaptive strategies score, mean±SD	41.08±10.29	43.23±6.94	0.298
Maladaptive strategies score, mean±SD	21.46±4.77	26.38±4.55	**<0.001**
T-DSM-IV-S			
Inattention score, mean±SD	12.67±5.21	17.29±5.93	**0.002**
Hyperactivity/impulsivity score, mean±SD	11.88±7.78	12.21±7.55	0.862
ODD score, mean±SD	9.58±6.47	11.73±5.94	0.165
CD score, mean±SD	1.46±2.40	2.67±3.75	0.102

Significant p values are shown in bold.
SD: Standard deviation, GRC: Global Rating of Change Scale, MBI: Maslach Burnout Inventory, DASS-21: Depression Anxiety Stress Scale-21, MSPSS: Multidimensional Scale of Perceived Social Support Scale, T-DSM-IV-S: Atilla Turgay DSM-IV Based Screening and Evaluation Scale for Behavioral Disorders in Children and Adolescents, ODD: Oppositional defiant disorder, CD: Conduct disorder

Table 4. Correlation coefficients between scales

	1	2	3	4	5	6	7	8	9	10
1. MBI score	-									
2. DASS-21 score	**0.603****	-								
3. GRC score	**-0.523****	**-0.559****	-							
4. MSPSS score	-0.220	**-0.324****	0.096	-						
5. Adaptive COPE score	0.074	-0.047	-0.020	**0.327****	-					
6. Maladaptive COPE score	**0.424****	**0.603****	**-0.382****	-0.073	**0.420****	-				
7. Inattention score	**0.514****	**0.346****	**-0.404****	-0.142	0.130	**0.325****	-			
8. Hyperactivity/impulsivity score	**0.352****	0.145	-0.093	-0.184	-0.072	0.113	**0.589****	-		
9. ODD score	**0.487****	**0.261***	**-0.241***	**-0.331****	-0.001	0.197	**0.571****	**0.552****	-	
10. CD score	**0.333****	0.090	**-0.240***	**-0.281***	0.024	0.056	**0.333****	0.321**	**0.569****	-

Bold values are significant. *p<0.05, **p<0.01.
MBI: Maslach Burnout Inventory, DASS-21: Depression Anxiety Stress Scale-21, GRC: Global Rating of Change Scale, MSPSS: Multidimensional Scale of Perceived Social Support Scale, ODD: Oppositional defiant disorder, CD: Conduct disorder

between the subscales of T-DSM-IV-S and the total scores of MBI, DASS-21, and GRC scales, particularly with the inattention subscale (r=0.51, p<0.001; r=0.35, p=0.003; r=-0.40, p=0.004, respectively). Correlation coefficients between the scales are provided in Table 4.

Predictors of maternal mental health

Logistic regression analysis was applied to determine the risk factors that predict deteriorated mental health from the pandemic in the mothers of children with ADHD. GRC was determined as the dependent variable (1 = low; 0 = high), and the independent variables were education level (College education or above = 1; Senior high school or below = 0), medical background of the mother (1 = present; 0 = absent), frequency of going out (1 = at least 1 day per week; 0 = less than 1 per week), maladaptive coping score, comorbid psychiatric disorder in child (1 = present; 0 = absent), and inattention subscale score based on T-DSM-IV-S. Before the analysis, the logistic regression analysis assumptions were tested. Education level [adjusted odds ratio (AOR)=5.12, 95% confidence interval (CI)=1.19-21.99, p=0.028], frequency of outdoor activities (AOR=0.22, 95% CI=0.06-0.83, p=0.025), maladaptive coping score (AOR=1.31, 95% CI=1.10-1.57, p=0.003), and inattention subscale score (AOR=1.13, 95% CI=1.0-1.27, p=0.045) were independently associated with low GRC. Education level was noted as the strongest predictor among these variables (Table 5). There was no significant relationship found between the medical background of the mother (AOR=0.10, 95% CI=0.1-1.05, p=0.054), the presence of comorbid psychiatric disorder in the child (AOR=1.39, 95% CI=0.19-10.17, p=0.744), and GRC. The regression model explained the variance in the GRC between 36.5% (Cox & Snell R^2) and 50.7% (Nagelkerke R^2), [$\chi^2=32.67^{(4)}$, p<0.001].

Table 5. Logistic regression analysis to explore the variables independently associated with low GRC

	OR (95% CI)	p
Education status (1: College education or above)	5.12 (1.19-21.99)	**0.028**
Medical background of the mother (1: Present)	0.10 (0.10-1.05)	0.054
Outdoor activities (1: At least 1 day a week)	0.22 (0.06-0.83)	**0.025**
Cope maladaptive score	1.31 (1.10-1.57)	**0.003**
Comorbid psychiatric disorders in child (1: Present)	1.39 (0.19-10.17)	0.744
Inattention score	1.13 (1.00-1.27)	**0.045**

Significant p values are shown in bold.
OR: Odds ratio, CI: Confidence interval, GRC: Global Rating of Change Scale

Discussion

One noteworthy aspect of this study is its distinction as one of the rare studies that uniquely focuses on mothers who have children with ADHD during distance learning. Moreover, this is the first published study in Türkiye to investigate this population from this perspective. Our findings showed that distance learning and home confinement are associated with deteriorated mental health in children with ADHD but also in their mothers. The mother's maladaptive coping strategies, frequency of outdoor activities, education level, and attention deficit level were identified as independent predictors of deteriorated mental health in mothers.

According to a recent study, children with ADHD exhibited shorter attention spans during distance learning, leading to increased academic challenges specifically for these students (24). A meta-analysis study has indicated that children with ADHD are facing disproportionate and adverse effects on a global scale during the pandemic (25). Furthermore, studies have highlighted an increase in ADHD symptoms (25-27). Our findings suggest that symptoms of inattention, as opposed to symptoms of conduct and hyperactivity/impulsivity, have a stronger association with the mental health of mothers who have children with ADHD. Furthermore, as the severity of inattention symptoms increases, maternal levels of burnout and general distress also increase. These results can be attributed to the demands of distance learning, which necessitate heightened attention and parental support. Parents of children with higher levels of inattention symptoms may encounter difficulties in providing sufficient support in this context.

One study conducted with parents of children with ADHD during the COVID-19 pandemic identified the primary challenge faced by children in distance learning as difficulties in maintaining focus and motivation (28). Furthermore, parents encountered substantial obstacles in effectively balancing the demands of distance learning with their work responsibilities (28). Another study revealed that parents expressed feelings of inadequacy in meeting the educational needs of their children with ADHD during the distance education process, which subsequently led to heightened experiences of frustration and guilt (29). Consistent with our findings, Yousef et al. (30) identified symptoms of depression (53.7%), anxiety (61%), and elevated stress levels (53.7%) among a significant proportion of mothers with children with ADHD.

In response to stressful events, individuals often seek social support and use various coping strategies to alleviate psychological distress (31). However, our study findings indicate a stronger association between maladaptive coping strategies and parental mental health, which contradicts the significance of social support and adaptive coping strategies. These results are consistent with the findings of Achterberg et al. (32) and provide additional support for this notion. Achterberg et al. (32) have posited that, in contrast to other disaster situations, the "stay-at-home" measures implemented during the pandemic may lead to reduced anticipation of social support among individuals. However, it was determined that going out less than once a week for physical activity was a risk factor for mothers with an ADHD child, which is consistent with the findings of a previous study (33). According to Merrill et al. (34), these parents require support for distance learning, and they proposed a behavioural parent training program as a potentially beneficial intervention.

In the current study, the education level of the mother was the strongest predictor of deteriorated mental health during the pandemic. The risk of deteriorated mental health was 5.1 times higher for university or higher graduate individuals than for those with a lower education level. This finding is in contrast to pre-pandemic studies, which reported no significant relationship between education level and the mental well-being of mothers of children with ADHD (35,36). Another study conducted with mothers of children with developmental disabilities during the pandemic reported that those with higher education levels experienced more parenting stress (37). These results suggest that more educated parents may have more difficulties adapting to the increased needs of parenting in the distance learning process.

Study Limitations

The findings of this study should be evaluated within some limitations. The critical limitation of the current study is the

absence of data on the mental health status of mothers and children in the pre-pandemic period, which makes it difficult to discriminate the core impact of the pandemic and distance learning conditions on the obtained results. Second, the cross-sectional design of the study precludes the establishment of a causal relationship between distance learning in children with ADHD and the deterioration of parental mental health. Third, fathers were not included in the study because we intended to explore mothers as central care providers for children with ADHD in the local context. Fourth, due to the differences in economic, social, and cultural factors across countries, in the measures taken during the pandemic, the results may not be generalized to other populations.

Conclusion

In conclusion, the results indicate that distance learning during the COVID-19 pandemic is associated with deteriorated mental health among mothers with children with ADHD. The study identified several independent factors that predict deteriorated maternal mental health, including maladaptive coping strategies, frequency of outdoor activities, education level, and severity of attention-deficit symptoms. In accordance with the results, parents may be informed about appropriate coping strategies, and mental health professionals should closely monitor the mental health of both children and parents during distance learning. This highlights the imperative of engaging mental health professionals and educators in multidisciplinary planning to develop distance learning programs and strengthen home support systems for this vulnerable group. Future studies incorporating larger sample sizes, longitudinal designs that encompass both children and fathers and comparison groups will provide a more comprehensive understanding of the psychological impacts of online education experiences on disadvantaged children and their parents.

Ethics

Ethics Committee Approval: The study was approved by Başkent University, Medical and Health Sciences Research and Ethics Committee (project number: KA21/223, date: 28.04.2021).

Informed Consent: All study participants provided written informed consent.

Authorship Contributions

Concept: D.K., H.A.T., B.A.S., A.E., Design: D.K., H.A.T., B.A.S., B.A., Data Collection or Processing: DK., Analysis or Interpretation: D.K., Literature Search D.K., H.A.T., B.A.S., A.E., B.A., Writing DK., H.A.T., B.A.S., A.E., B.A.

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Preeclampsia severity and associated factors in Kelantan, Malaysia

Nurdiyana Farhana Mat Tamizi[1], Sarimah Abdullah[1], Siti Azrin Ab Hamid[1], Fauziah Jummaat[2], Wan Nor Asyikeen Wan Adnan[1]

[1]Universiti Sains Malaysia, School of Medical Sciences, Biostatistics and Research Methodology Unit, Kelantan, Malaysia
[2]Universiti Sains Malaysia, Advanced Medical and Dental Institute, Penang, Malaysia

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Corresponding Author:
Siti Azrin Ab Hamid, M.D., Universiti Sains Malaysia, School of Medical Sciences, Biostatistics and Research Methodology Unit, Kelantan, Malaysia
+609-7676832
ctazrin@usm.my

ORCID:
orcid.org/0000-0002-8212-9849

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ABSTRACT

Aims: Preeclampsia is a significant disorder that poses serious risks to maternal and fetal health. This study aimed to identify the severity of preeclampsia and associated factors in a University Hospital in Malaysia.

Methods: A cross-sectional study was conducted among preeclampsia patients at the Hospital Universiti Sains Malaysia (USM), Kelantan, Malaysia, between 2011 and 2016. Preeclampsia patients with chronic hypertension, hemolysis, elevated liver enzymes, low platelet (HELLP) syndrome, and eclampsia were included, whereas patients with chronic kidney disease were excluded. Preeclampsia severity was classified into mild, moderate, and severe.

Results: The study included 202 patients [mean (standard deviation) maternal age: 30.49 (6.18) years]. Most patients were multigravida (134) and multipara (134). Sixty-five (32.2%) patients had a history of preeclampsia. The proportion of patients with mild preeclampsia was 35% [95% confidence interval (CI): 30%, 40%], moderate preeclampsia was 30% (95% CI: 25%, 35%), and severe preeclampsia was 35% (95% CI: 30%, 40%). High levels of uric acid [adjusted odds ratio (OR): 1.05, 95% CI: 1.02, 1.07, p=0.001], chronic hypertension (adjusted OR: 2.36, 95% CI: 1.28, 4.33, p=0.006), and gestational diabetes mellitus (adjusted OR: 0.53, 95% CI: 0.30, 0.96, p=0.035) were the factors associated with the severity of preeclampsia.

Conclusions: Higher levels of uric acid, chronic hypertension, and gestational diabetes mellitus were significantly associated with the severity of preeclampsia among patients in the USM.

Introduction

Preeclampsia is defined as hypertension accompanied by proteinuria that occurs after 20 weeks of pregnancy (1). The parameters for initial identification of preeclampsia are a systolic blood pressure of 140 mmHg and higher or diastolic blood pressure of 90 mmHg and higher twice at least 4 hours apart; or shorter interval timing of systolic blood pressure of 160 mmHg and higher or diastolic blood pressure of 110 mmHg and higher, after 20 weeks of gestation (2).

Other significant findings that may or may not be a part of the clinical presentation include proteinuria, signs of end-organ damage, such as thrombocytopenia, impaired liver function, severe persistent right upper quadrant or epigastric pain (excluding all other alternative diagnoses), new-onset headache unresponsive to all forms of management, pulmonary edema, or renal insufficiency with abnormal laboratory values (3).

The prevalence of preeclampsia varies from 0.2% to 6.7% in Asia, 0.5% to 2.3% in Africa, 2.8% to 5.2% in Europe, 2.8% to 9.2% in Oceania, 1.8% to 7.7% in South America and the Caribbean, and 2.6% to 4.0% in North America (4). In Asian regions the prevalence of preeclampsia was reported by 2.1% in China, 1.2% in Japan, 2.2% in Thailand, and 0.6% in Nepal (5). Based on the Malaysia Clinical Research Centre review of hypertensive

disorders in pregnancy from 2011 to 2012, the incidence of preeclampsia was 19.6% (6). Globally, preeclampsia is diagnosed in 8.5 million women annually (7). Preeclampsia affects 1-5% of pregnancies, resulting in severe maternal and neonatal morbidity and mortality (8,9). Annually, preeclampsia results in 70,000 maternal deaths and more than 500,000 fetal and neonatal deaths worldwide (9). Thus, preeclampsia poses serious risks to the health of women.

Risk factors for preeclampsia include obesity, diabetes and chronic hypertension. Other risk factors for the illness include nulliparity, adolescent pregnancy, and disorders that cause hyperplacentation and large placentas (e.g. twin pregnancy) (10). In Malaysia, primigravid women carrying multiple pregnancies, older than 35 years, had their last pregnancy more than 10 years ago, had a body mass index (BMI) greater than 35 kg/m^2, or had a family history of preeclampsia are categorized as having intermediate risk (11,12). A pregnant woman is accepted at high risk with the presence of at least one high-risk factor, such as chronic kidney disease, systemic lupus erythematosus, antiphospholipid syndrome, type 1 or type 2 diabetes mellitus, or chronic hypertension (11,12).

Preeclampsia and associated issues should be prevented by early detection and early intervention in women at high risk (13,14). Primary health clinics that offer prenatal care are essential for the early detection and prevention of steps to manage preeclampsia and its consequences (13). Additionally, Malaysia offers pre-pregnancy care that includes preconception counseling, reliable contraception, the management of underlying comorbid diseases, and treatment alterations before conception, all of which are crucial precautions for women at risk of preeclampsia (13).

Preeclampsia continues to be a major cause of morbidity and mortality in both mothers and infants. However, information on the factors associated with preeclampsia based on its severity is limited among pregnant Malaysian women. Therefore, this study aimed to assess the severity of preeclampsia and associated factors among hospitalized pregnant women in a university hospital.

Methods

Study design and sample size

This retrospective cross-sectional study was conducted among pregnant women who received routine antenatal care and delivered at the Universiti Sains Malaysia (USM) Hospital in Kelantan, Malaysia from 1st January 2011 until 31 December 2016. The study included hospitalized patients aged between 15 and 49 years who had chronic hypertension with superimposed preeclampsia, hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome, and had eclampsia.

This study involved patients with a minimum age of 15 years. Muslim girls below the age of 18 are still allowed to marry with the Syariah court's permission. Under Malaysian law, underage marriage for non-Muslims is governed by civil marriage law, whereas underage marriage for Muslims is governed by Islamic family law in the Syariah courts. Under this dual legal system, the legal minimum age of marriage for Muslim men is 18 and 16 for women; for non-Muslim men and women, the legal minimum age is 18. This study excluded patients with chronic kidney disease and incomplete data.

Patients with preeclampsia were identified in the medical record unit and hospital database known as the Laboratory Information System and recruited using a simple random sampling method. There were 756 preeclampsia patients at the Hospital USM. Using Power and Sample Size Calculation (PS) software, the required sample was calculated as 216 patients.

Based on this number, we first entered all preeclampsia patients into the software and then randomly selected 216 patients. After applying the inclusion and exclusion criteria, 202 patients were considered eligible for the study.

Outcome variable

The outcome variable was the severity of preeclampsia. The severity of preeclampsia was categorized into mild, moderate, and severe (15). Mild preeclampsia is defined as a systolic blood pressure of 140 to 149 mmHg and/or 90 to 99 mmHg diastolic blood pressure with proteinuria (15). Moderate preeclampsia is defined as systolic blood pressure of 150 to 159 mmHg and/or 100 to 109 mmHg diastolic blood pressure with proteinuria (15). Severe preeclampsia is defined as systolic blood pressure higher than 160 mmHg and/or diastolic blood pressure higher than 110 mmHg with proteinuria (16). Significant proteinuria in pregnancy is defined as ≥300 mg protein in a 24-h urine sample or a protein-creatinine ratio of 30 mg/mmol in a spot urine sample, or at least persistent 1+ dipstick proteinuria (17).

Statistical Analyses

Stata Special Edition version 14 was used for data entry and analysis (StataCorp, 2015. Stata Statistical Software, Release 14. College Station, TX, USA: StataCorp LP). Results are presented as frequency (n) and percentage (%) for categorical variables and mean and standard deviation (SD) for numerical variables. Descriptive analyses were performed for sociodemographic characteristics, clinical characteristics, and laboratory parameters.

The independent variables were sociodemographic characteristics (maternal age, BMI, gravidity, parity, number of fetuses, marital status), clinical characteristics (history of preeclampsia, family history of hypertension, family history of diabetes mellitus, history of small for gestational age, gestational diabetes mellitus, chronic hypertension, patients on

hypertension medications, history of abortion), and laboratory parameters [uric acid, creatinine, albumin, platelet, aspartate aminotransferase, alanine transaminase, urea, and hemoglobin (Hb)].

The severity of preeclampsia was assessed as a ratio and 95% confidence interval (CI). The factors associated with the severity of preeclampsia were studied using simple and multiple ordinal logistic regression analysis.

Simple ordinal logistic regression models followed by the selection of backward and backward stepwise methods were used to select the variables (p=0.05 for entry and p=0.10 for removal). Variables showing an association at p<0.25 in the univariate analysis or clinically associated variables with the outcome were included in the full model. Dummy variables were created for categorical variables with more than two levels. Multicollinearity, interactions, and linear-to-logit transformations (if applicable) were examined. Assumptions, goodness of fit, outliers, and influential cases were appropriately assessed. The crude and adjusted odds ratio (OR), regression coefficient (b), 95% CI, and p-value were reported. P<0.05 was accepted as statistically significant.

Results

Characteristics of the patients

The study included 202 patients [mean (SD) maternal age: 30.49 (6.18) years]. Mean (SD) pre-pregnancy BMI of 28.83 (5.60) kg/m^2. The majority of patients were in their first marriage (94.1%), multigravida (66.3%), and multiparous (59.9%). Eight patients had twins during their pregnancy. A history of preeclampsia was recorded in 65 patients, whereas the remaining 137 had preeclampsia for the first time. A total of 73 patients were diagnosed with chronic hypertension. More than half of the preeclampsia patients had no family history of hypertension or diabetes mellitus. Thirty-seven patients had a history of a small gestational age in a previous pregnancy (Table 1).

Laboratory parameters

Uric acid, creatinine, and urea levels were higher in patients with severe preeclampsia, with mean (SD) values of 433.64 (105.23) µmol/L, 76.97 (13.28) µmol/L, and 3.99 (1.49) mmol/L, respectively (Table 2). Overall, the mean (SD) albumin level was 32.59 (3.18) g/L. The mean Hb level was lower in patients with mild preeclampsia**.**

Associated factors for more severe preeclampsia

The proportion of patients with mild preeclampsia was 35% (95 % CI: 30%, 40%), moderate preeclampsia was 30% (95% CI: 25%, 35%), and severe preeclampsia was 35% (95% CI: 30%, 40%). High uric acid levels, chronic hypertension, and gestational diabetes mellitus were factors associated with the severity of preeclampsia. Every 10 µmol/L increase in uric acid was associated with a 5% higher risk of developing more severe preeclampsia versus mild preeclampsia (adjusted OR; 1.05; 95% CI: 1.02, 1.07; p=0.001) after controlling for chronic hypertension and gestational diabetes mellitus (Table 3).

Pregnant women with chronic hypertension had 2.4 fold higher risk of developing severe preeclampsia versus mild preeclampsia than those without chronic hypertension (adjusted OR: 2.36, 95% CI: 1.28, 4.33, p=0.006) with adjustment for uric acid level and gestational diabetes mellitus.

Those with gestational diabetes mellitus had 47% lower odds of severe preeclampsia versus mild preeclampsia after controlling for chronic hypertension and uric acid.

Discussion

The study revealed that uric acid levels were significantly associated with the severity of preeclampsia, demonstrating that individuals with elevated uric acid levels were at a greater risk of developing severe preeclampsia than those with mild preeclampsia. This study was similar to that of Ugwuanyi et al. (18) who showed that higher levels of uric acid were associated with the severity of preeclampsia and associated with poorer perinatal outcomes. Women with abnormal uric acid levels were four times more likely to have severe preeclampsia than those with normal serum uric acid levels (18). In addition, previous research conducted in India, Bangkok, and Southeast Nigeria revealed that uric acid was a good marker of the severity of preeclampsia (19-21).

Furthermore, uric acid levels were significantly higher in women with preeclampsia than without preeclampsia (22). In addition, a previous study involving 877 preeclampsia women and 580 normotensive women reported that an increase in uric acid levels was seen only in patients with preeclampsia, whereas, among normotensive women who developed preeclampsia later, serum uric acid was not significantly high (23). The study pointed out that uric acid may increase because of preeclampsia but is not a biomarker or risk factor for preeclampsia (23). Uric acid synthesis may be increased in preeclampsia patients due to hypoxia and ischemia of the placenta, and increased cytokine production (24).

A higher uric acid level may be associated with poor maternal and fetal outcomes (25). Preterm birth, intrauterine growth restriction, fetal death, and neonatal death are all possible outcomes (23,26). Therefore, monitoring uric acid levels throughout pregnancy may help in the early detection of preeclampsia and reduce its serious effects.

In this study, chronic hypertension was significantly associated with severe preeclampsia. The findings of the current study were in line with a previous meta-analysis that concluded that patients with chronic hypertension were at a more than two-fold higher risk of developing preeclampsia than those without

Table 1. Characteristics of preeclampsia patients based on the severity (n=202)

Variables	Severity of preeclampsia		
	Mild	Moderate	Severe
Gravidity, n (%)			
Primigravida	24 (35.3)	19 (27.9)	25 (36.8)
Multigravida	46 (34.3)	42 (31.3)	46 (34.3)
Parity, n (%)			
Nullipara	28 (34.6)	24 (29.6)	29 (35.8)
Multipara	42 (34.7)	37 (30.6)	42 (34.7)
Number of fetuses, n (%)			
Singleton	67 (34.5)	59 (30.6)	68 (35.1)
Multiple	3 (37.5)	2 (25.0)	3 (37.5)
Marital status, n (%)			
Unmarried	1 (33.3)	1 (33.3)	1 (33.3)
First marriage	65 (34.2)	56 (29.5)	69 (36.3)
Second marriage	4 (44.4)	4 (44.4)	1 (11.2)
History of preeclampsia, n (%)			
No	49 (35.6)	43 (31.4)	45 (32.9)
Yes	21 (32.3)	18 (27.7)	26 (40.0)
Chronic hypertension, n (%)			
No	47 (36.4)	44 (34.1)	38 (29.5)
Yes	23 (31.5)	17 (23.3)	33 (45.2)
Family history of hypertension, n (%)			
No	32 (31.4)	37 (36.3)	33 (32.3)
Yes	38 (38.0)	24 (24.0)	38 (38.0)
Family history of diabetes mellitus, n (%)			
No	37 (31.4)	37 (31.4)	44 (37.2)
Yes	33 (39.3)	24 (28.6)	27 (32.1)
History of small gestational age, n (%)			
No	61 (37.0)	49 (29.7)	55 (33.3)
Yes	9 (24.3)	12 (32.4)	16 (43.2)
Gestational diabetes mellitus, n (%)			
No	40 (32.3)	34 (27.4)	50 (40.3)
Yes	30 (38.5)	27 (34.6)	21 (26.9)
History of abortion, n (%)			
No	56 (34.6)	50 (30.8)	56 (34.6)
Yes	14 (35.0)	11 (27.5)	15 (37.5)
Patients on medication, n (%)			
No	48 (35.3)	38 (27.9)	50 (36.8)
Yes	22 (33.3)	23 (34.9)	21 (31.8)

Table 2. Laboratory assessment of preeclampsia patients based on the severity (n=202)

Variables	Severity of preeclampsia		
	Mild	Moderate	Severe
Uric acid (μmol/L), mean (SD)	375.42 (90.58)	403.98 (112.56)	433.64 (105.23)
Creatinine (μmol/L), mean (SD)	73.40 (15.38)	73.63 (11.73)	76.97 (13.28)
Albumin (g/L), mean (SD)	32.91 (2.56)	32.97 (2.64)	31.98 (3.97)
Platelet (10^3/UL), mean (SD)	247.16 (97.09)	253.62 (70.24)	229.82 (85.72)
Hemoglobin (g/dL), mean (SD)	11.95 (1.54)	12.02 (1.53)	12.42 (1.70)
Urea (mmol/L), mean (SD)	3.43 (1.47)	3.50 (1.48)	3.99 (1.49)
AST (U/L), mean (SD)	20.0 (12.0)	20.0 (8.0)	26.0 (13.0)
ALT (U/L), mean (SD)	13.0 (9.0)	11.0 (7.0)	18.0 (12.0)

SD: Standard deviation, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

Table 3. Factors associated with the severity of preeclampsia (n=202)

Variables	b (SE)	Adjusted OR (95% CI)	p-value
Uric acid (μmol/L)	0.04 (0.01)	1.05 (1.02, 1.07)	0.001
Chronic hypertension	0.86 (0.31)	2.36 (1.28, 4.33)	0.006
Gestational diabetes mellitus	-0.63 (0.30)	0.53 (0.30, 0.96)	0.035

[b]Coefficient regression.
SE: Standard error, OR: Odds ratio, CI: Confidence interval

chronic hypertension (27). However, patients may develop other types of chronic diseases, such as diabetes mellitus and renal disease, which can affect the maternal end organs. Patients who have chronic hypertension or any other pre-existing medical condition should be paid more attention during routine antenatal care visits, and additional precautions should be taken even before pregnancy to avoid serious consequences (27).

Paré et al. (28) indicated that chronic hypertension was a significant predictor of severe preeclampsia. Women with chronic hypertension had a 3.2 higher chance of developing severe preeclampsia than those without prior hypertension. This finding is consistent with a study conducted by Direkvand-Moghadam et al. (29), in which they reported that chronic hypertension was significantly associated with the severity of preeclampsia. They claimed that chronic hypertension was the strongest predictor of preeclampsia in the Iranian population. However, they found that chronic hypertension was only a significant factor in women with mild preeclampsia; there was no association between chronic hypertension and severe preeclampsia.

The current study also found that women with gestational diabetes mellitus had 47% lower odds of developing severe preeclampsia than those with mild preeclampsia. A link between gestational diabetes mellitus and preeclampsia has been reported in a study from the German birth registry (30). This finding was consistent with another birth registry study in Alberta, Canada, which confirmed that gestational diabetes mellitus was a significant risk factor for preeclampsia (31).

In contrast, a study using national perinatal data of Slovenian pregnant mothers found that gestational diabetes mellitus was not associated with the development of preeclampsia (1). The contrast in the results was explained by the fact that the insult that causes preeclampsia most likely occurs early in pregnancy, whereas gestational diabetes mellitus develops during mid-gestation (9,32). Furthermore, Slovenian pregnant mothers received appropriate antenatal care through a targeted program for diabetic pregnancies beginning in the early 1980s, which included preconception consulting, management of gestational diabetes mellitus, and regular screening for gestational diabetes mellitus.

Study Limitations

Several limitations of the current study should be addressed. Medical records were incomplete or not applicable for some patients, resulting in deviations in the results obtained. In addition, the findings of this study may not be applicable to the general population because this was a hospital-based study. On the other hand, these findings could provide new information on the severity of preeclampsia in the local population because, to our knowledge, there is no similar study in the literature on the factors associated with the severity of preeclampsia in Kelantan.

Conclusion

This study showed that uric acid, chronic hypertension, and gestational diabetes mellitus were significantly associated with the severity of preeclampsia. Pregnant mothers should be encouraged to seek medical attention so that preeclampsia can be diagnosed early to prevent complications.

Acknowledgment

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Ethics

Ethics Committee Approval: Ethical approval was obtained from the Human Research Ethics Committee of the School of Medical Sciences Universiti Sains Malaysia (USM) with JEPeM code USM/JEPeM/16090273 on 9th January 2017. Permission to review the medical records was obtained from the Director of Hospital USM.

Informed Consent: Consent form was filled out by all participants.

Authorship Contributions

Concept: N.F.M.T., S.A., S.A.A.H., F.J., W.N.A.W.A., Design: N.F.M.T., S.A., S.A.A.H., F.J., W.N.A.W.A., Data Collection or Processing: N.F.M.T., S.A.A.H., W.N.A.W.A., Analysis or Interpretation: N.F.M.T., S.A., S.A.A.H., W.N.A.W.A., Literature Search: N.F.M.T., S.A.A.H., W.N.A.W.A., Writing: S.A.A.H., W.N.A.W.A.

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Relationship of shoulder position sense with trunk control, balance, and walking speed in patients with multiple sclerosis

Mustafa Ertuğrul Yaşa[1], Taşkın Özkan[2], Buse Korkmaz[1], Nezehat Özgül Ünlüer[1], Gönül Vural[3]

[1]University of Health Sciences Türkiye, Gülhane Faculty of Physiotherapy and Rehabilitation, Ankara, Türkiye
[2]Giresun University, Vocational School of Health Services, Giresun, Türkiye
[3]Ankara Yıldırım Beyazıt University Faculty of Medicine, Department of Neurology, Ankara, Türkiye

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Corresponding Author:
Mustafa Ertuğrul Yaşa, Ph.D.,
University of Health Sciences Türkiye,
Gülhane Faculty of Physiotherapy and Rehabilitation, Ankara, Türkiye
+90 312 567 15 57
mustafaertugrul.yasa@sbu.edu.tr

ORCID:
orcid.org/0000-0002-7796-2588

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ABSTRACT

Aims: This study examined the relationship between altered shoulder joint position sense (JPS) and trunk control, balance, and walking speed in patients with multiple sclerosis (MS).

Methods: This case-control study included patients with MS and healthy controls. A digital inclinometer was used to determine shoulder JPS. Balance was measured using the Berg Balance Scale (BBS) and single-leg stance (SLS) test, trunk control using the Trunk Impairment Scale (TIS), and walking speed using the 10 meter walk test (10 mWT).

Results: The study included 40 MS patients (mean age: 44.68±8.71 years, 75% were females) and 40 healthy control subjects (mean age: 43.56±9.91 years, 65% were females). The BBS and SLS scores were significantly lower and the 10 mWT time was longer in the patients compared with the controls ($p<0.001$). The planar and total JPS scores (error rates) for both sides were higher in the MS group ($p<0.001$). Negative correlations were observed in the MS group between the total JPS score and the BBS, SLS, and total TIS score ($r=-0.770$, $r=-0.619$, $r=-0.665$; respectively) ($p<0.001$), and a positive correlation was observed between the total JPS score and the 10 mWT ($r=0.456$, $p=0.003$) at the dominant side. Total JPS score correlated with BBS, SLS and 10 mWT ($r=-0.658$, $r=-0.522$, $r=0.531$, respectively) ($p<0.001$) at the non-dominant side.

Conclusions: This study showed that decreased shoulder JPS relates to impaired trunk control, balance, and walking speed in MS patients.

Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease characterized by progressive neuronal loss and demyelination in the central nervous system (1). Together with sensorial impairments such as pain, fatigue, visual deficits, and impaired somatosensory system, various physical functions, such as trunk control, gait, and balance, are affected in MS (2-4). Approximately 85% of MS patients describe gait abnormalities as their primary symptom, which causes a fear of falling and kinesiophobia, which restricts social participation and ultimately puts patients on a path to depression (5,6). Therefore, investigation of the causes of these impaired physical functions is crucial for a proper management.

The upper extremities function in reaching, holding, releasing, throwing, and writing, but more complicated processes such as postural control, trunk stability, and gait are also closely related to the upper extremities. The shoulder joint is of particular significance because it serves as a bridge between the upper extremities and the axial skeleton and being able to adjust the position of the hand in space is important to perform different upper extremity functions. The position of the upper extremity segments in the trunk is directly related to the position of the shoulder joint, for which there must be a strong proprioceptive capability to enable these actions (7,8).

Upper extremity reactions are a part of the whole-body response to keep the center of mass within the base of support

against sudden balance changes (9). For example, falls are frequent in some neurological conditions such as hemiplegia, Parkinson's disease, and MS, in which upper extremity functions are impaired; therefore, the required upper extremity positioning cannot be made against the disruptive effect (2,10,11). Individuals with shoulder pain may experience balance problems, which can be explained by the lateral inhibition of pain in the sensory feedback that forms the afferent part of the balance function (12). In addition, torsional forces created by reciprocal lower extremity movements during walking are neutralized by rhythmic arm swings, which is the basis for the walking economy. In some specific diseases known to have proprioceptive disorders, the effects of impaired rhythmic arm swings on gait function have been reported (13).

The shoulder joint functions as a bridge between the upper extremity distally and the trunk proximally. Any deterioration in the shoulder joint may have a two-way effect in both the distal and proximal directions. Upper extremity dysfunctions may occur in at least two-thirds of MS patients (14). To date, only the distal reflection of the effects of decreased shoulder joint position sense (JPS) has been reported in MS (15). To the best of our knowledge, there has been no previous investigation of the effects of decreased shoulder JPS on trunk performance and lower extremities, which are the basis for gait and balance. Therefore, this study examined the effects of decreased shoulder JPS on trunk control, balance, gait, and functional mobility in MS patients.

Methods

Study design and participants

Patients diagnosed with MS and age-matched healthy control subjects who volunteered to participate were included in this case-control study. The inclusion criteria were (a) diagnosis of MS verified by a neurologist according to the 2017 revisions of the McDonald criteria (1), (b) age between 18 and 60 years, (c) no MS attacks in the past three months, and (d) ability to walk independently. The inclusion criteria for healthy control subjects were (a) aged between 18 and 60 years and (b) absence of any disease that can affect physical function. Patients and controls were excluded if they (a) had any disease other than MS that would affect physical functioning, (b) had Meniere's syndrome or any other disease of the inner ear, (c) were pregnant, and (d) had a score of <24 points in the Mini-Mental State Examination. The study was approved by the Gülhane Scientific Research Ethics Committee (decision no: 2022-305, date: 17/10/2022). All the procedures were in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Demographic characteristics were recorded on a standard form. The disability status was determined by a neurologist using the Expanded Disability Status Scale (EDSS) (16). Measurements were performed in the same order in a well-lit, quiet environment by the same physiotherapist with 10 years of experience in the field. Fatigue was consistently followed throughout the measurements and appropriate breaks were allowed.

The same procedures were followed in the control group. Demographic characteristics were recorded on a standard form, and measurements were performed in the same order by the same physiotherapist. Appropriate rest intervals were provided if needed.

Outcome measures

Shoulder position sense: A digital inclinometer (Dualer IQ Pro™ Digital Inclinometer, JTECH Medical; USA) was used to assess shoulder JPS as measured by repositioning errors. Both shoulders were assessed, and arm dominance was determined by asking the patient. Shoulder JPS was tested in four positions: 30°-60° flexion and 30°-60° abduction (17). Only the primary part of the inclinometer was used. It was secured with a Velcro strap to the lateral side of the subject's wrist, just proximal to the ulnar styloid process for the abduction measurement, and the anterior side of the humerus, on the biceps brachii belly for the flexion measurement. The participants were guided to move their arms to the target angle and were then instructed to hold the position for 3 seconds. The movement was demonstrated twice to ensure that the participants understood and remembered the target angle. Then, they were asked to repeat the previously achieved position five times consecutively. The absolute difference between the target position degree and the other five attempts was recorded. The lowest and highest results were discarded, and the average of the remaining 3 results was recorded as the angular JPS score.

A total JPS score was calculated for each shoulder joint for correlation analysis. First, the average of the angular scores at 30° and 60° was used to determine the planar proprioception score (flexion and extension), and the average of the planar proprioception scores was then used to calculate the total JPS score.

Trunk control: The Trunk Impairment Scale (TIS), a valid tool for measuring trunk performance in MS, was used to evaluate motor impairment of the trunk (18). This scale consists of 17 items under 3 sub-sections: static sitting balance, dynamic sitting balance, and coordination of trunk movement. Each item is scored according to the patient's ability to perform the assigned task. From a maximum of 23, higher scores indicate better trunk performance.

Balance: Dynamic and static aspects of balance were measured separately.

Dynamic balance*:* Dynamic balance was evaluated using the Berg Balance Scale (BBS), which is a 14-item, 56-point scale designed to measure balance through the assessment

of functional tasks (19). This scale measures balance abilities during tasks involving sitting and standing, in addition to anticipatory balance during activities commonly performed in daily functions, including transfers, turning, and retrieving objects from the floor. A 5-point scale from 0 (unable to perform) to 4 points (normal performance) is used to score the patient's ability to perform the task assigned safely and independently. The total score is calculated as the total of the scores of each item, with higher scores from a maximum of 56, indicating better functional balance.

Static balance: Static balance was evaluated using the single-leg stance (SLS) test (20). In the SLS test, each subject was instructed to stand on one leg with the arms resting on the hips. Once the participant is in the test position, the test is timed (in seconds) until the other foot contacts the ground or until the arms are separated from the hips. The test was repeated in the same manner for both legs. If the participant failed to concentrate on the test, the session was invalidated and postponed. The maximum time allowed for the test was 60 seconds.

Walking speed: The 10 mWT was used to determine the ability to increase walking speed (21). Paying attention to the acceleration and deceleration phases of walking the participants were instructed to walk 14 m along a straight corridor, in which the middle 10 m were marked with tape. The subjects were asked to walk as fast as possible without running, but safely, and the time taken to cover the middle 10 m was recorded in seconds. The physiotherapist walked with the subject during the test to guard against the risk of falling.

Statistical Analyses

Data were analyzed using IBM Statistical Package for the Social Sciences statistics version 21.0 software (IBM Corp. released in 2012., Armonk, NY, USA). Normal distribution was tested using the Shapiro-Wilk test. Values are expressed as mean±standard deviation for normally distributed variables or median (minimum-maximum) values for non-normally distributed variables. Categorical data are stated as number (n) and percentage (%). Comparisons between the two groups of continuous data were tested using the independent samples t-test and the Mann-Whitney U test. The chi-square test was used to compare categorical variables. Correlations between shoulder JPS and other measurements were calculated using Spearman's correlation coefficients. A value of $p<0.05$ was considered statistically significant.

Results

Demographic and clinical characteristics

The study included 40 MS patients (44.68±8.71 years old, 75% female) and 40 age-matched healthy control subjects (43.56±9.91 years old, 65% female). The mean age, gender, body mass index, and dominant extremity were similar in the two groups. Disease duration ranged between 1 and 18 years, and the mean EDSS score of the patients was 2.5 (range, 1-4). The demographic and clinical characteristics of the groups are given in Tables 1 and 2.

Table 1. Demographic characteristics

Characteristic		MS (n=40)		Control (n=40)		t, Z	p
Age, years, mean±SD		44.68±8.71		43.56±9.91		0.537	0.592[a]
BMI, kg/m², median (min-max)		24.24 (19.33-31.25)		24.92 (18.65-31.22)		-0.933[b]	0.353[b]
Gender		n	%	n	%	χ^2	
Female		30	75	26	65	0.536	0.464[c]
Male		10	25	14	35		
Dominant hand	Right	34	85	37	92.5	-	0.481[d]
	Left	6	15	3	7.5		
Dominant foot	Right	34	85	36	90	0.114	0.735[c]
	Left	6	15	4	10		

[a]Independent samples t-test, [b]Mann Whitney U-test, [c]Continuity correction, [d]Fisher's exact test.
BMI: Body mass index, min-max: Minimum-maximum, MS: Multiple sclerosis

Table 2. Clinical characteristics of multiple sclerosis patients

Expanded Disability Status Scale, median (min-max)	2.5 (1-4)
Duration of diagnosis, years, median (min-max)	8 (1-18)
Number of attacks, median (min-max)	3 (0-20)
Number of falls in the last year, median (min-max)	0.5 (0-8)

min-max: Minimum-maximum

Comparison of the groups

Significant differences between the groups were apparent across all measurements. BBS score, SLS for both sides, and TIS scores were significantly lower, and the 10 mWT time was significantly longer in MS patients than in controls (p<0.001). The planar and total JPS scores (error rates) for both sides were significantly higher in the MS group (p<0.001). The differences between the groups in all measurements are shown in Table 3.

Correlation analyses

Moderate to strong correlations were observed between the total JPS scores and BBS, SLS, and 10 mWT (p<0.05). Negative correlations were found between the total JPS score and BBS, and SLS, showing that balance and trunk control deteriorated as shoulder JPS decreased (p<0.001). A positive correlation was observed between the total shoulder JPS score and the 10 mWT time, indicating that as shoulder JPS decreased, walking speed also decreased (p<0.05). The results of the correlation analyses are presented in Table 4.

Table 3. Comparison analyses

		MS (n=40)	Control (n=40)	t, Z	p
Berg Balance Scale, median (min-max)		50 (33-56)	56 (54-56)	-7.353[b]	**<0.001***
Single-leg stance (D), median (min-max)		4.23 (0-60)	60 (28.35-60)	-7.582[b]	**<0.001***
Single-leg stance (ND), median (min-max)		6.76 (0-60)	60 (18.50-60)	-7.583[b]	**<0.001***
10-meter walk test, median (min-max)		9.10 (4.67-28.75)	5.81 (4.17-8.22)	-6.967[b]	**<0.001***
Trunk Impairment Scale	Static sitting, median (min-max)	7 (5-7)	7 (6-7)	-2.715[b]	**0.007***
	Dynamic sitting, median (min-max)	6 (0-10)	10 (8-10)	-5.566[b]	**<0.001***
	Coordination, median (min-max)	6 (2-6)	6 (6-6)	-4.925[b]	**<0.001***
	Total score, median (min-max)	18.50 (11-23)	23 (20-23)	-6.044[b]	**<0.001***
Shoulder JPS	Sagittal JPS Score (D), mean±SD	3.25±1.19	0.87±0.45	2.380[a]	**<0.001***
	Sagittal JPS Score (ND), median (min-max)	3.32 (1-8.45)	1.47 (0.45-2.95)	-6.502[b]	**<0.001***
	Frontal JPS Score (D), median (min-max)	3.32 (1.60-8)	1.12 (0.30-2.15)	-7.589[b]	**<0.001***
	Frontal JPS Score (ND), mean±SD	3.40±1.42	1.58±0.45	1.821[a]	**<0.001***
	Total JPS Score (D), median (min-max)	3.16 (1.70-7.25)	0.97 (0.30-1.63)	-7.700[b]	**<0.001***
	Total JPS Score (ND), median (min-max)	3.23 (1.23-7.95)	1.55 (0.80-2.38)	-6.675[b]	**<0.001***

[a]Independent samples t-test, [b]Mann-Whitney U test, *p<0.05, min-max: Minimum-maximum, JPS: Joint position sense, D: Dominant, ND: Non-dominant, MS: Multiple sclerosis

Table 4. Correlation analyses

			Total JPS score (D)	Total JPS score (ND)
Berg Balance Scale		rho	-0.770	-0.658
		p	**<0.001***	**<0.001***
Single-leg stance (D)		rho	-0.619	-0.500
		p	**<0.001***	**0.001***
Single-leg stance (ND)		rho	-0.653	-0.522
		p	**<0.001***	**0.001***
10-meter walk test		rho	0.456	0.531
		p	**0.003***	**<0.001***
Trunk Impairment Scale	**Static sitting**	rho	-0.374	-0.290
		p	**0.017***	0.070
	Dynamic sitting	rho	-0.587	-0.472
		p	**<0.001***	**0.002***
	Coordination	rho	-0.414	-0.382
		p	**0.008***	**0.015***
	Total score	rho	-0.665	-0.517
		p	**<0.001***	**0.001***

rho: Spearman's rank correlation coefficient, *p<0.05. JPS: Joint position sense, D: Dominant, ND: Non-dominant

Discussion

We observed that both sagittal and planar shoulder proprioception were significantly lower in MS patients compared with the controls. Decreased shoulder proprioception was also associated with trunk control, balance, and walking speed. To the best of our knowledge, this is the first study investigating the relationship between impaired shoulder JPS and proximal reflections in MS.

MS is a chronic and progressive neurodegenerative disease. During disease progression, many motor functions are affected due to axon loss and decreased nerve conduction velocity. Reduced trunk and lower extremity performance can lead to impairment in tasks such as balance and walking, ultimately restricting the independence of patients in daily life. However, the symptoms of MS extend beyond those related to motor issues. A range of deep and superficial sensation deficits are also evident in MS due to lesions on various ascending pathways in the spinal cord, thalamus, and sensory cortex (1). The effect of JPS on function may be dramatic and warrants further investigation. Accordingly, the impact of decreased shoulder JPS was investigated in this study because of the significance of the proprioceptive function of the shoulder joint, which connects two major body components with a structure rich in proprioceptors (8). Numerous previous investigations have been conducted to determine proprioceptive abnormalities in patients with MS and other neurological conditions (3,15,22,23). Consistent with the findings of those investigations, the current study also showed that shoulder position sense was reduced in MS patients compared with controls.

Arm reactions are a strategy used to maintain postural control. Reactive arm movements against a destabilizing effect as a part of balance reactions may provide extra moments to keep the center of gravity within the support surface (positioning arms to absorb disruptive momentum) or stabilize the body (reach and grasp) (9). Compared with the general healthy population, people with MS have impaired reactive balance (24). Aruin et al. (25) compared the onset of muscle activation of the trunk and lower extremities against an external disruption and observed a delay in anticipatory postural adjustments in MS. Suhaimy et al. (26) also reported that subjects with MS had significantly delayed response initiation compared with healthy control subjects, suggesting an impairment in balance. These reports can clarify the connection between reduced shoulder proprioception sense and balance-related measurements (TIS, SLST and BBS) in MS patients in the current study. A slower spinal somatosensory conduction speed in MS may cause impairments in the reception and response processes (27). Consequently, the protective and corrective responses of MS patients to various disruptive forces and situations will be reduced and delayed.

Walking speed and economy are two significant characteristics of walking capacity, and walking capacity is compromised in various types of neurological diseases. There have been reports of abnormal physiological responses during walking performance in patients with MS. Goldman et al. (28) observed a significantly lower mean walking speed in the 6-min walk test that is closely correlated with the 10 mWT in MS patients (21). In addition, the results of a recent systematic review indicate that MS subjects walk more slowly and with higher energy expenditure than healthy subjects (29). A lower walking speed may indicate a more cautious walking strategy that requires more energy. These changes in walking capacity can be attributed to inadequacies such as disability level, balance confidence, motor planning, cardiopulmonary capacity, and muscle performance (28,30). Arm swings during human locomotion are known to reduce the energy cost of walking and facilitate leg movements (31). Previous studies have stated that arm movement during walking improves stability (32). Central pattern generators in the medulla spinalis control and regulate arm swing in human locomotion based on accurate and adequate proprioceptive perception (31). In addition to being one of the primary structures affected by MS itself, reduced shoulder joint proprioceptive feedback could compromise the control of arm swings (33). Although the current study did not include such a methodology, future studies investigating the association between decreased shoulder proprioception and arm movements in MS patients may provide additional insights on this topic.

Study Limitations

There are some limitations to this study that could present potential for additional research. One limitation of this study is that it was conducted in a single center. Multicenter studies allow for the expansion of the sample population and evaluation of the results with a larger sample size. In addition, the cross-sectional design of our study limited the results to patients who came to our clinic within a certain period. Further multicentral studies are warranted to determine how proprioceptive changes affect MS patients in various joints with larger sample sizes.

Conclusion

All functions in the human body coordinated by the nervous system operate with feedback control, which allows for fine adjustments, such as the amplitude and timing of the response. It is well known that decreased feedback flow in many neurological diseases affects the quality of the response. In this study, we focused on the proprioceptive feedback integrity of the junction between the trunk and upper extremity in MS patients with well-defined sensory symptoms. This study demonstrated that decreased positional feedback flow in the shoulder joint, which has some auxiliary roles in complex functions such as balance and walking, is associated with the worsening of these functions. Based on these results, new rehabilitation strategies can be developed to strengthen the positional relationship between the shoulder and trunk in MS patients.

Ethics

Ethics Committee Approval: Approval for this study was granted by the Gülhane Scientific Research Ethics Committee (decision no: 2022-305, dated: 17/10/2022).

Informed Consent: All participants provided written informed consent.

Authorship Contributions

Concept: M.E.Y., T.Ö., N.Ö.Ü., Design: M.E.Y., T.Ö., G.V., Data Collection or Processing: M.E.Y., T.Ö., B.K., G.V., Analysis or Interpretation: M.E.Y., T.Ö., B.K., Literature Search: M.E.Y., T.Ö., N.Ö.Ü., Writing: M.E.Y., N.Ö.Ü.

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Vitamin B12 and folate levels in children with primary nocturnal enuresis

Cengiz Zeybek

University of Health Sciences Türkiye, Gülhane Faculty of Medicine, Department of Pediatric Nephrology, Ankara, Türkiye

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Corresponding Author:
Cengiz Zeybek, M.D., University of Health Sciences Türkiye, Gülhane Faculty of Medicine, Department of Pediatric Nephrology, Ankara, Türkiye
+90 532 684 26 99
zeybekcengiz@yahoo.com

ORCID:
orcid.org/0000-0002-4820-0373

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ABSTRACT

Aims: Primary nocturnal enuresis (PNE) is associated with the maturation of the central nervous system in children. Vitamin B12 and folate are involved in the metabolism, development, and maturation of the nervous system. We examined vitamin B12, folate, and ferritin levels in children with PNE.

Methods: This retrospective study included children with and without PNE from a tertiary pediatric nephrology clinic. PNE was defined as nighttime bedwetting (≥2 nights per week) in children aged >5 years. Children with chronic diseases or neurological, psychiatric or urological abnormalities were excluded. Vitamin B12, folate, hemoglobin, hematocrit, and ferritin levels were obtained from medical health records.

Results: The study included 86 patients with PNE and 90 age- and sex-matched controls. The PNE group had lower vitamin B12 (229 vs. 264 pg/mL; p=0.001) and folate (7.9 vs. 12.4 ng/mL; p=0.001) levels than the control group. Vitamin B12 deficiency was more common in children with PNE than in controls (40.7% vs. 25.6%; p=0.037). None of the children with PNE or controls had folate deficiency. The hemoglobin, hematocrit, and ferritin levels were similar between the two groups.

Conclusions: Compared with controls, children with PNE had lower vitamin B12 and folate levels and a higher prevalence of vitamin B12 deficiency. Further studies are needed to determine whether vitamin B12 and folate supplementation can improve PNE symptoms.

Introduction

Primary nocturnal enuresis (PNE) is characterized by involuntary bedwetting during sleep in children aged >5 years without any congenital or acquired defects in the central nervous system (CNS) (1). Children with PNE have a lifelong inability to achieve continence, whereas those with secondary enuresis have experienced continence for ≥6 months (2). The exact cause of PNE remains unclear; several mechanisms have been proposed, including small bladder capacity, abnormal sleep patterns, excessive urine production during sleep, and delayed functional maturation of the CNS (3). The prevalence of PNE decreases rapidly with increasing age, which is likely associated with neuroanatomical development (4).

Vitamin B12 plays a crucial role in the maturation of peripheral nerves, optic nerves, brain tissue, and the posterior and lateral columns of the spinal cord (5). Vitamin B12 deficiency results in the accumulation of methylmalonyl-CoA, which replaces acetyl-CoA and leads to the formation of unstable myelin. Myelin instability can cause neurological damage to the nervous system in children (5).

Folate is an essential nutrient for CNS development (6). In fact, folate deficiency during preconception and early pregnancy is a major risk factor for neural tube defects (7). The precise mechanism by which folate prevents neural tube defects is not understood but may be related to methionine and nucleotide biosynthesis (8). Mice fed a folate-poor diet exhibited impairments in the hypothalamic serotonin and dopamine systems and behavioral changes (6).

Iron plays a crucial role in early brain development and is essential for myelination and neurotransmitter function (9). Iron deficiency leads to impaired myelination, neurotransmission, synaptogenesis, and dendritogenesis, particularly during infancy (10). Ferritin, a form of iron, is not only crucial for brain

development but also plays a significant role in spinal cord development and repair (11).

Because vitamin B12, folate, and iron are involved in nervous system development and PNE is associated with delayed CNS maturation, we explored whether vitamin B12, folate, hemoglobin, hematocrit, and ferritin levels are associated with PNE.

Methods

This single-center, retrospective study included 176 children with PNE, and age- and sex-matched controls admitted to the Pediatric Nephrology Clinic of Gülhane Training and Research Hospital between November 2016 and December 2022. The medical records of children with PNE and controls were obtained from the hospital information system. This study included children with and without PNE who underwent testing for vitamin B12, folate, and ferritin levels and complete blood count on family request or physician advice during routine check-ups. Children who did not undergo the aforementioned laboratory tests or had incomplete data were excluded. PNE was diagnosed in children aged >5 years with two or more nighttime enuretic episodes per week and no daytime urinary incontinence, no history of continence lasting for >3 months, no neurological, psychiatric (e.g. autism spectrum disorder, attention deficit disorder, and hyperactivity), urological abnormalities, and no chronic diseases such as diabetes mellitus or diabetes insipidus (1). The control group included children aged >5 years who had normal blood and urine tests, no neurological, psychiatric, or urological abnormalities, no bacterial growth in urine culture, no chronic diseases, and no history of PNE.

Some inflammatory markers (e.g. C-reactive protein and systemic immune inflammation index) may increase in acute otitis media (AOM) (12). Furthermore, recurrent AOM may be associated with adenotonsillar hypertrophy (ATH), which is associated with nocturnal enuresis (13). Non-steroidal anti-inflammatory drugs (NSAIDs), such as indomethacin, can be used for treating nocturnal enuresis (14). Therefore, we excluded children who were taking NSAIDs and had ATH and/or a history of recurrent AOM from both the PNE and control groups.

Hemoglobin, hematocrit, ferritin, vitamin B12, and folate levels were obtained from hospital records. The UniCel DxH 800 Hematology Analyzer (Beckman Coulter, Miami, FL, USA) was used for complete blood count analysis, and the AU680® analyzer (Beckman Coulter) was used for biochemical tests during the study period.

Vitamin B12 levels <200, 200-300, and >300 pg/mL indicate vitamin B12 deficiency, borderline vitamin B12 deficiency, and normal vitamin B12 levels, respectively (15). Folate levels <2, 2-4, and >4 ng/mL indicate folate deficiency, borderline folate deficiency, and normal folate levels, respectively (16). Ferritin levels <10 ng/mL indicated low ferritin levels (17).

The study protocol was approved by the Local Ethics Committee of the University of Health Sciences Türkiye, Gülhane Training and Research Hospital (date: 15.03.2023, decision no: 35).

Statistical Analysis

Statistical analyses were performed using Statistical Package for the Social Sciences software (version 22; IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov test was used to test for the normality of the data distribution. Normally distributed continuous variables were compared using Student's t-test, whereas non-normally distributed continuous variables were compared using the Mann-Whitney U test. Continuous variables are presented as medians and interquartile ranges. Categorical variables were compared using the chi-square or Fisher's exact test and are presented as numbers and percentages. P values <0.05 were considered indicative of statistical significance.

Results

The study included 176 patients [median age=9 (6-14) years], including 86 children (49 boys and 37 girls) in the PNE group and 90 children (42 boys and 48 girls) in the control group. As shown in Table 1, the median age and sex were not significantly different between the groups.

The median vitamin B12 level was 229 (165-295) pg/mL in children with PNE and 264 (178-351) pg/mL in controls, with significant differences between the groups (p=0.001) (Table 1, Figure 1). The number of children with PNE with borderline vitamin B12 deficiency and vitamin B12 deficiency was 39 and 35, respectively, compared with 27 and 23, respectively, in the control group. There were statistically significant differences between the children with PNE and controls regarding the number of children with borderline vitamin B12 deficiency and vitamin B12 deficiency (p=0.034 and p=0.037, respectively) (Table 1).

The median folate level was 7.9 (6.5-10.5) ng/mL in the children with PNE group and 12.4 (9.7-15) ng/mL in the control group; the difference was statistically significant (p=0.001) (Table 1, Figure 2). The number of children with PNE with borderline folate deficiency was significantly higher than that of the controls (6 and 1, respectively; p=0.041).

There were no statistically significant differences in hemoglobin, hematocrit, or ferritin levels between the two groups (p=0.566, p=0.729, and p=0.806, respectively). Although the proportion of children with PNE with low ferritin levels was higher than that of controls, the difference was not statistically significant (15.1% and 7.8%, respectively; p=0.136) (Table 1).

A comparison of the cases and controls according to sex did not reveal any significant differences in age or hemoglobin, hematocrit, or ferritin levels (p>0.05) (Table 2).

Table 1. Demographic characteristics and comparison of hemoglobin, hematocrit, vitamin B12, folate and ferritin levels

	PNE (n=86)	Control (n=90)	p-value
Sex, female/male	37/49	48/42	0.184**
Age, years, median (IQR)	9 (6-13)	9 (7-13)	0.144*
Hemoglobin, g/dL, median (IQR)	13.2 (12.8-13.9)	13.3 (12.9-14.1)	0.566*
Hematocrit, %, median (IQR)	39.7 (38.1-41.2)	39.5 (37.9-41.5)	0.729*
Vitamin B12, pg/mL, median (IQR)	229 (165-295)	264 (178-351)	**0.001***
Borderline vitamin B12 deficiency, n (%)	39 (45.3)	27 (30.0)	**0.034****
Vitamin B12 deficiency, n (%)	35 (40.7)	23 (25.6)	**0.037****
Folate, ng/mL, median (IQR)	7.9 (6.5-10.5)	12.4 (9.7-15)	**0.001***
Borderline folate deficiency, n (%)	6 (6.9)	1 (1.1)	**0.041*****
Folate deficiency, n (%)	0	0	1.000***
Ferritin level, ng/mL, median (IQR)	20.5 (12.2-33.4)	20.4 (14.8-32.3)	0.806*
Low ferritin level, n (%)	13 (15.1)	7 (7.8)	0.136**

*Mann-Whitney U test, **Chi-squared test, ***Fisher's exact test.
PNE: Primary nocturnal enuresis, IQR: Interquartile range

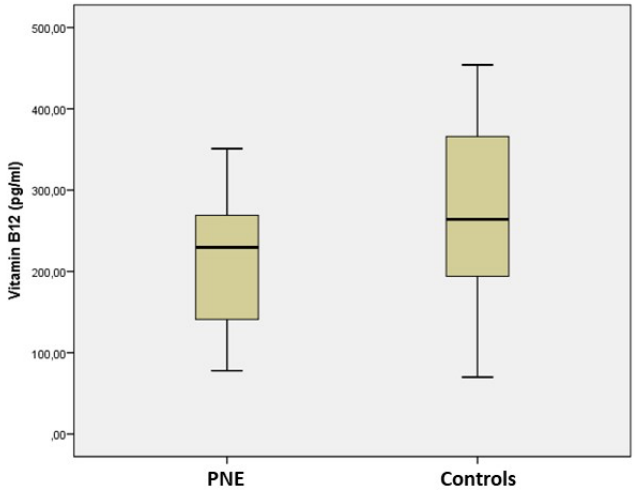

Figure 1. Boxplot diagram of serum vitamin B12 levels in PNE patients and control subjects
PNE: Primary nocturnal enuresis

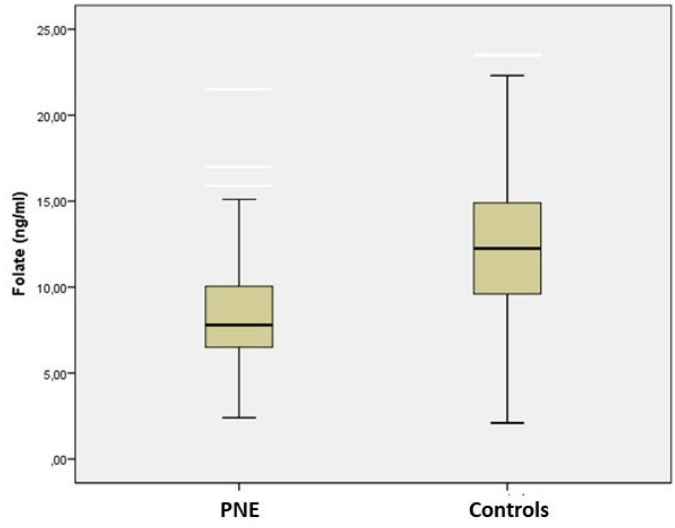

Figure 2. Boxplot diagram of serum folate levels in PNE patients and control subjects
PNE: Primary nocturnal enuresis

Table 2. Comparison of demographic characteristics, hemoglobin, hematocrit, vitamin B12, folate and ferritin levels according to sex

	Female			Male		
Parameters	**PNE (n=37)**	**Control (n=48)**	**p-value**	**PNE (n=49)**	**Control (n=42)**	**p-value**
Age, years, median (IQR)	8 (5-12)	9 (6-13)	0.065*	8 (6-11)	9 (6-12)	0.113*
Hemoglobin, g/dL, median (IQR)	12.9 (12.5-13.3)	13.0 (12.6-13.4)	0.435*	13.4 (12.8-13.8)	13.7 (13.1-16.2)	0.225*
Hematocrit, %, median (IQR)	38.8 (37.6-40.1)	38.8 (37.5-40.1)	0.645*	40.4 (38.8-42.2)	40.3 (38.1-43.1)	0.337*
Vitamin B12, pg/mL, median (IQR)	228 (137-248)	256 (186-355)	**0.004***	220 (133-285)	287 (229-420)	**0.001***
Borderline vitamin B12 deficiency, n (%)	19 (51.4)	14 (29.2)	**0.037****	20 (40.1)	13 (31.0)	0.329**
Vitamin B12 deficiency, n (%)	14 (37.8)	14 (29.2)	0.437**	21 (40.7)	9 (25.6)	**0.030****
Folate, ng/mL, median (IQR)	7.9 (5.9-10.5)	11.0 (8.8-14.7)	**0.001***	7.8 (6.6-10.2)	13.3 (10.4-15.5)	**0.001***
Borderline folate deficiency, n (%)	2 (5.4)	0 (0)	0.059***	4 (8.2)	1 (2.4)	0.228***
Folate deficiency, n (%)	0	0	1.000***	0	0	1.000***
Ferritin level, ng/mL, median (IQR)	19.2 (10.1-27.9)	18.4 (13.3-25.8)	0.946*	25.1 (14.1-34.0)	27.8 (16.0-38.1)	0.337*
Low ferritin level, n (%)	8 (21.6)	5 (10.4)	0.181**	5 (11.2)	2 (4.8)	0.442**

*Mann-Whitney U test, **Chi-squared test, ***Fisher's exact test.
PNE: Primary nocturnal enuresis, IQR: Interquartile range

Among females in the case and control groups, the vitamin B12 levels were 228 (137-248) and 256 (186-355) pg/mL, respectively, whereas among males, the levels were 220 (133-285) and 287 (229-420) pg/mL, respectively. Vitamin B12 levels were significantly different between the female and male cases and controls (p=0.004 and=0.001, respectively). Similarly, a statistically significant difference was found between the cases and controls among both males and females with folate levels ((both p=0.001). Among females in the case and control groups, the folate levels were 7.9 (5.9-10.5) and 11.0 (8.8-14.7) ng/mL, respectively, whereas the levels were 7.8 (6.6-10.2) and 13.3 (10.4-15.5) ng/mL among male cases and controls, respectively. These results suggest that serum vitamin B12 and folate levels were significantly lower in both female and male patients than in controls.

The number of female patients with borderline vitamin B12 deficiency was significantly higher in the case group than in the control group (p=0.037), whereas there was no significant difference in males (p=0.329) (Table 2). Although there were no significant differences in the number of female cases and controls with vitamin B12 deficiency (p=0.437), the number of male children with vitamin B12 deficiency was higher in the case group than in the control group (p=0.030). Finally, there were more females and males with borderline folate deficiency in the case group than in the control group, although the differences were not statistically significant (p=0.059 and p=0.228, respectively) (Table 2).

Discussion

In this study, children with PNE had significantly lower vitamin B12 and folate levels than healthy controls. The prevalence of vitamin B12 deficiency and borderline vitamin B12 deficiency was significantly higher in children with PNE than in controls. Similarly, borderline folate deficiency was significantly more common in children with PNE than in controls. Conversely, there were no significant differences between the groups in ferritin, hemoglobin, or hematocrit levels. Although the prevalence of low ferritin levels was higher in the PNE group than in the controls, the difference was not statistically significant.

Several studies have evaluated vitamin B12 and folate levels in children with PNE (18-21). However, most studies included fewer than 50 participants (18-20). Our study included 86 children with PNE, making it the second-largest study. We found that vitamin B12 and folate levels were significantly lower in the PNE group than in the controls, which is in line with most previous studies (18-20), although Keles et al. (21) did not find a significant difference in folate levels between children with and without PNE.

The lower urinary tract, including the bladder, is innervated by three types of peripheral nerves. Pelvic parasympathetic nerves, which are located at the level of the sacral spinal cord, provide sensory innervation to the bladder and relax the urethra by increasing the levels of the neurotransmitter acetylcholine (22). Lumbar sympathetic nerves inhibit contraction of the bladder body and provide sensory innervation to the bladder base and urethra by increasing the levels of the neurotransmitter norepinephrine (22). Pudendal nerves play crucial roles in opening and closing the external urethral sphincter by increasing the levels of the neurotransmitter acetylcholine (22).

Certain vitamin deficiencies are involved in the pathogenesis of some diseases; for example, vitamin D deficiency is involved in the pathogenesis of celiac disease (23). Similarly, vitamin B12 deficiency is primarily associated with impaired neurotransmitter production, myelin damage, subacute combined degeneration of the spinal cord, polyneuritis, neuropathy, myelopathy, optic nerve atrophy, and cognitive dysfunction (24). The main cause of neuronal demyelination is reduced levels of S-adenosylmethionine (SAM), a universal methyl donor (5). Vitamin B12 deficiency plays a crucial role in SAM synthesis, and SAM plays important roles in the nervous system, including myelination and neurotransmitter synthesis (5). Myelinated Aδ-afferent fibers participate in the normal micturition reflex (25). In cases of vitamin B12 deficiency, these Aδ-afferent fibers also undergo demyelination (26).

Folate has crucial functions in the nervous system, including multiple CNS methylation reactions (27). Folate deficiency can lead to subacute combined spinal cord degeneration (28). It enters the nervous system in the form of methyl folate (27). Methyl folate donates its methyl group via SAM (7). Mouse studies have shown that folate contributes to myelination by promoting oligodendrocyte survival and differentiation (29).

Therefore, both vitamin B12 and folate are involved in the regulation of myelination of nerves that innervate the urinary system and promote neurotransmitter synthesis for normal micturition (24,29).

In our study, vitamin B12 and folate levels were significantly lower in the PNE group than in the controls. The prevalence of borderline vitamin B12 deficiency was significantly lower in females than in males. Margalit et al. (30) also found a higher rate of vitamin B12 deficiency in males than in females and attributed this difference to genetic variations rather than dietary habits or estrogen. In the present study, although only six children with PNE had borderline folate deficiency, it was more common in males than in females, which is consistent with previous studies (31).

Although several case reports have described improvements in enuresis in adults with vitamin B12 supplementation (32,33), limited evidence exists for children (34). In an 18-year-old autistic patient, oral vitamin B12 supplementation administered to improve psychobehavioral status also improved nocturnal enuresis (34). Furthermore, the same patient experienced a recurrence of enuresis after discontinuation of supplements.

Kurabayashi et al. (32) and Lindenbaum et al. (33) showed that vitamin B12 supplementation improved urinary incontinence in adults. Conversely, Campellone et al. (35) found no improvement in vitamin B12 deficiency-related neurogenic bladder dysfunction after intramuscular vitamin B12 administration. Although the aforementioned evidence is useful, the patients in previous studies were much older than our study population and did not have PNE.

PNE is more common in patients with anemias, including sickle cell disease and thalassemia major (36). However, the increased incidence of these diseases was not associated with nephropathy or hypostenuria, which may be due to anemia, but rather to factors such as younger age and positive family history in the general population (36,37). We observed no statistically significant differences in ferritin levels between children with PNE and controls, which is in agreement with previous studies (19,21). Moreover, there were no significant differences between the children with PNE and controls regarding the prevalence of anemia or in the hemoglobin or hematocrit levels. Therefore, iron deficiency may not play a role in the etiopathogenesis of PNE.

Study Limitations

Our study had several limitations. First, this was a retrospective study. Second, we did not analyze serum methylmalonic acid or homocysteine levels, which are more sensitive indicators of vitamin B12 or folate deficiency, particularly in patients with borderline levels. Third, we did not evaluate the history of iron deficiency anemia during infancy, which can be related to PNE because of the role of iron in myelination and neurotransmitter functions. Fourth, the study had a small sample size, which may have been insufficient to determine the role of vitamin B12, folate, and ferritin levels in the etiopathogenesis of PNE.

Conclusion

Children with PNE had lower vitamin B12 and folate levels than controls. No significant differences were observed in the hemoglobin, hematocrit, or ferritin levels between children with PNE and controls. Further studies are needed to determine whether vitamin B12 and folate deficiencies contribute to the etiopathogenesis of PNE and to establish whether vitamin B12 and/or folate supplementation can improve PNE in children.

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Ethics

Ethics Committee Approval: The study protocol was approved by the Local Ethics Committee of the University of Health Sciences Türkiye, Gülhane Training and Research Hospital (date: 15.03.2023, decision no: 35).

Informed Consent: Retrospective study.

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Preoperative, operative, and postoperative pathological features in thyroid papillary carcinoma with and without capsule invasion

Burak Uçaner[1], Mehmet Zeki Buldanlı[1], İbrahim Ali Özemir[2], Mehmet Sabri Çiftçi[1], Sacit Altuğ Kesikli[1], Murat Özkara[1], Ertuğrul Çelik[3], Oğuz Hançerlioğulları[1]

[1]University of Health Sciences Türkiye, Gülhane Training and Research Hospital, Clinic of General Surgery, Ankara, Türkiye
[2]İstanbul Göztepe Prof. Dr. Süleyman Yalçın City Hospital, Clinic of General Surgery, İstanbul, Türkiye
[3]University of Health Sciences Türkiye, Gülhane Training and Research Hospital, Clinic of Medical Pathology, Ankara, Türkiye

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Corresponding Author:
Burak Uçaner, M.D., University of Health Sciences Türkiye, Gülhane Training and Research Hospital, Clinic of General Surgery, Ankara, Türkiye
+90 312 304 50 10
burakucaner@hotmail.com

ORCID:
orcid.org/0000-0002-5420-3810

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ABSTRACT

Aims: Approximately 80-85% of thyroid malignancies are papillary thyroid cancer (PTC). This study evaluated the relationship between thyroid capsule invasion (TCI) in PTC and macroscopic histopathological findings.

Methods: A single-center, retrospective study was conducted using the medical records of adult patients who underwent PTC surgery. The patients were classified as TCI (+) or TCI (-) based on the postoperative pathological examination. Tumor localization, calcification, and multifocality were evaluated between the two groups.

Results: The study included 236 patients (mean age: 44.3±12.0 years, female sex: 76.7%). Preoperative basic characteristics, comorbidities, thyroid function status, nodule calcification, halo border irregularity, and nodule diameter on ultrasonography were similar between the two groups. However, more TCI (+) patients had positive or suspicious fine needle aspiration biopsy findings preoperatively. The duration of surgery was longer in TCI (+) patients (86 minutes vs. 75 minutes, p<0.001), whereas the length of hospital stay was similar. Surgical margin >1 mm was more common in TCI (+) vs. TCI (-) patients (47.3% vs. 81.8%, p<0.001). Postoperative macroscopic pathological reports showed that middle lobe tumor localization was more common in TCI (+) (38.2%) than in TCI (-) (23.2%) patients (p=0.028), whereas tumor localization in the upper pole, lower pole, and isthmus was not different. Multifocal involvement (41.8% vs. 38.1%) was also similar between the two groups.

Conclusions: This study showed that fine needle biopsy positivity and nodule localization in the middle thyroid gland were more common in TCI (+) PTC patients as detected postoperatively. The other macroscopic pathological findings were not different.

Introduction

The thyroid gland, which consists of two connected lobes, is one of the largest endocrine glands in the human body, weighing 20-30 g in adults. Malignant diseases of the endocrine system are common, and the thyroid lesions are the most frequent by 4-7% (1). Approximately 80-85% of thyroid malignancies are papillary thyroid cancer (PTC) (2). 'Papillary microcarcinomas' of ≤10 mm, classified as a subgroup of PTCs, comprise 30% of all papillary cancers and are considered more moderate than PTCs of >10 mm (3). Despite high survival rates, local recurrence and metastases may still occur and radical surgery may be required in such patients (4). Treatment of PTC is usually bilateral total thyroidectomy (BTT) and based on the tumor size and lymph node involvement, central or lateral lymph node dissection is performed (5).

Similar to tumor characteristics such as lymphovascular invasion and extrathyroidal extension, capsule invasion is also associated with clinical outcomes in PTC (6).

The relationship between capsule invasion and clinicopathological characteristics has been previously studied in thyroid follicular cancer (6). However, the data in this regard are sparse in PTC (6). Therefore, this study aimed to investigate the relationship of capsule invasion with preoperative clinical and laboratory parameters and postoperative histopathological findings in PTC.

Methods

Study population and recruitment

This was a retrospective analysis in a tertiary-care training and research hospital. This study was conducted in accordance with the Declaration of Helsinki as revised in 2013 and, was approved by the University of Health Sciences Türkiye, Gülhane Training and Research Hospital, Clinical Research Ethics Committee (decision no: 2022/145, date: 24.11.2022).

A total of 236 patients who were above eighteen years old and operated with the diagnosis of PTC in the general surgery clinic of a single center between January 2017 and June 2022 were included in this study. Patients under 18 years of age and those who were operated on for recurrence were excluded from this study.

Variables and data collection

In addition to demographic variables, comorbidities, the American Society of Anesthesiologists (ASA) score, and body mass index (BMI) were recorded. Biochemical parameters were analyzed, including preoperative thyroxine levels (T4, ng/dL) and thyroid-stimulating hormone levels (mU/mL) and postoperative calcium (Ca) (Ca, mg/dL), thyroglobulin (Tg) (Tg, ng/mL) and anti-Tg antibody (anti-Tg, IU/mL). Preoperative ultrasonography (USG) findings and histopathological reports of fine-needle aspiration biopsies (FNAB) were investigated. Postoperative first-day Ca values were analyzed. In addition, Tg and anti-Tg levels were assessed in the third month in high-risk patients and in the sixth month in low-intermediate-risk patients. Surgical findings, postoperative complications, and histopathological findings were examined. Based on capsule invasion on pathological examination, the patients were grouped into thyroid capsule invasion (TCI) (+) and TCI (-).

Statistical Analysis

All analyses were performed using the IBM Statistical Package for the Social Sciences Statistics® Statistical Software Program version 22.0 (IBM Corporation, 1 New Orchard Road, Armonk, New York, United States). Descriptive statistics are expressed as numbers, percentages, mean, standard deviation, and median (minimum-maximum). The conformity of the variables to the normal distribution was examined using visual (histograms and probability graphs) and analytical methods ("Kolmogorov-Smirnov", "Shapiro-Wilk tests"). Numerical variables with normal distribution were analyzed using the independent samples t-test between the two groups, and non-normally distributed variables were analyzed using the Mann-Whitney U test". Chi-square analysis and Fisher's exact test were used to compare the categorical data. A p-value of <0.05 was considered statistically significant.

Results

Basic characteristics of the sample and prevalence of TCI

The mean age of 236 patients included in the study was 44.3±12.0 years (18-75 years), and 76.7% were female. In the whole sample, most of the nodules were solid on preoperative USG evaluation (n=175, 74.2%), whereas most nodules were malignant on FNAB (n=162, 68.6%). The ratio of TCI in the whole sample was 23.3% (n=55).

Preoperative characteristics of TCI (+) versus TCI (-) patients

As shown in Table 1, age, sex, BMI, chronic comorbidities, USG nodule classification, diameter, calcification, and halo border irregularity were similar in the two groups. Preoperative thyroid function status was also not different; however, malignant FNAB findings were more frequent in TCI (+) (80.0%) than in TCI (-) (65.2%) (0.015). The preoperative ASA classification was similar in the two groups.

Intraoperative and postoperative clinical characteristics

As shown in Table 2, BTT + central lymph node dissection (CLND) was performed more frequently in TCI (+) (63.6%) vs. TCI (-) (38.1%) (p=0.001). The duration of surgery was longer in patients with TCI. As expected, increased Tg and RAI therapy were more common in TCI (+) patients, but serum Ca levels were not different. Surgery-related complications, length of hospital stay, duration of follow-up, and lymph node metastasis during follow-up were similar in the two groups (Table 2).

Macroscopic and histopathological examination characteristics

As shown in Table 3, the middle lobe localization of tumors was more frequent in TCI (+) patients. Lower pole localization was also more frequent, but the difference was not significant. In contrast, although there was no significance, isthmus and upper pole tumors were more frequent in TCI (-) patients. Multifocal involvement was not different between the two groups. The tumor type was papillary microcarcinoma by 44.1% in the whole group and was not different between the two groups, as were tumor grade and tumor pattern. The rate of lymphovascular invasion was 13.9% in the whole group, which was similar in TCI (+) and TCI (-) patients. More patients with TCI had surgical margins <1 mm. The number of metastatic lymph nodes was significantly lower in TCI (+) patients (Figures 1, 2, Table 3).

Table 1. Demographic and preoperative characteristics of patients with and without thyroid capsule invasion

	Total (n=236)	TCI (+) (n=55)	TCI (-) (n=181)	p-value
Age, mean±SD	44.3±12.0	44.6±12.3	44.2±12.0	0.841
Sex, female, n (%)	181 (76.7)	40 (72.7)	141 (77.9)	0.427
BMI (kg/m^2)	26 (16-42)	26 (16-38)	26 (18-42)	0.437
Hypertension, n (%)	53 (22.5)	14 (25.5)	39 (21.5)	0.543
Diabetes mellitus, n (%)	26 (11.0)	7 (12.7)	19 (10.5)	0.644
Coronary artery disease, n (%)	2 (0.8)	0	2 (1.1)	0.587
USG nodule classification, n (%)				
Solid	175 (74.2)	38 (69.1)	137 (75.7)	0.309
Semisolid	48 (20.3)	14 (25.5)	34 (18.8)	
Cystic	8 (3.4)	3 (5.5)	5 (2.8)	
Solid with cystic areas	5 (2.1)	0	5 (2.8)	
USG nodule diameter (millimeter), median (minimum-maximum)	14 (2-59)	13 (3-48)	15 (2-59)	0.781
Nodule calcification (+)	61 (25.8)	13 (23.6)	48 (26.5)	0.669††
Halo border irregularity (+)	62 (26.2)	16 (29.1)	46 (25.4)	0.587††
Preoperative TSH, median (minimum-maximum)	1.57 (0.01-24.5)	1.47 (0.02-7.70)	1.61 (0.01-24.5)	0.666
Preoperative T4, median (minimum-maximum)	0.88 (0.12-1.77)	0.88 (0.12-1.41)	0.92 (0.41-1.77)	0.440
Preoperative TSH classification, n (%)				
Euthyroid	211 (89.4)	48 (87.3)	163 (90.1)	0.462
Hyperthyroid	18 (7.6)	4 (7.3)	14 (7.7)	
Hypothyroid	7 (3.0)	3 (5.5)	4 (2.2)	
FNAB, n (%)				
Malignant	162 (68.6)	44 (80.0)	118 (65.2)	**0.015**
Suspicion of malignancy	40 (16.9)	7 (12.7)	33 (18.2)	
AUS-FLUS	6 (2.5)	0	6 (3.3)	
Benign	23 (9.7)	2 (3.6)	21 (11.6)	
Follicular lesion	2 (0.8)	2 (3.6)	0	
Atypia of undetermined significance	3 (1.3)	0	3 (1.7)	
ASA classification, n (%)				
I	140 (59.3)	32 (58.2)	108 (59.7)	0.899
II	74 (31.4)	17 (30.9)	57 (31.5)	
III	22 (9.3)	6 (10.9)	16 (8.8)	

††Fisher's exact test.
TCI: Thyroid capsule invasion, BMI: Body mass index, ASA: American Society of Anaesthesiologists, USG: Ultrasonography, FNAB: Fine needle aspiration biopsy, AUS/FLUS: Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance, TSH: Thyroid stimulating hormone, T4: Thyroxine, SD: Standard deviation

Discussion

Consistent with the literature, most PTCs were diagnosed as a solid or semisolid mass, and a minority were in cystic form. Approximately 10% of the patients may present with metastatic disease (7), but we observed a slightly higher rate of lymphovascular involvement of 13.9%, which can be explained by the level of the facility that accepts more complicated patients as a referral center.

The College of American Pathologists specifies that capsular invasion, extrathyroidal extension, and lymphovascular invasion in thyroid carcinomas are key features in malignancy based on histopathological classification and emphasizes the importance of reporting (8). Capsular invasion is among the gross pathophysiological findings and may affect the aggressive behavior pattern of the tumor, locoregional spread, and both central and lateral lymph node metastasis, particularly in follicular-patterned carcinomas (9,10). It may also be associated with many parameters during the postoperative period (11,12).

We observed no difference in demographic findings, co-morbidities and preoperative thyroid function tests between patients with and without TCI, which is consistent with the literature. However, consistent with the literature, more patients

Table 2. Intraoperative and postoperative clinical characteristics

	Total (n=236)	TCI (+) (n=55)	TCI (-) (n=181)	p-value
Surgical procedure				
Bilateral total thyroidectomy, n (%)	132 (55.9)	20 (36.4)	112 (61.9)	**0.001**††
Bilateral total thyroidectomy + Central lymph node dissection, n (%)	104 (44.1)	35 (63.6)	69 (38.1)	
Duration of surgery (minutes)[a]	80 (40-176)	86 (55-146)	75 (40-176)	**<0.001**[ŧ]
Postoperative calcium level[b]	8.4±0.6	8.4±0.6	8.5±0.6	0.265[†]
Postoperative Tg (>1 ng/mL), n (%)	14 (5.9)	7 (12.7)	7 (3.9)	**0.023**[ŧŧ]
Radioactive iodine therapy, n (%)	115 (48.7)	42 (76.4)	73 (40.3)	**<0.001**††
Complications				
Transient hypocalcemia, n (%)	79 (33.5)	18 (32.7)	61 (33.7)	0.893††
Transient hoarseness, n (%)	28 (11.9)	10 (18.2)	18 (9.9)	0.098††
Bleeding, n (%)	2 (0.8)	1 (1.8)	1 (0.6)	0.413[ŧŧ]
Presence of cheilosis leakage , n (%)	1 (0.4)	1 (1.8)	0	0.233[ŧŧ]
Length of hospital stay[a]	2 (2-10)	2 (2-10)	2 (2-7)	0.212[ŧ]
Follow-up duration (months)[a]	24 (12-54)	24 (12-54)	24 (12-52)	0.743[ŧ]
Lymph node metastasis during postoperative follow-up	9 (3.8)	3 (5.5)	6 (3.3)	0.439[ŧŧ]

[a]Median (minimum-maximum), [b]mean±standard deviation. [ŧ]Mann-Whitney U test, ††chi-square test, [ŧŧ]Fisher's exact test, Tg: Thyroglobulin, Anti-Tg: Anti-thyroglobulin antibody, TCI: Thyroid capsule invasion

Table 3. Macroscopic and histopathological examination characteristics

	Total (n=238)	TCI (+) (n=55)	TCI (-) (n=181)	p-value
Location, n (%)				
Upper pole	100 (29.7)	21 (38.2)	79 (43.6)	0.473††
Lower pole	91 (27.2)	25 (45.5)	66 (36.5)	0.230††
Isthmus	82 (24.4)	14 (25.5)	68 (37.6)	0.098††
Middle	63 (19.4)	21 (38.2)	42 (23.2)	**0.028**††
Multifocal involvement (+)	92 (38.7)	23 (41.8)	69 (38.1)	0.623††
Tumor type, n (%)				
Papillary microcarcinoma	104 (44.1)	23 (41.8)	81 (44.8)	0.701††
Papillary carcinoma	132 (55.9)	32 (58.2)	100 (55.2)	
Tumor grade, n (%)				
T1a	103 (43.6)	22 (40.0)	81 (44.8)	0.652††
T1b	81 (34.3)	21 (38.2)	60 (33.1)	
T2	44 (18.6)	9 (16.4)	35 (19.3)	
T3	8 (3.4)	3 (5.5)	5 (2.8)	
Tumor pattern, n (%)				
Papillary	76 (32.2)	17 (30.9)	59 (32.6)	0.872††
Follicular	20 (8.5)	5 (9.1)	15 (8.3)	
Papillary and follicular	138 (58.5)	33 (60.0)	105 (58.0)	
Focal papillary and follicular	2 (0.8)	0	2 (1.1)	
Lymphovascular invasion	33 (13.9)	10 (18.2)	23 (12.7)	0.305††
Surgical margin, n (%)				
>1 mm	174 (73.7)	26 (47.3)	148 (81.8)	**<0.001**††
<1 mm	62 (26.3)	29 (52.7)	33 (18.2)	
Number total of lymph nodes[a]	2 (0-23)	6 (0-23)	2 (0-21)	**0.002**[ŧ]
Number of metastatic lymph nodes[a]	0 (0-18)	0 (0-13)	0 (0-18)	**0.008**[ŧ]

[a]Median (minimum-maximum). [ŧ]Mann-Whitney U test, ††chi-square test.
TCI: Thyroid capsule invasion

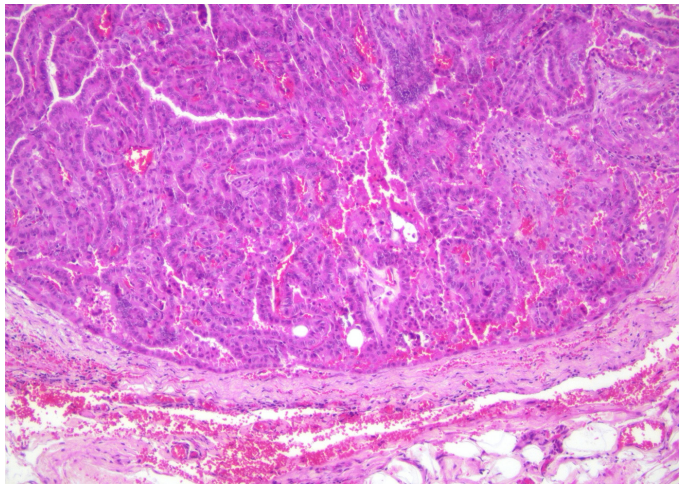

Figure 1. There is no capsular infiltration, although there is subcapsular localization of the tumor (hematoxylin and eosin staining, x100 magnification)

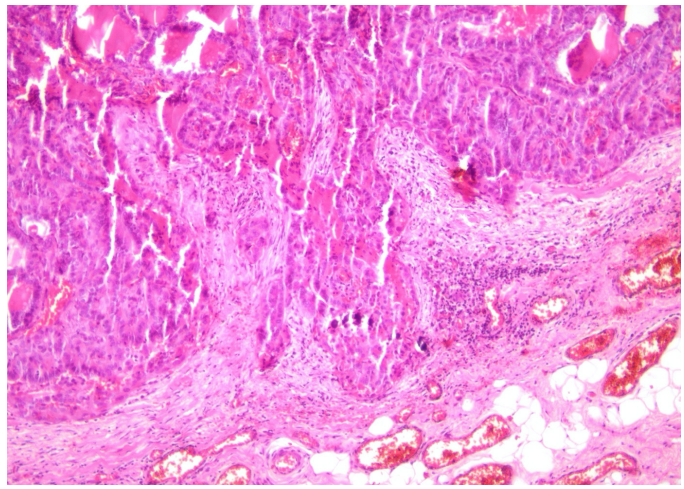

Figure 2. Tumor infiltration outside the capsule is seen (hematoxylin and eosin staining, x100 magnification)

with TCI had malignancy features in FNAB. Our findings suggest that the more malignant features in the pre-operative biopsy, the greater the probability of capsule invasion postoperatively. Although it is difficult to predict the behavior of papillary thyroid carcinomas using diagnostic methods in the pre-operative period (13), confirmation by FNAB may suggest postoperative local-advanced histopathological findings (13,14).

Previous authors suggested that irregular borders and hypoechogenicity were the fundamental USG findings in PTC, and postoperative capsular invasion, extrathyroidal extension, and preoperative USG findings were correlated (15). However, we observed no difference in USG findings between patients with and without TCI in the current study, which may have been caused by the fact that a relatively higher ratio of advanced disease was observed.

Li et al. (16) reported that large tumor size, multifocality, and vascular invasion were significant risk factors for central lymph node metastasis and surgical time in PTC. In addition, a significant relationship was observed for capsular invasion in patients who underwent CLND in addition to BTT because of central lymph node metastasis. We also observed a significant correlation between TCI and total and malignant lymph node counts in the histopathological evaluation.

The tumor grade, tumor pattern, and multicentricity did not differ according to capsule invasion in PTC in our study. However, previous authors have shown associations of multifocal PTCs with lymph node metastasis, recurrence, and low survival (17). The association of multifocality with advanced pathological classification, capsule invasion, and extrathyroidal extension has been reported (17,18). Our findings which contradict these data in the literature warrant future observations.

Isthmus tumors, but not other regions, were found to be more likely to present with capsular invasion in PTC (19). We could not confirm this association. On the other hand, as a novel finding, we observed a significantly higher proportion of middle-localization nodules in PTC with capsular invasion. The upper pole, lower pole, and isthmus tumor localizations were not different in patients with and without capsular invasion. Nodules in the middle-lower pole were also associated with malignancy and nodule aggressiveness (20). However, to the best of our knowledge, this is the first study to show a relationship between the middle lobe localization of a nodule and capsular invasion in PTC. Nevertheless, these findings await confirmation in larger studies that perform adjusted comparisons.

Capsule invasion is a predictor of worse prognosis, even in low-stage PTCs (20,21). However, we observed no significant differences in surgery-related complications in patients with PTC with and without capsular invasion. This is also a new finding. On the other hand, higher postoperative Tg levels and RAI therapy were more frequent in TCI (+) patients, which agrees with previous studies that showed Tg levels are effective predictors of prognosis (22) and may correlate with histopathological parameters (22,23). In addition, a previous study showed that early complications after BTT and subtotal thyroidectomy in differentiated thyroid cancers are similar in the BTT and subtotal thyroidectomy groups (24).

Thyroid nodules with Bethesda categories III and IV have an overall malignancy risk of 15-40% (25,26). If Bethesda III or IV lesions are found malignant, the most common histopathological subtype is the follicular variant of PTC, which is generally less aggressive than the classical PTC (26). Furthermore, this subtype has a lower risk of lymph node metastases, recurrence, and local extension, especially when encapsulated (26). The findings of the current study are consistent with the literature showing that Bethesda III and IV tumors were more aggressive, as shown by the lower margin and Tg level.

Study Limitations

This study has some limitations. The design was retrospective and single-center. The sample size was relatively small, and the follow-up duration was short. Several variables, such as the risk factors for thyroid cancer, could not be obtained from the patient files. We were also not able to explore the potential variables that may be related to capsule involvement, such as radiation history, dietary iodine intake, a history of benign thyroid disease, and BRAF V600E mutation.

Conclusion

In patients with PTC, capsular invasion was significantly more common in tumors in the middle of the thyroid gland. Furthermore, metastatic lymph nodes and increased postoperative Tg levels, which determine aggressiveness and worse outcomes, were more frequent in patients with capsule invasion.

Ethics

Ethics Committee Approval: This study was approved by the University of Health Sciences Türkiye, Gülhane Training and Research Hospital, Clinical Research Ethics Committee (decision no: 2022/145, date: 24.11.2022).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: M.Ö., O.H., Concept: B.U., Design: B.U., M.Z.B., İ.A.Ö., E.Ç., O.H., Data Collection or Processing: B.U., M.S.Ç., Analysis or Interpretation: S.A.K., M.Ö., Literature Search: B.U., M.Z.B., Writing: B.U., M.Z.B.

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Mean platelet volume and platelet distribution width in the prediction of treatment response in immune thrombotic thrombocytopenic purpura with severe ADAMTS13 deficiency: a multicenter study

Selim Sayın[1], Murat Yıldırım[1], Ahmet Kürşad Güneş[2], Merih Reis Aras[3], Esra Şafak Yılmaz[4], Murat Albayrak[3], Gülsüm Özet[2], Meltem Aylı[1]

[1]University of Health Sciences Türkiye, Gülhane Training and Research Hospital, Clinic of Hematology, Ankara, Türkiye
[2]Ankara Bilkent City Hospital, Clinic of Hematology, Ankara, Türkiye
[3]University of Health Sciences Türkiye, Dışkapı Yıldırım Beyazıt Training and Research Hospital, Clinic of Hematology, Ankara, Türkiye
[4]University of Health Sciences Türkiye, Gülhane Training and Research Hospital, Clinic of Medical Informatics, Ankara, Türkiye

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Corresponding Author:
Selim Sayın, M.D., University of Health Sciences Türkiye, Gülhane Training and Research Hospital, Clinic of Hematology, Ankara, Türkiye
+90 505 538 27 43
sayinselim@hotmail.com

ORCID:
orcid.org/0000-0002-7197-6890

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ABSTRACT

Aims: Lactate dehydrogenase (LDH) and platelet count are routinely used to evaluate response to treatment and discontinuation of treatment in thrombotic thrombocytopenic purpura (TTP). This study aimed to evaluate the mean platelet volume (MPV) and platelet distribution width (PDW) in immune TTP (iTTP) as markers of treatment response.

Methods: This retrospective, multicenter study included patients diagnosed with iTTP with severe ADAMTS13 deficiency. We studied the correlations of MPV and PDW values with platelet count, LDH, total bilirubin hematocrit and mean corpuscular volume, which are used to evaluate the response to total plasma exchange (TPE) or relapse in iTTP. The study variables were recorded at the time of diagnosis, 1st week of TPE treatment, and the time of the last TPE. The correlation analyses were performed between the values before the initial TPE, and after the first week and last TPE.

Results: The study included 28 patients, 20 females with iTTP [median age (minimum-maximum): 45 (23-74) years]. MPV correlated positively with LDH (r=0.533, p=0.002) and negatively with hematocrit (r=-0.445, p=0.002) and platelet count (r=-0.560, p=0.002). PDW also correlated positively with LDH (r=0.339, p=0.008) and negatively with hematocrit (r=-0.244, p=0.032) and platelet count (r=-0.285, p=0.022).

Conclusions: The results showed that MPV and PDW correlated with LDH and platelet count, which are currently used to evaluate the response to treatment in iTTP. Changes in MPV and PDW may serve as a surrogate of treatment response in these patients as an indicator of response to plasmapheresis.

Introduction

Platelets are the main component of blood that plays a role in primary hemostasis; thus, the biological functions of platelets are far beyond hemostasis and thrombosis. They are associated with inflammation, atherosclerosis, autoimmunity, and tumor immunology (1-4). Platelet indices such as mean platelet volume (MPV), plateletcrite (PCT), platelet-large cell ratio (P-LCR), platelet distribution width (PDW), and immature platelet fraction (IPF) are widely used in the investigation of the etiology of thrombocytopenia because of the low cost of the procedure, non-invasiveness, and faster results (4,5).

Genetic and acquired factors such as race, age, smoking status, alcohol consumption, and physical activity can alter platelet count and MPV (5). Platelet size correlates with cell activity and can be assessed using volume indices. Immature/young platelets are released from the bone marrow into the circulation, with a larger size and greater RNA content than mature platelets (6). The release of immature platelets increases when platelet consumption increases, which manifests as a higher MPV and IPF ratio in the whole blood count (7,8). Some platelet indices have been sought as potential indicators of platelet recovery with clinical improvement in patients who undergo bone marrow transplantation, and it was observed that MPV and PCT values decreased after thrombocyte engraftment was achieved (9-11).

In many studies, MPV value was found to decrease in thrombocytopenia that develops due to low production, and on the contrary, it increases in thrombocytopenia that develops due to increased consumption of platelets (6,8). Thrombocytopenia with high MPV is observed in patients with immune thrombocytopenic purpura (iTTP), disseminated intravascular coagulation, sepsis, preeclampsia, thrombocytopenia with low MPV, aplastic anemia, B12 deficiency, and myelodysplastic syndrome (6,12,13).

PDW is a parameter that defines the differences in platelet volume due to increased production and/or platelet activation. It has been reported that the normal reference range for PDW varies between 10-18% in healthy individuals and between 8.3% and 56% in individuals with various diseases (5,14-20). MPV and PDW show a proportional relationship with each other. The distribution of the PDW value in sick people is much above the standard deviation compared with normal individuals. Therefore, it was investigated as a useful parameter in the follow-up of diabetes, coronary artery disease, sickle cell anemia crises, acute cholecystitis, appendicitis, Crohn's disease, and non-malignant tumors (14-20).

Thrombotic thrombocytopenic purpura (TTP) is a thrombotic microangiopathy (TMA) caused by the accumulation of ultra-large von Willebrand factor with platelets due to significantly reduced ADAMTS activity. Many patients do not have a distinct TTP clinic until the signs and symptoms of specific organ damage caused by microthrombotic ischemia occur; therefore, many clinicians have difficulty diagnosing TMA/TTP at first admission (21). The first laboratory findings of the disease were thrombocytopenia, Coombs-negative hemolytic anemia, and platelet-rich thrombi in small vessels. During the treatment of patients presenting with microangiopathy, it is desirable to find laboratory parameters that can assist in management and follow-up (21,22).

The fact that platelet indices are cheap and very easy to measure has enabled them useful in research in various fields, such as autoimmune diseases, inflammation, malignancies, and cardiovascular diseases. In microangiopathic hemolytic anemia, including TTP, thrombocytopenia develops through consumption. Changes caused by TTP in platelet parameters in the acute and remission periods and their relationship with the need for plasmapheresis have not been investigated before. The primary purpose of this study was to evaluate the significance of decreases in MPV and PDW to follow the treatment response in iTTP.

Methods

Study type and patients

This was a retrospective, multicenter study that included patients diagnosed with iTTP by three different hematology units and underwent total plasma exchange (TPE) between January 2011 and June 2020. Patients with congenital TTP, secondary TTP, pregnancy, missing data or who died within 1 week after the initiation of treatment were excluded.

This study was approved by the Etlik Zübeyde Hanım Training and Research Hospital Ethics Committee (decision no: 2021/9, date: 13.01.2021).

Data collection

Hospital records and patient files were screened in all centers. MPV and PDW measurements and other laboratory results were recorded before the first TPE and after the 1st week and last TPE separately. PDW and MPV were measured using a fully automated analyzer (Sysmex automated hematology analyzer) at all centers. Platelet counts, hemoglobin, hematocrit, mean corpuscular volume (MCV), lactate dehydrogenase (LDH), and total/indirect bilirubin were also recorded.

ADAMTS13 activity and ADAMTS13 antibody measurements were performed before the first TPE in all centers. ADAMT13 activities were <10% and ADAMT13 antibodies were positive (≥15 U/mL) in all patients.

TPE protocol

All patients received TPE with a preliminary clinical diagnosis of TTP without waiting for the ADAMTS13 activity test result. TPE procedures were performed using Fresenius COM. TEC and/or Spectra Optia apheresis systems at all centers. Following the

same protocol, daily TPE was continued with 1-2 volumes until the platelet count exceeded 150,000/mm^3 for 2 consecutive days and the LDH level returned to the normal range. Fresh frozen plasma specific to the ABO type was used as the replacement fluid in all procedures. The frequency of TPE was reduced and completed according to the response.

Statistical Analysis

Statistical analyses were performed using R 3.5.0 (R Core Team, 2018) software. Because TTP is very rare, a sufficient sample size could not be reached. Previous literature has reported that parametric tests lose their strength, and permutation tests produce more reliable results in small sample sizes and under assumptions that cannot be provided (23,24). Therefore, we used permutation test equivalents instead of the t-test for dual or multiple comparisons of continuous and categorical variables. Correlation coefficients between MPV and PDW values and platelet count, LDH, total bilirubin, hemoglobin/hematocrit, and MCV were also calculated using permutation tests. The type 1 error rate was accepted as alpha 0.05.

Results

Clinical and laboratory features

We identified 30 patients (20 females and 10 males; median age 45 years; range, 23 to 74 years). Two patients were excluded because of early death. Therefore, 28 patients had available data at the time of diagnosis, after the 7th TPE, and after the last TPE (Table 1). ADAMTS13 activity was below 5% [mean 0.2% (0-5)] and ADAMTS13 antibodies were positive [mean 56 U/mL (26.9-90)] in the whole sample. Neurological symptoms, fever, renal involvement, and cardiac involvement were observed in 16 (57.1%), 14 (50.0%), 6 (21.4%), and 3 (10.7%) patients, respectively.

The mean response time of the patients to TPE was 9 (4-23) days, and 21.7% (n=5) of the patients responded in the first week. The mean number of TPEs was 21.5 and the mean length of hospital stay was 28.5 days. All patients were administered standard oral prednisone (1 mg/kg/day) because of ADAMTS13 antibody positivity. Intravenous methylprednisolone 500/1000 mg/day for three days was administered to 2 patients with serious neurological involvement. Thirteen (46.4%) patients received weekly rituximab (375 mg/m^2) for 4 consecutive weeks. Of the patients who received rituximab, 5 relapsed and 8 showed exacerbation under TPE treatment. Other therapeutic approaches were recorded much less and only 2 patients received vincristine treatment before rituximab, but rituximab was also added to these patients' treatment because of insufficient response. The median follow-up period was 48.5 months.

Correlations between MPV and PDW values and laboratory variables

MPV showed a positive correlation with LDH, total bilirubin, and indirect bilirubin reduction during the treatment. In addition, MPV reduction negatively correlated with increases in hemoglobin/hematocrit and platelet counts. There was a positive correlation between MPV and PDW, but it did not reach statistical significance. There was no correlation between MPV and MCV.

There was a positive correlation between the decrease in PDW and LDH and a negative correlation between hemoglobin/hematocrit and platelet count (Table 2). The changes in MPV and PDW values by week are shown in Figures 1 and 2.

Because the mean MPV of the patients at the time of diagnosis was within normal limits, no cut-off value could be established for the diagnosis of the disease. We observed that the decrease in MPV during treatment in patients who benefited from the treatment according to the baseline value was significant and correlated with other response parameters. Similarly, the mean PDW value was above the normal limits even when the treatment was completed, and we determined that the decrease in the PDW value was a correlation of response to the treatment.

Table 1. The laboratory results of patients; at the time of diagnosis, after the 7th and last TPE

Variable*	At the time of diagnosis	After 7th TPE	After last TPE
Hb (gr/dL)	8.55 (5.3-12.8)	9.8 (7.6-12.6)	11.5 (8.6-15.7)
Hct (%)	24.75 (15.5-35.1)	29.1 (22.9-36.8)	33.9 (25.1-46.3)
MCV (fL)	88 (76.6-129.3)	92.3 (82-103.2)	92.2 (83.9-104)
LDH (U/L)	1103 (311-3200)	354 (177-945)	197.5 (150-374)
Plt (/mm^3)	11000 (3000-74000)	107500 (10000-463000)	304500 (195000-604000)
Total bilirubin (mg/dL)	2.7 (1.2-5.2)	1.3 (0.2-7.9)	0.7 (0.2-1.4)
Indirect bilirubin (mg/dL)	2.2 (0.4-4.6)	0.9 (0.1-2.9)	0.3 (0.1-0.8)
MPV (fL)	10.4 (7.3-14.6)	8.8 (6.1-12.8)	7.9 (5.9-11.4)
PDW (%)	21.9 (15.2-44)	17.5 (12.6-40)	16.1 (10-36)

*Data are presented as median (minimum-maximum).
TPE: Total plasma exchange, Hb: Hemoglobin, Hct: Hematocrit, MCV: Mean corpuscular volume, LDH: Lactate dehydrogenase, Plt: Platelet, MPV: Mean platelet volume, PDW: Platelet distribution width

Table 2. Correlation matrix of MPV and PDW

	MPV	PDW	Hct	MCV	PLT	LDH	Total bilirubin
MPV	1.000						
PDW	0.082 p=0.494	1.000					
Hct	-0.455 **p=0.002**	-0.244 **p=0.032**	1.000				
MCV	0.066 p=0.538	-0.148 p=0.204	0.019 p=0.898	1.000			
Plt	-0.560 **p=0.002**	-0.285 **p=0.022**	0.633 **p=0.002**	0.176 p=0.096	1.000		
LDH	0.533 **p=0.002**	0.339 **p=0.008**	-0.563 **p=0.002**	0.036 p=0.65	-0.587 **p=0.002**	1.000	
Total bilirubin	0.462 **p=0.002**	0.242 p=0.064	-0.514 **p=0.002**	-0.283 **p=0.008**	-0.626 **p=0.002**	0.622 **p=0.002**	1.000

MPV: Mean platelet volume, PDW: Platelet distribution width, Hct: Hematocrit, MCV: Mean corpuscular volume, Plt: Platelet, LDH: Lactate dehydrogenase

Discussion

Studies have claimed that platelet indices such as MPV and PDW can be used for discriminating hyperdestructive thrombocytopenia from hypoproductive thrombocytopenia, and they are sensitive and specific for their diagnostic predictive value. In these studies, MPV and PDW values were higher in hyperdestructive thrombocytopenias such as TTP than in hypodestructive thrombocytopenia (7,25,26). However, there is no study on MPV and/or PDW alteration due to TTP treatment. Platelet counts have been routinely used as laboratory variables with LDH to monitor clinical responses to TTP therapy; however, MPV and PDW may be more specific objective measurements to define the clinical outcomes in TTP. To the best of our knowledge, this is the first study that investigated the change in MPV and PDW in the follow-up of TPE response in patients with severe ADAMTS13 deficiency. We found that MPV and PDW were high in iTTP patients at the beginning of treatment and decreased in correlation with the response to TPE. We also found that the decrease in MPV and PDW negatively correlated with platelet count and positively correlated with LDH. In some of our cases, we observed an increase in MPV and/or PDW one or two days before the decrease in platelet count in relapses or exacerbations. However, because there were a few patients, we were unable to reach a firm conclusion in this regard.

The two main causes of thrombocytopenia in TTP are increased destruction or peripheral consumption. A decrease in platelet production is not an expected situation in TTP, except for several bone marrow diseases that can lead to secondary TTP, such as bone marrow transplantation or tumor infiltration. Immature platelets which are newly synthesized in the bone marrow are larger than the reticulocytes. They contain higher amounts of cytoplasmic RNA, and as they age, their RNA content

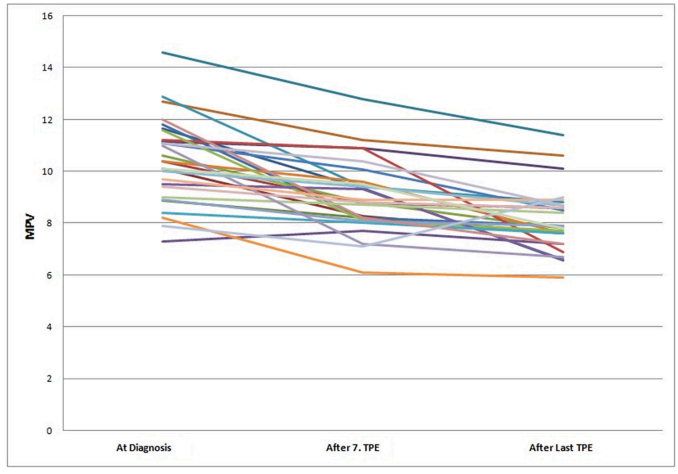

Figure 1. The change of MPV values by weeks
MPV: Mean platelet volume, TPE: Total plasma exchange

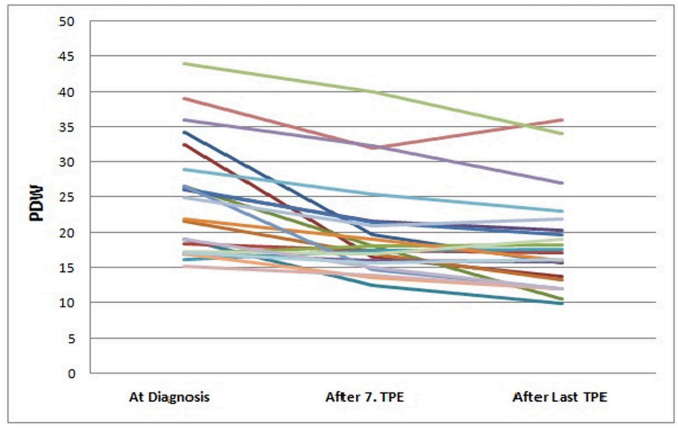

Figure 2. The change of PDW values by weeks
PDW: Platelet distribution width, TPE: Total plasma exchange

and size decrease. From this point, theoretically, in the recovery period, bone marrow platelet production reduces due to the decrease in disease activity in patients with TTP, accompanied by lower MPV and PDW. As it is known in TTP patients, a platelet count of 150,000/mm^3 is a criterion of response and termination of TPE treatment. This study was designed to determine whether MPV and PDW values that indicate an indirect reduction of platelet destruction could be a predictor of treatment response or an indication of early relapse and exacerbation.

Although some studies suggested cut-off values for MPV and PDW in hyperdestructive thrombocytopenias and others, their sensitivity and specificity are low (7,25,27). Previous studies also showed a statistically significant difference in MPV, PDW, and P-LCR according to race/skin color. This situation supports the idea that following up the level of MPV and PDW in the treatment process will be more valuable than determining a limit value for these in TTP patients, as in our study (26,28).

The other platelet parameters that can be measured with a fully automated hematology analyzer are P-LCR and absolute immature platelet count (A-IPC). MPV, PCT, and PDW are more frequently available parameters, and A-IPC and IPF measurements cannot be routinely performed with the automatic hematology analyzer used by all three centers. In previous studies, these platelet parameters were usually evaluated for the differentiation of immune thrombocytopenia from healthy participants or diseases in which the bone marrow production of platelets decreased (7). However, no study has examined the change in MPV and PDW in the treatment process of TTP patients and its relationship with other parameters such as platelet count and LDH level used in response to treatment. In a case report, Kier et al. (29) suggested that IPF can be used to follow the efficacy and response to treatment modalities in TMA hemolytic anemia. Additionally, Zheng et al. (30) reported that A-IPC could be useful in the prediction of clinical response in idiopathic TTP patients and serves as a potential indicator for TPE cessation.

The only study that examined A-IPC in the diagnosis and monitoring of the clinical course of TTP was performed by Hong et al. (31) who found that all iTTP patients had significantly increased IPF and decreased A-IPC and platelet count at presentation. During follow-up, their patients with TTP who responded to TPE showed a decrease in IPF in correlation with an increase in the number of platelets. Their study included 12 patients with iTTP, routine measurement of ADAMTS13 antigen during treatment, and assessment of correlations between IPF and ADAMTS13 antigen levels. They found that A-IPC positively correlated with ADAMTS13 activity at presentation but negatively correlated with ADAMTS13 activity during recovery. Another study (32) found that PDW, MPV, and P-LCR in children with iTTP were significantly higher, and, as in our study, they found that after the treatment, PLT and PCT gradually increased while PDW, MPV, and P-LCR gradually decreased.

Study Limitations

This study has several limitations. First, MPV and/or PDW could not be studied in some periods because of the change in automated hematology analyzers in the three participating centers, leading to a decrease in the number of patients included. Second, the daily change in MPV and PDW in patients with relapse or exacerbation and their relationships with platelet count could not be demonstrated due to insufficient data.

Conclusion

In conclusion**,** MPV and PDW are easily accessible, non-invasive tests of thrombopoiesis that can be used to monitor disease improvement and treatment responses of iTTP. The decrease in MPV and PDW values during treatment is an indicator of the response to plasmapheresis. They also correlate with the number of platelets and LDH levels, which are used in treatment response. Prospective clinical trials on the correlation of platelet parameters such as PCT, PDW, MPV, P-LCR, and A-IPC with disease progression and remission in patients with TTP are warranted.

Ethics

Ethics Committee Approval: This study was approved by the Etlik Zübeyde Hanım Training and Research Hospital Ethics Committee (decision no: 2021/9, date: 13.01.2021).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: S.S., M.Y., A.K.G., M.R.A., M.A., G.Ö., M.A., Concept: S.S., M.Y., Design: S.S., M.Y., M.A., G.Ö., M.A., Data Collection or Processing: M.Y., A.K.G., M.R.A., Analysis or Interpretation: E.Ş.Y., Literature Search: S.S., M.Y., Writing: S.S., M.Y.

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Relationship between ultrasonographically assessed biceps brachii muscle mass and complete blood cell and blood chemistry

Nezahat Müge Çatıkkaş

University of Health Sciences Türkiye, Hamidiye Faculty of Medicine; Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital, Clinic of Internal Medicine, Division of Geriatrics, İstanbul, Türkiye

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Corresponding Author:
Nezahat Müge Çatıkkaş, M.D., University of Health Sciences Türkiye, Hamidiye Faculty of Medicine; Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital, Clinic of Internal Medicine, Division of Geriatrics, İstanbul, Türkiye
+90 536 711 12 48
nz444mg@hotmail.com

ORCID:
orcid.org/0000-0003-2494-1625

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ABSTRACT

Aims: Relationships between muscle mass measured using different methods at different sites and several biochemical variables have been reported. The biceps brachii muscle mass can easily be assessed by ultrasonography (USG). This study investigated the relationship between ultrasonographically assessed biceps brachii muscle mass and complete blood cell count and blood chemistry variables in older palliative care patients.

Methods: A cross-sectional observational study was conducted between June 2023 and August 2023 in an institutional palliative care setting. Demographic characteristics, comorbidities, Nutritional Risk Screening-2002 score, feeding route, and pressure ulcers were recorded. Biceps brachii muscle thickness (BBMT) and cross-sectional area (BBCSA) were assessed using USG. The relationship between muscle mass and target laboratory variables was evaluated by correlation and multiple regression analyses.

Results: The study included 214 patients (age, mean±standard deviation: 78.4±9.1 years, female: 55.1%). BBMT was positively correlated with serum albumin (r=0.160, p=0.019) and creatinine (r=0.182, p=0.008) levels. BBCSA was positively correlated with serum albumin level (r=0.216, p=0.001). Controlling for age, sex, malnutrition risk, and C-reactive protein, only serum albumin level was independently associated with BBMT (ß=0.238, p=0.002) and BBCSA (ß=0.258, p<0.001).

Conclusions: This study showed for the first time that serum albumin levels were independently associated with BBMT and BBCSA in older palliative care patients. Multi-center, longitudinal studies on multiple body regions are warranted to generalize these findings.

Introduction

Sarcopenia, characterized by the progressive loss of muscle strength, muscle mass, and/or physical performance (1), increases with aging and leads to increased risks of falls, fractures, disability, prolonged length of hospital stay, rehospitalization, and death, especially in patients with chronic diseases (2,3). Several mechanisms have been identified for sarcopenia, including nutritional deficiencies, insulin resistance, oxidative stress, atherosclerosis, smoking, changes in age-related sex hormones, neuromuscular dysfunction, endocrine abnormalities, chronic inflammation, and inadequate physical activity (4,5).

Because muscle mass is critical in assessing sarcopenia, measurement techniques have attracted great interest. Various imaging modalities, such as computed tomography (CT), magnetic resonance imaging (MRI), dual-energy X-ray absorptiometry (DXA), and bioelectrical impedance analysis (BIA), can predict muscle mass (1). However, these methods

have some limitations in daily practice. Although CT and MRI are the gold standards, they are time-consuming, costly, and require special equipment. CT can also emit radiation. DXA can also cause radiation, cannot be performed at the bedside, and may not always be available in primary care hospitals (3). BIA measurements may be affected by alterations in body water (6). However, the utility of ultrasonography (USG) is promising because it is feasible, inexpensive, portable, and radiation-free, yielding results comparable to CT, MRI, and DXA (7). It can be used at the bedside, especially for bedridden patients in palliative and intensive care units (2). USG is also a valid and reliable tool for assessing muscle mass in older people (7). While most studies conducted on USG have focused on the lower limbs, primarily the rectus femoris and gastrocnemius muscles (8), few studies have investigated the upper limbs (2).

Several markers have been identified in recent years that may be useful for tracking muscle decline. The most widespread indicators are those related to the inflammatory response [e.g., C-reactive protein (CRP), tumor necrosis factor-α, and interleukin-6], biochemical parameters [e.g., albumin, white blood cell (WBC), neutrophil-to-lymphocyte ratio (NLR), platelet (PLT), platelet-to-lymphocyte ratio (PLR), hemoglobin, blood urea nitrogen, creatinine, alanine aminotransferase (ALT), and aspartate aminotransferase (AST)], hormones (e.g., vitamin D and testosterone), oxidative stress markers (e.g., advanced glycation end products and low-density lipoproteins), or antioxidants (e.g., α tocopherol and carotenoids) (4,9-11). Although muscle mass assessed by different methods correlates with various biochemical variables, potential associations between ultrasonographically assessed biceps brachii muscle mass and laboratory variables in the palliative care setting are unknown. Therefore, this study investigated the relationship of ultrasonographically assessed biceps brachii muscle mass with complete blood cell count and blood chemistry variables in older patients receiving palliative care.

Methods

Design, setting, and study population

A cross-sectional, observational study was conducted in the palliative care unit of University of Health Sciences Türkiye, Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital, İstanbul, Türkiye, from June 2023 to August 2023. The inclusion criteria were age 60 or older and residing in the palliative unit for at least 24 hours. The exclusion criteria were hemiplegia/quadriplegia, upper limb amputation, contractures, fractures, edema (2), missing relevant medical information (3), and refusal to participate. The study protocol was approved by the University of Health Sciences Türkiye, Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital Local Ethics Committee (code: 2022/152, date: 14.12.2022). Patients and/or their carers provided signed informed consent, and the procedures were in accordance with the Declaration of Helsinki.

Study variables

Demographic characteristics and comorbidities were collected from patient records. The Charlson Comorbidity Index (CCI) score, a predictor of mortality, with higher scores indicating a higher risk for mortality and more severe comorbid conditions, was calculated as previously defined (12-14). Nutritional status was assessed by Nutritional Risk Screening-2002 (NRS-2002), a validated tool in Turkish (15,16). A score of ≥3 indicates malnutrition risk (MNR). The feeding route was recorded as oral, enteral (nasogastric tube/percutaneous endoscopic gastrostomy tube), or parenteral. Pressure ulcers were classified using the system proposed by the European Pressure Ulcer Advisory Panel/Pan Pacific Pressure Injury Alliance/National Pressure Ulcer Advisory Panel (17).

After 8 h of fasting, blood samples were collected at 08:00-A.M. Complete blood cell count variables included WBC, neutrophil, lymphocyte, hemoglobin, hematocrit, mean corpuscular volume, and PLT levels. NLR was calculated by dividing the neutrophil count by the lymphocyte count. PLR was calculated by dividing the PLT count by the lymphocyte count. Blood chemistry variables included glucose, HbA1c, sodium, potassium, magnesium, calcium, phosphorus, albumin, urea, creatinine, uric acid, alkaline phosphatase, gamma-glutamyl transferase, AST, ALT, lactate dehydrogenase, CRP, procalcitonin, erythrocyte sedimentation rate, thyroid-stimulating hormone, vitamin B12, folate, and 25 (OH) vitamin D levels. The albumin/creatinine ratio was calculated by dividing serum albumin level by creatinine level. The estimated glomerular filtration rate was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation. All measurements were performed using Mindray BC6800, Alifax Test 1, and Roche Cobas 6000 autoanalyzers.

Muscle mass measurement (muscle thickness and cross-sectional area)

The biceps brachii muscle mass was assessed using USG. The same clinician (NMC) who was blinded to the patient's medical condition performed the measurements using a linear probe of 7.5 MHz with 5 cm width in B mode (Philips Affiniti 50). Biceps brachii MT (BBMT) and CSA (BBCSA) were measured with the patients lying down and the limbs outstretched and relaxed. After a 5-minute rest, three consecutive measurements were obtained, and the mean value was recorded. The biceps brachii muscle was determined at the center point of the distance between the olecranon and acromion. The probe was orientated vertically to the horizontal axis of the humerus at a minimum pressure. The layer between the superficial and deep fascias of the biceps brachii muscle was referred to as muscle thickness in millimeters (mm), whereas the area between the superficial and deep fascias of the biceps brachii muscle was expressed as the cross-sectional area in mm^2 (Figure 1).

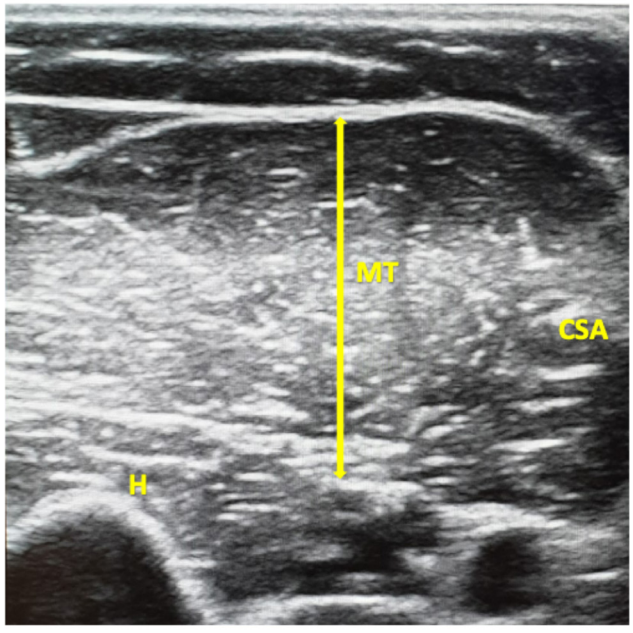

Figure 1. Transverse ultrasonographic image of biceps brachii muscle
CSA: Cross-sectional area, H: Humerus, MT: Muscle thickness

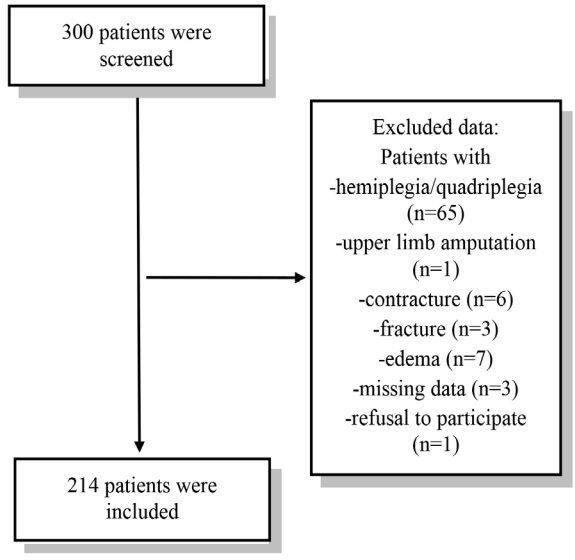

Figure 2. Flow chart of patient selection

To check intraobserver reliability, intraclass correlation coefficients (ICCs) were calculated by taking two images obtained 15 min apart from 15 healthy volunteers. The ICCs for BBMT and BBCSA were 0.95 and 0.99, respectively.

Statistical Analysis

The Statistical Package for Social Sciences version 21 for Windows (IBM Corp., Armonk, NY) was used to perform statistical analyses. The Kolmogorov-Smirnov test was used to test the data distribution. Continuous variables are presented as mean±standard deviation (SD) for those with a normal distribution or median (minimum-maximum) for those without a normal distribution. Categorical variables are presented as numbers and percentages [n (%)]. Correlations between biceps brachii muscle mass and complete blood cell count and blood chemistry variables were tested using Pearson's or Spearman's rho correlation coefficients. Unadjusted (Model 1) and multiple regression analyses were performed to explore the factors independently associated with BBMT and BBCSA. Model 2 was adjusted for age and gender. Model 3 included the variables in Model 2 and MNR. Model 4 included CRP in addition to the variables in Model 3. A p-value of <0.05 was considered statistically significant.

Results

Patient selection

There were 300 eligible patients during the study period. Following excluding patients with hemiplegia/quadriplegia (n=65), upper limb amputation (n=1), contracture (n=6), fracture (n=3), edema (n=7), missing data (n=3), and unwillingness to participate (n=1) the study included 214 patients in the final analysis (Figure 2).

Demographic and clinical variables

The mean age of the patients included in the study was 78.4±9.1 years and 55.1% were female. The majority of patients had hypertension (60.3%), cerebrovascular accident (36.9%), diabetes mellitus (32.2%), and dementia (31.3%). The median number of diseases was three (minimum-maximum: 0-8) and the median CCI score was four (minimum-maximum: 0-15). The mean±SD NRS-2002 score was 5±1.6, 93% had MNR, and 51.5% were on enteral nutrition. Of the included patients, 55.1% had pressure ulcers. Table 1 shows the characteristics of the study population.

Correlation analyses

BBMT positively correlated with serum albumin (r=0.160, p=0.019) and creatinine serum (r=0.182, p=0.008) levels, whereas BBCSA positively correlated only with serum albumin level (r=0.216, p=0.001) (Table 2).

Multiple regression analyses

Multiple regression analysis showed that after adjusting for age, sex, MNR, and CRP, albumin level was independently associated with both BBMT (ß=0.238, p=0.002) and BBCSA (ß=0.258, p<0.001) (Tables 3, 4).

Table 1. Characteristics of the study population (n=214)	
Age, years, mean±SD	78.4±9.1
Sex, female, n (%)	118 (55.1)
Comorbidities, n (%)	
Hypertension	129 (60.3)
Cerebrovascular accident	79 (36.9)
Diabetes mellitus	69 (32.2)
Dementia	67 (31.3)
Malignancy	59 (27.6)
Coronary artery disease	39 (18.2)
Chronic heart failure	35 (16.4)
Chronic obstructive pulmonary disease	33 (15.4)
Number of diseases, median (min.-max.)	3 (0-8)
Charlson Comorbidity Index score, median (min.-max.)	4 (0-15)
Nutritional Risk Screening-2002 score, mean±SD	5±1.6
Malnutrition risk, n (%)	199 (93)
Feeding routes, n (%)	
Oral	
Enteral	90 (42)
Nasogastric tube	110 (51.5)
Percutaneous endoscopic gastrostomy	74 (34.7)
Parenteral	36 (16.8)
Pressure injuries, n (%)	14 (6.5)
Complete blood cell and blood chemistry	118 (55.1)
White blood cell, µL, median (min.-max.)	8.370 (890-31.430)
Neutrophil, µL, median (min.-max.)	6.075 (780-23.500)
Lymphocyte, µL, median (min.-max.)	1.320 (60-6.350)
Neutrophil-to-lymphocyte ratio§	4.190 (1-33.470)
Hemoglobin, g/dL, mean±SD	9.7±1.6
Hematocrit, %, mean±SD	30.4±5.1
Mean corpuscular volume, fL, mean±SD	88.4±7
Platelet, µL, median (min.-max.)	238.500 (14.000-650.000)
Platelet-to-lymphocyte ratio§	153.792 (80-674.698)
C-reactive protein, mg/L, median (min.-max.)	43.5 (0.7-269.7)
Procalcitonin, median (min.-max.)	0.1 (0.04-100)
Erythrocyte sedimentation rate, mm/h, median (min.-max.)	52 (2-120)
Glucose, g/dL, median (min.-max.)	117.3 (68.4-385.8)
Sodium, mmol/L, mean±SD	136.9±5.8
Potassium, mmol/L, mean±SD	4±0.6
Magnesium, mg/dL, mean±SD	1.8±0.3
Calcium, mg/dL, mean±SD	8.5±1.1
Phosphorus, mg/dL, mean±SD	3.1±0.9
Albumin, g/L, mean±SD	28±5.8
Urea, mg/dL, median (min.-max.)	52 (13-299)
Creatinine, mg/dL, median (min.-max.)	0.8 (0.2-8.7)
Albumin/creatinine ratio, g/mmol, median (min.-max.)	36 (2.4-128.7)
Uric acid, mg/dL, median (min.-max.)	4.7 (0.8-18.5)
Estimated glomerular filtration rate, mL/min/1.73 m^2, median (min.-max.)	85.6 (5.2-171.9)
Alkaline phosphatase, U/L, median (min.-max.)	89.5 (12-1.603)
Gamma-glutamyl transferase, U/L, median (min.-max.)	32 (3-1.041)
Aspartate aminotransferase, U/L, median (min.-max.)	19.6 (5-393)
Alanine aminotransferase, U/L, median (min.-max.)	14 (3-147)
Lactate dehydrogenase, U/L, median (min.-max.)	257 (38-1.561)
Thyroid-stimulating hormone, mIU/L, median (min.-max.)	1.5 (0.005-37.6)
Vitamin B12, pmol/L, median (min.-max.)	545 (128-2.000)
Folate, µg/L, median (min.-max.)	6.1 (0.8-20)
25 (OH) vitamin D, ng/mL, median (min.-max.)	12.8 (3-144)
Muscle mass measures	
Biceps brachii muscle thickness, mm, mean±SD	14±3.4
Biceps brachii cross-sectional area, mm^2, mean±SD	42±10.8

SD: Standard deviation, min.-max.: Minimum-maximum

Table 2. Correlations coefficients between biceps brachii muscle mass and complete blood cell count and blood chemistry variables

Variable	BBMT	BBCSA
White blood cell	r=-0.089, p=0.195	r=-0.068, p=0.321
Neutrophil	r=-0.086, p=0.210	r=-0.065, p=0.348
Lymphocyte	r=-0.017, p=0.803	r=0.019, p=0.787
Neutrophil-to-lymphocyte ratio	r=-0.080, p=0.245	r=-0.105, p=0.127
Hemoglobin	r=0.073, p=0.2837	r=0.113, p=0.100
Hematocrit	r=0.051, p=0.461	r=0.82, p=0.234
Mean corpuscular volume	r=0.096, p=0.163	r=0.095, p=0.164
Platelet	r=-0.031, p=0.652	r=-0.004, p=0.957
Platelet-to-lymphocyte ratio	r=0.012, p=0.865	r=0.036, p=0.600
C-reactive protein	r=0.001, p=0.989	r=-0.021, p=0.758
Procalcitonin	r=-0.085, p=0.218	r=-0.060, p=0.384
Erythrocyte sedimentation rate (mm/h)	r=-0.056 p=0.414	r=-0.105, p=0.125
Glucose	r=-0.041, p=0.549	r=0.011, p=0.868
Sodium	r=-0.060, p=0.380	r=-0.098, p=0.151
Potasium	r=0.053, p=0.444	r=0.035, p=0.615
Magnesium	r=-0.053, p=0.443	r=-0.019, p=0.779
Calcium	r=0.132, p=0.054	r=0.090, p=0.191
Phosphorus	r=0.131, p=0.055	r=0.024, p=0.728
Albumin	r=0.160, p=0.019*	r=0.216, p=0.001*
Urea	r=-0.060, p=0.381	r=-0.085, p=0.213
Creatinine	r=0.182, p=0.008*	r=0.050, p=0.467
Albumin/creatinine ratio	r=-0.105, p=0.127	r=-0.120, p=0.081
Uric acid	r=0.071, p=0.301	r=0.043, p=0.532
Estimated-glomerular filtration rate	r=-0.077, p=0.329	r=-0.018, p=0.821
Alkaline phosphatase	r=0.033, p=0.634	r=0.068, p=0.324
Gamma-glutamyl transferase	r=0.033, p=0.635	r=0.058, p=0.399
Aspartate aminotransferase	r=0.064, p=0.350	r=-0.051, p=0.454
Alanine aminotransferase	r=0.088, p=0.198	r=0.047, p=0.496
Lactate dehydrogenase	r=0.076, p=0.267	r=0.047, p=0.491
Thyroid-stimulating hormone	r=0.052, p=0.448	r=-0.003, p=0.962
Vitamin B12	r=-0.043, p=0.527	r=-0.118, p=0.084
Folate	r=-0.037, p=0.590	r=-0.032, p=0.642
25 (OH) vitamin D	r=0.018, p=0.788	r=-0.063, p=0.356

*p<0.05.
BBMT: Biceps brachii muscle thickness, BBCSA: Biceps brachii cross-sectional area

Discussion

This study showed a positive correlation between BBMT and serum albumin and creatinine levels, whereas BBCSA positively correlated only with serum albumin levels. Serum albumin level was the only parameter independently associated with BBMT and BBCSA in older palliative care patients.

Several studies have suggested that serological tests could serve as diagnostic markers for sarcopenia. A recent study reported higher PLT counts and PLT-to-WBC ratios in sarcopenic patients (4). Another study showed a higher AST-to-ALT ratio in middle-aged and older adults with sarcopenia (11).

Table 3. Multivariate multiple regression analysis for the independently associated factors of biceps brachii muscle thickness

Model	Variable	Unstandardized coefficients		Standardized coefficients	t	Significance	95% confidence interval	
		Beta	Standard error	Beta			Lower bound	Upper bound
1	Albumin level	0.013	0.004	0.214	3.161	**0.002**	0.005	0.021
	Creatinine level	0.043	0.027	0.109	1.608	0.109	-0.010	0.096
2	Age, years	0.001	0.003	0.014	0.205	0.838	-0.005	0.006
	Sex, female	0.221	0.048	0.317	4.564	**<0.001**	0.125	0.316
	Albumin level	0.013	0.004	0.217	3.358	**0.001**	0.005	0.020
	Creatinine level	0.027	0.026	0.068	1.034	0.302	-0.024	0.078
3	Age, years	0.001	0.003	0.016	0.223	0.824	-0.005	0.006
	Sex, female	0.222	0.049	0.318	4.562	**<0.001**	0.126	0.318
	Albumin level	0.013	0.004	0.219	3.361	**0.001**	0.005	0.021
	Creatinine level	0.027	0.026	0.068	1.034	0.302	-0.024	0.078
	Malnutrition risk	0.024	0.087	0.018	0.273	0.785	-0.149	0.196
4	Age, years	0.001	0.003	0.018	0.264	0.792	-0.005	0.006
	Sex, female	0.219	0.049	0.314	4.458	**<0.001**	0.122	0.315
	Albumin level	0.014	0.004	0.238	3.163	**0.002**	0.005	0.023
	Creatinine level	0.026	0.026	0.065	0.993	0.322	-0.025	0.077
	Malnutrition risk	0.030	0.088	0.022	0.337	0.736	-0.144	0.204
	C-reactive protein level	<0.001	<0.001	0.039	0.514	0.607	-0.001	0.001

Dependent variable: biceps brachii muscle thickness, independent variables: albumin level, creatinine level, age, sex, malnutrition risk, and C-reactive protein.
Model 1: unadjusted, Model 2: adjusted for age and sex, Model 3: adjusted for age, sex, and malnutrition risk, Model 4: adjusted for age, sex, malnutrition risk, and C-reactive protein

Table 4. Multivariate multiple regression analysis for the independently associated factors of biceps brachii cross-sectional area

Model	Variables	Unstandardized coefficients		Standardized coefficients	t	Significance	95% confidence interval	
		Beta	Standard error	Beta			Lower bound	Upper bound
1	Albumin level	0.040	0.012	0.216	3.220	**0.001**	0.015	0.064
2	Age, years	0.005	0.008	0.044	0.654	0.514	-0.010	0.021
	Sex, female	0.851	0.145	0.392	5.885	**<0.001**	0.566	1.136
	Albumin level	0.042	0.012	0.225	3.619	**<0.001**	0.019	0.064
3	Age, years	0.006	0.008	0.049	0.728	0.468	-0.010	0.021
	Sex, female	0.862	0.145	0.398	5.948	**<0.001**	0.577	1.148
	Albumin level	0.043	0.012	0.231	3.693	**<0.001**	0.020	0.065
	Malnutrition risk	0.281	0.264	0.067	1.065	0.288	-0.239	0.802
4	Age, years	0.006	0.008	0.052	0.780	0.436	-0.009	0.022
	Sex, female	0.848	0.146	0.391	5.788	**<0.001**	0.559	1.136
	Albumin level	0.048	0.013	0.258	3.554	**<0.001**	0.021	0.074
	Malnutrition risk	0.307	0.267	0.073	1.152	0.251	-0.218	0.833
	C-reactive protein level	0.001	0.001	0.055	0.745	0.457	-0.002	0.004

Dependent variable: biceps brachii cross-sectional area, independent variables: albumin level, age, sex, malnutrition risk, and C-reactive protein.
Model 1: unadjusted, Model 2: adjusted for age and sex, Model 3: adjusted for age, sex, and malnutrition risk, Model 4: adjusted for age, sex, malnutrition risk, and C-reactive protein

NLR and PLR as systemic inflammatory response parameters were reported as predictors of sarcopenia among gastric cancer patients (18). Indeed, numerous studies have established an independent inverse relationship between muscle mass and inflammatory markers. Serum albumin is a negative acute phase protein that may indicate not only inflammatory status but also nutritional status. A positive relationship between serum albumin level and animal protein intake has been reported, and hypoalbuminemia has been proposed as a predictor of all-cause mortality, postoperative complications, and cardiovascular disease (19-21). In a study by Chen et al. (22), serum albumin levels were positively correlated with muscle mass in males but negatively correlated with muscle mass in females, whereas another study reported reduced total protein and albumin levels in patients with sarcopenia compared with controls (23). In a recent study on the severity of sarcopenia in patients with liver cirrhosis, the mid-upper arm and mid-thigh ultrasonographic muscle thicknesses on both sides were positively correlated with serum albumin, bilirubin, and creatinine levels and international normalized ratio (3). In a more recent study, higher serum albumin levels were associated with higher temporal and masseter muscle thickness in patients with large vessel occlusion after endovascular thrombectomy (24). Serum albumin levels and muscle mass were negatively correlated with inflammation and positively correlated with adequate nutrition. In the current study, in line with the literature, both BBMT and BBCSA were positively correlated and independently associated with serum albumin levels, regardless of age, sex, nutrition, and inflammation status. Ninety-eight percent of creatinine is stored in muscles (10). Although its production may depend on muscle quantity/quality, its measurement is also sensitive to impaired renal function, which restricts its utility in assessing muscle mass (10). Even so, circulating creatinine may still serve as an alternative to estimate muscle mass (25). In the current study, BBMT was positively correlated with serum creatinine levels, a finding that is consistent with the literature. Nevertheless, there was no correlation between muscle mass and complete blood cell counts and blood chemistry variables other than serum albumin and creatinine levels, which may be caused by the altered characteristics of muscle fibers and other comorbidities among older patients receiving palliative care.

The judgment of muscle strength and physical performance is challenging in palliative care patients with severe dementia, delirium, reduced cooperation, and immobilization. Therefore, muscle mass measurement is preferable in hospitalized palliative patients. USG may allow clinicians to monitor muscle mass quickly and cheaply, such as muscle thickness, cross-sectional area, pennation angle, fascicle length, and echogenicity (8). However, upper limb muscles have received less interest so far than lower limbs because lower limb muscles are measured more easily and are more relevant to mobilization and activities of daily living than the trunk or skull muscles (26). In addition, senescence-related volumetric alterations in the upper limb muscles may also limit their utility in assessing muscle mass (27). However, in the clinical setting, different authors have linked upper limb muscles with handgrip strength (28), DXA-assessed muscle mass (29), CT-assessed muscle mass (30), sarcopenia (2), and mortality (31). In a recent study, upper limb muscle thickness was correlated with upper limb muscle cross-sectional area, quadriceps femoris muscle thickness, and rectus femoris muscle cross-sectional area, allowing screening for low muscularity during intensive care unit admission (32). Nevertheless, data on the relationship between ultrasonographically measured upper limb muscles and laboratory values in older palliative care patients are insufficient.

The NRS-2002 tool has been validated in hospitalized patients (33). In the current study, the average NRS-2002 score was 5±1.6, the MNR rate was 93%, and 51.5% had enteral nutrition. In a recent study among geriatric palliative care patients, the mean±SD NRS-2002 score was 4±1, the MNR rate was 93.9%, and 58.3% were on enteral feeding (34). In another study comparing the mini-nutritional assessment, NRS-2002, and Global Leadership Initiative on Malnutrition criteria in hospitalized palliative care patients, 93.2% were found to be at risk of malnutrition based on the NRS-2002 test (35). In this context, the results obtained in this study are consistent with the literature.

Pressure ulcers are among the indicators of quality care provision (36-38). The prevalence of pressure ulcers varies in the literature. Although the prevalence ranges between 3% and 14% for inpatients, this rate can reach 70% in certain patient groups. In a systematic review of pressure ulcers in patients receiving palliative care, the overall prevalence was 12.4%, ranging from 9.9% to 54.7% (39). In a more recent study, 42% of hospitalized palliative care patients had pressure ulcers (40). In the current study, 55.1% of patients had pressure ulcers, which could be attributed to advanced age, malnutrition, comorbidities, and previous hospitalizations.

Study Limitations

This study has some limitations. The study focused only on the biceps brachii, and muscle quality measures were unavailable. Causality could not be determined because of the cross-sectional design, and the results may not be generalized to other settings, given the unique characteristics of palliative care patients. In contrast, prospective patient enrollment and the inclusion of a variety of laboratory values in older palliative care patients were the major strengths.

Conclusion

In conclusion, this study revealed that serum albumin levels were independently associated with BBMT and BBCSA in older

palliative care patients. There is a gap in the literature regarding the potential associations of ultrasonographically assessed biceps brachii muscle mass with complete blood cell counts and blood chemistry in older palliative patients. Ultrasonographically assessed biceps brachii muscle mass may serve as a simple and reliable marker of muscle health and nutritional status in patients receiving palliative care. However, further multi-center, longitudinal studies that include multiple body regions are warranted to generalize the observed findings.

Ethics

Ethics Committee Approval: The study protocol was approved by the University of Health Sciences Türkiye, Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital Local Ethics Committee (code: 2022/152, date: 14.12.2022).

Informed Consent: All patients and/or their formal caregivers provided signed informed consent.

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Post COVID-19 subacute thyroiditis-de Quervain: a case series

Merita Emini Sadiku[1], Izet Sadiku[2], Mimoza Ramadani Piraj[3]

[1]University of Prishtina Faculty of Medicine, University Clinical Center of Kosova, Clinic of Endocrinology, Endoclinic-Medical Laboratory, Prishtina, Kosova
[2]University Clinical Center of Kosova, Clinic of Infectious Diseases, Endoclinic-Medical Laboratory, Prishtina, Kosova
[3]University Clinical Center of Kosova, Clinic of Endocrinology, Prishtina, Kosova

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Corresponding Author:
Izet Sadiku, M.D., University Clinical Center of Kosova, Clinic of Infectious Diseases, Endoclinic-Medical Laboratory, Prishtina, Kosova
+38344124029
izadoc@yahoo.com

ORCID:
orcid.org/0000-0002-8682-948X

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ABSTRACT

The Coronavirus disease of 2019 (COVID-19) pandemic had a public impact on various dimensions, including multisystemic complications. One such complication is subacute thyroiditis (SAT), also called subacute granulomatous or de Quervain thyroiditis, in which the severe acute respiratory syndrome-Coronavirus-2 virus affects thyroid tissue. Here we report seven patients with SAT and positive for COVID-19 and real-time quantitative-polymerase chain reaction. Neck pain was the most common symptom, followed by headache, fatigue, lethargy, and fever. Ultrasound revealed typical findings of the SAT. Corticosteroid treatment resulted in a complete improvement of inflammatory parameters and normalization of thyroid hormone levels. Our findings highlight the importance of considering rare cases of SAT as a potential complication of COVID-19. This report can also help physicians from various disciplines to identify such complications and provide better care for COVID-19 patients.

Introduction

Subacute thyroiditis (SAT) is an isolated disease of viral origin, with possible pathogens including mumps virus, hepatitis B and C viruses, cytomegalovirus, enterovirus, and coxsackie viruses A and B (1). SAT is characterized by a three-phase clinical course of thyrotoxicosis, followed by euthyroidism and sometimes hypothyroidism (2). Clinically, SAT is associated with severe pain that is localized to the anterior part of the neck and may radiate up to the jaw or ears, tenderness of the thyroid gland upon palpation, and specific manifestations including fever, fatigue, asthenia, tremor, and sweating as thyrotoxicosis symptoms (3).

The Coronavirus disease of 2019 (COVID-19) pandemic was announced in Kosovo in March 2020. Multiorgan effects of the virus were shown on diabetes very early, while its consequences in other endocrine glands remained unclear until recently. However, shortly after May 2020, the first case of SAT after a COVID-19 record was reported, prompting further observation of its spread.

We investigated the clinical and laboratory characteristics and follow-up data of patients with SAT in COVID-19 patients in Kosovo.

Case Presentation

This patient series included 7 individuals diagnosed and treated with SAT. All patients were admitted to the Outpatient Clinic of Endocrinology of the University Clinical Center of Kosovo or the Endoclinic Policlinic between May 2020 and May 2021. Demographic, clinical, biochemical, and imaging records were available in the medical health records. We informed the patients about this case series presentation and obtained their informed consent.

Of the seven cases, six were women and the age range was 17 to 65 years. All but one were young and did not have comorbidities. One patient had undergone thyroid lobectomy a few years previously. Six individuals presented with neck pain, and one patient showed atypical symptoms such as fear and no neck pain. Headache, odynophagia, fatigue, laziness, fever, and signs of thyrotoxicosis (e.g., tachycardia, anxiety, and insomnia) were common. The time between the onset of SAT symptoms and diagnosis was 5 to 30 days after COVID-19. Six patients had confirmed past real-time quantitative-polymerase chain reaction positive COVID-19, whereas one patient was diagnosed through serological tests (IgM and IgG).

Biochemical tests showed increased levels of C-reactive protein (CRP), erythrocyte sedimentation rate, total and free fractions of thyroxine (FT4) and triiodothyronine (FT3), and reduced thyroid-stimulating hormone (TSH). The gland was soft but painful on palpation, as confirmed by ultrasound examination. Typical ultrasonography findings of SAT were identified, including heterogeneous parenchyma and central hypoechoic areas (Figure 1) and reduction of radioiodine uptake in SAT scintigraphy (Figure 2).

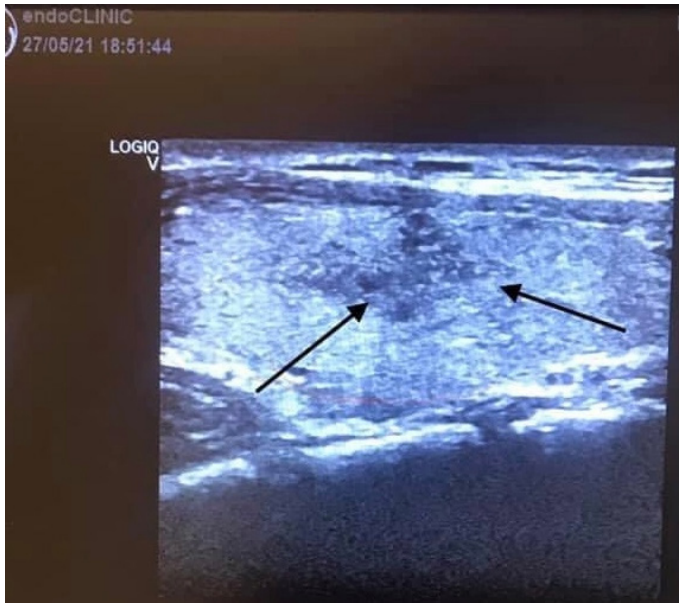

Figure 1. Ultrasound signs of SAT: longitudinal view of thyroid left lobe with hypoechoic field in the center (patient number 1 from Table 1)
SAT: Subacute thyroiditis

Almost all patients were treated with corticosteroids or non-steroidal anti-inflammatory drugs, leading to complete improvement of inflammatory parameters and normalization of thyroid hormones (Table 1). Vitamin D supplementation at a dosage of 2000-5000 IU/day was prescribed for most patients.

Discussion

In this study, we found that almost all patients with SAT after COVID-19 had a typical clinical presentation and responded well to corticosteroid treatment. Because large clinical studies with patients with SAT after COVID-19 have not been published, case reports or series and systematic reviews dominate the literature. The most plausible theory explaining direct cellular damage in thyroid tissues due to COVID-19 is that the Severe acute respiratory syndrome-Coronavirus-2 virus identifies angiotensin-converting enzyme-2 and TMPRSS2 mRNA receptors as cellular entrance receptors, and these receptors are expressed in follicular thyroid cells (4-6). Direct follicular cell damage can spread thyroid hormones into the plasma, explaining the thyrotoxic clinical features (e.g., tachycardia, anxiety, insomnia).

In terms of clinical, imaging, and laboratory features, SAT after COVID-19 is unlikely to differ from SAT of other viral origins (mumps, rubella, influenza, coxsackie, adenovirus, varicella-zoster virus, cytomegalovirus, Epstein-Barr virus, hepatitis E, and HIV) (7). SAT after COVID-19 also presents with classical clinical features of neck pain, anxiety, and thyrotoxicosis (high FT3 and FT4 and suppressed TSH) and typical ultrasound findings (8), as well as high inflammatory markers such as CRP, as shown in our cases.

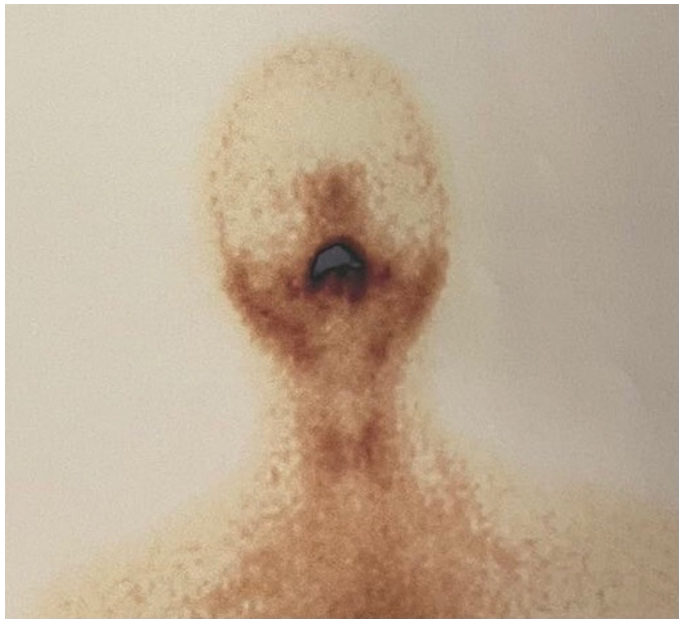

Figure 2. Reduction of radioiodine uptake in SAT scintigraphy (patient number 7 from Table 1)
SAT: Subacute thyroiditis

Table 1. Demographic, clinical, biochemical, imaging and treatment data in patients with SAT after COVID-19

Patients no-years/ gender	COVID-19 diagnosis	Time (days) onset of SAT after COVID-19	Pulses/ minute	Thyroid ultrasound/ imaging	Specific therapy	Before treatment thyroid hormones	Other analyses	After treatment thyroid hormones	Other analyses
1-65/M	RT-PCR	30	100	Heterogeneous parenchyma and central hypoechoic areas	Prednisone 25 mg/ day, indomethacin 150 mg/day, propranolol 80 mg/ day	T4 265 nmol/L FT3 3.48 pmol/L TSH 0.05 mIU/mL	CRP 104 mg/L ES 57 mm/h	FT4 14.75 pmol/L FT3 3.04 pmol/L TSH 0.6 mIU/mL	CRP 4.0 mg/L ES 13 mm/h
2-39/F	RT-PCR	30	120	Heterogeneous parenchyma	Prednisone 40 mg/ day, propranolol 80 mg/day	T4 237.5 nmol/L FT3 6.4 pmol/L TSH 0.05 mIU/mL	CRP 60 mg/L ES 80 mm/h	T4 136 nmol/L FT3 2.4 pmol/L TSH 1.0 mIU/mL	CRP 6.0 mg/L ES 30 mm/h
3-42/F	RT-PCR	5	110	Heterogeneous echo texture and central hypoechoic areas, enlarged thyroid	Prednisone 25 mg/ day, propranolol 40 mg/day	FT4 42 pmol/L FT3 7.2 pmol/L TSH 0.04 mIU/mL	CRP 70 mg/L ES 40 mm/h	FT4 15.7 pmol/L FT3 4.5 pmol/L TSH 0.97 mIU/mL	CRP 2 mg/L ES 15 mm/h
4-26/F	RT-PCR	7	90	Central hypoechoic areas	Indomethacin 150 mg/day	FT4 39 pmol/L FT3 8 pmol/L TSH 0.01 mIU/mL	CRP 90 mg/L ES 50 mm/h	FT4 18.1 pmol/L FT3 5.2 pmol/L TSH 1.2 mIU/mL	CRP 3 mg/L ES 12 mm/h
5-47/F	RT-PCR	14	125	Heterogeneous echo texture and central hypoechoic areas	Prednisone 25 mg/ day, propranolol 40 mg/day	FT4 41 pmol/L FT3 7.5 pmol/L TSH 0.09 mIU/mL	CRP 100 mg/L	FT4 14.2 pmol/L FT3 4.1 pmol/L TSH 0.97 mIU/mL	CRP 4 mg/L
6-17/F	IgM and IgG SARS-CoV-2	30	105	Heterogeneous echo texture	Indomethacin 150 mg/day	FT4 28 pmol/L FT3 7 pmol/L TSH 0.1 mIU/mL	CRP 88 mg/L ES 55 mm/h	FT4 19.51 pmol/L FT3 4.29 pmol/L TSH 1.5 mIU/mL	CRP 5 mg/L
7-42/F	RT-PCR	7	100	Hypoechoic areas	Prednisone 40 mg/ day, propranolol 20 mg/day	FT4 30 pmol/L FT3 7.1 pmol/L TSH 0.02 mIU/mL	CRP 60 mg/L	FT4 12.8 pmol/L FT3 1.4 pmol/L TSH 2.6 mIU/mL	CRP 4 mg/L ES 10 mm/h

This table describes the data related to the time of onset of the SAT, which varied from 5-30 days after the COVID-19 infection, followed by the clinical and imaging data at admission as well as the laboratory data at the beginning and after the treatment, which apparently show the improvement of inflammatory parameters and thyroid hormones.
RT-PCR: Real-time quantitative-polymerase chain reaction, M: Male, F: Female, COVID-19: Coronavirus disease-2019, SARS-CoV-2: Severe acute respiratory syndrome-Coronavirus-2, SAT: Subacute thyroiditis, CRP: C-reactive protein, ES: Erythrocyte sedimentation, FT4: Thyroxine, FT3: Triiodothyronine, TSH: Thyroid-stimulating hormone

Similar to other etiologies, the treatment of SAT after COVID-19 includes short-term corticosteroids, beta-adrenergic receptor blockers, and analgesics (9). On the other hand, in most cases, corticosteroids with a lower average dose of 25-30 mg/day have been used compared with SAT of other viral origins. Increased predisposition to systemic infections, impaired immune responses, or even the development of autoimmune diseases due to vitamin D insufficiency have been reported (10). Nevertheless, more studies on the benefits and prognostic role of vitamin D supplements in COVID-19 are warranted (11).

Our series included more women than men; no patient required hospitalization or developed hypothyroidism, and all patients showed mild COVID-19. Similar results were reported in a study that compared patients with mild or severe COVID-19 pneumonia. None of the hospitalized patients with mild pneumonia had hypothyroidism, whereas 3.2% of those with severe pneumonia did have hypothyroidism (12).

The presentation of such cases is crucial for physicians of different specialties to identify potential complications of COVID-19, especially rare cases such as SAT that may be overlooked during routine clinical practice. Recognition of this clinical entity is important because timely treatment can lead to a complete return to normal thyroid function. However, it is essential to investigate the possibility of hypothyroidism at a later stage. Adequate follow-up after recovery from COVID-19 is necessary because SAT, except in the early phase, may be found even several months after the initial infection.

Conclusion

Our findings contribute to the growing body of evidence on the impact of COVID-19 on the endocrine system and can help physicians identify and manage potential complications such as SAT.

Ethics

Informed Consent: We informed the patients about this case series presentation and obtained their informed consent.

Authorship Contributions

Surgical and Medical Practices - Concept - Design - Data Collection or Processing - Analysis or Interpretation - Literature Search - Writing: M.E.S., I.S., M.R.P.

Conflict of Interest: No conflict of interest was declared by the authors.

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