



Annals of Medical Research

The Official Journal of Inonu University Faculty of Medicine

Ann Med Res

Volume: 32

Issue: 2

February 2025

Original Articles

Ischemia modified albumin levels in patients with insulin resistance

Seker et al.

Evaluation of knowledge, attitude and behavioral features of our hospital staff about family practice

Canpolat et al.

Comparing the effect of office hysteroscopy with endometrial injury versus office hysteroscopy in gonadotropin-induced cycles before intrauterine insemination: A randomized controlled trial

Ege et al.

Investigating the effects of percussion massage therapy on pain, functionality, kinesiophobia, and quality of life in individuals with non-specific low back pain

Gorguluer et al.

Effects of sertraline on episodic memory in experimental model of chronic mild stress model of depression

Solak et al.

Evaluation of Chat Generative Pre-trained Transformer's responses to frequently asked questions about psoriatic arthritis:

A study on quality and readability

Kılıçoğlu et al.

Effect of Sleeper One® 5 anesthesia device on heart rate

Doğan et al.



Annals of Medical Research

The Official Journal of Inonu University Faculty of Medicine

Editorial Board

Volume: 32

Issue: 2

February 2025

Owner

Mehmet Aslan (Dean)

Inonu University Faculty of Medicine,
Department of Pediatrics, Malatya, Türkiye

Editor-in-Chief

Nurettin Aydoğdu, PhD

İnönü University, Faculty of Medicine,
Department of Physiology, Malatya, Türkiye

Section Editors

Ahmet Sami Akbulut, MD, PhD

İnönü University, Faculty of Medicine,
Department of General Surgery and
Liver Transplant Institute, Malatya, Türkiye

Ahmet Sarıcı, MD

İnönü University, Faculty of Medicine,
Department of Hematology,
Malatya, Türkiye

Barış Otlı, PhD

İnönü University, Faculty of Medicine,
Department of Medical Microbiology,
Malatya, Türkiye

Cem Azılı, MD

Ministry of Health, Ankara Training and
Research Hospital, Clinic of Surgical
Oncology, Ankara, Türkiye

Cem Çankaya, MD

İnönü University, Faculty of Medicine,
Department of Ophthalmology,
Malatya, Türkiye

Cuma Mertoğlu, MD, PhD

İnönü University, Faculty of Medicine,
Department of Biochemistry,
Malatya, Türkiye

Emrah Gündüz, MD

İnönü University, Faculty of Medicine,
Department of Otolaryngology Surgery,
Malatya, Türkiye

Ercan Yılmaz, MD

İnönü University, Faculty of Medicine,
Department of Obstetrics and
Gynecology, Malatya, Türkiye

Esra İşçi Bostancı, MD

Gazi University, Faculty of Medicine,
Department of Obstetrics and Gynecology,
Ankara, Türkiye

Lokman Hekim Tanrıverdi, MD, PhD

İnönü University, Faculty of Medicine,
Department of Medical Pharmacology,
Malatya, Türkiye

Neslihan Çelik, MD

Department of Pediatric Surgery,
Ankara, Türkiye

Nurettin Taştekin, MD

Trakya University, Faculty of Medicine,
Department of Physical Medicine and
Rehabilitation, Edirne, Türkiye

Nurullah Dağ, MD

İnönü University, Faculty of Medicine,
Department of Radiology, Malatya, Türkiye

Okan Aslantürk, MD

İnönü University, Faculty of Medicine,
Department of Orthopaedics
and Traumatology, Malatya, Türkiye

Osman Kurt, MD

İnönü University, Faculty of Medicine,
Department of Public Health,
Malatya, Türkiye

Tevfik Tolga Şahin, MD, PhD

İnönü University, Faculty of Medicine,
Department of General Surgery,
Malatya, Türkiye

Biostatistics Editors

Cemil Colak, PhD

Inonu University, Faculty of Medicine,
Biostatistics and Medical Informatics,
Malatya, Türkiye

Harika Gozde Gozukara Bag, PhD

Inonu University Faculty of Medicine,
Biostatistics and Medical Informatics,
Malatya, Türkiye

Ethics Editor

Mehmet Karataş, MD., PhD

Inonu University,
Faculty of Medicine, Department
of History of Medicine and Medical
Ethics, Malatya, Türkiye

Language Editors

Murat Kara, PhD

Siirt University, Faculty of
Veterinary Medicine,
Parasitology, Siirt, Türkiye

Publications Coordinator

Neala Bozkurt Dışkaya

Inonu University Faculty of Medicine,
Annals of Medical Research,
Malatya, Türkiye

Web and Social Media Editor

Mustafa Karakaplan, PhD

Inonu University Faculty of Medicine,
Dijital Office Manager,
Malatya, Türkiye

**Adel Hamed Elbaih**

Suez Canal University Faculty of Medicine,
Emergency Medicine, Ismailia, Egypt

Ayşe Seval Özgu Erdinc

Ministry of Health, Ankara City Hospital,
Gynecology and Obstetrics, Ankara, Türkiye

Aysegül Taylan Özkan

Department of Medical Microbiology
Faculty of Medicine,
TOBB University of Economics and Technology,
Ankara, Türkiye

Cemal Karakurt

Inonu University Faculty of Medicine,
Pediatric Cardiology Malatya, Türkiye

Erdem Topal

Inonu University Faculty of Medicine, Pediatric,
Malatya, Türkiye

Gökçe Şimşek

Kirikkale University, Faculty of Medicine,
Otorhinolaryngology, Kirikkale, Türkiye

Hakan Parlakpınar

Inonu University Faculty of Medicine,
Medical Pharmacology, Malatya, Türkiye

İbrahim Topçu

Inonu University, Faculty of Medicine,
Urology, Malatya, Türkiye

Kamran Kazımoglu Musayev

Merkezi Klinika, Cardiovascular Surgery,
Baku, Azerbaijan

Mehmet Hamamcı

Bozok University, Faculty of Medicine,
Neurology, Yozgat, Türkiye

Mehmet Kilic

Firat University Faculty of Medicine,
Pediatric Immunology and Allergy, Elazığ, Türkiye

Meltem Kurus

Katip Celebi, University, Faculty of Medicine,
Histology and Embology, İzmir, Türkiye

Mustafa Canpolat

Inonu University Faculty of Medicine,
Anatomy, Malatya, Türkiye

Neslihan Yücel

Inonu University, Faculty of Medicine,
Emergency Medicine, Malatya, Türkiye

Numan Karaarslan

Istanbul Medeniyet University Faculty of Medicine,
Neurosurgery, Tekirdağ, Türkiye

Ozkan Özger

Istanbul Rumeli University,
Neurosurgery, İstanbul, Türkiye

Rauf Melekoglu

Inonu University Faculty of Medicine,
Gynecology and Obstetrics, Malatya, Türkiye

Reni Kalfin

Institute of Neurobiology,
Bulgarian Academy of Sciences,
Sofia, Bulgaria

Rizaldi Taslim

Pinzon Universitas Kristen Duta Wacana
UKDW Neurology, Yogyakarta, Indonesia

Siho Hidayet

Inonu University Faculty of Medicine,
Cardiology, Malatya, Türkiye

Yusuf Yakupoğulları

Inonu University, Faculty of Medicine,
Clinic Microbiology, Malatya, Türkiye

Yücel Duman

Inonu University Faculty of Medicine,
Clinic Microbiology, Malatya, Türkiye



Original Articles

047-050 Ischemia modified albumin levels in patients with insulin resistance

Rabia Seker, Elif Ayca Sahin, Aziz Sener

051-056 Evaluation of knowledge, attitude and behavioral features of our hospital staff about family practice

Esra Kurt Canpolat, Isil Gonenc

057-060 Comparing the effect of office hysteroscopy with endometrial injury versus office hysteroscopy in gonadotropin-induced cycles before intrauterine insemination: A randomized controlled trial

Serhat Ege, Numan Cim

061-068 Investigating the effects of percussion massage therapy on pain, functionality, kinesiophobia, and quality of life in individuals with non-specific low back pain

Sema Gorguluer, Burak Menek

069-078 Effects of sertraline on episodic memory in experimental model of chronic mild stress model of depression

Hatice Solak, Zulfikare Isik Solak Gormus, Raviye Ozen Koca, Ayse Ozdemir, Mehmet Sinan Iyisoy, Ercan Kurar, Selim Kutlu

079-084 Evaluation of Chat Generative Pre-trained Transformer's responses to frequently asked questions about psoriatic arthritis: A study on quality and readability

Mehmet Serkan Kılıçoğlu, Ozan Volkan Yurdakul, Teoman Aydın

085-091 Effect of Sleeper One® 5 anesthesia device on heart rate

Özgür Doğan, Fatma Duygu Dertli, Özge Doğan, Suat Serhan Altıntepe Doğan, Gözde Ülker



Ann Med Res

Current issue list available at AnnMedRes

Annals of Medical Research

journal page: www.annalsmedres.org

Ischemia modified albumin levels in patients with insulin resistance

Rabia Seker^a, Elif Ayca Sahin^b, Aziz Sener^{a,*}

^aAnkara Etlik City Hospital, Department of Medical Biochemistry, Ankara, Türkiye

^bGazi University, Faculty of Medicine, Department of Medical Microbiology, Ankara, Türkiye

ARTICLE INFO

Keywords:

Insulin resistance

Oxidative stress

Ischemia-modified albumin

HOMA-IR

Type 2 diabetes mellitus

Received: Aug 27, 2024

Accepted: Jan 09, 2025

Available Online: 26.02.2025

DOI:

[10.5455/annalsmedres.2024.08.178](https://doi.org/10.5455/annalsmedres.2024.08.178)

Abstract

Aim: Insulin resistance (IR) is a crucial characteristic of type 2 diabetes mellitus (T2DM) and can also occur independently or in other conditions such as obesity and metabolic syndrome. Oxidative stress significantly contributes to IR pathology. Ischemia-modified albumin (IMA) is a biomarker of oxidative stress and has been studied for its potential link to IR. This study aimed to identify the relationship between IMA levels and IR.

Materials and Methods: We conducted a prospective study involving 101 IR patients without diabetes mellitus and 101 healthy controls. HOMA-IR was used to classify IR and control groups. IMA levels were measured using a colorimetric method. The groups' IMA values were compared, and correlations between IMA and other parameters were determined.

Results: There was no statistically remarkable difference in IMA levels between the IR and control groups ($p=0.27$). In addition, no significant correlation was observed between IMA and HOMA-IR ($p=0.60$). Significant differences were found in glucose, insulin, and HOMA-IR levels between the IR and control groups ($p<0.001$ for insulin and HOMA-IR, $p<0.01$ for glucose).

Conclusion: Despite higher IMA levels in the IR group, the difference was not statistically notable, and no significant correlation with HOMA-IR was found. This may be due to the finite number of patients with high HOMA values, the need for more precise data on body mass index, and longer follow-up. Further prospective studies are necessary to define the connection between IMA and IR.



Copyright © 2025 The author(s) - Available online at www.annalsmedres.org. This is an Open Access article distributed under the terms of Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

Introduction

Insulin resistance (IR) is elucidated as the lack of appropriate response by muscle, fat, and liver cells to insulin. Therefore, increased blood glucose levels are observed because of decreased glucose uptake by tissues [1]. The pancreas tries to compensate by producing more insulin to help absorb the glucose. Over time, this leads to beta-cell problems and can result in type 2 diabetes (T2DM). IR is a hallmark feature of T2DM, but it can also be observed in obesity, hypothyroidism, Gestational diabetes mellitus, and metabolic syndrome [2]. The extant research evidence substantiates a strong and direct relationship between oxidative stress and insulin resistance (IR) [3, 4]. Oxidative stress is characterized as a state where an overabundance of reactive oxygen species (ROS) surpasses the inadequate cellular antioxidant defenses, significantly contributing to the pathophysiology of IR. These ROS interfere with insulin signaling pathways through oxidative modifications,

either by tyrosine nitration or serine phosphorylation of key proteins [5, 6]. Consequently, there is an impairment in either the insulin receptor itself or its downstream signaling molecules, which reduces glucose uptake in both adipose tissues and muscle. Moreover, the production of ROS also amplifies the existing inflammatory reaction by triggering nuclear factor kappa B, thereby enhancing the production of pro-inflammatory cytokines [7]. This inflammatory milieu also contributes to the worsening of insulin resistance. This chronic oxidative environment may lead to endoplasmic reticulum stress and mitochondrial dysfunction, thereby promoting cellular damage [8]. Thus, these mechanisms, taken together, disrupt metabolic homeostasis and participate in the progressive nature of insulin resistance as well as its complications.

Ischemia-modified albumin (IMA) is a changed form of serum albumin that arises under an ischemic background owing to oxidative stress. The N-terminal region of albumin undergoes structural changes during ischemia, reducing its metal-binding capacity, particularly for cobalt,

*Corresponding author:

Email address: dr.azizsenerr@gmail.com (Aziz Sener)

making IMA a valuable biomarker for ischemic events and oxidative stress [9]. Various studies have shown that IMA has both diagnostic and prognostic value in diseases characterized by oxidative stress [10]. Studies have also showed a connection between IMA levels and IR [11, 12]. The close relationship between IR and oxidative stress and the fact that IMA indicates oxidative stress explain the possible relationship between IR and IMA.

In this study, we aimed to evaluate IMA levels in a cohort with insulin resistance and in a control group. In this way, we aimed to assess the relation between IMA and IR.

Materials and Methods

Patient selection

This prospective study included 101 IR patients without DM (serum glucose levels between 70-100 mg/dL and HbA1c level between 4%-6%) and 101 healthy controls between the ages of 15 and 60. The exclusion criteria for the study included a documented history of DM, the use of medications such as corticosteroids that induce insulin resistance, ongoing pharmacological treatments, a history of malignancy, recent or chronic infections, and the presence of any other underlying diseases.

Study was conducted in accordance with the principles outlined in the Declaration of Helsinki. Ethical approval was granted by the Ethics Committee of Ankara Dr. Sami Ulus Training and Research Hospital on April 20, 2022, under protocol number E-2022/04-326. Informed consent was not obtained from patients, as the research involved a retrospective analysis of anonymized blood test results. These results were used solely for disease monitoring and included waste blood samples collected for ischemia-modified albumin (IMA) measurements.

Grouping and cut-off values

HOMA-IR values were calculated using fasting insulin and fasting glucose levels in the patient and control groups for both sexes. Individuals with HOMA-IR values exceeding 2.5 formed the insulin resistance (IR) patient group, while those with HOMA-IR values at 2.5 or lower made up the control group.

Laboratory measurements

Glucose levels were measured using the hexokinase method on an AU 5800 analyzer (Beckman Coulter), while insulin levels were determined through the chemiluminescence method on an Advia Centaur XPT system (Siemens Healthcare).

Blood samples from all subjects were collected by venipuncture in serum separating tube, and instantly centrifuged at 4,000 X g for 10 min at 4 C in terms of their routine blood analysis. Waste serum samples for IMA measurement were frozen at -20°C up to the working day. On the day of the study, all samples were brought to room temperature, and the study was completed on the same day, and the results were evaluated. A colorimetric determination method based on the cobalt binding property of albumin was used to determine the IMA level, and the colored compound formed by dithiothreitol and cobalt II,

which does not bind to albumin, was measured colorimetrically. Measurements were made on a Human Huma-lyzer 2000 spectrophotometer at 470 nm, and the results were obtained using an absorbance unit (ABSU) [13]. The intra- and inter-study coefficient of variation (CV) determined for this method was lower than 3.6%.

Statistical analysis

All statistical analyses were examined with IBM SPSS Statistics for Windows, Version 24.0. (Armonk, NY: IBM Corp.). The normal distribution was assessed through the Kolmogorov-Smirnov test, kurtosis and skewness of the data distribution. An independent samples t-test was conducted to evaluate statistically notable differences between the groups. Chi-square test was conducted to assess the gender distribution differences along the groups. The relationship between IMA and the parameters was investigated using the Pearson correlation test. Descriptive statistics were expressed as mean ± standard deviation. Statistical significance was set at $p < 0.05$ for all analyses. To determine the appropriate sample size, a priori power analyses were conducted using G*Power software (version 3.1.9.7). For the independent samples t-test, with parameters set at $\alpha = 0.05$, an effect size of $d = 0.5$, and a statistical power of 0.95, the minimum required sample size to compare two groups was calculated as 176. The study included 202 participants, exceeding the required threshold to ensure sufficient power for the analysis. Similarly, for the Pearson correlation analysis, with a significance level of $\alpha = 0.05$, an effect size of $r = 0.30$, and a power of 0.80, the minimum sample size needed was 178. Since the actual sample size was 202, the study had adequate power to detect meaningful correlations.

Results

The mean age was 30.42 ± 11.41 years in the control group and 27.76 ± 11.52 years in the group with insulin resistance. The mean IMA was 0.37 ± 0.10 (ABSU) in the control group and 0.39 ± 0.17 (ABSU) in the group with insulin resistance (Table 1).

Table 1. Comparison of parameters between groups.

Parameters	Insulin resistant group (n=101)	Control group (n=101)	p-value
Age (years)	27.76 ±11.52	30.42±11.41	0.10 ^a
Gender (Female/Male)	75/26	76/25	0.99 ^b
IMA (ABSU)	0.39±0.17	0.37±0.10	0.27 ^a
Glucose (mg/dL)	91.94±7.98	88.64±5.94	<0.01 ^a
Insulin (pmol/L)	21.56±10.06	7.39±2.51	<0.001 ^a
HOMA-IR	4.89±2.39	1.61±0.55	<0.001 ^a

Parameters were compared between the two groups. The gender parameter was compared with Chi-square test and other tests were compared with Independent T-Test. P-values less than 0.05 are considered statistically significant for Independent T-Test^a and Chi-Square test^b and are shown in bold. Continuous variables are presented as mean ± standard deviation. Abbreviations: IMA, ischemia modified albumin; HOMA-IR, homeostasis model assessment for insulin resistance; ABSU, absorbance unit.

Table 2. Correlation analysis of IMA with other parameters.

Parameters	r-value	p-value
Age	-0.02	0.81
Glucose	-0.11	0.11
Insulin	-0.02	0.74
HOMA-IR	-0.04	0.60

Pearson correlation test was used to determine relation between IMA and parameters. P-values less than 0.05 are considered statistically significant for Pearson correlation test. None of the parameters showed statistically significant correlation with IMA. Abbreviations: IMA, ischemia modified albumin; HOMA-IR, homeostasis model assessment for insulin resistance; r-value. Pearson correlation coefficient.

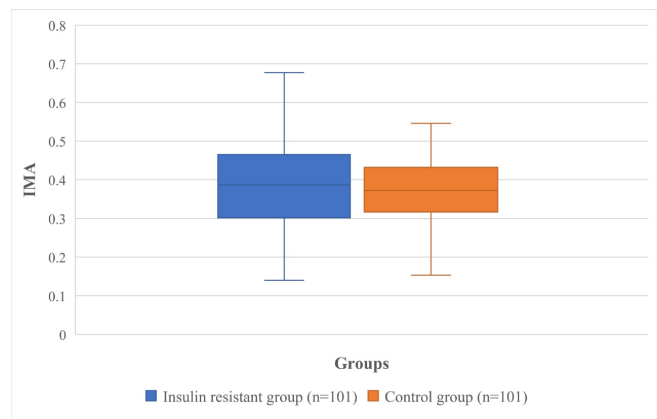


Figure 1. Box plot showing the distribution of IMA values according to groups. Abbreviations: IMA, ischemia modified albümin.

It is found that there was no statistically remarkable difference between the control group and the group with insulin resistance regarding age and IMA ($p=0.10$ and $p=0.27$ respectively) (Figure 1). There was a statistically remarkable disparity between the control group and the group with insulin resistance regarding glucose, insulin, and HOMA-IR parameters ($p<0.01$ for glucose, $p<0.001$ for insulin and HOMA-IR). It is found that there was no statistically notable correlation between IMA and age, glucose, insulin, and HOMA-IR ($p=0.81$, $p=0.11$, $p=0.74$ and $p=0.60$ respectively) (Table 2).

Discussion

Although IR is a hallmark of T2DM, IR can be seen in many clinical conditions and on its own. Oxidative stress plays a vital role in the pathology of IR. IMA draws attention as an important parameter to indicate oxidative stress. This study inspected the relation between IMA and IR in a cohort aged 15-60 years. Although the IMA levels were higher in the IR group than in the control group, the disparity was not statistically significant. There was also no notable correlation between IMA and HOMA-IR. IR alone has been established as a separate risk factor for endothelial dysfunction, cellular damage and cardiovascular events, even in patients without T2DM [12, 14]. Gast KB et al. conducted a meta-analysis of 65 (involving 516,325 participants) published between 2011 and 2012 and found possible relationships between cardiovascular

disease (CVD) and fasting glucose, fasting insulin and HOMA-IR values. This study found that cardiovascular disease (CVD) risk was greater when HOMA-IR increased by one standard deviation compared to when fasting glucose or insulin concentration increased by one standard deviation. Additionally, research suggests that including HOMA-IR in a cardiovascular risk prediction model could be advantageous [15]. Mottillo and colleagues also evaluated the relationship between metabolic syndrome and CVD. They conducted a meta-analysis of 87 studies (involving 951,083 participants) published until 2009. They found that all components of CVD and metabolic syndrome were separately related. They reported that the overall risk is higher than the sum of the individual risks of the components [16]. In a study conducted by Yigitbasi et al., they examined IMA and oxidative stress parameters in obese and IR, obese and non-IR, and control groups. They found that obesity or IR had no effect on IMA and no notable disparity between the groups regarding the oxidative stress index [17]. In a study conducted by Ates et al. in obese women with and without IR, it was found that the IMA value was lower in the group with IR. They also found no significant correlation between IMA and IR [18]. In a study by Kazanis et al. on postmenopausal women, IMA was positively correlated with body mass index, insulin and HOMA-IR. They also found that obesity was the strongest determinant of IMA levels in regression analysis [19]. There are varying results in studies in the literature. In our study, we found no significant difference among the groups regarding IMA and no correlation between IMA and HOMA-IR. This may be attributed to the insufficient number of patients with high HOMA values. The mean HOMA in our patient group is $4.89 (\pm 2.39)$. Only 5 of our patients have an HOMA value above 10. In addition, we could have obtained more precise results if we had included the body mass indexes of our sample group in the study. Moreover, we could have obtained more precise results if we could have followed the patients for a while. Previous studies have reported elevated IMA levels in various conditions associated with insulin resistance and diabetes mellitus (DM). Reddy et al. found high IMA levels in diabetic patients, particularly in those with retinopathy [20]. D’Souza et al. also reported remarkably increased IMA levels in T2DM patients, both with and without complications [21]. Similarly, Gulpamuk B et al. observed higher IMA values in diabetic patients with proliferative retinopathy, suggesting its utility as an indicator for monitoring tissue ischemia in DM [22]. Recent studies have highlighted the relationship between IMA and inflammation, as evidenced by high levels of high-sensitivity C-reactive protein (hs-CRP). In patients with T2DM, both IMA and hs-CRP levels were observed to be significantly elevated in relation to healthy controls, suggesting that hyperglycemia and inflammation may contribute to the observed increase in IMA levels. The positive correlation between hs-CRP and IMA levels indicates that inflammation, alongside oxidative stress, plays a significant act in the pathophysiology of ischemia and related complications in diabetic patients. This association underscores the complex interplay between oxidative stress, in-

flammation, and the progression of insulin resistance [23]. Considering that the IR pattern is well established in DM and that conditions other than IR, such as accumulation of advanced glycation end products and chronic hyperglycemia-induced activation of the polyol pathway, contribute to oxidative stress, IMA is expected to be elevated in DM patients. Most studies on this topic analyze the connection between IR and IMA in patients with DM. The novelty and strong aspect of our study is that it applied the diagnosis of DM as an exclusion criterion and inspected the connection between IR and IMA only in the presence of IR. It is the first study in this category. In addition, applying strict exclusion criteria is another strong aspect of our study. We think that our study will also shed light on future prospective studies.

Conclusion

In conclusion, IR is a serious condition. IR can lead to complications even when seen independently rather than as part of T2DM or other clinical conditions. More comprehensive and prospective studies are required.

Ethical approval

Ethical approval was granted by the Ethics Committee of Ankara Dr. Sami Ulus Training and Research Hospital on April 20, 2022, under protocol number E-2022/04-326.

References

- Lee SH, Park SY, Choi CS. Insulin Resistance: From Mechanisms to Therapeutic Strategies. *Diabetes Metab J*. 2022 Jan;46(1):15-37.
- Szablewski L. Insulin Resistance: The Increased Risk of Cancers. *Curr Oncol*. 2024 Feb 13;31(2):998-1027.
- Onyango AN. Cellular Stresses and Stress Responses in the Pathogenesis of Insulin Resistance. *Oxid Med Cell Longev*. 2018(7):1-27. doi: 10.1155/2018/4321714.
- Sędzikowska A, Szablewski L. Insulin and Insulin Resistance in Alzheimer's Disease. *Int J Mol Sci*. 2021 Sep 15;22(18):9987.
- Li H, Ren J, Li Y, Wu Q, Wei J. Oxidative stress: The nexus of obesity and cognitive dysfunction in diabetes. *Front Endocrinol (Lausanne)*. 2023 Apr 3;14:1134025. doi: 10.3389/fendo.2023.1134025.
- Erdemli Z, Erdemli ME, Gul M, Altinoz E, Gul S, Kocaman G, Kustepe EK, Gozukara Bag H. Ameliorative effects of crocin on the inflammation and oxidative stress-induced kidney damages by experimental periodontitis in rat. *Iran J Basic Med Sci*. 2021 Jun;24(6):825-832.
- Hussain T, Tan B, Yin Y, Blachier F, Tossou MC, Rahu N. Oxidative Stress and Inflammation: What Polyphenols Can Do for Us? *Oxid Med Cell Longev*. 2016 Sep 22;2016:7432797. doi: 10.1155/2016/7432797.
- Kasai S, Kokubu D, Mizukami H, Itoh K. Mitochondrial Reactive Oxygen Species, Insulin Resistance, and Nrf2-Mediated Oxidative Stress Response-Toward an Actionable Strategy for Anti-Aging. *Biomolecules*. 2023 Oct 19;13(10):1544.
- Coverdale JPC, Katundu KGH, Sobczak AIS, Arya S, Binda-dauer CA, Stewart AJ. Ischemia-modified albumin: Crosstalk between fatty acid and cobalt binding. *Prostaglandins Leukot Essent Fatty Acids*. 2018 Aug;135:147-157.
- Shevtsova A, Gordienko I, Tkachenko V, Ushakova G. Ischemia-Modified Albumin: Origins and Clinical Implications. *Dis Markers*. 2021 Jul 19;2021:9945424. doi: 10.1155/2021/9945424.
- Kaefer M, Piva SJ, De Carvalho JAM, Da Silva DB, Becker AM, Coelho AC, et al. Association between ischemia modified albumin, inflammation and hyperglycemia in type 2 diabetes mellitus. *Clin Biochem*. 2010;43(4-5):450-454.
- Ormazabal V, Nair S, Elfeky O, Aguayo C, Salomon C, Zuñiga FA. Association between insulin resistance and the development of cardiovascular disease. *Cardiovasc Diabetol*. 2018 Aug 31;17(1):122.
- Zoroddu S, Zinellu A, Carru C, Sotgia S. Analytical Insights into Methods for Measuring Ischemia-Modified Albumin. *Molecules*. 2024;29(19):4636.
- Laakso M, Kuusisto J. Insulin resistance and hyperglycaemia in cardiovascular disease development. *Nat Rev Endocrinol*. 2014 Mar;10(5):293–302. doi: 10.1038/nrendo.2014.29
- Gast KB, Tjeerdema N, Stijnen T, Smit JW, Dekkers OM. Insulin resistance and risk of incident cardiovascular events in adults without diabetes: meta-analysis. *PLoS One*. 2012;7(12):e52036.
- Mottillo S, Filion KB, Genest J, Joseph L, Pilote L, Poirier P, et al. The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. *J Am Coll Cardiol*. 2010 Sep 28;56(14):1113-32.
- Yigitbasi T, Baskin Y, Akgol E, Calibasi Kocal G, Ellidokuz H. Association of ischemia-modified albumin with oxidative stress status and insulin resistance in obese patients. *Rev Rom Med Lab*. 2017;25(3).
- Ates E, Set T, Karahan SC, Bicer C, Erel O. Thiol/Disulphide Homeostasis, Ischemia Modified Albumin, and Ferroxidase as Oxidative Stress Markers in Women with Obesity with Insulin Resistance. *J Med Biochem*. 2019 Mar 26;38(4):445-451.
- Kazanis K, Dalamaga M, Kassi E, Nounopoulos C, Manolis AS, Merantzi G, et al. Serum levels of ischemia modified albumin in overweight/obese postmenopausal women: a potential biomarker of atherosclerotic burden associated with oxidative stress. *Maturitas*. 2011 Oct;70(2):182-7.
- Reddy VS, Agrawal P, Sethi S, Gupta N, Garg R, Madaan H, et al. Associations of FPG, A1C and disease duration with protein markers of oxidative damage and antioxidative defense in type 2 diabetes and diabetic retinopathy. *Eye (Lond)*. 2015 Dec;29(12):1585-93.
- D'Souza JM, D'Souza RP, Vijin VF, Shetty A, Arunachalam C, Pai VR, et al. High predictive ability of glycated hemoglobin on comparison with oxidative stress markers in assessment of chronic vascular complications in type 2 diabetes mellitus. *Scand J Clin Lab Invest*. 2016;76(1):51-7.
- Gulpamuk B, Tekin K, Sonmez K, Inanc M, Neselioglu S, Erel O, et al. The significance of thiol/disulfide homeostasis and ischemia-modified albumin levels to assess the oxidative stress in patients with different stages of diabetes mellitus. *Scand J Clin Lab Invest*. 2018 Feb-Apr;78(1-2):136-142.
- Kaefer M, Piva SJ, De Carvalho JA, Da Silva DB, Becker AM, Coelho AC, et al. Association between ischemia modified albumin, inflammation and hyperglycemia in type 2 diabetes mellitus. *Clin Biochem*. 2010 Mar;43(4-5):450-4.



Ann Med Res

Current issue list available at AnnMedRes

Annals of Medical Research

journal page: www.annalsmedres.org

Evaluation of knowledge, attitude and behavioral features of our hospital staff about family practice

Esra Kurt Canpolat^{a,*}, Isil Gonenc^b

^aDiyarbakir Gazi Yasargil Training and Research Hospital, Department of Family Medicine, Diyarbakir, Türkiye

^bHaydarpaşa Training and Research Hospital, Department of Family Medicine, Istanbul, Türkiye

Abstract

ARTICLE INFO

Keywords:

Family practice
Knowledge
Attitude
Behavior
Satisfaction

Received: Aug 27, 2024

Accepted: Jan 10, 2025

Available Online: 26.02.2025

DOI:

[10.5455/annalsmedres.2024.08.179](https://doi.org/10.5455/annalsmedres.2024.08.179)

Aim: This study aimed to assess the knowledge level, attitudes, and satisfaction with family medicine services among healthcare workers at Haydarpaşa Numune Education and Research Hospital (HNEAH).

Materials and Methods: A face-to-face questionnaire was administered to healthcare personnel at HNEAH to collect data on sociodemographic characteristics, knowledge, attitudes, behaviors, and satisfaction with family medicine practices. The questionnaire included items designed to measure participants' familiarity with family physicians, utilization of family medicine services, and satisfaction levels.

Results: Among the participants, 63% were under 30 years of age, 57.8% were female, and 37.2% were doctors. Approximately 80.4% of the employees were enrolled in the family medicine system, and 75% knew their assigned family physician. Despite this high enrollment rate, 45.5% of participants had not visited their family physician in the past year. The primary reasons for visiting family physicians included proximity (56.7%), while the most cited barrier was the mismatch between working and service hours (44.5%).

Conclusion: The study highlights that while healthcare workers reported high levels of satisfaction with family medicine services, the knowledge of family medicine practices was significantly influenced by age and the frequency of visits to family physicians in the past year. These findings underscore the need for targeted educational initiatives to improve awareness of family medicine and encourage greater utilization of primary care services. Future research should focus on exploring participant preferences and barriers to optimize the effectiveness of family medicine services.



Copyright © 2025 The author(s) - Available online at www.annalsmedres.org. This is an Open Access article distributed under the terms of Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

Introduction

The primary objective of primary health care services is to provide comprehensive curative and preventive health services that do not require hospitalization. These services represent the first point of contact for individuals seeking treatment and play a critical role in ensuring accessible, equitable, and effective healthcare delivery [1]. In Turkey, primary health care institutions include health houses, family health centers, medical practices, tuberculosis dispensaries, maternal and child health centers, and community health centers [2].

Family medicine, also referred to as general practice, is an academic and scientific discipline rooted in the principles of primary care. It encompasses a structured program and curriculum, an evidence base, and practical clinical

applications [3]. Family physicians, as the cornerstone of family medicine, are uniquely equipped to manage the disease process at all stages, provide holistic care, and build meaningful patient relationships. They bear significant responsibility not only for individual health but also for the well-being of society as a whole [4,5].

Previous studies have highlighted varying levels of knowledge and attitudes toward family medicine practices in different populations, emphasizing their critical impact on the effective utilization of primary care services. Limited awareness and negative perceptions of family medicine have been associated with underutilization of preventive services, delayed healthcare-seeking behavior, and increased strain on secondary and tertiary healthcare systems. Understanding these dynamics is essential to addressing barriers to care, improving patient outcomes, and optimizing the role of family medicine within the healthcare system.

Assessing knowledge and attitudes about family medicine

*Corresponding author:

Email address: esra.kurtcanpolat@saglik.gov.tr (Esra Kurt Canpolat)

practices is essential for enhancing public engagement with these services, promoting preventive care, and reducing the burden on higher-level healthcare institutions. Investigating these factors not only contributes to the growing body of literature on primary care but also provides actionable insights for policymakers, educators, and healthcare practitioners.

The purpose of this study is to evaluate the knowledge, attitudes, and behavioral characteristics of healthcare personnel regarding family medicine practices. By exploring these dimensions, the study aims to identify gaps in knowledge, examine the factors influencing attitudes, and offer evidence-based recommendations for strengthening primary healthcare delivery.

The increase in patients seeking secondary health care without first consulting a family physician can be attributed to multiple factors. One significant reason is the absence of a mandatory referral system, which has been shown in previous studies to effectively regulate patient flow and promote the use of primary care services in countries with similar healthcare structures [6]. Additionally, individuals may perceive their conditions as chronic and opt for secondary care to access more comprehensive diagnostic and therapeutic services. The general approach of health institutions, the design of the social security system, and the demands of a technology-driven, modern lifestyle further exacerbate the preference for secondary care services. Furthermore, perceived deficiencies in staffing and technological infrastructure within primary care facilities discourage patients from seeking family medicine services, contributing to the underutilization of primary care [7].

This study evaluates the knowledge, attitudes, and practices of healthcare professionals at HNEAH regarding family practice, with the aim of identifying barriers to primary care utilization and proposing strategies for improving the effectiveness of family medicine services.

Materials and Methods

Patient protocol

The study admitted to the ethical standards summarized in the Declaration of Helsinki, and the Haydarpasa Numune Training and Research Hospital Clinical Research Ethics Committee granted approval (Protocol No: HNEAH-KAEK 2016/KK/89, Date: 26.09.2016). Participant Selection: The participants were selected from a total of 301 health personnel (doctors, nurses, midwives, health officers, psychologists, dieticians and other allied health personnel) working at HNEAH between 26/10/2016 and 26/12/2016. The patient group comprised volunteers aged 18 and above who had consented to participate in the study. No selection was made in the research, and the general scope of the research was included. Prior to the evaluation, the patients were informed about the study and provided written consent. The questionnaire comprised two sections: a sociodemographic data form and a series of questions designed to assess the knowledge, attitudes and behaviours of the employees in relation to family practice. The aforementioned questions were answered in a face-to-face interview format.

Participant selection

The participants were selected from a total of 301 health personnel (doctors, nurses, midwives, health officers, psychologists, dieticians and other allied health personnel) working at HNEAH between 26/10/2016 and 26/12/2016. The patient group comprised volunteers aged 18 and above who had consented to participate in the study. No selection was made in the research, and the general scope of the research was included. Prior to the evaluation, the patients were informed about the study and provided written consent. The questionnaire comprised two sections: a sociodemographic data form and a series of questions designed to assess the knowledge, attitudes and behaviours of the employees in relation to family practice. The aforementioned questions were answered in a face-to-face interview format.

Inclusion criteria for the study

The following individuals were eligible to participate in the study

1. Being a health personnel working in our hospital
2. Being over 18 years of age
3. Being a volunteer
4. Having completed the questionnaire form in its entirety.

Exclusion criteria

1. Those not employed as health personnel within our hospital
2. Those under the age of 18
3. Those not volunteering.

The sociodemographic characteristics of the patients selected according to these criteria were recorded by questioning age, sex and occupation, respectively.

Technique

Evaluation

The responses provided by the participants to the information questions were evaluated on a scale of 1 (affirmative) to 0 (negative or unknown). Their knowledge levels were subsequently scored. The knowledge score regarding the duties of family practice exhibited considerable variation, with a range of 0 to 46. A total of 46 points will be awarded when all questions are answered correctly. An intermediate-level knowledge score was accepted as 23, with 23–33 points classified as moderate, 33–40 points as good, and above 40 points as very good. A higher score indicates a higher level of knowledge. In order to ascertain the level of satisfaction with family practice, participants were invited to assign a score between 1 and 5, with 1 indicating the lowest level of satisfaction and 5 indicating the highest level of satisfaction.

Statistical examination

The data obtained from the study were subjected to analysis using the SPSS (Statistical Package for Social Sciences) for Windows 22.0 software. Descriptive statistical methods, including number, percentage, mean, and standard deviation, were employed for the evaluation of the data.

A two-sample t-test was employed to evaluate the statistical significance of quantitative continuous data between two independent groups. Similarly, a one-way analysis of variance (ANOVA) test was utilized to assess the statistical differences between more than two independent groups. A Scheffe test was employed as a supplementary post-hoc analysis to ascertain the discrepancies subsequent to the ANOVA test. Pearson correlation and regression analyses were conducted to examine the relationship between the continuous variables under investigation.

Hypothesis testing methods and assumptions

Independent Samples t-Test

Purpose: To compare quantitative continuous data between two independent groups.

Assumptions: The data of the groups should follow a normal distribution, tested using the Kolmogorov-Smirnov test ($n > 50$) or the Shapiro-Wilk test ($n \leq 50$). Variances between the groups should be homogeneous, assessed using Levene’s test.

One-Way ANOVA Test

Purpose: To compare quantitative continuous data among more than two independent groups.

Assumptions: The data of the groups should follow a normal distribution. Variances between the groups should be homogeneous, tested using Levene’s test. If the ANOVA result indicated a significant difference, the Scheffe test was applied to identify which groups differed.

Pearson correlation analysis

Purpose: To examine the linear relationship between continuous variables.

Assumptions: Both variables should follow a normal distribution. There should be a linear relationship between the variables.

Regression analysis

Purpose: To evaluate the effect of independent variables on the dependent variable.

Assumptions: There should be a linear relationship between the independent variables and the dependent variable. Residuals should follow a normal distribution. There should be no multicollinearity among the independent variables.

Normal distribution and variance homogeneity checks

- *Normal distribution compliance;*
- The Kolmogorov-Smirnov test was applied when the number of participants was > 50 .
- The Shapiro-Wilk test was applied when the number of participants was ≤ 50 .
- *Skewness and Kurtosis values* were considered acceptable if they fell within the range of $+2.0$ to -2.0 .
- *Variance homogeneity* was evaluated using Levene’s test.

Evaluation of results

The findings were evaluated with a 95% confidence interval and a 5% significance level ($p < 0.05$).

Results

The study population was predominantly young, with approximately 63% of participants aged 30 years or younger. Women constituted 57.8% of the sample, and the majority of participants were physicians (37.2%) (Table 1).

An evaluation of the health institution to which the participants of our study applied, with the exception of cases of emergency, revealed that approximately half of the participants (49.8%) had applied to the Training and Research Hospital. Upon inquiry as to the number of visits to a family physician in the preceding year, 45.5% of respondents indicated that they had not consulted with a family physician. As the study was conducted in HNEAH, it was anticipated that the initial institution to which the participants applied, with the exception of emergencies, would be EAH (Table 2).

Approximately 75% of the participants indicated that they had a preexisting relationship with their family physician. Despite participants demonstrating adequate knowledge regarding vaccination and newborn examination, the level of knowledge about cancer screening by family physicians remained below 50%. While 80% of respondents were aware that childhood vaccinations were conducted in ASMs, more than 70% were also cognizant of the fact that family physicians performed chronic disease follow-up screening and pregnancy follow-up (Table 3).

The most significant reasons for the participants’ preference, as identified through evaluation, can be classified into three primary categories: proximity to one’s residence (56.7%), accessibility to minor health concerns (52.4%), and the influence of a medical prescription (49.4%). The least common reason for attending was to monitor the progress of a pregnancy. The primary reason cited by those who did not attend a family physician appointment

Table 1. Distribution of demographic characteristics of participants.

Tables	Groups	Frequency (n)	Percentage (%)
Age	18-25 years	84	27.9
	26-30 age	106	35.2
	31-35 age	36	12.0
	36-40 years	35	11.6
	41-45age	23	7.6
	Over 45	17	5.6
	Total	301	100.0
Gender	Woman	174	57.8
	Male	127	42.2
	Total	301	100.0
Profession	Doctor	112	37.2
	Nurse	89	29.6
	Midwife	15	5.0
	Other	85	28.2
	Total	301	100.0

Table 2. Behavioural status of people in family practice.

Tables	Groups	Frequency (n)	Percentage (%)
Health Institution Referred to Other Than Emergency	Training and Research Hospital	150	49.8
	University Hospital	9	3.0
	State Hospital	44	14.6
	Private Hospital Private Practice	30	10.0
	Registered Family Physician	52	17.3
	Any Family Doctor	3	1.0
	Nowhere	13	4.3
	Total	301	100.0
Number of visits to the family physician in the last one year	Never been there	137	45.5
	Once	70	23.3
	Between 1-5	75	24.9
	More than 5	19	6.3
	Total	301	100.0

Table 3. Distribution of knowledge about family practice and duties of family physicians.

Tables	Groups	Frequency (n)	Percentage (%)
Familiarity with the family physician	Yes	225	74.8
	No	76	25.2
	Total	301	100.0
The family doctor performs immunisation in infancy and childhood	Yes	242	80.4
	No	20	6.6
	No opinion	39	13.0
	Total	301	100.0
The family doctor screens for colorectal, breast and cervical cancer	Yes	127	42.2
	No	73	24.3
	No opinion	101	33.6
	Total	301	100.0
The family physician performs the necessary examinations and follow-ups in pregnant women	Yes	241	80.1
	No	30	10.0
	No opinion	30	10.0
	Total	301	100.0
The family physician carries out chronic disease follow-up and screening of people registered with him/her (such as diabetes, hypertension, thyroid disease, heart disease)	Yes	217	72.1
	No	26	8.6
	No opinion	58	19.3
	Total	301	100.0

was the incompatibility of the appointment time with their work schedule (44.5%) (Table 4).

The responses provided by the participants to the information questions were scored as either "1" (affirmative) or "0" (negative or "no idea"). These scores were then used to assess the participants' knowledge levels. A total of 46 points can be obtained when all questions are answered correctly. In our study, the mean score for the level of knowledge about the duties of family physicians was found to be 27.203 ± 10.531 , indicating a moderate level of knowledge. Upon inquiring of the participants as to the extent of their satisfaction with the family physician, a score between 1 and 5 was requested, with 1 representing the lowest level of satisfaction and 5 the highest. The results indicated a high level of satisfaction (3.880 ± 1.101).

When calculating the level of satisfaction with family prac-

tice, it can be stated that even when only those who gave a rating of "4" or "5" are considered, the percentage of satisfaction is 62.8% (Table 5).

Discussion

The objective of our study was to evaluate the knowledge, attitudes and behaviours of healthcare professionals working in HNEAH with regard to family practice. In our study, approximately half of the participants initially sought care at the PHC, except in cases of emergency. In contrast, the study conducted by Oyan [8] in 2013 on patients applying to Istanbul Faculty of Medicine observed that a significant proportion (38%) initially sought care at the State Hospital, while 35% initially sought care at the University Hospital. The high number of applications to EAH observed in our study can be attributed to the fact that the study was conducted on Haydarpaşa Numune

Table 4. Attitudes towards family practice.

Tables	Groups	Frequency (n)	Percentage (%)
The most important reasons for choosing a family doctor	Close by location	93	56.7
	Trust in the physician	51	31.1
	Minor health problems can be dealt with there	86	52.4
	Having laboratory facilities	29	17.7
	Dressing, injections	44	26.8
	Protective services	30	18.3
	Family planning	23	14.0
	Printing a prescription	81	49.4
	Pregnancy follow-up	20	12.2
	Infant and child follow-up	35	21.3
	Health report	62	37.8
	Other	1	0.6
Reasons for not visiting the family doctor	I do not trust	3	2.2
	I don't like the family doctor	2	1.5
	Inadequate laboratory facilities	17	12.4
	Not suitable for my working hours	61	44.5
	I don't know where he is	21	15.3
	Other	52	38.0

Table 5. Average level of knowledge and satisfaction with family practice.

	N	Centre	Ss	Min.	Max	Ranj
Level of knowledge about the duties of family practice	301	27.203	10.531	1.000	46.000	0-46
Satisfaction with family practice	164	3.880	1.101	1.000	5.000	1-5

EAH employees.

While 18.3% of our participants proceeded to the registered FH or any FH as the initial institution consulted when they encountered a health issue, this figure was 14.1% in Oyan’s study [8], which aligns with our findings. In a study conducted in Kayseri province in 2011 among patients who applied to ASMs [9], it was observed that approximately half of the individuals preferred to initiate the first step in addressing their health concerns. It can be concluded from these findings that there is a parallel between the location of the studies and the initial institution to which patients apply. It was established that the location of the study was the primary point of contact for individuals seeking healthcare services. Nevertheless, it would be erroneous to extrapolate these findings to the entire country.

In our study, 45.5% of participants reported never having visited an FH centre in the previous year, representing approximately half of the total sample. In Oyan’s study [8], the corresponding figure was 42.1%, which is consistent with our own findings.

The majority of participants (80.4%) were registered in the family practice system, and 75% of them had a known family physician. In a study conducted on 1,016 students at Gümüşhane University in 2010 [10], only 20% of the participants were registered with the family practice system, and only 7% were aware of their family physician. The pilot implementation of family practice commenced in Düzce province in 2005 and was subsequently implemented in all provinces across Turkey in 2014. As our study was con-

ducted towards the end of 2016, it is anticipated that the level of enrolment in the family practice system and the level of recognition of the family physician will be higher than that reported in a study conducted in 2010.

In our study, the most common reasons for preferring a family physician were that it was conveniently located and that they could address minor health concerns at the FHC and obtain a prescription (Table 5). In another study [8], the most common reasons for visiting a family physician were to have a prescription written and to have tests and treatment when ill, which are similar to those reported in our study. In our study, the percentage of individuals who selected a family physician for the follow-up of healthy children remained at 21.3%. This figure contrasts with the findings of a UK-based study, in which the primary reason for visiting a family physician was for the examination of healthy children [11]. In a separate study conducted in Turkey [9], it was observed that the majority of participants sought only therapeutic services, with minimal demand for preventive services such as vaccination, pregnancy follow-up, and family planning. Furthermore, the desired level of success in preventive services was not achieved in this study.

The level of satisfaction with family practice in our study was 62.8%, while a study conducted on 1,001 patients who applied to GATA revealed that 73.4% of participants were satisfied with family practice services. This satisfaction rate is comparable to those observed in numerous other studies on family practice satisfaction [12].

Limitations

1. As the study was conducted in an ECAH, the findings cannot be generalised to healthcare professionals working outside of this context.
2. As the study was conducted on a sample of 301 individuals, it is not possible to generalise the results to the entire population.

Conclusion

The findings of our study, along with those of numerous other studies in this field, indicate that the majority of individuals prefer to seek care from specialists or other healthcare providers, rather than from family physicians, when they experience a health issue. This results in an increased financial and personnel burden on higher-level health institutions, effectively nullifying the impact of initiatives and expenditures made to strengthen primary care. In light of the proven efficacy of primary care services in countries such as the United Kingdom and the Netherlands, where a referral system is in place, the introduction of a similar system in our country could ensure a more optimal utilisation of primary care resources. It is our contention that the implementation of specific regulatory measures at the state level, aimed at encouraging the utilisation of the step system in health services, will facilitate a more optimal utilisation of primary care resources and mitigate the burden on higher-level health services.

Ethical approval

The study admitted ethical clearance from the Scientific Research Ethics Committee of Haydarpasa Numune Training and Research Hospital (Protocol No: HNEAH-KAEK 2016/KK/89).

This study is based on the findings of our 2017 thesis, entitled "Evaluation of Knowledge, Attitude and Behavioral

Features of Our Hospital Staff About Family Practice" (Thesis number 463991).

References

1. Karadağ Z. The effect of family medicine practice on customer/patient satisfaction. [Master's Thesis]. *Ankara: Gazi University Institute of Social Sciences*; 2007.
2. Ağdemir H. A research on the place and importance of family practice in the health sector and the satisfaction status of service beneficiaries and service providers. [Master's Thesis]. *Mersin: Çağ University Institute of Social Sciences*; 2012.
3. The European Definition of General Practice/Family Practice. WONCA Europe. *Barcelona: WONCA Europe Office Publication*; 2002:55-56.
4. European Definition of Family Practice. WONCA; 2005. Available from: <http://www.tahud.org.tr> [Accessed: 18 May 2017].
5. Kara IH. Definition and principles of family medicine, family practice in the world and in Turkey. Available from: <http://ailehekimligi.gov.tr> [Accessed: 20 May 2017].
6. Aydın S. The story of the increase in the number of applications to physicians. *SD (Health Thought and Medical Culture) Journal*. 2015;35:6-13. Available from: <http://www.sdplatform.com/Yazilar/Kose-Yazilari/408/Hekime-basvuru-sayisinin-artis-hikyesi.aspx> [Accessed: 14 June 2017].
7. Aksakoğlu G, Kılıç B, Uçku R. Family practice model/system is not suitable for Turkey. *Toplum ve Hekim*. 2003;18(4):251-7.
8. Oyan NK. Information and thoughts of patients applying to the medical faculty hospital about family medicine practice. [Specialist Thesis]. *İstanbul: İstanbul Faculty of Medicine*; 2013.
9. Çetinkaya F, Baykan Z, Naçar M. Adults' thoughts on family medicine practice and application status to family physicians. *TAF Prev Med Bull*. 2013;12(4):417-24.
10. Kıvanç A, Şahinöz T. Determination of knowledge status of Gümüşhane University students on family medicine. *Gümüşhane Univ J Health Sci*. 2012;1(1):41.
11. Green LA, Phillips RL, Fryer GE, et al. The nature of primary medical care. In: Jones R, Britten N, Culpepper L, et al., editors. *Oxford Textbook of Primary Medical Care*. 1st ed. New York: Oxford University Press; 2004:3-10.
12. Aydoğan Ü. Patient satisfaction in family practice. [Specialist Thesis]. *Ankara: GATA Faculty of Medicine*; 2005.



Ann Med Res

Current issue list available at AnnMedRes

Annals of Medical Research

journal page: www.annalsmedres.org

Comparing the effect of office hysteroscopy with endometrial injury versus office hysteroscopy in gonadotropin-induced cycles before intrauterine insemination: A randomized controlled trial

 Serhat Ege^{a,*}, Numan Cim^b^aDicle University, Faculty of Medicine, Department of Obstetrics and Gynecology, Diyarbakır, Türkiye^bIstanbul Haseki Training and Research Hospital, Department of Obstetrics and Gynecology, Istanbul, Türkiye

ARTICLE INFO

Keywords:

Office hysteroscopy
Intrauterine insemination
Endometrial injury
Gonadotropin
Infertility

Received: Oct 03, 2024

Accepted: Jan 10, 2025

Available Online: 26.02.2025

DOI:

[10.5455/annalsmedres.2024.09.196](https://doi.org/10.5455/annalsmedres.2024.09.196)

Abstract

Aim: Our aim in this study was to evaluate the implantation success in local endometrial injury in patients undergoing intrauterine insemination following induction of ovulation with gonadotropins.

Materials and Methods: In this prospective randomized controlled study, 62 patients undergoing gonadotropin-assisted ovulation induction after intrauterine insemination were included. Thirty patients with hysteroscopy-related injuries were included in Group 1 and 32 control patients who underwent hysteroscopy alone were included in Group 2.

Results: The rates of clinical pregnancy (16.6% [5/30] vs. 18.7% [6/32]; $p = 0.8$), abortion (3.3% [1/30] vs. 3.1% [1/32]; $p = 0.9$), and multiple pregnancy (3.3% [1/30] vs. 3.1% [1/32]; $p = 0.9$) were similar between the two groups.

Conclusion: There were no significant differences in clinical pregnancy, multiple pregnancy, or abortion rates between the groups. The intervention group underwent hysteroscopic injury in the follicular phase before gonadotropin-induced intrauterine insemination, while the control group underwent hysteroscopy only.



Copyright © 2025 The author(s) - Available online at www.annalsmedres.org. This is an Open Access article distributed under the terms of Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

Introduction

Creating mature follicles, ensuring ovulation, and eliminating cervical factors can increase pregnancy rates in intrauterine insemination (IUI) performed with ovulation induction. Since IUI is cheap, easy, and noninvasive, it is the first method that should be used in cases of unexplained infertility [1,2]. Implantation failure is a possible cause of unexplained infertility [3]. Blastocyst invasion of the endometrium occurs during the implantation window [4]. During this period, there is a reciprocal interaction between the embryo and the endometrium.. If the uterus can not become receptive, this reciprocation is impaired, and implantation fails [5]. Many different methods are mentioned to increase the success of insemination [6,7].

Endometrial injury (EI) has attracted much attention for improving implantation success. This method aims to increase pregnancy rates by causing injury in a patient's IUI cycles before initiating assisted reproductive techniques.

Interfering with the endometrium may increase the secretion of cytokines, growth factors, and adhesive molecules by modulating gene expression, hence providing the basis for implantation [7-11].

The current study aims to investigate the local implantation success of EI in patients having IUI after gonadotropin-induced ovulation.

Materials and Methods

Sixty-two infertile patients admitted to the infertility polyclinic in a tertiary center hospital, Gynaecology and Obstetrics department participated in this prospective randomized controlled trial (RCT).

According to World Health Organization (WHO) rules [12], male factor infertility was defined as sperm count less than 15×10^6 /mL, overall motility less than 40%, or normal forms less than 4%. Aberrant semen parameters with $>5\%$ normal morphology and $>5 \times 10^6$ /mL motile spermatozoa recovered following sperm preparation were characterized as mild male factor infertility [13].

The following patients were excluded from the study:

*Corresponding author:

Email address: serhatege782@gmail.com (Serhat Ege)

Table 1. The demographic and clinical characteristics of the patients in the study groups.

	Group 1 (n = 30)	Group 2 (n = 32)	P-value
Age (years) (mean ± SD)	26.5±3.6	26.15±4.4	0.463
Duration of infertility (years)	3.08±1.04	3.22±1.05	0.313
BMI (kg/m ²)	24.51±1.71	24.29±1.88	0.353
Basal FSH (mIU/mL)	4.63±1.16	4.63±1.25	0.974
Basal LH (mIU/mL)	4.85±0.89	4.65±1.12	0.13
E2 (pg/mL)	47.92±5.89	47.21±5.21	0.7078
TSH (ng/dL)	2.8±1.1	3.08±1.47	0.107
PRL (ng/mL)	14.46±2.63	14.35±1.68	0.709
Sperm count ×10 ⁶ /mL	24.33±6.48	25.32±3.48	0.151
Follicles >18 mm(milimeter) on hCG administration	1.23±0.5	1.51±0.5	0.862
Endometrium thickness on day of hCG (mm)	11.48±1.52	11.61±1.61	0.538
Total gonadotropin (IU/L)	524.77±68.32	527.11±52.96	0.771

Values presented as mean ± SD. BMI: body mass index, LH: luteinizing hormone, FSH: follicle stimulating hormone, TSH: thyroid stimulating hormone, PRL: prolactin, E2: estradiol.

Table 2. Summary of the outcome and success of both intervention groups in the study.

	Hysteroscopy with injury (n = 30)	Hysteroscopy (n = 32)	P-value
Clinical pregnancy	5/30 (16.6%)	6/32 (18.7%)	0.8
Multiple pregnancy	1/30 (3.3%)	1/32 (3.1%)	0.9
First-trimester abortion	1/30 (3.3%)	1/32 (3.1%)	0.9

Values are presented as mean ± SD or n (%).

those with male factor infertility, those with anovulatory cycles, those over 39 or under 18 years of age, smokers, women with a history of chronic medication or disease, and those who had not undergone IUI or canceled their appointment.

Patients who met these criteria and applied to the outpatient clinic were assigned to the intervention, the clinic nurse assigned participants to two groups: the intervention group (Group 1) and the control group (Group 2). Group 1 included 30 patients who received hysteroscopic endometrial injury, while Group 2 consisted of 32 patients who underwent hysteroscopy without injury. Patients who met the criteria but had no follicle development during follow-up were also excluded from the study.

Treatment protocol

On the third day of the menstrual cycle, ovulation induction was started in both groups with 75 IU gonadotropins (Gonal-F pen, follitropin alfa, Merck & Co., Inc.). In all patients, hysteroscopic scissors (Karl Storz Endoscopy) were used to create an injury on the posterior endometrial wall during the follicular phase, specifically 5 to 7 days before the IUI cycle.

EI was conducted by filling the uterine cavity with normal saline solution at 100–120 mmHg of pressure without using a speculum or tenaculum and without anesthesia or analgesia. In the control group, only office hysteroscopy was performed, and no EI was carried out.

Transvaginal ultrasonography was used to measure the number and size of follicles at 3- to 5-day intervals after ovulation induction. Afterwards, serum estradiol lev-

els were assessed, and gonadotropin dosage adjustments were made periodically. In the absence of ovarian hyperstimulation (E2 > 3000) or multiple pregnancy (follicular count >4) risk, a single dosage (250 mcg/0.5 mL) of human chorionic gonadotropin (hCG) (Ovitrelle, Merck & Co., Inc.) was given subcutaneously when a dominant follicle (18 mm and above) was produced.

The same researcher used sterile procedures to do IUI 36 hours following ovulation. Vaginal progesterone (Crinone 8%, Merck & Co., Inc.) was used to support the luteal phase following IUI. Fourteen days following IUI, serum beta-hCG levels were assessed to identify pregnancies. Transvaginal ultrasonography was used to diagnose pregnancy by detecting the presence of the fetal heartbeat and gestational sac during 5th and seventh week of gestation. A clinical diagnosis of pregnancy was made if both were present. Early loss of pregnancy occurring before 12 weeks of gestation was considered an abortion.

All volunteers signed informed consent and also gave verbal consent to participate in our study. The Declaration of Helsinki’s ethical guidelines for medical research involving human participants were followed (ethics committee no. 147).

Statistical analysis

The statistical data was analyzed using Statistical software Package for Social Sciences version 18 (SPSS ve 18.0) (IBM,USA), > Continuous (mean ± SD). When comparing quantitative data, normally distributed variables were compared using the Student’s t-test, while the data that showed non-normal distribution were compared using the

Mann-Whitney U test. The qualitative data were compared using a chi-square test. Any p value less than 0.05 was considered to be statistical significant.

Results

Sixty-two patients undergoing intrauterine insemination (IUI) were enrolled in this study and randomly assigned to two groups: an intervention group receiving office hysteroscopy (OH) with endometrial injury (EI) ($n = 30$) and a control group undergoing OH only ($n = 32$). Baseline characteristics, including age, BMI (kg/m^2), duration of infertility, basal LH (mIU/mL), basal E2 (pg/mL), basal FSH (mIU/mL), and sperm count, were similar between the groups. No statistically significant differences were found between groups regarding the day of hCG administration, total gonadotropin dose per cycle (IU/L), the number of mature follicles (≥ 18 mm), and endometrial thickness on the day of hCG administration (Table 1).

As shown in Table 2, there were no significant differences between groups in the rates of multiple pregnancies (3.3% [1/30] vs. 3.1% [1/32]; $p = 0.9$), clinical pregnancies (16.6% [5/30] vs. 18.7% [6/32]; $p = 0.8$), and abortions (3.3% [1/30] vs. 3.1% [1/32]; $p = 0.9$).

Discussion

In our study, no significant difference was found in the clinical pregnancy, live pregnancy and abortion rates among the study groups.

IUI with ovulation induction should be the first choice in cases of unexplained infertility [1]. Pregnancy rates have been reported to be 7% for each cycle in IUI conducted with clomiphene citrate (CC) and 12% for IUI conducted with gonadotropins [14].

Many studies have been performed to increase the pregnancy rates slightly using EI. Soliman et al. [15] concluded that EI performed with CC and human menopausal gonadotropin (Merional) in the follicular phase increased the pregnancy rate after an IUI deficiency. Wadhwa et al. [16] evaluated the effect of EI on IUI outcome, they concluded that EI in the follicular phase was associated with a better clinical pregnancy rate compared to injury in the luteal phase of the previous menstrual cycle. Bahaa Eldin et al. [17] assessed EI performed with a Pipelle catheter in the follicular phase of the stimulation cycle combined with IUI and found that the results were significantly better. In a study conducted by Zarei et al. [18], EI performed in the follicular phase showed no significant difference in the live pregnancy and abortion rates of the control groups. We studied hysteroscopic injury with gonadotropins, and we found no statistical difference in the treatment group compared with the control group in terms of the outcomes of insemination.

A systematic review on EI with IUI stated that 8 RCTs [15–22] with total of 1,871 IUI cycles (and 1,523 participants) were included. Patients got EI in 998 IUI cycles, while no intervention was carried out in 873 IUI cycles the EI had significantly superior results over the control groups in terms of. In terms of clinical pregnancy rate (CPR), ongoing pregnancy rate (OR) = 2.27; $p < .00001$; data from 1,871 IUI cycles]. In addition, follicular-EI

was associated with a statistically significantly higher CPR (OR = 2.57; $p < .00001$) in comparison with the controls.

Various studies report superior results in patients who were injured in the follicular phase and the controls [15, 17, 20]. Our results did not support these observations. We believe that the number of studies regarding this area of research are insufficient and that more comprehensive studies are needed in future. Furthermore, studies vary in the type of patients included, the type and timing of intervention, and the number of IUI cycles. Therefore, comparing and drawing conclusions is problematic due to the large number of confounding factors.

The reviews published on this subject have no definitive results on whether the endometrial injury improves CPR, LBR, or ongoing pregnancy rates in women undergoing IUI or having sexual intercourse. The quality of evidence was rated as low or very low [23]. Furthermore, another review stated that that endometrial injury improved CPR (odds ratio (OR) 2.27, $P < 0.00001$) and ongoing pregnancy rates (OR 2.04, $P = 0.004$) in patients undergoing IUI. Endometrial injury did not increase the risk of multiple pregnancy, miscarriage, or ectopic pregnancy [24].

Key advantages of this study include the use of hysteroscopy in both groups, allowing for the detection of intrauterine pathologies potentially missed by ultrasound (USG) and enabling intervention for minor pathologies such as polyps. A limitation is that hysteroscopic scissor injury may cause greater pain than injuries induced by other devices (e.g., pipelle, Karman cannula, aspiration catheter, or endometrial brush).

Conclusion

Clinical pregnancy, multiple pregnancy, and abortion rates did not differ significantly between the intervention group (hysteroscopic injury in the follicular phase before gonadotropin-induced IUI) and the control group (hysteroscopy only).

Conflict of interest

All of the authors declare that they do not have any conflicts of interest.

Acknowledgements

We would like to thank Fesih Aktar his assistance with the statistics used in this report.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Ethical approval

Ethical approval was obtained for this study from the Diyarbakır Gazi Yaşargil Training and Research Hospital Clinical Research Ethics Committee (Date: 05.10.2018, Number: 147).

References

- Wallach EE, Allen NC, Herbert CM, Maxson WS, Rogers BJ, Diamond MP, et al. Intrauterine insemination: a critical review. *Fertil Steril*. 1985 Nov;44(5):569-80. doi:10.1016/S0015-0282(16)48969-7.
- Alanya Tosun S, Ergun B, Gökmen Karasu AF, Özkaya E, Gürbüz T. The utility of detecting ovulation to predict success in ovulation induction and intrauterine insemination cycles - a prospective observational study. *Ginek Pol*. 2021;92(12):860-864. doi: 10.5603/GP.a2021.0131.
- Antamaria X, Simón C. Endometrial Factor in Unexplained Infertility and Recurrent Implantation Failure. *Semin Reprod Med*. 2021 Nov;39(5-06):227-232. doi: 10.1055/s-0041-1735199. Epub 2021 Aug 23.
- Achache H, Tsafir A, Prus D, Reich R, Revel A. Defective endometrial prostaglandin synthesis identified in patients with repeated implantation failure undergoing in vitro fertilization. *Fertil Steril*. 2010;94(4):1271-8. doi:10.1016/j.fertnstert.2009.07.1668.
- Donaghy M, Lessey BA. Uterine receptivity: alterations associated with benign gynecological disease. *Semin Reprod Med*. 2007;25(6):461-475. doi: 10.1055/s-2007-991044.
- Sahin Gulec E, Oztekin D, Yildirim Karaca S. Could laparoscopic cystectomy improve intrauterine insemination with controlled ovarian hyperstimulation outcomes in women with endometrioma? *Ginek Pol*. 2022;93(8):650-654. doi: 10.5603/GP.a2022.0040.
- Huang, S., Wang, C.-J., Soong, Y.-K., Wang, H.-S., Wang, M., Lin, C, et al. Site-specific endometrial injury improves implantation and pregnancy in patients with repeated implantation failures. *Reprod Biol Endocrinol*. 2011;9:140. doi: 10.1186/1477-7827-9-140.
- Dekel N, Gnainsky Y, Granot I, Mor G. Inflammation and implantation. *American Journal of Reproductive Immunology*. 2010 Jan;63(1):17-21. doi:10.1111/j.1600-0897.2009.00792.x.
- Haider S, Knöfler M. Human tumour necrosis factor: physiological and pathological roles in placenta and endometrium. *Placenta*. 2009 Feb;30(2):111-23. doi.org/10.1016/j.placenta.2008.10.012.
- Kalma, Y., Granot, I., Gnainsky, Y., Or, Y., Czernobilsky, B., Dekel, N, et al. Endometrial biopsy-induced gene modulation: first evidence for the expression of bladder-transmembranal uroplakin Ib in human endometrium. *Fertil Steril*. 2009;91(4):1042-9. doi: 10.1016/j.fertnstert.2008.01.043.
- Almog B, Shalom-Paz E, Dufort D, Tulandi T, et al. Promoting implantation by local injury to the endometrium. *Fertil Steril*. 2010;94(6):2026-9. doi: 10.1016/j.fertnstert.2009.12.075.
- World Health Organization. In: WHO laboratory manual for the examination of human sperm and sperm-cervical mucus interaction, 4th ed., New York, NY: Cambridge University Press; 1999, p. 4-33. 60-61.
- Colpi GM, Francavilla S, Haidl G, Link K, Behre HM, Goulis DG, Krausz C, Giwercman European Academy of Andrology guideline Management of oligo-astheno-teratozoospermia. *A. Andrology*. 2018 Jul;6(4):513-524. doi: 10.1111/andr.12502.
- Jasovic V, Jasovic-Siveska E. Success rate of intrauterine insemination in patients with unknown infertility. *Vojnosanit Pregl*. 2012;69(4):301-307. doi: 10.2298/vsp1204301j.
- Soliman BS, Harira M. Local endometrial scratching under ultrasound guidance after failed intrauterine insemination and cycle outcome: a randomized controlled trial. *Middle East Fertil Soc J*. 2017;22(1):60-6. doi.org/10.1016/j.mefs.2016.06.006.
- Wadhwa L, Pritam A, Gupta T, Gupta S, Arora S, Chandoke R. Effect of endometrial biopsy on intrauterine insemination outcome in controlled ovarian stimulation cycle. *J Hum Reprod Sci*. 2015;8(3):151-8. doi: 10.4103/0974-1208.165144.
- Bahaa Eldin AM, Abdelmaabud KH, Laban M, Hassanin AS, Tharwat AA, Aly TR, et al. Endometrial injury may increase the pregnancy rate in patients undergoing intrauterine insemination: an interventional randomized clinical trial. *Reprod Sci*. 2016;23(10):1326-31. doi: 10.1177/1933719116638191.
- Zarei A, Alborzi S, Dadras N, Azadi G. The effects of endometrial injury on intrauterine insemination outcome: a randomized clinical trial. *Iran J Reprod Med*. 2014;12(9):649-52.
- Goel T, Mahey R, Bhatla N, Kalaivani M, Pant S, Kriplani A. Pregnancy after endometrial scratching in infertile couples undergoing ovulation induction and intrauterine insemination cycles: a randomized controlled trial. *J Assist Reprod Genet*. 2017;34:1051-8.
- Ashrafi M, Tehraninejad ES, Haghiri M, Masomi M, Sadatmahalleh SJ, Arabipour A. The effect of endometrial scratch injury on pregnancy outcome in women with previous intrauterine insemination failure: a randomized clinical trial. *J Obstet Gynaecol Res*. 2017;43:1421-7.
- Abdelhamid AM. The success rate of pregnancy in IUI cycles following endometrial sampling: a randomized controlled study: endometrial sampling and pregnancy rates. *Arch Gynecol Obstet*. 2013;288:673-8. doi: 10.1007/s00404-013-2785-0.
- Maged AM, Al-Inany H, Salama KM, Souidan II, Abo Ragab HM, Elnassery N. Endometrial scratch injury induces higher pregnancy rate for women with unexplained infertility undergoing IUI with ovarian stimulation: a randomized controlled trial. *Reprod Sci*. 2016;23:239-43.
- Lensen SF, Manders M, Nastri CO, Gibreel A, Martins WP, Templer GE, Farquhar C. Endometrial injury for pregnancy following sexual intercourse or intrauterine insemination. *Cochrane Database Syst Rev*. 2021 Mar;18(3):CD011424. doi: 10.1002/14651858.CD011424.pub3
- Vitagliano A, Noventa M, Saccone G, Gizzo S, Vitale SG, Laganà AS, Litta PS, Saccardi C, Nardelli GB, Di Spiezio Sardo A. Endometrial scratch injury before intrauterine insemination: is it time to re-evaluate its value? Evidence from a systematic review and meta-analysis of randomized controlled trials. *Fertil Steril*. 2018 Jan;109(1):84-96.e4. doi: 10.1016/j.fertnstert.2017.09.021.



Investigating the effects of percussion massage therapy on pain, functionality, kinesiophobia, and quality of life in individuals with non-specific low back pain

Sema Gorguluer^a, Burak Menek^{b,*}

^aIstanbul Medipol University, Institute of Health Sciences, Department of Physiotherapy and Rehabilitation, Istanbul, Türkiye

^bIstanbul Medipol University, Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation, Istanbul, Türkiye

Abstract

Aim: This study aimed to investigate the effects of percussion massage therapy applied to the paravertebral muscles on pain, functionality, flexibility, kinesiophobia, and quality of life in individuals with mechanical low back pain.

Materials and Methods: The study enrolled 40 individuals diagnosed with non-specific low back pain. Participants were randomly assigned to one of two groups: percussion massage therapy (PMT) or conventional therapy (CT). Both groups participated in a 6-week treatment program. Pain levels were evaluated using the Visual Analog Scale (VAS), functionality was assessed with the Oswestry Disability Scale (ODS), flexibility was measured via the Sit-and-Reach Test, kinesiophobia was assessed using the Tampa Scale for Kinesiophobia (TSK), and quality of life was measured with the Short Form-36 (SF-36). Assessments were performed both before and after the treatment period. The CT group engaged in exercises focused on strengthening, stabilization, and stretching of the lumbar region, while the PMT group received percussion massage therapy targeting the paravertebral muscles in addition to conventional physiotherapy.

Results: Post-treatment, both groups significantly improved all assessment parameters ($p < 0.05$). However, the PMT group demonstrated greater effectiveness compared to the CT group in terms of ODS, TSK, VAS, Sit-and-Reach Test, and the physical role limitation, emotional function, social function, pain, and general health subscales of the SF-36 ($p < 0.05$).

Conclusion: This study concluded that incorporating percussion massage therapy, a novel approach in literature, into the rehabilitation program for individuals with mechanical low back pain may be an effective treatment addition.

ARTICLE INFO

Keywords:

Exercise
Mechanical low back pain
Local vibration
Percussion massage therapy

Received: Aug 26, 2024

Accepted: Jan 27, 2025

Available Online: 26.02.2025

DOI:

[10.5455/annalsmedres.2024.08.177](https://doi.org/10.5455/annalsmedres.2024.08.177)



Copyright © 2025 The author(s) - Available online at www.annalsmedres.org. This is an Open Access article distributed under the terms of Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

Introduction

Non-specific low back pain (NLBP) is a significant health issue and one of the leading causes of chronic pain problems in adults, with no established specific rehabilitation program for its treatment. Epidemiological studies have shown that psychosocial and work-related factors play a significant role in the occurrence and recurrence of low back pain [1]. Low back pain is a common condition with various etiologies, affecting an estimated 70-80% of the global population. The majority of these cases (approximately 95%) are attributed to mechanical causes [2]. Unlike other types of low back pain, mechanical low back pain typically worsens with movement and improves with rest. Causes include poor posture, excessive strain, and

structural predisposition. Non-mechanical causes of low back pain, such as infections, tumors, fractures, metabolic disorders, and referred pain from internal organs, must be excluded before a diagnosis of mechanical low back pain can be made [3]. Medical interventions and physiotherapy are frequently employed in the management of NLBP, with the primary goal of both approaches being to optimize pain relief and improve the patient's quality of life [4]. A systematic review found that, among pharmacological interventions for NSLBP, NSAIDs and muscle relaxants offer the most favorable risk-benefit ratio for pain and disability management. However, exercise and manual therapy are considered more effective first-line treatments and should be prioritized over pharmacological options [5]. Exercise therapy is an effective treatment for NLBP, with studies demonstrating its ability to reduce chronic pain and improve functional capacity. Recommended exercises in-

*Corresponding author:

Email address: bmenek@medipol.edu.tr (Burak Menek)

clude strengthening, flexibility, and stabilization exercises [6]. Vibration therapy has become increasingly common in rehabilitation settings over the past decade. This therapy can be applied using WBV platforms, which deliver vibrations to the entire body, or through localized percussion massage devices for more targeted treatment [7]. Percussion massage therapy (PMT) is a widely used myofascial intervention that has been shown to reduce pain, increase blood circulation, enhance scar tissue healing, reduce muscle spasms, improve lymphatic drainage, inhibit the Golgi tendon reflex, and increase joint range of motion [8]. Despite their growing popularity among athletes and therapists, the evidence supporting the purported benefits of percussion massage devices remains limited. These devices, which combine traditional massage with vibration, are thought to enhance flexibility, performance, and recovery, but further research is needed to establish their definitive effects [9]. Percussion massage devices, typically powered by electricity or batteries, deliver rapid, compressive forces to myofascial tissue using a variety of attachments. Popular brands like Theragun and Hypervolt offer devices for both self-massage and professional use. These devices operate at varying frequencies, with some models reaching up to 53 Hz, and offer interchangeable heads to target specific tissues [10]. A review of the literature indicates that studies on percussion massage therapy have predominantly involved healthy individuals and athletes, with a primary focus on assessing the effects of this therapy on flexibility, muscle strength, and range of motion [11]. Percussion massage devices are increasingly used in sports and clinical settings, but there is a gap between their popularity and the available evidence to guide their use. Limited research, a lack of clinical practice guidelines, and scarce information on how health professionals utilize these devices contribute to this gap. To date, most studies have focused on the effects of percussion massage therapy on joint range of motion and muscle stiffness [9,11–13]. This study investigated the effects of adding percussion massage therapy to a conventional exercise program for the management of NSLBP. We hypothesized that this combined approach would be superior to exercise alone in improving pain, flexibility, kinesiophobia, functionality, and quality of life.

Materials and Methods

Study design and participants

This single-blind, randomized controlled trial included 42 participants with non-specific low back pain (NSLBP) recruited from Yeni Huzur Hospital. The study protocol was approved by the Non-interventional Ethics Committee at Istanbul Medipol University (Approval Number: E-10840098-202.3.02-238/3), and all participants provided written informed consent. Participants were randomly assigned (1:1 ratio) to either a percussion massage therapy (PMT) group or a conventional therapy (CT) group using randomizer.org. A physical medicine and rehabilitation specialist diagnosed NSLBP based on clinical evaluation and exclusion of "red flag" indicators. Individuals with specific pathologies (e.g., disc herniation, spinal stenosis, fractures) or those who had received treatment for low back pain within the past six months were excluded. As-

sessments were conducted before and after the 6-week intervention period. The study flow diagram is presented in Figure 1.

Conventional therapy group

All participants received a standardized treatment program consisting of stretching and strengthening exercises, transcutaneous electrical nerve stimulation (TENS), and ultrasound (US) therapy. TENS was applied to the paravertebral muscles at 100 Hz for 20 minutes, while US was administered to the lumbar region at 1.5 W/cm² for 6 minutes. Stretching exercises targeted the quadratus lumborum, hamstrings, multifidus, and iliocostalis muscles, with each stretch held for 15 seconds and repeated five times. Strengthening exercises focused on the hip extensors, lumbar extensors, and abdominal muscles, consisting of three sets of 10 repetitions. The complete program was conducted over 6 weeks, with three sessions per week, and was tailored to individual tolerance and progression under the supervision of a physiotherapist.

Percussion massage therapy group

In addition to the conventional therapy program, the PMT group received percussion massage therapy using a Hypervolt device (Hyperice, CA, USA). The treatment was applied to the paravertebral muscles at 40 Hz and medium intensity (level 2) for 5 minutes per side, twice a week for 6 weeks, following the manufacturer's instructions [11,14,15] (Figure 2). The soft-top attachment (number 5 head) was used in a distal-to-proximal direction, with gentle skin contact and no additional pressure. This intervention was performed prior to the exercise program with participants in the prone position. After the study, participants in the CT group were offered the option to receive PMT.

Outcome measures

Outcome measurements were conducted before and after the 6-week treatment period by an independent, experienced physiotherapist blinded to group allocation.

Pain

Participants' pain levels during activity were assessed using the Visual Analog Scale (VAS), a self-reported measure of pain intensity. Participants marked their pain level on a 10-cm line, with 0 indicating "no pain" and 10 representing "unbearable pain." Higher VAS scores correspond to greater pain intensity [16,17].

Functionality

The Oswestry Disability Index (ODI) was used to assess functionality. This self-reported questionnaire evaluates the impact of low back pain on daily activities. It consists of ten items, each with six response options ranging from 0 to 5, with higher scores indicating greater disability. The total score is calculated as: ((total score / 10) x 5) x 100, with a maximum possible score of 50 [18].

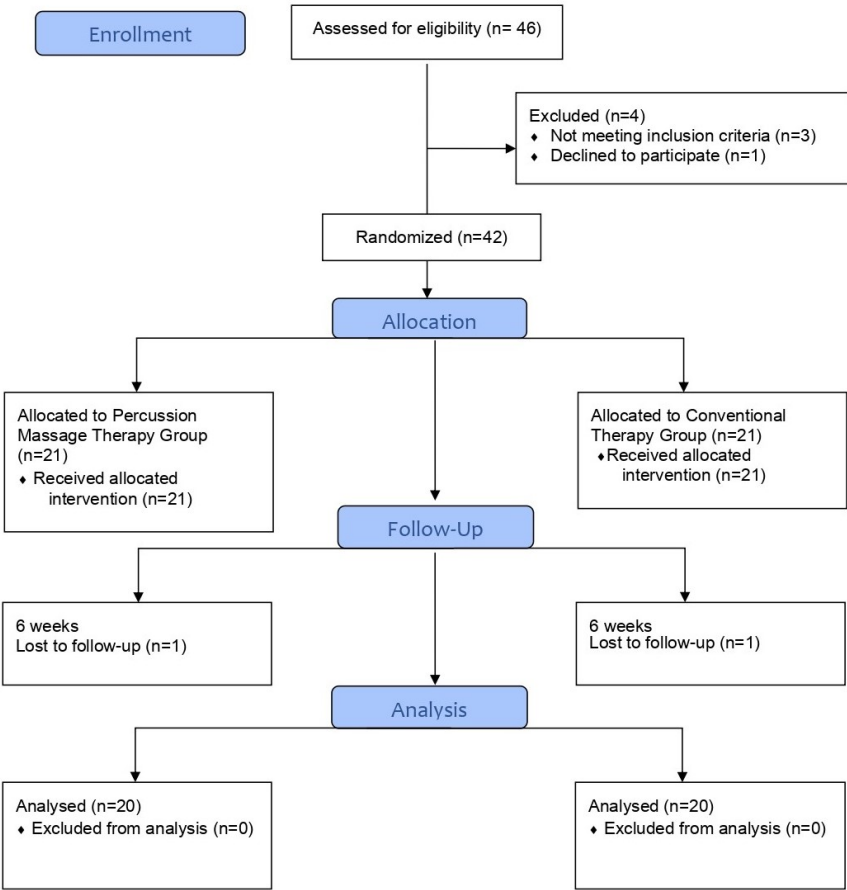


Figure 1. Design and flow chart of the study.



Figure 2. Percussion massage therapy.

Flexibility

The Sit-and-Reach Test was used to assess flexibility. Participants sat on the floor with their feet flat against a testing bench and reached forward as far as possible while keeping their knees extended. The distance reached beyond the edge of the bench was recorded in centimeters (positive values for distances beyond the bench, negative values for distances short of the bench). The highest of two attempts was recorded [19,20] (Figure 3).

Kinesiophobia

Kinesiophobia was assessed using the TSK, a 17-item questionnaire designed to evaluate fear of movement in individuals with chronic musculoskeletal pain. Responses are scored on a 4-point Likert scale (1 = strongly disagree to 4 = strongly agree), with a total score ranging from 17 to 68. Higher scores indicate greater kinesiophobia [21,22].



Figure 3. Sit and reach test.

Quality of life

The SF-36, a widely used health survey, was used to assess overall health status and quality of life. This tool evaluates eight health domains: physical functioning, social functioning, role limitations (physical and emotional), mental health, vitality, pain, and general health perception. Each domain is scored from 0 to 100, with higher scores indicating better health and functional capacity [23,24].

Statistical analysis

This study hypothesized that adding percussion massage therapy (PMT) to a conventional exercise program would be more effective than conventional therapy alone in managing non-specific low back pain (NSLBP) by improving pain, flexibility, kinesiophobia, functionality, and quality of life. Using G*Power, a sample size of 36 participants was estimated to achieve 80% power with an effect size of 0.25 ($\alpha = 0.05$, $1-\beta = 0.80$) [25]. To account for potential dropout, the study was initiated with 42 participants (21 per group). Data were analyzed using IBM SPSS Statistics 26.0 (IBM Corp., Armonk, NY, USA). Skewness and kurtosis values were within the acceptable range for normal distribution (± 2.0 for skewness, < 7.0 for kurtosis), thus parametric tests were used [26]. Categorical characteristics were compared using chi-square tests (Pearson chi-square/Fisher’s exact test). Independent samples t-tests were used to compare baseline characteristics and post-treatment outcomes between groups. Paired samples t-tests were used for within-group comparisons. Statistical significance was set at $p < 0.05$.

Results

Forty participants with NSLBP completed this study, with 20 in the PMT group and 20 in the CT group. The groups were comparable in terms of demographic characteristics (Table 1). Both groups showed significant improvements in all outcome measures after treatment ($p < 0.05$) (Table 2). No treatment-related complications occurred. However, one participant in each group withdrew due to unrelated medical or logistical reasons. Between-group comparisons revealed that the PMT group experienced significantly

greater improvements in most measured parameters, including the ODI, TSK, VAS, and Sit-and-Reach Test, as well as the physical role limitation, emotional function, social function, pain, and general health subscales of the SF-36 (Table 3). No significant differences were found between groups for the physical function, vitality, and mental health components of the SF-36 ($p < 0.05$).

Discussion

This study compared the effects of conventional exercise alone versus conventional exercise combined with percussion massage therapy (PMT) on pain, flexibility, kinesiophobia, functionality, and quality of life in individuals with mechanical low back pain. Both groups showed improvements in all parameters after treatment. However, the PMT group demonstrated significantly greater improvements in pain, flexibility, disability, kinesiophobia, and several aspects of quality of life.

These findings are consistent with previous research on vibration therapy for low back pain. Jung et al. found that core stabilization exercises combined with vibration were more effective in reducing pain in adolescents with mechanical low back pain [27]. Similarly, studies have shown that whole-body vibration combined with exercise is more effective in reducing pain in individuals with lumbar instability [28]. Mansuri et al. also reported that vibration therapy using a Theragun device effectively reduced low back pain in bus drivers [29]. This study demonstrated that adding percussion massage therapy (PMT) to a conventional exercise program resulted in greater improvements in pain, flexibility, disability, kinesiophobia, and several aspects of quality of life compared to conventional therapy alone in individuals with mechanical low back pain. These findings are consistent with previous research on vibration therapy for low back pain. For example, Jung et al. found that core stabilization exercises combined with vibration were more effective in reducing pain in adolescents with mechanical low back pain. Similarly, whole-body vibration combined with exercise has been shown to be more effective than exercise alone in reducing pain in individuals with lumbar instability. Furthermore, Mansuri et al. reported that vibration therapy using a Theragun device effectively reduced low back pain in bus drivers. Yang et al. [15] also found that six weeks of percussion massage therapy reduced pain and improved functionality in firefighters with chronic non-specific low back pain, concluding that it is an effective and safe treatment strategy [15]. Similarly, in our study, the reduction in pain levels in the PMT group was more pronounced and statistically significant, consistent with the literature. Pozo-Cruz et al. indicated that 20 Hz vibration therapy administered over 12 weeks was an effective treatment for improving lumbar region functionality in individuals with mechanical low back pain [30]. Welling and colleagues [31] demonstrated the effectiveness of short-term PMT in reducing pain and improving lumbar function in individuals with NSLBP. Importantly, they found that PMT’s benefits were not solely attributable to a placebo effect, suggesting a genuine therapeutic impact [31]. In another study, 30 Hz vibration therapy applied to office workers with chronic low back pain was reported to contribute more to improv-

Table 1. Distribution of demographic and physical characteristics of the study population.

Variables	PMT (mean±SD)	CT (mean±SD)	p-value
Age (years)	48.65 ± 6.05	47.10 ± 4.54	0.366
(Min-Max)	48.00 (40-60)	46.50 (41-56)	
Gender, n (%)			0.749
Male	8 (40%)	9 (45%)	
Female	12 (60%)	11 (55%)	
Height (cm)	166.65 ± 8.67	171.45 ± 7.66	0.071
(Min-Max)	168.00 (150-186)	172.00 (155-183)	
Weight (kg)	73.95 ± 10.90	73.35 ± 11.02	0.863
(Min-Max)	75.00 (48-89)	75.50 (55-89)	

PMT, Percussion massage therapy group; CT: conventional therapy, SD: standard deviation, kg: kilogram, cm: centimeter.

Table 2. Comparison of changes in outcome measures within and between the groups.

Variables	PMT (mean±SD)		p-value	Cohen d (%95 CI)	CT (mean±SD)		p-value	Cohen d (%95 CI)
	Pre	Post			Pre	Post		
VAS	6.30±0.92	2.40±1.35	<0.001 ^t	4.277 (2.853; 5.688)	5.85±0.81	2.45±1.05	<0.001 ^t	3.852 (2.555; 5.137)
ODS	36.53±10.17	13.62±5.09	<0.001 ^t	2.509 (1.596; 3.406)	30.53±8.22	15.79±4.93	<0.001 ^t	2.481 (1.576; 3.370)
Sit and Reach Test	12.80±6.41	6.25±7.45	<0.001 ^t	-2.814 (-3.796; -1.816)	8.05±6.65	5.60±6.96	<0.001 ^t	-1.806 (-2.516; -1.078)
TKS	44.65±6.70	32.75±6.18	<0.001 ^t	2.001 (1.224; 2.761)	41.60±7.42	34.15±8.49	<0.001 ^t	1.622 (0.939; 2.286)
SF-36/ Physical Function	65.00±18.85	85.00±9.46	<0.001 ^t	-1.605 (-2.266; -0.926)	52.75±15.17	72.25±13.81	<0.001 ^t	-1.622 (-2.287; -0.939)
SF-36/ Role Physical	22.50±21.31	63.92±23.43	<0.001 ^t	-2.826 (-3.812; -1.825)	30.63±21.64	55.00±24.80	<0.001 ^t	-1.528 (-2.170; -0.867)
SF-36/ Bodily Pain	29.00±17.29	59.50±14.11	<0.001 ^t	-2.017 (-2.781; -1.235)	34.00±15.90	54.13±16.33	<0.001 ^t	-2.057 (-2.832; -1.265)
SF-36/ General Health	40.75±15.75	59.13±14.49	<0.001 ^t	-1.669 (-2.345; -0.975)	36.75±15.75	47.80±12.56	<0.001 ^t	-1.494 (-2.129; -0.841)
SF-36/ Vitality	45.13±8.05	55.38±10.83	<0.001 ^t	-1.277 (-1.864; -0.673)	43.25±15.41	52.50±16.90	<0.001 ^t	-1.628 (-2.294; -0.943)
SF-36/ Social Function	38.00±18.22	64.48±15.23	<0.001 ^t	-1.840 (-2.559; -1.104)	35.00±12.57	53.75±12.89	<0.001 ^t	-2.471 (-3.357; -1.569)
SF-36/ Role Emotional	32.48±30.80	73.30±25.61	<0.001 ^t	-1.669 (-2.345; -0.975)	36.63±23.92	63.30±26.28	<0.001 ^t	-1.299 (-1.890; -0.690)
SF-36/ Mental Health	52.20±6.42	61.80±10.76	<0.001 ^t	-1.133 (-1.689; -0.558)	52.40±10.37	58.60±14.29	<0.001 ^t	-0.569 (-1.036; -0.089)

PMT: percussion massage therapy, CT: conventional therapy; VAS: visual analog scale, ODS: oswestry disability scale, TKS: tampa kinesiphobia scale, SF-36: short form-36, SD: standart deviation. P-values were calculated using the Independent Samples t-test, with $p < 0.05$ deemed significant.

ing functionality compared to a conventional exercise program, suggesting potential benefits of vibration therapy as an adjunct to exercise [32]. Our findings regarding the functional improvements associated with PMT are consistent with some studies in the literature. For example, Jung et al. found that core stabilization exercises combined with vibration were more effective in reducing pain and improving function in adolescents with mechanical low back pain. Similarly, whole-body vibration combined with exercise has been shown to be more effective than exercise alone in improving function in individuals with lumbar instability. However, Özsoy et al. [25] found no significant difference between exercise alone and exercise combined with vibration therapy in older adults with mechanical low back pain, although both groups experienced functional improvement. This discrepancy may be due to age-related differences in response to vibration therapy or differences in the specific vibration protocols used [25]. Another study reported that vibration therapy applied at 20 Hz for 2 minutes twice a week had a similar effect on improving functionality levels in individuals with chronic low back pain compared to conventional methods. The different results observed in these studies compared to ours

may be attributed to the limited application of vibration therapy, only 2 minutes twice a week, and the insufficient duration of the treatment [33]. The fear of movement is another factor influencing the outcomes of parameters assessed in mechanical low back pain [34]. In a study by Bunzli et al, in kinesiphobia was more prevalent in individuals with mechanical low back pain. The main reason for this belief is the fear that engaging in painful activities may lead to further damage or that the pain experienced during these activities could worsen functional limitations and increase discomfort. Relieving the symptoms of mechanical low back pain can reduce both kinesiphobia and anxiety related to pain [35]. Another study reported that local vibration therapy is effective in improving impaired proprioception and alleviating low back pain in patients with chronic low back pain [36]. In a systematic review conducted by Zafer and colleagues, it was reported that vibration therapy positively affects pain, functionality, and the proprioceptive system in individuals with non-specific chronic low back pain [37]. The findings of this study suggest that vibration therapy also enhances the proprioceptive mechanism. In our study, the vibration therapy was administered using a Percussion Massage Gun. We

Table 3. Differences within groups before and after treatment and comparison of differences between groups.

Variables	PMT (mean±SD)	CT (mean±SD)	p-value	Cohen d (%95 CI)
VAS	3.90±0.91	3.40±0.88	0.046 ^t	0.557 (1.186; 0.079)
ODS	22.91±9.13	14.74±5.94	0.002 ^t	1.061 (1.718; 0.391)
Sit and Reach Test	6.55±2.33	2.45±1.36	<0.001 ^t	2.152 (1.358; 2.929)
TKS	11.90±5.95	7.45±4.59	0.012 ^t	0.838 (1.480; 0.185)
SF-36/ Physical Function	20.00±12.46	19.50±12.02	0.898 ^t	0.041 (-0.579; 0.660)
SF-36/ Role Physical	41.42±14.65	24.38±15.95	0.001 ^t	1.112 (0.438; 1.774)
SF-36/ Bodily Pain	30.50±15.12	20.13±9.78	0.014 ^t	0.815 (0.164; 1.456)
SF-36/ General Health	18.38±11.01	11.05±7.39	0.018 ^t	0.781 (0.132; 1.420)
SF-36/ Vitality	10.25±8.03	9.25±5.68	0.652 ^t	0.144 (-0.478; 0.763)
SF-36/ Social Function	26.48±14.39	18.75±7.59	0.040 ^t	0.672 (0.030; 1.305)
SF-36/ Role Emotional	40.82±24.46	26.67±20.52	0.048 ^t	0.627 (0.012; 1.259)
SF-36/ Mental Health	9.60±8.48	6.20±10.89	0.278 ^t	0.348 (-0.279; 0.971)

PMT: percussion massage therapy, CT: conventional therapy; VAS: visual analog scale, ODS: oswestry disability scale, TKS: tampa kinesiophobia scale, SF-36: short form-36, SD: standart deviation. P-values were calculated using the Paired Samples t-test, with p<0.05 considered significant.

hypothesize that the observed improvement in kinesiophobia in the PMT group is related to the enhancement of proprioceptive mechanisms by PMT, which can positively influence the reduction of fear of movement.. While research on the effects of vibration therapy on kinesiophobia is limited, this study explored the impact of PMT on fear of movement. We observed improvements in kinesiophobia scores with both PMT and conventional therapy, suggesting that symptom reduction may be associated with decreased kinesiophobia. This is consistent with Konrad et al. [38], who reported that 5 minutes of percussion massage using a massage gun increased range of motion and may have indirectly influenced kinesiophobia by improving physical function. Similarly, a study by Skinner and colleagues have stated that PMT therapy can be used to improve range of motion following a reduction in tissue stiffness [39]. A systematic analysis by Remer et al. [40] noted that vibration therapy at 30 Hz was more effective in enhancing proprioception compared to high-frequency vibration therapy at 50 Hz. Additionally, they suggested that the pain reduction achieved through vibration could improve functionality and potentially reduce kinesiophobia.

This is a thorough discussion of your study's findings, potential mechanisms, and limitations. However, it could benefit from some restructuring and conciseness. Here's a revised version:

In our study, both groups showed reduced kinesiophobia after treatment, but the reduction was more pronounced in the PMT group. This may be attributed to PMT's enhancement of proprioceptive mechanisms. The localized vibration from PMT may stimulate the tonic vibration reflex, enhancing motor control and neural adaptation. This, in turn, could lead to improved proprioceptive feedback, potentially reducing fear of movement and increasing confidence in physical abilities. Additionally, vibration therapy may desensitize pain receptors, modulate nociceptive input, and promote endorphin release, contributing to pain reduction. Furthermore, by stimulating mechanoreceptors like muscle spindles and Golgi tendon organs, PMT may improve proprioceptive feedback, further reducing fear of movement and enhancing range of motion and flexibility.

Compared to conventional therapy, which primarily involves manual techniques, PMT offers a more dynamic approach by simultaneously addressing pain, flexibility, and proprioception. The multifaceted nature of PMT, coupled

with the neural and physiological adaptations induced by vibration therapy, may explain its superior effectiveness in reducing kinesiophobia. Importantly, PMT was well-tolerated in our study, with no adverse events reported, supporting its safety and feasibility in clinical practice.

Limitations

This study has limitations, including the small sample size, single-center design, lack of long-term follow-up, and the absence of a placebo control group. Future studies with larger, more diverse samples, long-term follow-up assessments, and a placebo control are needed to confirm these findings and further evaluate the efficacy and sustained impact of PMT.

Conclusion

The findings demonstrated that both treatment approaches significantly improved pain, flexibility, kinesiophobia, functionality, and quality of life. Notably, PMT proved more effective than conventional therapy, particularly in enhancing functionality, reducing kinesiophobia and pain, improving flexibility, and positively impacting the physical role limitation, emotional function, social function, pain, and general health subscales of the SF-36. These results align with existing literature and suggest that integrating percussion massage therapy into rehabilitation programs for individuals with mechanical low back pain could offer considerable benefits. Further research is needed to examine the long-term effects of percussion massage therapy and other therapeutic modalities in larger randomized controlled trials.

Ethical approval

Ethical approval was obtained for this study from Istanbul Medipol University Non-Interventional Clinical Research Ethics Committee (Date: 04.01.2024, Decision no: 3).

References

- Karadağ M, Çalışkan N, İşeri Ö (pekin), Sarıtaş S. Kronik Bel Ağrısı Olan Hastaların Kullanmış Oldukları Tamamlayıcı Tedavi Yöntemlerinin ve Hastalık Algılarının İncelenmesi. *HUHEM-FAD*. 2016 Dec 23;3(2):14–27.
- Altınbilek T, Çolak TK, Dereli EE, Pehlivan Y, Çavun SS. Mekanik özellikte kronik bel ağrısı olan hastaların tedavisinde bel ağrısı okulu programının etkinliği. *Marmara Med J*. 2014 May 20;27(2):107–11.
- Kinkade S. Evaluation and treatment of acute low back pain. *Am Fam Physician*. 2007 Apr 15;75(8):1181–8.
- Gökoğlu G, Ekici G. Mekanik Bel Ağrısı Olan Bireylerde 'Activity Pacing' Yaklaşımının Özür, Aktivite-Dinlenme Dengesi ve Yaşam Kalitesine Etkisinin İncelenmesi. *Ergoterapi ve Rehabilitasyon Dergisi*. 2021 Mar 16;9(1):15–20.
- Gianola S, Barger S, Castillo GD, Corbetta D, Turolla A, Andreano A, et al. Effectiveness of treatments for acute and sub-acute mechanical non-specific low back pain: a systematic review with network meta-analysis. *Br J Sports Med*. 2022 Jan 1;56(1):41–50.
- Hayden J, van Tulder MW, Malmivaara A, Koes BW. Exercise therapy for treatment of non-specific low back pain. *Cochrane Database Syst Rev*. 2005 Jul 20;2005(3):CD000335.
- Rittweger J. Vibration as an exercise modality: how it may work, and what its potential might be. *Eur J Appl Physiol*. 2010 Mar;108(5):877–904.
- Menek MY, Menek B. Effects of percussion massage therapy, dynamic stretching, and static stretching on physical performance and balance. *J Back Musculoskelet Rehabil*. 2024;37(1):183–93.
- Konrad A, Glashüttner C, Reiner MM, Bernsteiner D, Tilp M. The Acute Effects of a Percussive Massage Treatment with a Hypervolt Device on Plantar Flexor Muscles' Range of Motion and Performance. *J Sports Sci Med*. 2020 Dec;19(4):690–4.
- Davis HL, Alabed S, Chico TJA. Effect of sports massage on performance and recovery: a systematic review and meta-analysis. *BMJ Open Sport Exerc Med*. 2020;6(1):e000614.
- Sams L, Langdown BL, Simons J, Vseteckova J. The Effect Of Percussive Therapy On Musculoskeletal Performance And Experiences Of Pain: A Systematic Literature Review. *International Journal of Sports Physical Therapy*. 2023;18(2):309–27.
- Menek B, Tayboga UI. Examining acute impacts of soft tissue mobilization, percussion massage, and dynamic stretching on hamstring tightness. *Gazz Med Ital - Arch Sci Med*. 4(183):296–304.
- Cheatham SW, Baker RT, Behm DG, Stull K, Kolber MJ. Mechanical Percussion Devices: A Survey of Practice Patterns Among Healthcare Professionals. *Int J Sports Phys Ther*. 2021;16(3):766–77.
- Patel R, Patel A. Effect of Theragun on the improvement of back flexibility: A case study. *J Appl Dent Med Sci*. 2020;19(5):15–6.
- Yang C, Li Y, Sucharit W, Eungpinichpong W, Huang X. Effects of percussive massage therapy on fascia echo intensity and fascia thickness in firefighters with chronic non-specific low back pain: a randomized controlled trial. *BMC Complement Med Ther*. 2024 Nov 8;24(1):390.
- Hawker GA, Mian S, Kendzerska T, French M. Measures of adult pain: Visual Analog Scale for Pain (VAS Pain), Numeric Rating Scale for Pain (NRS Pain), McGill Pain Questionnaire (MPQ), Short-Form McGill Pain Questionnaire (SF-MPQ), Chronic Pain Grade Scale (CPGS), Short Form-36 Bodily Pain Scale (SF-36 BPS), and Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP). *Arthritis Care Res (Hoboken)*. 2011 Nov;63 Suppl 11:S240–252.
- Yaray O, Akesen B, Ocaklıoğlu G, Aydinli U. Validation of the Turkish version of the visual analog scale spine score in patients with spinal fractures. *Acta Orthop Traumatol Turc*. 2011;45(5):353–8.
- Yakut E, Düğer T, Oksüz C, Yörükan S, Ureten K, Turan D, et al. Validation of the Turkish version of the Oswestry Disability Index for patients with low back pain. *Spine (Phila Pa 1976)*. 2004 Mar 1;29(5):581–5; discussion 585.
- Choobsaz H, Ghotbi N, Mohamadi P. Comparison Between the Effects of Transfer Energy Capacitive and Resistive Therapy and Therapeutic Ultrasound on Hamstring Muscle Shortness in Male Athletes: A Single-Blind Randomized Controlled Trial. *Galen Med J*. 2023;12:1–9.
- Lemmink KAPM, Kemper HCG, Greef MHG, Rispens P, Stevens M. The Validity of the Sit-and-Reach Test and the Modified Sit-and-Reach Test in Middle-Aged to Older Men and Women. *Research Quarterly for Exercise and Sport*. 2003 Sep;74(3):331–6.
- Chen Y, Zhong G, Zhong S, Lin J, Lin Y. Effect of kinesiophobia on postoperative rehabilitation outcomes in patients with cervical spondylotic myelopathy: a cross-sectional study. *J Orthop Surg Res*. 2024 Aug 9;19(1):469.
- Yılmaz ÖT, Yakut Y, Uygur F, Uluğ N. Tampa Kinezyofobi Ölçeği'nin Türkçe versiyonu ve test-tekrar test güvenilirliği. *Fizyoterapi Rehabilitasyon*. 2011;22(1):44–9.
- White MK, Maher SM, Rizio AA, Bjorner JB. A meta-analytic review of measurement equivalence study findings of the SF-36® and SF-12® Health Surveys across electronic modes compared to paper administration. *Qual Life Res*. 2018 Jul;27(7):1757–67.
- Koçyiğit H, Aydemir Ö, Fişek G, Ölmez N, Memiş AK. Form-36 (KF-36)'nın Türkçe versiyonunun güvenilirliği ve geçerliliği. *İlaç ve Tedavi Dergisi*. 1999;12(2):102–6.
- Ozsoy G, Ilcin N, Ozsoy I, Gurpinar B, Buyukturan O, Buyukturan B, et al. The Effects Of Myofascial Release Technique Combined With Core Stabilization Exercise In Elderly With Non-Specific Low Back Pain: A Randomized Controlled, Single-Blind Study. *Clin Interv Aging*. 2019;14:1729–40.
- Kim HY. Statistical notes for clinical researchers: assessing normal distribution (2) using skewness and kurtosis. *Restor Dent Endod*. 2013 Feb;38(1):52–4.
- Jung KS, Jung JH, In TS, Cho HY. The Effectiveness of Trunk Stabilization Exercise Combined with Vibration for Adolescent Patients with Nonspecific Low Back Pain. *Int J Environ Res Public Health*. 2020 Sep 25;17(19):7024.

28. Park SH, Oh YJ, Seo JH, Lee MM. Effect of stabilization exercise combined with respiratory resistance and whole body vibration on patients with lumbar instability: A randomized controlled trial. *Medicine (Baltimore)*. 2022 Nov 18;101(46):e31843.
29. Mansuri U, Patel S. Effectiveness of Theragun and Ergonomic Advice in Patients with Low Back Pain among Bus Drivers - A Randomized Controlled Trial. *International Journal of Science and Research (IJSR)*. 2021 Sep 27;10:50–3.
30. del Pozo-Cruz B, Hernández Mocholí MA, Adsuar JC, Parraca JA, Muro I, Gusi N. Effects of whole body vibration therapy on main outcome measures for chronic non-specific low back pain: a single-blind randomized controlled trial. *J Rehabil Med*. 2011 Jul;43(8):689–94.
31. Welling A, Kage V, Gurudut P, Patil C, Diniz W, Fernandes L, et al. Investigating the Effects of Percussion Therapy on Ultrasonography-Diagnosed Thoracolumbar Fascia on Trigger Points and Hyperlordosis in Low Back Pain: A Placebo-Controlled Trial. *Altern Ther Health Med*. 2024 Sep;30(9):16–22.
32. Kaeding TS, Karch A, Schwarz R, Flor T, Wittke TC, Kück M, et al. Whole-body vibration training as a workplace-based sports activity for employees with chronic low-back pain. *Scand J Med Sci Sports*. 2017 Dec;27(12):2027–39.
33. Wegener V, Rarack S, Tiffe T, Grill E, Melcher C, Birkenmaier C, et al. Effects of Whole Body Vibration Therapy and Classic Physiotherapy on Postural Stability in People With Back Pain: A Randomized Trial. *Clin Spine Surg*. 2019 May;32(4):E214–20.
34. Sm AO, Rm N, S AA, N AS. The role of anticipation and fear of pain in the persistence of avoidance behavior in patients with chronic low back pain. *Spine*. 2000 Jan 5;25(9).
35. Bunzli S, Smith A, Watkins R, Schütze R, O’Sullivan P. What Do People Who Score Highly on the Tampa Scale of Kinesiophobia Really Believe?: A Mixed Methods Investigation in People With Chronic Nonspecific Low Back Pain. *Clin J Pain*. 2015 Jul;31(7):621–32.
36. Sakai Y, Morita Y, Kawai K, Fukuhara J, Ito T, Yamazaki K, et al. Targeted vibratory therapy as a treatment for proprioceptive dysfunction: Clinical trial in older patients with chronic low back pain. *PLoS One*. 2024;19(7):e0306898.
37. Zafar T, Zaki S, Alam MF, Sharma S, Babkair RA, Nuhmani S, et al. Effect of Whole-Body Vibration Exercise on Pain, Disability, Balance, Proprioception, Functional Performance and Quality of Life in People with Non-Specific Chronic Low Back Pain: A Systematic Review and Meta-Analysis. *J Clin Med*. 2024 Mar 13;13(6):1639.
38. Konrad A, Glashüttner C, Reiner MM, Bernsteiner D, Tilp M. The Acute Effects of a Percussive Massage Treatment with a Hypervolt Device on Plantar Flexor Muscles’ Range of Motion and Performance. *J Sports Sci Med*. 2020 Nov 19;19(4):690–4.
39. Skinner B, Dunn L, Moss R. The Acute Effects of Theragun™ Percussive Therapy on Viscoelastic Tissue Dynamics and Hamstring Group Range of Motion. *Journal of Sports Science & Medicine*. 2023;22(3):496.
40. Remer F, Keilani M, Kull P, Crevenna R. Effects of whole-body vibration therapy on pain, functionality, postural stability, and proprioception in patients with subacute and chronic non-specific low back pain: a systematic review. *Wien Med Wochenschr*. 2023;175(3). Doi: 10.1007/s10354-023-01026-4.



Ann Med Res

Current issue list available at AnnMedRes

Annals of Medical Research

journal page: www.annalsmedres.org

Effects of sertraline on episodic memory in experimental model of chronic mild stress model of depression

Hatice Solak^{a,*}, Zulfikare Isik Solak Gormus^b, Raviye Ozen Koca^b, Ayse Ozdemir^b,
 Mehmet Sinan Iyisoy^c, Ercan Kurar^d, Selim Kutlu^b

^aKütahya Health Science University, Faculty of Medicine, Department of Physiology, Kütahya, Türkiye

^bNecmettin Erbakan University, Meram Faculty of Medicine, Department of Physiology, Konya, Türkiye

^cNecmettin Erbakan University, Meram Faculty of Medicine, Department of Medical Education and Information, Konya, Türkiye

^dNecmettin Erbakan University, Meram Faculty of Medicine, Department of Medical Biology, Konya, Türkiye

ARTICLE INFO

Keywords:

BDNF

Hippocampus

Nestin

NeuN

Novel object recognition test

Received: Dec 02, 2024

Accepted: Feb 05, 2025

Available Online: 26.02.2025

DOI:

[10.5455/annalsmedres.2024.12.257](https://doi.org/10.5455/annalsmedres.2024.12.257)

Abstract

Aim: Individuals with depression have cognitive deficits, including diminished thinking and concentration ability, as well as memory difficulties. Certain antidepressants used for depression are recognized to influence cognitive functions, including learning and memory positively. We aimed to examine the impact of sertraline on hippocampus cell proliferation and cognitive functions, including learning and memory, within a chronic mild stress (CMS) model.

Materials and Methods: 48 rats were divided into four groups: C, CMS, CMS+S, and S. CMS groups were subjected to various stressors for 15 days. S was delivered at a dosage of 10 mg/kg/day for a duration of 15 days using an osmotic minipump. On day 15, a forced swim test (FST), open field test (OFT) were conducted. The OFT, elevated plus maze (EPM), FST and novel object recognition test (NORT) were conducted to assess the efficacy of S. Animals were beheaded, and hippocampal tissues were excised. qRT-PCR was used to assess the expression levels of genes (BDNF, NeuN, MASH1). One-way ANOVA was used for statistical analysis.

Results: In the CMS group, there was a significant decrease in the percentage and speed of OFT movement compared to the control ($p < 0.001$). There was a significant decrease in swimming, climbing and immobilization times in the depression group compared to the other groups. In the long-term memory analysis, a significant increase was observed in the recognition and discrimination index in the CMS+S group compared to the CMS group ($p < 0.01$). BDNF, NeuN and MASH1 gene expression levels in hippocampal tissues showed a significant decrease in the depression group and a significant increase in the CMS+S group ($p < 0.05$).

Conclusion: The study shows that sertraline in the treatment of depression is beneficial in improving cognitive abilities which is confirmed by the increasing gene expression. It is thought that serotonin improves long-term memory and may positively affect brain precursor cell formation.



Copyright © 2025 The author(s) - Available online at www.annalsmedres.org. This is an Open Access article distributed under the terms of Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

Introduction

Depression is a prevalent psychiatric condition that has a toll on 350 million individuals globally [1]. Depression is a mood dysfunction characterised by persistent feelings of sadness, emptiness or hopelessness. Depression can affect various aspects of daily life, including emotions, thoughts, sleep patterns, eating habits, and work productivity [2]. Although the precise origins of depression are not well comprehended, it is believed that a mix of psychological, environmental, and biological variables play a role in

its development [3]. These elements may include genetics, brain chemistry, trauma, life events, and other factors. Depression is linked to a spectrum of symptoms, including emotional manifestations such as sadness and despair as well as somatic manifestations such as alterations in food-eating behavior and disturbances in sleeping patterns [2]. Some symptoms can be operational and measurable, making them suitable for evaluation in laboratory animals. These could include behaviors like changes in eating patterns, sleep disturbances, and cognitive alterations [3]. The examination of depression in laboratory animals has provided researchers with valuable insights into the

*Corresponding author:

Email address: hatice.solak@ksbu.edu.tr (Hatice Solak)

biological foundations of the condition and facilitated the development of viable remedies. Animal models can mimic certain aspects of depression-like behavior, which can aid in testing new therapies and understanding the neurobiological mechanisms involved [4]. Stress is believed to be a major contributor to the development of depression. Prolonged exposure to stressful events, particularly when coupled with genetic vulnerability, can heighten the likelihood of developing severe depression [5]. Environmental stressors can affect biological systems, especially the HPA axis. The brain regions mentioned are linked to the regulation of emotions, mood, and cognitive abilities [4]. Alterations in these regions are associated with the emergence of depressive symptoms. This axis governs the body's physiological reaction to stress. Stress can lead to the excessive secretion of glucocorticoids, such as cortisol, which are crucial to the organism's stress response. Stress impacts both hormonal systems, notably the HPA axis, and cerebral regions, including the limbic system and cortical areas.

Research with animal models, specifically mice, has shown that chronic stress paradigms, including chronic unpredictable stress and CMS, can lead to behavioral changes that mimic hopelessness and cognitive deterioration, both of which are characteristic of depression. The effects are believed to be caused by the overactivity of the HPA axis and the increased production of corticosterone [6,7]. Researchers have demonstrated that rats subjected to chronic mild stress model protocols exhibit behavior-related, neurochemical, neuroendocrine system, and neuroimmune changes analogous to those seen humans with depression. Thus, the CMS model established by Willner et al. [8] effectively meets numerous essential criteria to an animal model of depressed [8, 1]. CMS exposure prolongs immobility duration in rats during the FST. Traditional antidepressants, such as tricyclic antidepressants, SSRIs, serotonin-norepinephrine reuptake inhibitors (SNRIs), and monoamine oxidase inhibitors (MAOIs), help alleviate symptoms of depression caused by CMS [9]. Recent studies have focused on understanding how the brain carries out learning and memory that are considered to be advanced cognitive processes. Additionally, researchers have investigated the impact of medicines on these cognitive functions. Depression causes cognitive impairments, including reduced cognitive abilities such as focused thought and sustained attention, which in turn leads to impaired memory. An evident correlation between depression and cognitive impairment has been documented [10]. Short-term memory allows a person to recall numbers or events after thinking about them continuously for a few seconds or a few minutes, such as remembering 7 to 10 digits in a telephone number. Short-term memory can be explained by presynaptic facilitation or inhibition. These events take place at synapses located on presynaptic endings and not on the next neuron. Neurotransmitters released from such endings cause facilitation or inhibition, often lasting seconds or even minutes. Such circuits can lead to short-term memory [11]. Short-term memory covers the time it takes for ongoing events to be consolidated and translated into remote, long-term memory. At this stage, short-term memory is highly susceptible to alteration and can be erased. On the other hand long-term

memory has remarkable durability against deletion and persists even under substantial brain trauma [12]. The episodic memory is a kind of the memory that is necessary to remember personally experienced events or where, when and what happened. [13]. Adult neurogenesis is a progressive process that occurs in various regions of the brain, including the subgranular zone (SGZ) of the hippocampus and the subventricular zone (SVZ) of the lateral ventricles. Neural progenitor cells from these regions proliferate and migrate to the granule cell layer of the olfactory bulb and dentate gyrus which is their final destination. In this process, they undergo differentiation to generate new neurons and then integrate into pre-existing circuits [14]. The processes governing the control of hippocampus neurogenesis are currently being studied. A recent study indicated that both the cAMP cascade and BDNF have a role in the regulation of neurogenesis, which is enhanced by persistent antidepressant medication. The activation of the cAMP pathway or exposure to BDNF has been documented to enhance neuronal differentiation of progenitor cells and promote neurite outgrowth in vitro [15]. This study sought to examine the potential effects of sertraline on the limbic system in rats exhibiting depression-like behaviour. The research assessed the expression levels of particular biomarkers linked to neurogenesis in the hippocampus within an animal model of chronic mild stress administered sertraline.

Materials and Methods

Statement of ethics

Approved by the Ethics Committee with reference number 2021-029.

Experimental protocol

Animals

In this study we used 48 adult male Wistar albino rats, each aged 6 months. The rats were classified into four separate groups: The control group (n=12, C) received a solvent (DMSO) subcutaneously using an osmotic minipump. The Chronic Mild Stress group (n=12, CMS) established a depression model in animals by the performing a CMS protocol. The DMSO solvent was administered subcutaneously via an osmotic minipump.

In the CMS + Sertraline group (n = 12, CMS+S), Sertraline was administered via osmotic minipump at a dose of 10 mg/kg per day with the CMS protocol.

The Sertraline group (n=12, S) received Sertraline (10 mg/kg/day) via an osmotic minipump (Alzet 2002, Alza Corp., Palo Alto, CA).

Chronic mild stress (CMS) protocol

The CMS procedure employed in the current investigation was adapted from the protocol proposed by Wilner [7]. The model's duration was 30 days, accompanied by behavioral testing. In Figure 1, it is seen that the antidepressant therapy persists for 15 days until the animals are decapitated. The CMS paradigm commenced on the second day of the trial. On the fifteenth day, the forced swimming stress training phase was conducted. This practice is also

regarded as a stressor. The stress model was maintained for 12 days following the installation of the osmotic pump. Behavioral assessments were conducted on the subsequent days. Stressors were not administered on the days when behavioral assessments were performed (Table 1). Information is provided in the Supplementary Methods [16].

Behavioral tests

The model was subjected to a series of behavioural evaluations, comprising the OFT, FST, EPM, and NORT. The tests were performed to assess locomotor activity, anxiety and depression-like behaviors, as well as learning and memory once the model was established and the pharmacological therapy was administered. These methodologies for assessing behavior have been recorded in prior studies. Consult further methods for further behavioral details [16]. The behavioral assessments were recorded and evaluated utilizing the Ethovision Video Monitoring System XT11 Netherlands.

New object recognition test (NORT)

NORT is performed in an open arena featuring two identical objects, usually composed of plastic, glass, or metal, and sufficiently substantial to resist displacement by the animal. In the spatial version, the animal explores the objects freely. After a delay, one object is moved to a new location within the arena. Rodents typically prefer to explore the moved object, indicating spatial memory. The non-spatial version is similar, but instead of moving an object, one object is replaced with a novel object. Rodents are anticipated to allocate increased time investigating the novel object, indicating recognition memory. In both versions, the duration of exploration of each object is recorded to assess memory [17].

The NORT assessed short-term and long-term episodic memory in rats, using a five-phase protocol: habituation, familiarization, long-term memory assessment, short-term memory assessment and a final recognition phase. Every phase consumed 5 minutes and was recorded using Ethovision XT11 software (Noldus, Netherlands) in a black Plexiglas arena (80 x 80 x 40 cm). During the short-term memory evaluation (four hours post-familiarization), one object was substituted with a novel object. In the long-term memory assessment (24 hours post-familiarization), the original object was substituted with a distinct novel object. Objects and the arena were sanitised with 70% ethanol between experiments. Discrimination and recognition indices were calculated using object interaction durations recorded by the software.

Recognition index = New object / (New object + Familiar object)

Discrimination index = (New object - Familiar object) / (New object + Familiar object).

Measurement of serum levels of corticosterone

Serum corticosterone levels were assessed using the methodology established by Sahin et al. [18].

Concluding the experiment and obtaining biological specimens

Following behavioral evaluations, the rats were weighed and then euthanized under anesthesia. Their hippocampi were dissected over frozen dry ice in accordance with the Paxinos and Watson rat brain atlas. Hippocampal tissues were stored in cryotubes and snap-frozen in fluid nitrogen before being transplanted to -80 degrees Celsius for a long time storage.

Analysis of gene expression

The procedures for total RNA extraction, quality assessment, and removal of gDNA contamination from hippocampal tissue samples were previously described and are detailed in the Supplementary Methods [16].

Primary sequence

The primers for the target and reference genes (PGK1, CycA) employed in quantitative real-time PCR (qRT-PCR) analysis are detailed in Table 2. The primer designs were created with the IDT PrimerQuest software (<https://eu.idtdna.com/site>) and sourced from the literature [19,20].

Reverse transcription reaction

cDNA was synthesized from the quality-controlled RNA samples using the manufacturer's technique. To produce single chain cDNA from 2 µg/20 µl total RNA, 1 µl Oligo dT and 1 µl Random hexamer were combined with 2 µg/20 µl total RNA and incubated in a water bath at +70 °C for 5 minutes. Subsequently, 8 µl of 5X cDNA reaction mixture, 2 µl RNase inhibitor, and 4 µl dNTP were added and maintained in a water bath at +25 °C for 5 minutes. Subsequently, 2 µl of Reverse Transcriptase enzyme was introduced and incubated in a water bath at +25 °C for 10 minutes, followed by incubation at +37 °C for 60 minutes. The reaction was halted at a +70 °C water bath for 10 minutes. The cDNA samples were preserved at -20 °C for future utilisation.

Real-time quantitative polymerase chain reaction (qPCR)

Quantitative measurement of target and reference gene expression was conducted utilizing a real-time PCR apparatus (Bio-Rad CFX Connect Real-Time PCR System). SyberGreen, a dye that attaches to double-stranded DNA, was utilized for the reaction. In summary, 10 µl of 2X SyberGreen master mix, 5 pmol of forward primer, 5 pmol of reverse primer (refer to Table 2), 2 µl of cDNA, and ddH₂O were combined to achieve a total volume of 20 µl, followed by the execution of the polymerase chain reaction. The reaction temperature profile consisted of an initial 10 minutes at 95 °C, followed by 40 cycles of 95 °C for 30 seconds, 60 °C for 30 seconds, and 72 °C for 30 seconds. Furthermore, a melting curve study was conducted by heating to 95 °C for 1 minute, followed by a progressive temperature increase back to 95 °C after cooling to 55 °C. Threshold cycle (Ct) values acquired from the real-time PCR apparatus were documented. To verify the accuracy of the products generated from real-time

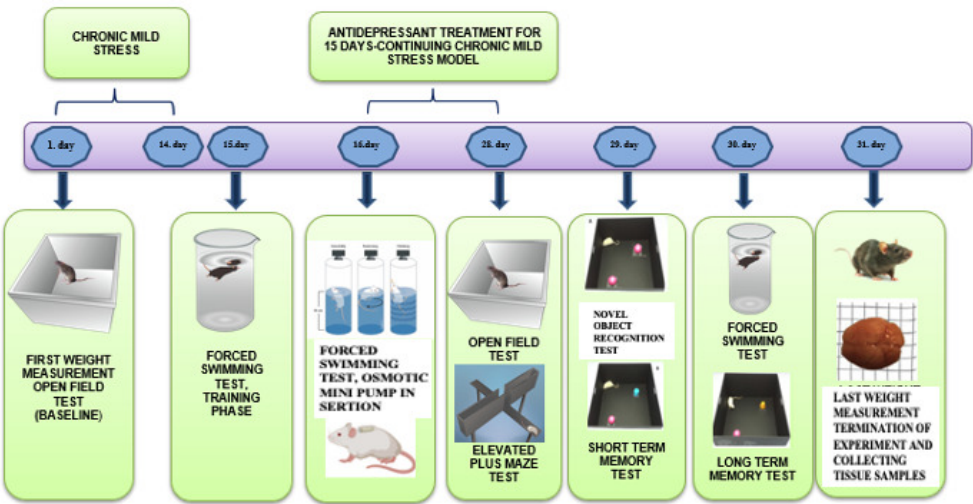


Figure 1. Schematic representation of experimental protocol.

Table 1. Scheme for the Chronic Mild Model Experiment.

Morning				Noon			Night		
Day	Hour	Stressor	Duration	Hour	Stressor	Duration	Hour	Stressor	Duration
1	09.00	Restraint stress	45 minutes	01.00	Noise	4 hours	04.00	Food deprivation	All night
2	10.00	Wet Cage	7 hours				04.00	Light on	All night
3	09.00	FST	10 minutes	03.00	Restraint stress	45 minutes			
4	11.00	Inclined Cage	7 hours						
5	09.00	Noise	4 hours	02.00	FST	10 minutes	04.00	Food deprivation	All night
6	09.00	Wet Cage	7 hours				04.00	Light on	All night
7	10.00	Inclined Cage	7 hours						

Table 2. Primer sequences of genes used in qPCR analysis of hippocampus tissues.

Gene	Primer sequence (5'→3')	PCR product (bc)	Reference
BDNF	CTGAGCGTGTGTGACAGTATTA GGGATTACACTTGGTCTCGTAG	153	[19]
Nestin	CACACCTCAAGATGTCCCTTAG AGGTACTGGTCCTCTGGTATC	166	[19]
NeuN	GGCAAATGTTCTGGGCAATTC GATCGTCCCATTTCAGCTTCTC	140	[19]
Neuritin	TCGCGGTGCAAATAGCTTAC CGGTCTTGATGTTCTGCTTGTC	152	[19]
CycA	TATCTGCACTGCCAAGACTGAGTG CTTCTTGCTGGTCTTGCCATTCC	126	[20]
PGK1	ATGCAAAGACTGGCCAAGCTAC AGCCACAGCCTCAGCATATTTC	104	[20]

PCR, the samples were subjected to electrophoresis on a 2% agarose gel at 120 volts for 30 minutes and thereafter analysed.

Statistical analysis

Continuous data are represented by mean and standard deviation, while categorical variables are represented by frequency and percentage. The continuous variables were analysed using ANOVA and mixed effects models. The

Posthoc Tukey test or mean- comparisons with Tukey modifications were employed. The studies were performed utilising R version 4.3.2 (R Core Team, 2024). A significance level of $p<0.05$ was considered statistically significant. The preliminary phase of analysing gene expression data involved normalising the Ct values of the target genes relative to the Ct values of the reference genes PGK1 and CycA. This facilitated the calculation of $2^{-\Delta Ct}$ values, indicative of the gene expression levels. The $2^{-\Delta Ct}$ val-

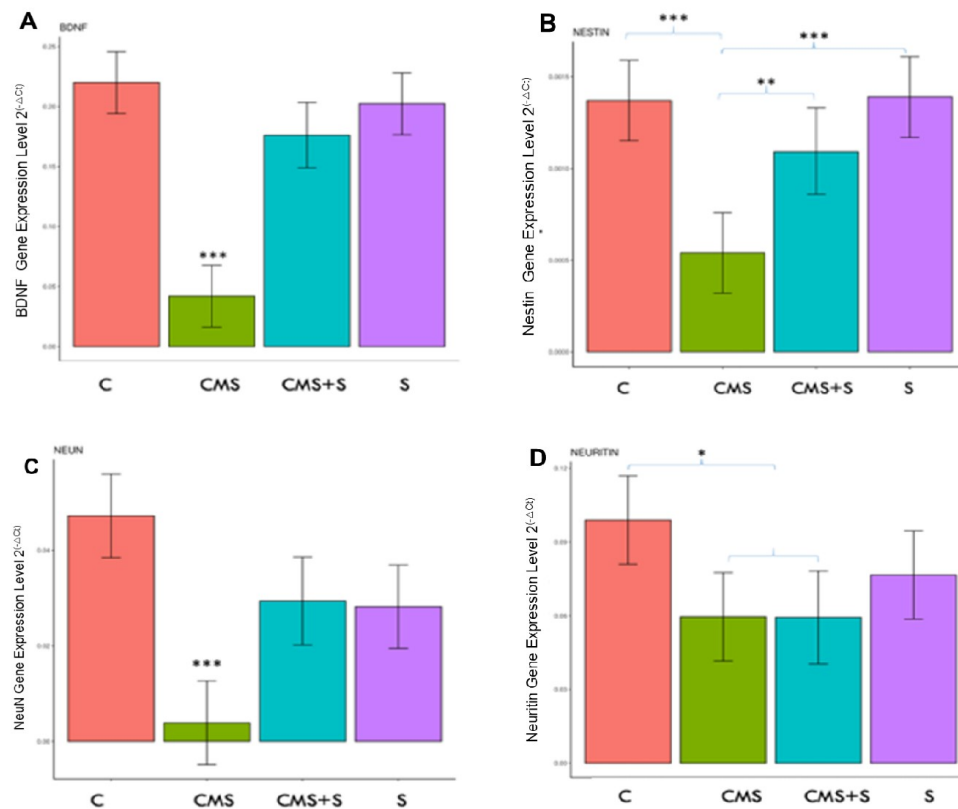


Figure 2. A BDNF expression levels in hippocampus tissue of control (C), CMS, CMS+sertraline (CMS+S), and sertraline (S) groups. Gene expression 2(-ΔCt) levels were compared by one-way analysis of variance (***p<0.001), (Comparison of CMS group with other groups). B Nestin expression levels in hippocampus tissue of control (C), CMS, CMS+sertraline (CMS+S), and sertraline (S) groups. Gene expression 2(-ΔCt) levels were compared by one-way analysis of variance (** p<0.01) (***p<0.001). C NeuN expression levels in hippocampus tissue of control (C), CMS, CMS+sertraline (CMS+S), and sertraline (S) groups. Gene expression 2(-ΔCt) levels were compared by one-way analysis of variance (***p<0.001), (Comparison of CMS group with other groups). D Neuritin expression levels in hippocampus tissue of control (C), CMS, CMS+sertraline (CMS+S), and sertraline (S) groups. Gene expression 2(-ΔCt) levels were compared by one-way analysis of variance (*p<0.05), (Comparison of CMS+ CMS+S group with other groups).

ues were analyzed for differences across groups using a one-way analysis of variance. Graphs illustrating the standard error of the mean squares for the relevant variables were generated. According to the post-hoc power analysis, the effect size (Cohen's *f*) obtained in the study was 0.901, the significance level was determined as ($\alpha=0.05$) and the targeted statistical power ($1-\beta$) was calculated as 99.4%. Post-hoc power analysis showed that the study had an extremely high statistical power of 99.4%. These results reveal that our study is strong and reliable in detecting differences between groups.

Results

Open field test (OFT) assessments on days 1 and 28 revealed a significant decrease in distance moved, velocity, and percentage of movement in the chronic mild stress (CMS) group compared to baseline (day 0) (Table 3). Post hoc analysis using Tukey-adjusted least squares means comparisons after mixed-effects models confirmed a significant reduction in distance moved between day 0 and day 28 in the CMS group ($p=0.017$). Pairwise comparisons also showed a significant decrease in distance moved in the CMS group at day 28 ($p=0.012$). Furthermore, a significant decrease in the percentage of movement was observed in the CMS group at day 28 ($p<0.001$).

Following the OFT, the EPM was administered. Analysis

of the percentage of time spent in open arms revealed a significant increase in both the sertraline-only group and the CMS+sertraline (CMS+S) group compared to the other groups. ($p<0.01$) (Table 4).

FST: In the FST, immobility time, swimming time, climbing time, and total movement were assessed on days 15 and 30 (Table 5). Tukey-adjusted post hoc least squares means comparisons following mixed-effects models revealed several significant differences:

Day 15:

- Swimming behavior was significantly decreased in the CMS and CMS+S groups compared to the control (C) and sertraline-only (S) groups ($p=0.043$).
- Climbing behavior was significantly decreased in the CMS and CMS+S groups compared to the C and S groups ($p=0.035$, 0.003 , 0.001 , and <0.001 , respectively).
- Immobility time was significantly increased in the CMS and CMS+S groups compared to the C and S groups ($p<0.001$).

Day 30:

- Swimming behavior was significantly increased in the CMS+S and S groups compared to the other groups ($p<0.001$).

Table 3. Summary of the results of the OFT tests.

	0 day Mean±SD	28 th day Mean±SD	p value
Control			
Distance Moved (cm)	1.142.91±426.18	985.86±362.94	0.462
Velocity(sn)	5.51±2.20	3.22±1.68	0.020*
Movement (%)	3.15±0.90	3.35±1.66	0.848
Chronic Mild Stress			
Distance Moved (cm)	894.50±197.18	523.53±346.01	0.017
Velocity(sn)	6.32±2.46	3.01±2.23	<0.001***
Movement (%)	3.52±1.27	1.07±1.14	<0.001***
Chronic Mild Stress +Sertraline			
Distance Moved (cm)	994.72±266.51	829.10±272.97	0.424
Velocity(sn)	7.51±2.90	4.30±2.00	0.001**
Movement (%)	3.40±1.08	1.89±0.53	<0.001***
Sertraline			
Distance Moved (cm)	1.192.47±325.65	948.84±404.94	0.160
Velocity(sn)	6.30±1.60	5.24±3.45	0.411
Movement (%)	3.29±0.71	2.39±0.53	0.042*

(*p<0.05). (**: p<0.01, ***: p<0.001). Tukey adjusted post hoc means comparisons after mixed effects models (n=48).

Table 4. Analysis of Serum Corticosterone Levels, Time Spent in Open Arms.

	C (n=12)	CMS (n=12)	CMS+ S (n=12)	S (n=12)	p value
Serum Corticosterone Levels	322.33±154.26	909.17±474.45	400.58±157.68	372.67±169.22	<0.001***
Time Spent in Open Arms	2.37±1.31	2.26±2.54	5.95±6.08	13.12±8.29	<0.001***

C: Control, CMS: Chronic Mild Stress, CMS+S: Chronic Mild Stress+Sertraline, (n=48).

- Immobility time was significantly decreased in the CMS+S group compared to the CMS group (p<0.001).

NORT: Analysis of the NORT revealed significant differences in long-term memory performance (Table 6). Specifically, both the discrimination index (p=0.005) and the recognition index (p=0.009) were significantly impaired in the CMS group compared to the control (C) group. Additionally, the long-term memory recognition index was significantly different between the CMS group and the sertraline-only (S) group (p=0.05). Further analysis of the long-term memory discrimination index showed significant differences between the C and CMS groups (p=0.005), as well as between the CMS and S groups (p=0.032).

Results of gene expression analysis

The expressions of BDNF, NESTIN, NEUN, and NEURITIN genes in hippocampus tissues were assessed and quantified using a one-way analysis of variance (Figure 2).

Assessment of serum corticosterone concentrations

Serum corticosterone levels were evaluated across the groups and are presented in Table 4.

Discussion

A great deal of research is being conducted using the continuous moderate stress paradigm to examine the effects of 5-HT activity in neurons and 5-HT1A autoreceptor functions in laboratory animals subjected to depression. These investigations have demonstrated that the spontaneous activity of 5-HT can exhibit significant variations across different brain areas [21, 22]. SSRIs have been found to promote developmental plasticity through a mechanism that relies on the neurotransmitter 5-HT. The treatment was found to elevate 5-HT levels, thus enhancing neuronal plasticity [23]. 5-HT modulates glutamate transmission in the brain. It can enhance N-methyl-D-aspartate receptor-mediated plasticity. 5-HT associates with the cell adhesion molecule. The cell adhesion molecule is crucial for cellular transformation and adaptability throughout development. 5-HT promotes the synthesis of the polysialylated variant of the neural cell adhesion molecule (PSA-NCAM), which is involved in synapse formation and neuronal reorganisation [23, 24]. According to Malberg et al., the administration of antidepressant drugs resulted in an increase in the number of newly formed cells after 14 and 28 days. A separate investigation demonstrated that 5-HT exerts a beneficial regulatory influence on the generation of neural precursor cells and the viability of recently formed neurons [15]. Research has indicated that stress situations are linked to heightened activity in the HPA axis and higher

Table 5. Summary of the results of the Forced swimming test.

	15 th day Mean±SD	30 th day Mean±SD	p value
Control			
Swimming (sn)	89.25±47.48	115.92±46.16	0.142
Climbing (sn)	126.92±31.32	126.50±32.85	0.974
Immobility (sn)	85.83±67.28	57.83±40.67	0.059
Movement (%)	71.91±22.25	81.27±14.32	0.064
CMS			
Swimming (sn)	41.33±20.12	47.25±27.03	0.742
Climbing (sn)	89.08±22.14	97.42±31.76	0.522
Immobility (sn)	169.50±29.67	155.75±41.40	0.346
Movement (%)	43.55±9.83	48.00±13.71	0.372
CMS+S			
Swimming (sn)	37.25±17.28	128.92±85.07	<0.001***
Climbing (sn)	77.92±18.93	99.08±53.56	0.108
Immobility (sn)	184.08±29.23	71.50±55.05	<0.001***
Movement (%)	38.64±9.60	76.07±18.40	<0.001***
Sertraline			
Swimming (sn)	99.67±23.05	137.67±66.77	0.039*
Climbing (sn)	143.33±25.29	84.42±39.79	<0.001***
Immobility (sn)	59.25±29.89	77.50±58.27	0.212
Movement (%)	80.44±10.23	74.08±19.43	0.203

CMS: Chronic Mild Stress, CMS+S: Chronic Mild Stress+Sertraline (n=48) (*p<0.05). (**: p<0.01, ***: p<0.001). Tukey adjusted post hoc lsmeans comparisons after mixed effects models

Table 6. New Object Recognition Test results.

	C n = 12	CMS n = 12	CMS+S n = 12	S n = 12	p-value
Recognition index in short-term	0.39±0.40	0.50±0.48	0.61±0.44	0.81±0.32	0.10
Discrimination index in short-term	-0.05±0.71	0.24±0.76	0.39±0.70	0.62±0.63	0.14
Recognition index in long-term	0.74±0.45	0.17±0.39	0.33±0.49	0.66±0.43	0.009
Discrimination index in long-term	0.72±0.44	0.00±0.60	0.33±0.49	0.59±0.42	0.005

(*: p<0.05, **: p<0.01) Tukey comparisons after One-way Anova (n=48).

levels of corticosterone in the bloodstream [25]. Guimaraes et al. contend that chronic stress activates the HPA axis, resulting in modifications to the serotonergic system in the hippocampus. This, in turn, increases the likelihood of developing depression. Our analysis indicated a significant increase in corticosterone levels within the CMS group [26]. In this study, we utilized the OFT to measure locomotor activity, the EPM to assess anxiety, and the FST asses depression. These tests were used to analyze the efficacy of sertraline, an antidepressant belonging to the SSRI group, in rats showing depressive-like behavior induced by the CMS model. NORT was applied to observe changes in episodic memory. To investigate the effectiveness of newly formed neural precursor cells in this change, BDNF, NESTIN, NEUN, and NEURITIN gene expression levels were examined to follow neurol gene formation.

Recent studies suggest that deficits in memory and attention in depression may be linked to inhibitory deficits, specifically the inability to disengage from negative stimuli. Rumination on negative emotions has been involved

in development, maintenance, and severity of depression. In depressed individuals, attentional biases towards negative stimuli can interfere with the processing of positive information. This is supported by the evidence that depressed individuals pay more attention to negative stimuli and less attention to positive stimuli. This attentional bias may be due to reduced activity in certain brain regions, including the right ventrolateral prefrontal cortex, right dorsolateral prefrontal cortex, and right superior parietal cortex. This diminished activity may impede the capacity to redirect attention from unfavourable stimuli, resulting in extended exposure to depressed influences [27]. Sertraline treatment has been shown to decrease immobility and increase swimming behavior in experimental animals, supporting its antidepressant-like properties [28]. The evidence demonstrates that the CMS concept induces heightened behavioral pessimism and cognitive deterioration [21]. Mice subjected to four weeks of stress have shown a notable rise in the duration of inactivity in the FST [29]. This study found that rats subjected to

a CMS treatment for four weeks demonstrated a marked increase in immobility duration relative to controls, suggesting a depressive-like condition. This effect persisted for two weeks. Treatment with sertraline (10 mg/kg/day) significantly reduced immobility time in the CMS group. Additionally, both the sertraline-only (S) and CMS+S groups showed increased swimming behavior compared to the other groups. These data validate that SSRIs can successfully alleviate depression-like behaviours and anxiety-inducing effects caused by CMS [21, 7, 1].

The 5-HT system is significant in the development of mood disorders [30]. Changes in 5-HT metabolism contribute to the onset of depression. In their investigation, Santiago et al. [31] discovered that serotonin reuptake inhibitors resulted in an increase in swimming time. The OFT was performed to evaluate the locomotor behaviours of the animals. The animals' velocity, distance moved, movement percentage, and parameters were evaluated in the OFT. At the outset, there were no notable discrepancies among the groups regarding distance travelled, velocity, or movement percentage in the OFT. However, by day 30, all groups showed a decrease in movement time. In the EPM, the sertraline-treated group spent significantly more time in the open arms, indicating reduced anxiety-like behavior. These findings suggest that sertraline may have anxiolytic effects [31]. Analysis of the OFT and EPM did not reveal significant differences in anxiety-like or avoidant behaviors between the control and CMS groups. While the control group exhibited reduced locomotion in the OFT, this may be attributable to individual behavioral variations during the test. EPM, the CMS group treated with sertraline spent significantly more time in the open arms, consistent with the findings of Peng et al., who reported a similar effect of fluoxetine in a rat model of depression. Li et al. [32] noticed notable augmentation in locomotion in both experimental groups following the injection of sertraline.

The NORT assesses short-term recognition memory in rats, capitalizing on their innate preference for novel objects. Rats typically explore objects through tactile and olfactory investigation using their mystacial vibrissae (whiskers). As nocturnal animals, they rely heavily on non-visual sensory input, including olfactory signals transmitted to the somatosensory cortex via the trigeminal nerve. Therefore, the NORT serves as both a visual and a somatosensory test. Successful performance requires intact visual and somatosensory systems; disruption of either would invalidate the assay as a screen for memory-enhancing drugs. Such disruption would be evident in altered exploration times in the second trial, regardless of object novelty [33]. In our study, there was an increase in discrimination and recognition index in short-term memory in the sertraline-treated groups compared to the CMS. There was a significant difference in discrimination index in long-term memory. A significant difference was observed between the treated group and the control group compared to the CMS group. Individuals suffering from depression have an extended duration in the processing of emotional stimuli, which may indicate a potential impairment in their ability to exert cognitive control over the limbic parts of the brain. Reduced activity in these specific brain regions may hinder the capacity of those suf-

fering from depression to control unpleasant and recurrent thoughts. The hippocampus, a key area for episodic memory, enhances the process of encoding and retrieving emotional events in individuals under physiological conditions. Depressed people had heightened activity in the amygdala, hippocampus, caudate, and putamen during the encoding process. This enhanced activity facilitated the recall of negative information while not affect the recall of good information. This discovery indicates that memory bias in individuals with depression may stem from heightened amygdala activity during the process of encoding information, as well as higher activity in the hippocampus, caudate, and putamen while recalling negative information, as compared to individuals without depression. Although the neural circuits underlying the pathology of depression remain unclear, the diverse symptoms of depression suggest that more than one brain region may be responsible. According to previous reports, learning and memory regions were the most affected brain regions after stress [27]. The slowing of neurogenesis, the absence of new neuron formation and the reduction of dendritic branching may be responsible.

Brain-derived neurotrophic factor (BDNF) is a crucial modulator of neuronal viability, synaptic plasticity, and memory development in the central nervous system (CNS). Many antidepressants, including sertraline, have been shown to increase BDNF expression in the brain. While depression is often associated with reduced hippocampal BDNF levels and impaired cognitive function, antidepressants can help restore BDNF and improve cognition. This study indicates that the observed elevation in BDNF after sertraline treatment may enhance cognitive abilities [34]. Wang et al. demonstrated that chronic stress negatively affects synaptic connections in the rat hippocampus, causing them to become shorter, thinner, and less efficient. BDNF therapy enhanced dendritic spine density in the CA1, CA3, and dentate gyrus (DG) regions of the hippocampus and decreased the diameter of the synaptic cleft in the CA1 region. These findings highlight the potential of BDNF to counteract the negative effects of stress on synaptic plasticity [35]. Moreover, nestin and doublecortin, indicators of neurogenesis, are frequently present in developing brain progenitor cells during adult neurogenesis [35, 36]. In our research, nestin levels were significantly decreased in rats with depressive behavior. Sertraline restored BDNF expression and up-regulated nestin, indicating that it leads to neurogenesis. Yang et al. [37] demonstrated a notable reduction in BDNF and NeuN levels following CUMS. They also examined NeuN expression using immunofluorescence and demonstrated a reduction in neuronal cells. After 3 weeks of fluoxetine administration, they observed an increase in new neurons. Decreased dendritic spine density and dendritic branching were associated with psychiatric disorders [37]. Our study, decrease in NeuN levels was found in the CMS group. Antidepressant drugs caused an observed increase. Our experimental model showed that it effectively decreased the growth of neural precursors in hippocampus by reducing the number of mature neurons (NeuN). Son et al. [38] showed a decrease in Neuritin levels in the depression group. Our study also shows that prolonged mild stress

leads to a decrease in neuritin levels in the hippocampus. Sertraline administration did not affect neuritin levels.

Our research offers significant insights into the impact of psychological stress on adult hippocampus neurogenesis and the possibilities for therapeutic intervention. Importantly, we expanded the analysis of subacute sertraline administration to two weeks, using a clinically relevant dosing regimen that aligns with potential therapeutic applications. This extended period enabled us to assess both the short- and long-term effects of sertraline on depressive behaviours and neurogenesis. A disadvantage of this study is that only gene expression in hippocampal tissue was examined. Due to budget constraints, protein analysis was not possible. Future studies should include protein-level measurements using techniques like Western blot or immunofluorescence.

Conclusion

Animal models are crucial for understanding the neurobiological mechanisms underlying mood disorders in humans. While the precise neural circuits involved in stress-induced mood and energy balance alterations remain incompletely understood, our study confirms previous findings that chronic stress suppresses hippocampal cell proliferation and neurogenesis. Furthermore, we demonstrate that sertraline effectively alleviates depressive symptoms in this model, as evidenced by behavioral measures [39]. Moreover, this suppression was more pronounced in the ventral hippocampus. Chronic sertraline administration, in accordance with prior findings, mitigated the CMS-induced inhibition of hippocampus cell growth and neurogenesis [40].

Ethical approval

This study protocol was approved by Necmettin Erbakan University Experimental Medicine Application and Research Center Experimental Animals Local Ethics Committee (No: 2021-029).

References

- Micheli L, Ceccarelli M, D'Andrea G, et al. Depression and adult neurogenesis: Positive effects of the antidepressant fluoxetine and of physical exercise. *Brain Res Bull.* 2018;143:181-93. doi:10.1016/j.brainresbull.2018.09.002
- Schweizer MC, Henniger MS, Sillaber I. Chronic mild stress (CMS) in mice: of anhedonia, 'anomalous anxiolysis' and activity. *PLoS One.* 2009;4(1):e4326. doi:10.1371/journal.pone.0004326.
- Remus J. Neuroimmune mechanisms of an animal model of recurrent depression. Doctoral Thesis, Kent State University, Ohio, 2015.
- Mahar I, Bambico FR, Mechawar N, Nobrega JN. Stress, serotonin, and hippocampal neurogenesis in relation to depression and antidepressant effects. *Neurosci Biobehav Rev.* 2014;38:173-192. doi:10.1016/j.neubiorev.2013.11.009.
- Jacobs BL. Adult brain neurogenesis and depression. *Brain Behav Immun.* 2002;16(5):602-609. doi:10.1016/s0889-1591(02)00015-6.
- David DJ, Samuels BA, Rainer Q, et al. Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. *Neuron.* 2009;62(4):479-493. doi:10.1016/j.neuron.2009.04.017.
- Willner P. The chronic mild stress (CMS) model of depression: History, evaluation and usage. *Neurobiol Stress.* 2016;6:78-93. Published 2016 Aug 24. doi:10.1016/j.ynstr.2016.08.002.
- Willner P, Towell A, Sampson D, Sophokleous S, et al. Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. *Psychopharmacology (Berl).* 1987;93(3):358-364. doi:10.1007/BF00187257.
- Yan HC, Cao X, Das M, et al. Behavioral animal models of depression. *Neurosci Bull.* 2010;26(4):327-337. doi:10.1007/s12264-010-0323-7.
- Burt DB, Zembar MJ, Niederehe G. Depression and memory impairment: a meta-analysis of the association, its pattern, and specificity. *Psychol Bull.* 1995;117(2):285-305. doi:10.1037/0033-2909.117.2.285.
- Hall JE. Guyton and Hall Textbook of Medical Physiology. In: Grulow R, ed. Behavioral and motivational mechanisms of the brain-the limbic system and the hypothalamus. *USA: Saunders Elsevier.* 2011:711-20.
- Ganong, W.F. Medical Physiology Turkish 17th Edition Translation Turkish Physiological Sciences Association Baris Bookstore, 1996.
- Dere E, Kart-Teke E, Huston JP, et al. The case for episodic memory in animals. *Neurosci Biobehav Rev.* 2006;30(8):1206-1224. doi:10.1016/j.neubiorev.2006.09.005.
- Sousa-Ferreira L, de Almeida LP, Cavadas C. Role of hypothalamic neurogenesis in feeding regulation. *Trends Endocrinol Metab.* 2014;25(2):80-88. doi:10.1016/j.tem.2013.10.005.
- Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J Neurosci.* 2000;20(24):9104-9110. doi:10.1523/JNEUROSCI.20-24-09104.2000.
- Solak H, Gormus ZIS, Koca RO, et al. Does Sertraline Affect Hypothalamic Food Intake Peptides in the Rat Experimental Model of Chronic Mild Stress-Induced Depression? *Neurochem Res.* 2022;47(5):1299-1316. doi:10.1007/s11064-022-03529-9.
- Morellini F. Spatial memory tasks in rodents: what do they model? *Cell Tissue Res.* 2013;354(1):273-286. doi:10.1007/s00441-013-1668-9.
- Sahin Z, Solak H, Koc A, et al. Long-term metabolic cage housing increases anxiety/depression-related behaviours in adult male rats. *Arch Physiol Biochem.* 2019;125(2):122-127. doi:10.1080/13813455.2018.1441314.
- Sak CKZ. Effect of apelin on behavior in socially isolated young rats. Master's thesis, Necmettin Erbakan University, Konya, 2019.
- Seol D, Choe H, Zheng H, et al. Selection of reference genes for normalization of quantitative real-time PCR in organ culture of the rat and rabbit intervertebral disc. *BMC Res Notes.* 2011;4:162. Published 2011 May 26. doi:10.1186/1756-0500-4-162.
- Bambico FR, Nguyen NT, Gobbi G. Decline in serotonergic firing activity and desensitization of 5-HT1A autoreceptors after chronic unpredictable stress. *Eur Neuropsychopharmacol.* 2009;19(3):215-228. doi:10.1016/j.euroneuro.2008.11.005.
- Kraus C, Castrén E, Kasper S, Lanzenberger R. Serotonin and neuroplasticity - Links between molecular, functional and structural pathophysiology in depression. *Neurosci Biobehav Rev.* 2017;77:317-326. doi:10.1016/j.neubiorev.2017.03.007.
- Lesch KP, Waider J. Serotonin in the modulation of neural plasticity and networks: implications for neurodevelopmental disorders. *Neuron.* 2012;76(1):175-191. doi:10.1016/j.neuron.2012.09.013.
- Klempin FC. Adult Brain Plasticity: Serotonin receptor subtypes mediate opposing effects on adult hippocampal neurogenesis. Doctoral Thesis. *Humboldt-Universität zu Berlin*, Berlin, 2008.
- Neurauter G, Schröcksnadel K, Scholl-Bürgi S, et al. Chronic immune stimulation correlates with reduced phenylalanine turnover. *Curr Drug Metab.* 2008;9(7):622-627. doi:10.2174/138920008785821738.
- Guimarães FS, Del Bel EA, Padovan CM, et al. Hippocampal 5-HT receptors and consolidation of stressful memories. *Behav Brain Res.* 1993;58(1-2):133-139. doi:10.1016/0166-4328(93)90098-b.
- Disner SG, Beevers CG, Haigh EA, Beck AT. Neural mechanisms of the cognitive model of depression. *Nat Rev Neurosci.* 2011;12(8):467-477. Published 2011 Jul 6. doi:10.1038/nrn3027.
- Rogöz Z, Skuza G. Mechanism of synergistic action following co-treatment with pramipexole and fluoxetine or sertraline in the forced swimming test in rats. *Pharmacol Rep.* 2006;58(4):493-500.

29. Haridas S, Kumar M, Manda K. Melatonin ameliorates chronic mild stress induced behavioral dysfunctions in mice. *Physiol Behav.* 2013;119:201-207. doi:10.1016/j.physbeh.2013.06.015.
30. Chiba S, Numakawa T, Ninomiya M, et al. Chronic restraint stress causes anxiety- and depression-like behaviors, downregulates glucocorticoid receptor expression, and attenuates glutamate release induced by brain-derived neurotrophic factor in the prefrontal cortex. *Prog Neuropsychopharmacol Biol Psychiatry.* 2012;39(1):112-119. doi:10.1016/j.pnpbp.2012.05.018.
31. Santiago RM, Zaminelli T, Bassani TB, et al. The mechanism of antidepressant-like effects of piroxicam in rats. *J Pharmacol Pharmacother.* 2015;6(1):7-12. doi:10.4103/0976-500X.149133.
32. Li P, Huang W, Chen Y, et al. Acupuncture Alleviates CUMS-Induced Depression-Like Behaviors by Restoring Prefrontal Cortex Neuroplasticity. *Neural Plast.* 2023;2023:1474841. Published 2023 May 2. doi:10.1155/2023/1474841.
33. Mathiasen JR, DiCamillo A. Novel object recognition in the rat: a facile assay for cognitive function. *Curr Protoc Pharmacol.* 2010;Chapter 5:Unit 5.59. doi:10.1002/0471141755.ph0559s49.
34. Heldt SA, Stanek L, Chhatwal JP, Ressler KJ. Hippocampus-specific deletion of BDNF in adult mice impairs spatial memory and extinction of aversive memories. *Mol Psychiatry.* 2007;12(7):656-670. doi:10.1038/sj.mp.4001957.
35. Wang G, An T, Lei C, et al. Antidepressant-like effect of ginsenoside Rb1 on potentiating synaptic plasticity via the miR-134-mediated BDNF signaling pathway in a mouse model of chronic stress-induced depression. *J Ginseng Res.* 2022;46(3):376-386. doi:10.1016/j.jgr.2021.03.005.
36. Chong PS, Poon CH, Roy J, et al. Neurogenesis-dependent antidepressant-like activity of *Herichium erinaceus* in an animal model of depression. *Chin Med.* 2021;16(1):132. Published 2021 Dec 7. doi:10.1186/s13020-021-00546-8.
37. Yang Y, Hu Z, Du X, et al. miR-16 and Fluoxetine Both Reverse Autophagic and Apoptotic Change in Chronic Unpredictable Mild Stress Model Rats. *Front Neurosci.* 2017;11:428. Published 2017 Jul 25. doi:10.3389/fnins.2017.00428.
38. Son H, Banasr M, Choi M, et al. Neuritin produces antidepressant actions and blocks the neuronal and behavioral deficits caused by chronic stress. *Proc Natl Acad Sci U S A.* 2012;109(28):11378-11383. doi:10.1073/pnas.1201191109.
39. Mineur YS, Belzung C, Crusio WE. Functional implications of decreases in neurogenesis following chronic mild stress in mice. *Neuroscience.* 2007;150(2):251-259. doi:10.1016/j.neuroscience.2007.09.045.
40. Malberg JE, Duman RS. Cell proliferation in adult hippocampus is decreased by inescapable stress: reversal by fluoxetine treatment. *Neuropsychopharmacology.* 2003;28(9):1562-1571. doi:10.1038/sj.npp.1300234.



Ann Med Res

Current issue list available at AnnMedRes

Annals of Medical Research

journal page: www.annalsmedres.org

Evaluation of Chat Generative Pre-trained Transformer's responses to frequently asked questions about psoriatic arthritis: A study on quality and readability

✉ Mehmet Serkan Kılıçoğlu^{a,*}, ✉ Ozan Volkan Yurdakul^a, ✉ Teoman Aydın^a

^aBezmialem Vakıf University, Faculty of Medicine, Department of Physical Medicine and Rehabilitation, Istanbul, Türkiye

ARTICLE INFO

Keywords:

ChatGPT
Artificial intelligence
Psoriatic arthritis
Quality information
Readability

Received: Nov 01, 2024

Accepted: Feb 11, 2025

Available Online: 26.02.2025

DOI:

[10.5455/annalsmedres.2024.10.231](https://doi.org/10.5455/annalsmedres.2024.10.231)

Abstract

Aim: The growing use of artificial intelligence (AI) in healthcare, especially through technologies such as Chat Generative Pre-trained Transformer (ChatGPT), has led to concerns regarding the quality and readability of AI-generated health data. This study aimed to evaluate ChatGPT's responses to frequently asked questions about psoriatic arthritis (PsA).

Materials and Methods: The quality of ChatGPT-generated responses was evaluated using the Ensuring Quality Information for Patients (EQIP) tool. Readability was assessed using the Flesch–Kincaid Reading Ease (FKRE) and Flesch–Kincaid Grade Level (FKGL) indices. The Kruskal–Wallis H test was used to compare subgroups, and Bonferroni correction was done for multiple comparisons.

Results: Significant differences were observed in EQIP scores across question subgroups, with treatment-related questions scoring lower than symptom-related questions. The FKRE and FKGL scores indicated that the information provided by ChatGPT could be challenging for patients with lower literacy levels.

Conclusion: Although ChatGPT provided relatively accurate information on PsA, its readability and ability to communicate complex medical information might be improved. These findings suggest the necessity for continual refinement of AI tools to address the diverse needs of patients.



Copyright © 2025 The author(s) - Available online at www.annalsmedres.org. This is an Open Access article distributed under the terms of Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

Introduction

Psoriatic arthritis (PsA) is a chronic inflammatory disorder that causes joint pain and affects approximately 30% of individuals with psoriasis [1]. The complexity of PsA, including several systems and a wide range of clinical symptoms, requires comprehensive and accessible patient education [2, 3]. With the proliferation of digital health information, patients are increasingly turning to online resources to better understand their diseases and treatment options [4]. However, concerns exist about the accuracy, comprehensiveness, and readability of AI-generated health information [5, 6].

The rapid advancement of artificial intelligence (AI) has facilitated the integration of large language models (LLMs), such as Chat Generative Pretrained Transformer (ChatGPT), into healthcare. AI-driven chatbots are increasingly employed to provide medical information, address

patient queries, and assist healthcare professionals in decision-making. While ChatGPT has shown promise in generating informative content, it is essential to evaluate the quality, reliability, and readability of AI-generated health data [5, 6]. Prior research indicates that AI tools often struggle to balance readability with medical accuracy, sometimes producing responses that are either overly simplistic or excessively technical [6, 7].

Several studies have assessed the performance of AI-based chatbots in various medical fields, including dermatology, rheumatology, and general medicine. Previous research has shown challenges in preservation of the readability of complex medical information generated by AI tools, particularly for patients with lower literacy levels [7].

This study aims to assess the quality and readability of Chat Generative Pre-trained Transformer's (ChatGPT's) responses to frequently asked questions related to PsA, thereby the study's findings could inform the development of AI algorithms designed to simplify medical jargon, use clearer language, and provide visual aids, thereby improving the accessibility and usefulness of digital health infor-

*Corresponding author:

Email address: dr.serkan.kilicoglu@gmail.com (✉ Mehmet Serkan Kılıçoğlu)

mation for a broader patient population.

Materials and Methods

Study design

This study was conducted on July 10, 2024, at the Department of Physical Medicine and Rehabilitation. This study did not involve human or animal participants; therefore, ethical approval and compliance with the Helsinki Declaration were not required. Google Trends was used to identify the most commonly searched terms for PsA. All browser-related data were cleared before starting the search to eliminate bias [8]. The terms “psoriatic arthritis,” “PsA,” and “arthritis psoriatica” were used, with all global and health subheadings selected as search criteria [9]. The “most important” question was selected from the relevant question section of the results, and regions of interest were organized by subregion [10]. Exclusion criteria included overlapping words, non-English terminology, and irrelevant questions. The study design process are outlined in Figure 1.

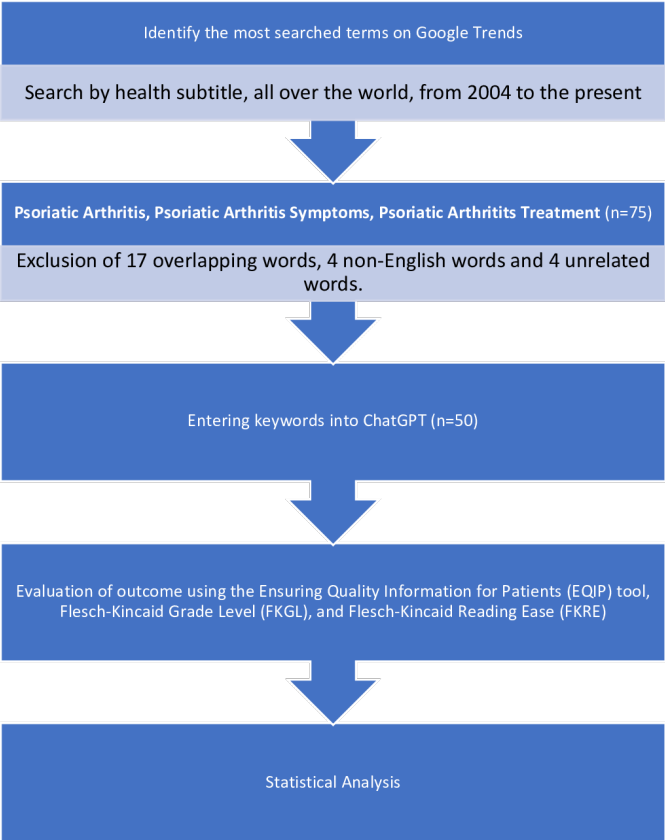


Figure 1. Flow chart of study design.

Each term was entered into ChatGPT to generate responses. This technique was developed to promote response diversity while avoiding redundancy. Subsequently, the generated responses were recorded for further analysis, which focused on quality, clarity, and readability [11].

Evaluation tools

The quality of the ChatGPT-generated responses was analyzed using the Ensuring Quality Information for Patients (EQIP) tool, which evaluated patient information across

various dimensions such as clarity, accuracy, completeness, and relevance [12]. The EQIP tool evaluates information on a scale of 0–100, with higher scores indicating higher quality. This tool uses a set of 20 criteria to assess the quality of the information, and the final EQIP score is expressed as a percentage, which represents the proportion of criteria satisfied by the information provided. Responses were independently examined by two physical and rehabilitation physicians (MSK and OVY), and inconsistencies were resolved by a third evaluator (TA) [13].

Readability assessment

Readability was assessed using the Flesch–Kincaid Reading Ease (FKRE) and Flesch–Kincaid Grade Level (FKGL) indices. The FKRE score is determined using sentence length and syllable count, with higher values indicating better readability [14]. The FKGL score indicates the U.S. school grade level necessary to understand the text [15].

Statistical analysis

The Kruskal–Wallis H test was used to compare EQIP, FKRE, and FKGL scores across several subgroups of PsA-related questions (symptoms, diagnosis, treatment options, and sequelae). Bonferroni post hoc correction was used for multiple comparisons, with an adjusted significance level of $p < 0.017$. Dunn’s post-hoc test with Bonferroni correction was used to identify pairwise differences between subgroups. Statistical significance for all analyses was determined at $p < 0.05$, which means any p value less than 0.05 was considered significant, not exactly 0.05.

- Kruskal–Wallis H test: It is used to examine if there are statistically significant differences in EQIP, FKRE, and FKGL scores among subgroups [16].
- Spearman’s rank correlation test: It is used to determine correlations between numerical variables in data [17].
- Bonferroni post hoc correction: It is used to correct for multiple comparisons by adjusting the significance level to prevent type I errors [18].

Results

The categorization of Psoriatic Arthritis topics by EQIP criteria is detailed in Table 1. The research indicated significant differences in EQIP scores across question subgroups, with treatment-related questions consistently scoring lower than those related to symptoms and diagnosis. Descriptive statistics of patient information quality and readability scores are presented in Table 2. Additionally, the FKRE and FKGL scores revealed that the readability of ChatGPT-generated responses might be challenging, particularly for patients with lower literacy levels. These findings indicate the areas where ChatGPT excels and struggles, notably in conveying complex medical information in an accessible manner.

The Kruskal–Wallis H test was used to compare EQIP scores, FKRE scores, and FKGL scores among different subgroups of questions about PsA.

Table 1. Categorization of Psoriatic Arthritis topics by EQUIP criteria.

Ranks	Keyword	Category of the based on EQUIP
1	What is psoriatic arthritis?	Event or Disease
2	What are the symptoms of psoriatic arthritis?	Event or Disease
3	What causes psoriatic arthritis?	Event or Disease
4	Is psoriatic arthritis hereditary?	Event or Disease
5	What are the early signs of psoriatic arthritis?	Event or Disease
6	What triggers psoriatic arthritis flare-ups?	Event or Disease
7	Can stress worsen psoriatic arthritis?	Event or Disease
8	Is psoriatic arthritis an autoimmune disease?	Event or Disease
9	Can children get psoriatic arthritis?	Event or Disease
10	What are the complications of psoriatic arthritis?	Event or Disease
11	How does psoriatic arthritis affect the skin?	Event or Disease
12	Is psoriatic arthritis related to psoriasis?	Event or Disease
13	What is the prognosis for psoriatic arthritis?	Event or Disease
14	What is the relationship between psoriatic arthritis and other autoimmune diseases?	Event or Disease
15	Can psoriatic arthritis cause disability?	Event or Disease
16	What are the risks of untreated psoriatic arthritis?	Event or Disease
17	Can psoriatic arthritis cause eye problems?	Event or Disease
18	Can psoriatic arthritis cause heart problems?	Event or Disease
19	What is dactylitis in psoriatic arthritis?	Event or Disease
20	Can psoriatic arthritis cause kidney problems?	Event or Disease
21	Can psoriatic arthritis cause lung problems?	Event or Disease
22	How is psoriatic arthritis treated during pregnancy?	Discharge or Postoperative Care
23	How to manage psoriatic arthritis pain?	Discharge or Postoperative Care
24	How to prevent psoriatic arthritis flare-ups?	Discharge or Postoperative Care
25	How does psoriatic arthritis affect daily life?	Discharge or Postoperative Care
26	How to manage fatigue in psoriatic arthritis?	Discharge or Postoperative Care
27	What lifestyle changes help with psoriatic arthritis?	Discharge or Postoperative Care
28	Can exercise help with psoriatic arthritis?	Discharge or Postoperative Care
29	What are the best exercises for psoriatic arthritis?	Discharge or Postoperative Care
30	What are the treatment options for psoriatic arthritis?	Medication, Drug, or Product
31	What are the best medications for psoriatic arthritis?	Medication, Drug, or Product
32	What is the role of biologics in treating psoriatic arthritis?	Medication, Drug, or Product
33	What are the common side effects of psoriatic arthritis medications?	Medication, Drug, or Product
34	What is the role of corticosteroids in treating psoriatic arthritis?	Medication, Drug, or Product
35	What is the role of methotrexate in psoriatic arthritis treatment?	Medication, Drug, or Product
36	What is the role of TNF inhibitors in treating psoriatic arthritis?	Medication, Drug, or Product
37	Are there natural remedies for psoriatic arthritis?	Medication, Drug, or Product
38	Can diet affect psoriatic arthritis?	Medication, Drug, or Product
39	Are there specific diets for psoriatic arthritis?	Medication, Drug, or Product
40	Can smoking affect psoriatic arthritis?	Medication, Drug, or Product
41	Can alcohol affect psoriatic arthritis?	Medication, Drug, or Product
42	How is psoriatic arthritis diagnosed?	Procedure, Test, Process, Study, or Method
43	What is enthesitis in psoriatic arthritis?	Procedure, Test, Process, Study, or Method
44	What is the difference between rheumatoid arthritis and psoriatic arthritis?	Procedure, Test, Process, Study, or Method
45	What is the difference between psoriatic arthritis and osteoarthritis?	Procedure, Test, Process, Study, or Method
46	What is the role of physical therapy in psoriatic arthritis?	Procedure, Test, Process, Study, or Method
47	What are the differences between psoriatic arthritis and osteoarthritis?	Procedure, Test, Process, Study, or Method
48	What is the role of TNF inhibitors in psoriatic arthritis treatment?	Procedure, Test, Process, Study, or Method
49	What are the risks of untreated psoriatic arthritis?	Procedure, Test, Process, Study, or Method
50	Can psoriatic arthritis cause disability?	Procedure, Test, Process, Study, or Method

These subgroups included following:

1. Symptoms: Questions related to PsA symptoms, such as joint pain, edema, and skin involvement.
2. Diagnosis: Questions focused on the diagnostic criteria and procedures used to detect PsA.
3. Treatment options: Questions about the different treatments for PsA, including medications and lifestyle changes.
4. Complications: Questions about the possible complications and long-term effects of PsA.

Table 2. Descriptive statistics of patient information quality and readability scores.

	Minimum	Maximum	Mean ± SD
Ensuring Quality Information for Patients Score	50	69	59 (5.2)
The Flesch-Kincaid Reading Ease Score	45	59	50 (4.1)
The Flesch-Kincaid Grade Level Score	8	12	9.5 (1.2)

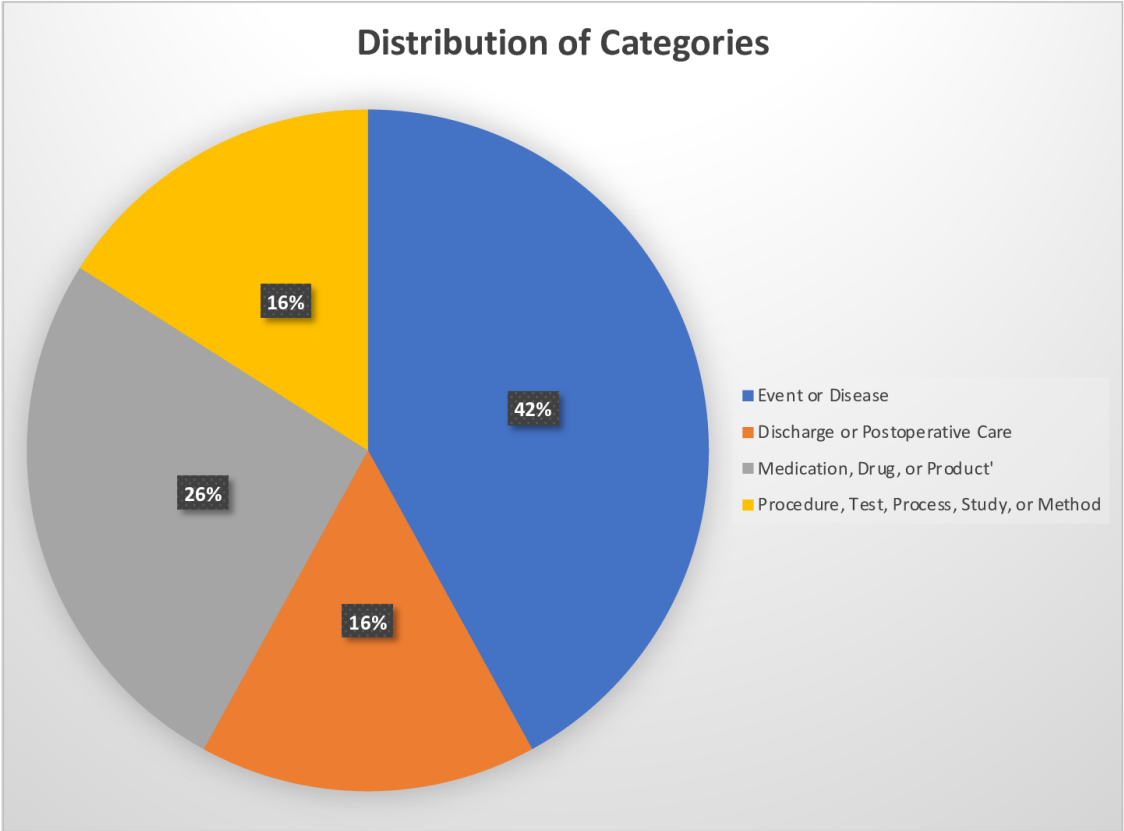


Figure 2. Distribution of categories in study analysis.

Significant differences in EQIP scores were observed among subgroups ($H(3) = 9.56, p < 0.05$). The treatment options subgroup had significantly lower EQIP scores than the symptoms and diagnosis subgroups, which indicated that ChatGPT’s responses were less clear and complete while presenting treatment regimens. The Bonferroni post hoc correction revealed a significant difference between treatment option and symptom subgroups ($p < 0.017$).

The distribution of categories in the study analysis is illustrated in Figure 2. Along with the overall analysis, a subgroup analysis was performed to evaluate the EQIP, FKRE, and FKGL scores within each question category (symptoms, diagnosis, treatment options, and complications). This analysis revealed that ChatGPT performed weaker in the treatment options category, with significantly lower EQIP scores (mean = 65.3, SD = 8.7) than in the symptoms category (mean = 78.2, SD = 5.4). The results indicated that, while ChatGPT accurately defined common PsA symptoms, such as joint pain and swelling, it was unable to provide detailed and clear information about treatment options.

The EQIP scores in the symptoms subgroup provided additional evidence for ChatGPT’s responses to common PsA symptoms, such as joint pain, swelling, and skin

patches. The high mean EQIP score in this subgroup (78.2) indicates that ChatGPT is reliable in reporting symptoms but less effective in answering treatment-related questions, as indicated by the lower EQIP scores in that subgroup.

Discussion

This study provides several significant insights into ChatGPT’s efficacy in providing health information for PsA. The EQIP scores reveal that while ChatGPT could provide high-quality information, there are notable deficiencies in several areas, particularly in the discussion of complex treatment options [19]. This finding is consistent with a previous study on AI-generated health information, which revealed that AI platforms fail to explain complex medical content [20].

One of the primary challenges identified in this study is the readability of the information generated by ChatGPT. The FKRE and FKGL scores indicate that the relatively high reading level is necessary to fully comprehend the content, which might limit its accessibility to patients with lower literacy levels [21]. Since health literacy is a significant predictor of health outcomes [22], patients with limited literacy skills might not fully understand the information

provided, which could limit their ability to make informed healthcare decisions [23].

ChatGPT's responses to questions concerning common PsA symptoms, such as joint pain, swelling, and skin involvement, were consistent with existing medical knowledge [24]. For example, in response to a question regarding the common PsA symptoms, ChatGPT accurately recognized the characteristic indicators of the condition, such as dactylitis and enthesitis, which are well-documented in medical literature [25]. Furthermore, ChatGPT identified that PsA can cause peripheral and axial joint involvement, which indicated its consistency with established clinical descriptions of the condition [26].

However, the study also identified areas where ChatGPT's responses were less consistent with medical knowledge, notably in terms of treatment strategies. While ChatGPT highlighted commonly prescribed medications such as NSAIDs and biologics, it often failed to provide detailed information on proper usage, possible side effects, and long-term management concerns, which are crucial for patient education and effective disease management [27].

The findings of this study are consistent with and build upon earlier studies on AI-generated health information. Studies on AI-generated content for conditions such as diabetes and hypertension have also shown challenges in preserving the accuracy and readability of complex medical information. Previous research has shown that, while AI tools can effectively provide general health information, they often struggle with more subtle issues that need detailed explanations or patient-specific guidance [28]. Comparing the EQIP and readability scores from this study with those reported in similar studies reveals that these issues are not unique to PsA but rather reflect broader limitations in current AI technologies [29].

Similar studies on AI-generated content for chronic conditions such as diabetes have found that, while the general information provided is accurate, the level of detail required for patient management often falls short, particularly in areas requiring personalized advice [30]. This comparison emphasizes the importance of continued development and refinement of AI tools to ensure they reach the high standards necessary for patient education and support [31].

The FKRE and FKGL scores presented in this study reveal a significant challenge in the accessibility of ChatGPT-generated health information. The FKGL scores, which show a reading level between high school and college, indicate that patients with lower literacy levels may struggle to understand the content [32]. This is particularly problematic considering that health literacy has a significant influence on patient outcomes [33]. Patients with limited literacy skills may struggle to interpret medical information offered at a higher reading level, potentially leading to miscommunication regarding their condition and treatment options [34]. This accessibility gap highlights the need to tailor AI-generated content to the demands of a diverse patient population, ensuring that all individuals, regardless of literacy level, have access to and benefit from accurate and comprehensible health information [35].

To improve the readability of ChatGPT's responses and

better meet the demands of a diverse patient population, several strategies might be used. First, AI algorithms could be programmed to reduce medical jargon using more common language and shorter sentences to make the content more understandable [36]. Additionally, developers might include a function that allows the complexity of the language to be modified based on the user's literacy level, ensuring the information is more accessible to a larger audience [37]. Another potential improvement is the use of visual aids, such as diagrams or videos, to help convey complex medical concepts more effectively [38]. These guidelines are intended to make AI-generated health information more inclusive, ensuring that patients of all literacy levels can access, comprehend, and act on the medical advice provided [39].

Several possible confounding factors might have impacted the EQIP and readability scores observed in this study. One important factor is the complexity of the medical content; more complex topics, such as treatment options or detailed diagnostic criteria, might have lower readability scores due to the need to use technical terminology and provide longer explanations. Additionally, the specific words used in the questions might influence the results. For example, unclear or broad questions elicit less detailed responses and reduce the EQIP score. In contrast, more specific questions might prompt responses that are more technical and less readable, thus increasing the difficulty of understanding as indicated by the FKRE and FKGL scores. These confounding factors emphasize the importance of careful attention when interpreting the results. Future studies should consider these characteristics to better understand how AI-generated content performs across diverse types of medical information.

While this study provides valuable insights into ChatGPT's ability to generate health information on PsA, several limitations must be acknowledged. First, our study did not examine the potential link between PsA and cardiovascular conditions such as subclinical atherosclerosis, a well-documented association in the literature [40]. Future research could explore how AI-generated responses address comorbidities associated with PsA, particularly cardiovascular risk factors.

Another limitation is that our readability assessment relied on standardized readability formulas, which do not fully capture comprehension levels across diverse patient populations. Future studies could incorporate patient surveys to evaluate real-world readability and comprehension. Additionally, AI-generated health information, it is crucial to investigate the broader implications of these results and potential solutions. One of the main challenges in improving AI for patient education is ensuring that the information provided is accurate and accessible to a diverse patient group. This includes addressing the technical aspects of AI, such as refining algorithms for better understanding and generating medical content along with considering the social and ethical components, such as providing equal access to information. Furthermore, AI tools must be designed to accommodate patients' diverse literacy levels, cultural backgrounds, and individual needs. Potential solutions include developing configurable AI systems that allow users to select desired level of detail or complexity

and incorporate feedback loops that allow patients to rate helpfulness or understandability of the information. Addressing these factors could help in optimizing AI to serve as a reliable and effective tool for patient education, eventually improving health outcomes and patient empowerment.

Conclusion

Although ChatGPT shows potential to provide accessible health information on PsA, this study highlights significant areas for improvement, notably in the readability and complexity of the information presented. By addressing these challenges, AI tools might be modified to effectively meet the demands of diverse patient populations, which could result in improved health outcomes.

Ethical approval

An ethics committee decision is not required for the study.

References

- Ogdie A, Weiss P. The epidemiology of psoriatic arthritis. *Rheum Dis Clin North Am*. 2015;41(4):545-568.
- Gladman DD, Antoni C, Mease P, et al. Psoriatic arthritis: epidemiology, clinical features, course, and outcome. *Ann Rheum Dis*. 2005;64(Suppl 2):iii14-7.
- Ritchlin CT, Colbert RA, Gladman DD. Psoriatic Arthritis. *N Engl J Med*. 2017;376(10):957-970.
- Wright JG, Feinstein AR. A comparative contrast of clinimetric and psychometric methods for constructing indexes and rating scales. *J Clin Epidemiol*. 1992;45(11):1201-1218.
- Uz C, Umay E. "Dr ChatGPT": Is it a reliable and useful source for common rheumatic diseases?. *Int J Rheum Dis*. 2023;26(7):1343-1349.
- Coskun BN, Yagiz B, Ocaoglu G, Dalkilic E, Pehlivan Y. Assessing the accuracy and completeness of artificial intelligence language models in providing information on methotrexate use. *Rheumatol Int*. 2024;44(3):509-515.
- Wang A, Wu Y, Ji X, et al. Assessing and Optimizing Large Language Models on Spondyloarthritis Multi-Choice Question Answering: Protocol for Enhancement and Assessment. *JMIR Res Protoc*. 2024;13:e57001.
- Rosenthal R. Meta-analytic procedures for social research. *Beverly Hills, CA: Sage*; 1984, 148 pp.
- Mann HB, Whitney DR. On a test of whether one of two random variables is stochastically larger than the other. *Ann Math Stat*. 1947;18(1):50-60.
- Holde M, Larsen JB. Validating tests for readability and comprehension: Implications for health literacy. *Health Educ Res*. 2006;21(5):648-657.
- Penchansky R, Thomas JW. The concept of access: definition and relationship to consumer satisfaction. *Med Care*. 1981;19(2):127-140.
- Smith AJ, Stevenson PM, Denberg T. Evaluating the Quality of Online Health Information for Patients with Chronic Diseases. *JAMA Netw Open*. 2019;2(11).
- Friedberg MW, Van Busum K, Wexler R, et al. A demonstration of AI-generated patient education materials. *BMJ Open*. 2021;11(2).
- Berland GK, Elliott MN, Morales LS, et al. Health information on the Internet: accessibility, quality, and readability in English and Spanish. *JAMA*. 2001;285(20):2612-2621.
- Wilson S, Randhawa G, Walker S, et al. Patient education materials for asthma: the importance of readability. *J Asthma*. 2007;44(9):675-681.
- Saluja S, Abraham A, Pandey A, et al. Impact of Language and Cultural Differences on the Accessibility of Online Health Information. *Health Commun*. 2019;34(6):685-691.
- Gilbert J, Tan B. AI in Healthcare: Democratizing Access or Perpetuating Inequity? *J Med Ethics*. 2022;48(1):3-9.
- Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics*. 1977;33(1):159-174.
- Kane D, Stafford L, Bresnihan B, et al. A prospective, clinical and radiological study of early psoriatic arthritis: an early synovitis clinic experience. *Rheumatology (Oxford)*. 2003;42(12):1460-1468.
- Husni ME, Schainker E, Thampy P, et al. Comparison of methods for assessing the severity of psoriatic arthritis in patients in the CORRONA registry. *Ann Rheum Dis*. 2014;73(10):1786-1792.
- de Vlam K, Cuvelier C, Mielants H, et al. Immunohistochemical characteristics of gut inflammation in spondyloarthropathy and ulcerative colitis: their potential role in diagnosis and understanding of pathogenesis. *Arthritis Rheum*. 1996;39(3):479-485.
- Wolfe F, Michaud K, Li T, et al. Chronic conditions and health problems in rheumatic diseases: comparisons with rheumatoid arthritis, noninflammatory rheumatic disorders, systemic lupus erythematosus, and fibromyalgia. *J Rheumatol*. 2010;37(2):305-315.
- Coates LC, FitzGerald O, Merola JF, et al. Group for Research and Assessment of Psoriasis and Psoriatic Arthritis: treatment recommendations for psoriatic arthritis 2015. *Arthritis Rheumatol*. 2016;68(5):1060-1071.
- Gladman DD, Tom BD, Mease PJ, et al. Informing the patient global assessment for psoriatic arthritis: a cross-sectional analysis of clinical data from the CORRONA Psoriatic Arthritis/ Spondyloarthritis Registry. *Arthritis Care Res (Hoboken)*. 2019;71(8):1011-1020.
- Callis Duffin K, Chandran V, Ogdie A, et al. Psoriatic arthritis: an update for dermatologists. *J Am Acad Dermatol*. 2016;74(2):289-306.
- Haroon M, Gallagher P, FitzGerald O. Diagnostic delay of more than 6 months contributes to poor radiographic and functional outcome in psoriatic arthritis. *Ann Rheum Dis*. 2015;74(6):1045-1050.
- Gladman DD, Farewell VT, Husted J, et al. The role of HLA antigens as indicators of disease progression in psoriatic arthritis: HLA markers indicate susceptibility and severity of psoriatic arthritis. *Arthritis Rheum*. 1998;41(10):1828-1835.
- Chandran V, Schentag CT, Gladman DD. Severity of psoriasis inversely correlates with improvement in joint disease activity in psoriatic arthritis: a short-term followup study. *Ann Rheum Dis*. 2006;65(1):26-29.
- Coates LC, Helliwell PS. Comparison of three screening tools to detect psoriatic arthritis in patients with psoriasis (CONTEST study). *Br J Dermatol*. 2013;168(4):802-807.
- Vlam K de, Mielants H, Veys EM. Gut inflammation and spondyloarthritis: immunohistologic features. *Curr Opin Rheumatol*. 2000;12(4):318-321.
- Tillett W, Jadon D, Shaddick G, et al. Smoking and delay to diagnosis are associated with poor functional outcome in psoriatic arthritis. *Ann Rheum Dis*. 2013;72(8):1358-1361.
- Ogdie A, Langan S, Love T, et al. Prevalence and treatment patterns of psoriatic arthritis in the UK: a population-based study. *Rheumatology (Oxford)*. 2013;52(3):568-575.
- Gelfand JM, Neimann AL, Shin DB, et al. Risk of myocardial infarction in patients with psoriasis. *JAMA*. 2006;296(14):1735-1741.
- Ritchlin CT, Kavanaugh A, Gladman DD, et al. Treatment recommendations for psoriatic arthritis. *Ann Rheum Dis*. 2009;68(9):1387-1394.
- Coates LC, Helliwell PS. Defining disease modification in psoriatic arthritis. *Rheumatology (Oxford)*. 2016;55(12):2105-2106.
- Van der Heijde D, Landewe R, Mease PJ, et al. Brief report: magnetic resonance imaging of the sacroiliac joints in the assessment of axial spondyloarthritis: data from the DESIR cohort. *Arthritis Rheumatol*. 2015;67(2):375-383.
- Mease PJ, Gladman DD, Papp KA, et al. A novel and evidence-based decision tool for guiding the treatment of patients with psoriatic arthritis: The GRAPPA Treatment Recommendations. *Arthritis Care Res (Hoboken)*. 2021;73(2):178-186.
- Smolen JS, Landewé R, Bijlsma J, et al. EULAR recommendations for the management of psoriatic arthritis with pharmacological therapies: 2019 update. *Ann Rheum Dis*. 2020;79(6):700-712.
- El-Miedany Y, Palmer D, Adebajo A, et al. Educating patients about biologics: perspectives of health professionals in rheumatology. *J Clin Rheumatol*. 2019;25(2):61-67.
- Atik I, Atik S, Gül E. Evaluation of Subclinical Atherosclerosis in Patients with Psoriatic Arthritis. *General Medicine Journal*. 2024;34(2):186-9.



Ann Med Res

Current issue list available at AnnMedRes

Annals of Medical Research

journal page: www.annalsmedres.org

Effect of Sleeper One® 5 anesthesia device on heart rate

Özgür Doğan^{a,*}, Fatma Duygu Dertli^b, Özge Doğan^c, Suat Serhan Altıntepe Doğan^d,
Gözde Ülker^a

^aAfyonkarahisar Health Sciences University, Faculty of Dentistry, Department of Pediatric Dentistry, Afyonkarahisar, Türkiye

^bSerdent Private Oral and Dental Health Polyclinic, Afyonkarahisar, Türkiye

^cTunceli State Hospital, Tunceli, Türkiye

^dAfyonkarahisar Health Sciences University, Faculty of Dentistry, Department of Periodontology, Afyonkarahisar, Türkiye

Abstract

ARTICLE INFO

Keywords:

Sleeper One® 5
Dental anxiety
Local anesthesia
Computer-controlled anesthesia
Dental pain

Received: Apr 18, 2024

Accepted: Feb 14, 2025

Available Online: 26.02.2025

DOI:

[10.5455/annalsmedres.2024.04.072](https://doi.org/10.5455/annalsmedres.2024.04.072)

Aim: This study compares the pulse parameter values before, during, and after anesthesia using computer-assisted local infiltration anesthesia and traditional local infiltration anesthesia methods in children.

Materials and Methods: The study included 100 children aged 7-11 who applied to the pediatric dentistry clinic. During the initial session, the children underwent intraoral examinations. The children were divided into two groups randomly. Traditional local infiltration and computer-assisted local anesthesia were applied to treat dentin caries in the maxillary primary molar teeth. The heart rate of the patients was measured using a pulse oximeter device.

Results: The average age of the patients was 8.21 ± 1.00 years, with 48 (48.0%) male and 52 (52.0%) female. The average heart rate (HR) was found to be 99.28 ± 14.68 . At the end of the study, 61 (61.0%) of the patients preferred the computer-assisted anesthesia method, and 39 (39.0%) preferred the traditional method. A notable distinction exists between the pre- and post-anesthesia heart rate values associated with each anesthesia technique, indicating significant variations when comparing different methods of anesthesia ($p < 0.001$). No significant differences were observed between the two methodologies across all measured values ($p > 0.05$).

Conclusion: Although there was no significant difference in heart rate parameters, 61% of the patients preferred the computer-controlled local anesthesia method, which showed effectiveness in reducing anxiety. Further studies are required to confirm these results using modern local anesthesia devices.



Copyright © 2025 The author(s) - Available online at www.annalsmedres.org. This is an Open Access article distributed under the terms of Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

Introduction

Dental anxiety and fear of dental treatment in children is a global public health problem that leads to neglect of dental care in many countries [1]. In studies conducted in various countries, the prevalence of childhood dental anxiety in Turkey ranges between 21.3% and 23.5% [2]. It has been reported that the percentage varies between 2.5% and 20% in other countries [3]. It is common for dental treatments that involve procedures such as local anesthesia applications to elicit fear and anxiety in patients. The resulting physiological reactions can make the experience even more unpleasant. That is why dental professionals must prioritize patient comfort and well-being. By addressing dental fear and anxiety, such as sedation or other relaxation techniques, dental professionals can help patients feel more at

ease and improve their overall experience [4]. Local anesthesia is frequently used in dentistry to prevent pain, but it can cause anxiety and fear in pediatric patients, making it difficult to manage their behavior [5]. During local infiltration anesthesia, using long needles, venting the syringe visibly, and administering inappropriate doses of anesthetics can trigger anxiety and fear of pain in children [6]. Several methods have been proposed to alleviate pain during injections, including topical anesthetics, warming the anesthetic solution to body temperature, and extending the injection time [7].

Local anesthesia devices have gained popularity in recent years. Various systems for administering local anesthesia include computer-aided applications, electronic dental anesthesia, intraoral lidocaine tape, jet injectors, and vibration devices [8]. Computer-controlled local anesthesia application systems are devices designed to reduce pain and anxiety. Excessive pressure and rapid injection of

*Corresponding author:

Email address: ozgurdogan1984@gmail.com (Özgür Doğan)

anesthetic solution cause pain during injection [9]. In computer-controlled anesthesia systems, an injection is administered gradually and at a speed controlled, eliminating the need for pressure. This device makes dental anesthesia more comfortable and painless for patients and pediatric dentists [10,11].

Sleeper One® 5 (DHT, Cholet, France) is a lightweight handpiece with an easy-to-grip design, wireless foot pedal, and control unit. It has a resistance analysis system that regulates the injection according to tissue density, and the foot pedal controls the injection speed. The device's hand unit allows for monitoring the amount of solution applied and the resistance to the solution, featuring three pre-programmed injection rates. The most important feature of Sleeper One® 5 is its double-curved needle, which makes it easier to penetrate bone (Figure 1) [12].



Figure 1. Sleeper One® 5 anesthesia device.

This study aims to evaluate using heart rate as a parameter for selecting anesthesia in children and detecting dental anxiety and fear. The study will compare the effectiveness of infiltrative anesthesia using classical dental syringes versus the Sleeper One® 5 anesthesia device. The study's null hypothesis is that there is no difference between patients' heart rate measurements during buccal infiltration anesthesia performed with the traditional technique and the Sleeper One® 5 device.

Materials and Methods

The study received approval from the Afyonkarahisar Health Sciences University Clinical Research Ethics Committee (decision numbered 2021/85) and the Ministry of Health Turkey Pharmaceuticals and Medical Devices Agency (decision numbered E-68869993-511.06-365560). All patients included in the study were treated according to the ethical principles of the Declaration of Helsinki. The study has been presented according to CONSORT guidelines for reporting trials.

Sample size determination

Patient groups were selected based on specified criteria from applicants to the pediatric dentistry department clinic between October 2021 and May 2022. The analysis for this study was conducted using the G*Power 3.1 software [13]. Drawing on power analyses from analogous re-

search, the minimum sample size was established at 88 patients, ensuring a 95% confidence interval with an achieved power of 80%. After detailed anamnesis and clinical and radiographic examinations, 100 patients (48 boys and 52 girls) were deemed appropriate for inclusion in the study.

Study population

A total of 100 patients, 48 (48.0%) males and 52 (52.0%) females, aged between 7 and 11, participated in the study voluntarily. The patients were randomly divided into two groups based on their preference for green or yellow.

The inclusion criteria

Individuals included in the study:

- Systemically healthy
- Score 3 or 4 on the Frankl scale, [14]
- Have caries in their upper jaw primary molars that have not reached the pulp and require treatment.

The exclusion criteria

Individuals excluded in the study:

- Who had a systemic disease,
- Children who were allergic to the anesthetic agent that would be used,
- Children who had an acute infection,
- Children or their guardians who refused to participate in the study,
- Patients who were unable or unwilling to attend the follow-up sessions.

Study design

Cross-over and split-mouth designs were used in the research. Patients were randomly divided into two groups according to their preference for green or yellow. It was determined that the green color represents traditional infiltration anesthesia, and the yellow color represents anesthesia performed with the help of computer technology using Sleeper One® 5. An independent researcher who was not involved in the study asked the participants to choose a color to ensure a double-blind randomization procedure. After each participant selected a color, they were informed about the relevant anesthesia technique and the groups were randomly assigned. Both anesthesia methods were administered to every patient (Figure 2). One hundred participants were thoroughly informed about the details of the study. Additionally, each participant completed the patient consent form to provide consent.

Clinical protocol

The first appointment

The first appointment is the session wherein child underwent an oral examination and became familiar with the environment and treatment. In this session, the children's heart rate was measured using a pulse oximeter (Yongkang, Xuzhou, China) before the intraoral examination with the help of HR determination.

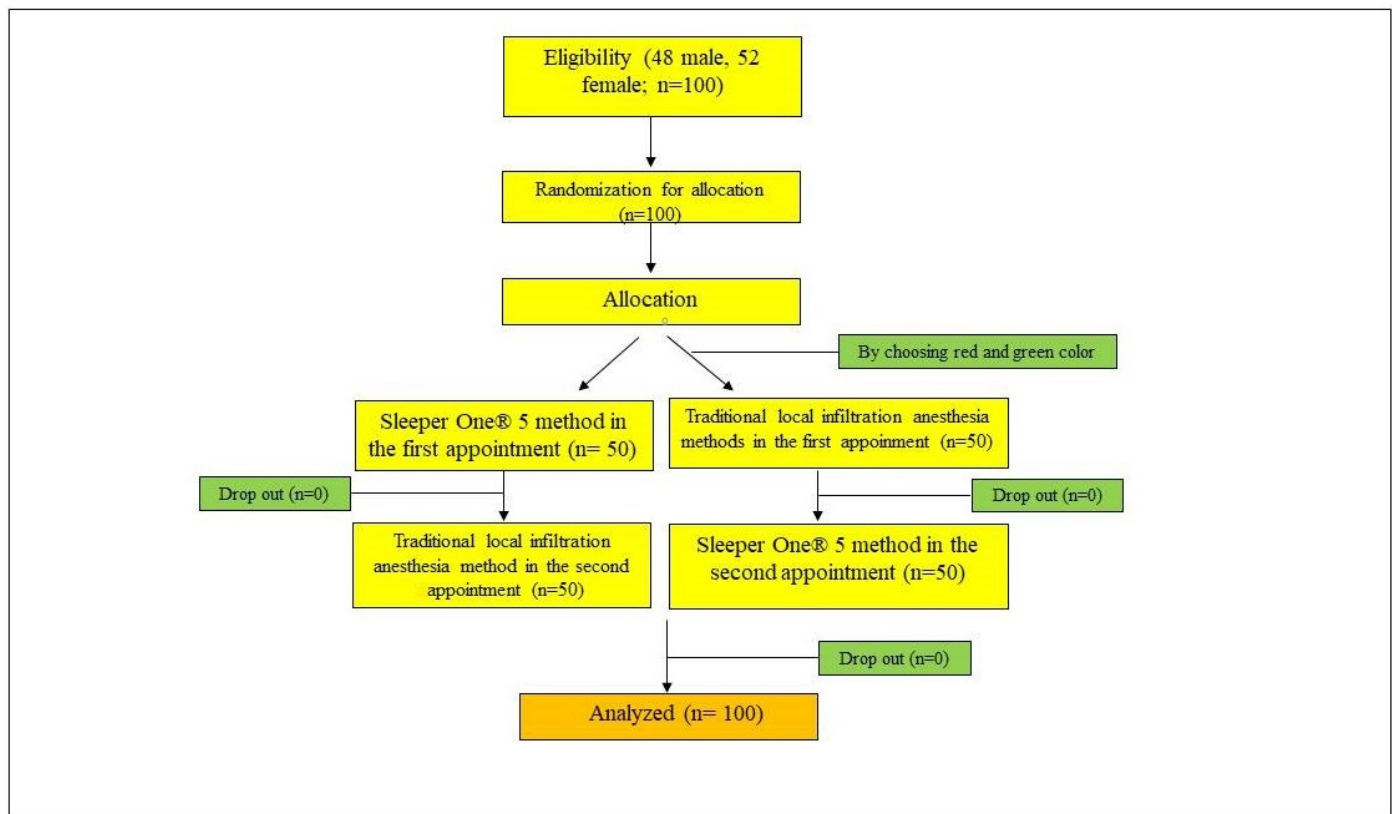


Figure 2. The study flowchart follows CONSORT guidelines for clinical trials.



Figure 3. Sleeper One® 5 anesthesia device.

The second appointment

A randomly selected anesthesia method was applied in this session. At the start of the session, a topical anesthesia sprays containing 2% lidocaine (Locanest spray, Axiva İlaç San. ve Tic. AŞ., İstanbul, Türkiye) was applied for 1 minute. Afterward, Sleeper One® 5 was used for children who chose the yellow color. At the same time, traditional local anesthesia was administered to children who chose the green color with a two-cc classic dental injector (Berika Teknoloji Medikal İml. İth. İhrc. Ticaret LTD. ŞTİ., Konya, Türkiye). During local anesthesia, 4% articaine was used as the active ingredient along with 1 part in 100000 epinephrine content mixed in Ultracaine DS Forte capsule (Hoechst Marion Roussel, Germany). In the buccal infiltrative injection, 1.5 cc of local anesthetic solution were injected into the tissue at 60 seconds using

the traditional method. However, the Sleeper One® 5 device took 145 seconds. After removing the caries and opening the cavities, teeth that required restorative treatment were restored.

The third appointment

After applying a 1-minute topical anesthetic, the anesthesia method used in the first session was changed to a different one. Local anesthesia was used for restorative treatment of decayed teeth in the other half of children's maxilla. The child was made to sit on a chair at the start of the three sessions. Afterward, the necessary information was given to the child, and then the finger apparatus of the pulse oximeter was attached to the index finger of the left hand. The apparatus remained on the finger until the end of the procedure (Figure 3). During the first session, only one measurement was recorded. However, four heart rate (HR) values were recorded in the second and third sessions. The first measurement was taken before starting the anesthesia, the second measurement was taken when half of the anesthetic capsule was reached, the third was taken immediately after the anesthesia procedure, and the fourth was taken one minute after the anesthesia process had ended. It was recorded that a single patient had 9 HR values in total. All the values were combined and averaged to produce a single value for statistical analyses.

Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics version 20.0 (IBM SPSS®, Chicago, USA). Each individual heart rate measurement was treated as a distinct statistical entity. Quantitative heart rate data

Table 1. Comparisons of repeated measurements for the conventional anesthesia method.

Variables	Mean±SD	95% CI for Mean (Lower Bound-Upper Bound)	p value
1. Heart Rate Measurements	98.18±14.31	95.34-101.02	<0.001 ^a
2. Heart Rate Measurements	95.29±15.89	92.14-98.44	
3. Heart Rate Measurements	96.86±15.96	93.69-100.03	
4. Heart Rate Measurements	103.20±13.27	100.57-105.83	

SD: Standard Deviation, CI: Confidence Interval, ^a: Analysis of Variance in Repeated Measurements test.**Table 2.** Comparisons of repeated measurements for the computer-assisted anesthesia method.

Variables	Mean±SD	95% CI for Mean (Lower Bound-Upper Bound)	p value
1. Heart Rate Measurements	99.82±14.52	96.94-102.70	<0.001 ^a
2. Heart Rate Measurements	95.17±15.35	92.12-98.22	
3. Heart Rate Measurements	96.80±15.03	93.82-99.78	
4. Heart Rate Measurements	101.52±13.43	98.86-104.18	

SD: Standard Deviation, CI: Confidence Interval, ^a: Analysis of Variance in Repeated Measurements test.**Table 3.** The comparison between computer-controlled anesthesia methods and traditional anesthesia methods for each measurement value.

Variables	Traditional		Computer-Controlled		p value
	Mean±SD	95% CI for Mean (Lower Bound-Upper Bound)	Mean±SD	95% CI for Mean (Lower Bound-Upper Bound)	
1. Heart Rate Measurements	98.18±14.31	95.34-101.02	99.82±14.52	96.94-102.70	0.094 ^b
2. Heart Rate Measurements	95.29±15.89	92.14-98.44	95.17±15.35	92.12-98.22	0.927 ^a
3. Heart Rate Measurements	96.86±15.96	93.69-100.03	96.80±15.03	93.82-99.78	0.968 ^a
4. Heart Rate Measurements	103.20±13.27	100.57-105.83	101.52±13.43	98.86-104.18	0.157 ^a

SD: Standard Deviation, CI: Confidence Interval, ^a: Paired-t test, ^b: Wilcoxon Sign Rank test.

were summarized using standard deviation and median (with minimum and maximum values), while qualitative data were presented as the number of patients along with their corresponding percentages. To compare heart rates between traditional and computer-assisted anesthesia techniques, the paired t-test was employed for normally distributed dependent quantitative variables, whereas the Wilcoxon signed-rank test was applied for non-normally distributed variables. For assessing variance across multiple measurements, the ANOVA test for repeated measures was utilized. A p-value threshold of <0.05 was established to denote statistical significance.

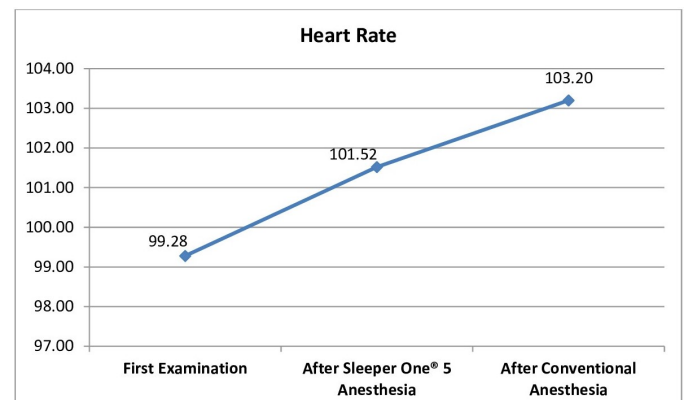
Results

Out of the total number of patients in the study (n=100), 48 (48.0%) were male and 52 (52.0%) were female. The mean age of the patients was 8.21±1.00 years. The average HR was calculated to be 99.28±14.68. At the end of the study, 61 out of 100 patients chose computer-assisted anesthesia, while 39 chose the traditional method.

Table 1 compares the repeated measurements obtained using the traditional anesthesia method. A significant difference was found in HR between the traditional anesthesia procedure (p<0.001). The HR measurements were taken at four different stages. It has been determined that there are considerable variations between certain pairs of measurement times. Significant differences were observed between certain dual measurement times: 1st-2nd, 1st-4th,

2nd-3rd, 2nd-4th, and 3rd-4th. The p-values were 0.001, <0.001, 0.041, <0.001, and <0.001, respectively.

In Table 2, comparisons were made for the computer-assisted anesthesia method, and a significant difference was found for HR (p<0.001) among repeated measurements. It has been determined that there are considerable variations between certain pairs of measurement times. Significant differences were observed between certain dual measurement times: 1st-2nd, 1st-3rd, 2nd-4th, and 3rd-4th. The p-values were 0.001, =0.019, 0.001, and <0.001, respectively.

**Figure 4.** Comparison of heart rate measurement during examination and measurements of anesthesia methods.

In Table 3, traditional anesthesia and computer-assisted methods were compared separately for each measurement value, and no significant difference was found between the two anesthesia methods for any measurement value ($p>0.05$) (Figure 4).

Discussion

The results of the study showed that although higher heart rate values were observed with the traditional method of administering local anesthesia during the upper jaw buccal infiltration technique as compared to the Sleeper One® 5 device at all measurement times, this difference was not statistically significant. It was found that the null hypothesis of the study was valid.

It is recommended to control the injection speed and the pressure applied to the tissues during injection, but manual control of this situation may be difficult in local anesthesia applications. Based on these considerations, The Wand® (later versions called Wand Plus® and CompuDent®) (Milestone Scientific, Livingston, New Jersey, USA), Quicksleeper® (Dental HiTec, Cholet, France), Sleeper One® 5 (Dental HiTec, Cholet, France) and Comfort Control Syringe® (Dentsply International, York, PA, USA) have been developed. [15,16] The Sleeper One® 5 device is controlled by a wireless foot pedal with three injection speeds. This device's asymmetrical triple-beveled needle detects tissue resistance, and the anesthetic dose and application rate are controlled to avoid exceeding the pain threshold [17]. In our study, we used disposable plastic dental syringes with a needle diameter of 0.40 mm and a length of 4-5 cm. On the other hand, the needle used in Sleeper One® 5 has a diameter ranging from 0.27-0.36 mm (30-27 Gauge) and a length of 0.9-1.2 cm. The Sleeper One® 5 injection device has a thinner and shorter needle than plastic dental syringes.

Dental anxiety in children is influenced by various physical and emotional factors such as age, gender, past dental experiences, family socioeconomic status, and parenting style. Some of the reasons why people experience fear and anxiety when it comes to dentistry are related to the tools used by the dentist, the procedures involved during the dental treatment, the sight of a syringe or the injection process, and the use of an aerator [18].

There are various methods to measure dental anxiety and fear, including observation-based techniques, psychometric scales, and physiological and projective measurements [19]. Physiological measurements use specialized equipment to indirectly obtain information about the severity of a patient's fear and anxiety by measuring pulse and blood pressure. In the current study, the researchers utilized physiological measurement techniques and preferred measuring HR through pulse oximetry due to its ease of clinical application. To eliminate the risk that a very short-term increase in HR may affect the general health of patients with cardiac disease, patients with any systemic disease were not included in our study. Dental fear and anxiety can cause an increase in cortisol levels, resulting in higher levels of catecholamines, which can lead to elevated blood pressure and heart rate [4,20].

During dental treatments, HR increases due to anxiety, such as during injections. In studies evaluating HR, base-

line HR values are measured before treatment and compared to subsequent values [15,21]. Rosenberg et al. conducted a study on 58 children, aged between 3 and 12, to investigate the relationship between heart rate (HR) and anxiety. Contrary to some studies that highlight HR as an important factor in measuring anxiety, Rosenberg et al. argued that HR cannot be a determinant of anxiety [22,23].

The treatment programs in our study consisted of three sessions with appointments scheduled 7-10 days apart. In a study by Wogelius et al. regular dentist visits without invasive treatment can prevent mild dental anxiety in children [24]. On the other hand, a study conducted by Hembrecht et al. revealed that children exhibited more anxious behavior in the second injection session with both The Wand and Sleeper One® 5 devices when compared to the first session. They suggested that this situation could be attributed to a conditioning effect resulting from repeated dental appointments [25].

Pulse oximeters are non-invasive devices that measure blood oxygen saturation and do not require calibration for HR measurement. The measurement is typically taken with a probe that is placed on the finger. It measures only the amount of oxygen present in the arteries during each heartbeat. Pulse oximetry may cause dental anxiety even in anxiety-free children and may produce inaccurate results [26]. It is important to consider any limitations that may have influenced the results obtained during their use.

Both computer-assisted anesthesia and traditional anesthesia techniques increased HR, with no significant difference found between them, according to a previous study [27,28].

This study measured heart rates four times during injections with both anesthesia methods. Significant differences were found. When comparing computer-assisted anesthesia devices to traditional anesthesia, the difference in average heart rate is insignificant. Computer-assisted anesthesia devices have an average heart rate of 98.32, and traditional anesthesia has an average heart rate of 98.38. HR increased after anesthesia in both methods, and there was no statistically significant difference between them.

One limitation of our study was that some patients had a phobia of the dental syringe. The sight of the needle caused behavioral disorders in some patients and made treatment difficult. One of the limitations of addressing dental anxiety is that it is not solely related to the use of a needle and anesthesia. Dentist anxiety is a common phenomenon that can be affected by a variety of factors. The sounds in the clinic environment, the behavior of other children receiving treatment, and even small details such as the dentist's coat can contribute to a person's anxiety level. It is important to keep in mind the limitations of this study when analyzing the results. Although physiological methods are important in evaluating pain and anxiety in school-age children, this study only evaluated the HR parameter. It should be noted that while HR can support physiological tests for anxiety and fear, it may not be enough on its own.

Conclusion

When anesthesia techniques are compared, heart rate values are considered, and there is a significant difference between each anesthesia technique's pre and post-anesthesia values. Our proposed study found no statistically significant difference in average heart rates between the two methods, leading us to accept the null hypothesis. 61% of patients preferred the anesthesia method performed with the Sleeper One® 5 devices for future dental procedures, while 39% preferred traditional anesthesia. However, further studies are necessary with a larger sample group of children using the Sleeper One® 5 anesthesia device to confirm the results obtained.

Acknowledgment

All participants in the study are thanked, including their parents and Assoc. Prof. Dr. Batuhan Bakırarar is acknowledged for his assistance with the statistical analysis section.

Conflict of interest

The authors have no specific funding or other conflicts of interest to disclose.

Funding

This study was supported by Afyonkarahisar Health Science University Scientific Research Projects Coordination Unit project number 21.DUS.006.

Ethical approval

This study received approval from the Afyonkarahisar Health Sciences University Clinical Research Ethics Committee (decision number: 2021/85).

References

1. D. Q. Taani, "Dental attendance and anxiety among public and private school children in Jordan," *Int Dent J*. 2002 Feb;52(1):25–29. doi: 10.1111/j.1875-595X.2002.tb00593.x.
2. D. Firat, E. P. Tunc, and V. Sar, "Dental Anxiety Among Adults in Turkey," *J Contemp Dent Pract*. 2006 Jul;7(3):75–82. doi: 10.5005/jcdp-7-3-75.
3. C. Hägglin, U. Berggren, M. Hakeberg, T. Hallstrom, and C. Bengtsson, "Variations in Dental Anxiety among Middle-aged and Elderly Women in Sweden: A Longitudinal Study between 1968 and 1996," *J Dent Res*. 1999 Oct;78(10):1655–1661. doi: 10.1177/00220345990780101101.
4. Akarslan Z and Erten H, "Diş Hekimliği Korkusu ve Kaygısı," *Hacettepe Diş Hekimliği Fakültesi Dergisi (Clinical Dentistry and Research)*. 2009;33(1):62–68.
5. V. Smail-Faugeron, M. Muller-Bolla, J.-L. Sixou, and F. Courson, "Split-mouth and parallel-arm trials to compare pain with intraosseous anaesthesia delivered by the computerised Quick-sleeper system and conventional infiltration anaesthesia in paediatric oral healthcare: protocol for a randomised controlled trial," *BMJ Open*. 2015 Jul;5(7):e007724. doi: 10.1136/bmjopen-2015-007724.
6. D. Locker, A. Liddell, L. Dempster, and D. Shapiro, "Age of Onset of Dental Anxiety," *J Dent Res*. 1999 Mar;78(3):790–796. doi: 10.1177/00220345990780031201.
7. Libonati A, Nardi R, Gallusi G, Angotti V, Caruso S, and Coniglione F, "Pain and anxiety associated with Computer-Controlled Local Anaesthesia: Systematic review and meta-analysis of cross-over studies," *Eur J Paediatr Dent*. 2018;19(4):324–332.
8. H. Çerçi Akçay and G. Aren, "Çocuk Hastalarda Lokal Anestezi Uygulamasında Kullanılan T," *Selcuk Dental Journal*. 2021 Dec;8(3):895–901. doi: 10.15311/selcukdentj.782836.
9. Tüloğlu DN and Bayrak Ş, "Çocuk Diş Hekimliğinde Kullanılan Lokal Anestezi Teknikleri ve Güncel Yaklaşımlar," *Atatürk Üniversitesi Diş Hekimliği Fakültesi Dergisi*. 2010;20(1):53–61.
10. J. Versloot, J. S. J. Veerkamp, and J. Hoogstraten, "Computerized anesthesia delivery system vs. traditional syringe: comparing pain and pain-related behavior in children," *Eur J Oral Sci*. 2005 Dec;113(6):488–493. doi: 10.1111/j.1600-0722.2005.00252.x.
11. Sumer M and Koyuturk AE, "Comparison of the Wand with a conventional technique," *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*. 2006;101(6):106–109.
12. Al-Obaida MI, Haider M, Hashim R, AlGheriri W, Celur SL, and Al-Saleh SA, "Comparison of perceived pain and patients' satisfaction with traditional local anesthesia and single tooth anesthesia: A randomized clinical trial," *World J Clin Cases*. 2019 Oct;7(19):2986–2994. doi: 10.12998/wjcc.v7.i19.2986.
13. F. Faul, E. Erdfelder, A. Buchner, and A.-G. Lang, "Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses," *Behav Res Methods*. 2009 Nov;41(4):1149–1160. doi: 10.3758/BRM.41.4.1149.
14. K. Baier, P. Milgrom, S. Russell, L. Mancl, and T. Yoshida, "Children's fear and behavior in private pediatric dentistry practices," *Pediatr Dent*. 2004;26(4):316–21.
15. San Martin-Lopez A, Garrigos-Esparza L, Torre-Delgadillo G, Gordillo-Moscoso A, Hernandez-Sierra J, and de Pozos-Guillen AJ, "Clinical comparison of pain perception rates between computerized local anesthesia and conventional syringe in pediatric patients," *Journal of Clinical Pediatric Dentistry*. 2005;29(3):239–243.
16. Sreenivas A, Raj A, Rose Puthenpurackal V, Haneef T, Pillai G, and Deepak J, "Pain perception in different injection techniques in paediatric dentistry: an original research," *Euro J Molec Clin Med*. 2020;7(9):3049–3055.
17. Tom K and Aps J, "Intraosseous anesthesia as a primary technique for local anesthesia in dentistry," *Clin Res Infect Dis*. 2015;2(1).
18. Erten H, Akarslan ZZ, and Bodrumlu E, "Dental fear and anxiety levels of patients attending a dental clinic," *Quintessence Int (Berl)*. 2006;37(4):304–10.
19. Raj S, Agarwal M, Aradhya K, Konde S, and Nagakishore V, "Evaluation of dental fear in children during dental visit using children's fear survey schedule-dental subscale," *Int J Clin Pediatr Dent*. 2013;6(1):12–5. Doi: 10.5005/jp-journals-10005-1178.
20. Schuller AA, Willumsen T, and Holst D, "Are there differences in oral health and oral health behavior between individuals with high and low dental fear?," *Community Dent Oral Epidemiol*. 2003;31(2):116–121.
21. Sullivan C, Schneider PE, Musselman RJ, Dummett Jr CO, and Gardiner D, "The effect of virtual reality during dental treatment on child anxiety and behavior," *ASDC J Dent Child*. 2000;67(3):193–196.
22. Aitken JC, Wilson S, Coury D, and Moursi AM, "The effect of music distraction on pain, anxiety and behavior in pediatric dental patients," *Pediatr Dent*. 2002;24(2):118–124.
23. Kuşçu Ö, "Çocuklarda Farklı İki Dental Enjektör İle Yapılan Lokal Anestezi Ve Tedavi Uygulamalarında Psikometrik, Fizyolojik Ve Gözleme Dayalı Yöntemlerle Kaygı Ve Ağrının İncelenmesi," Marmara Üniversitesi, 2006.
24. P. Wogelius, S. Poulsen, and H. Toft Sørensen, "Prevalence of dental anxiety and behavior management problems among six to eight years old Danish children," *Acta Odontol Scand*. 2003 Jan;61(3):178–183. doi: 10.1080/00016350310003468.
25. Hembrecht EJ, Nieuwenhuizen J, Aartman IHA, Krikken J, and Veerkamp JSJ, "Pain-related behaviour in children: A randomised study during two sequential dental visits," *European Archives of Paediatric Dentistry*. 2013;14(1):3–8.
26. K. Terp and A. Sjöström-Strand, "Parents' experiences and the effect on the family two years after their child was admitted to a PICU—An interview study," *Intensive Crit Care Nurs*. 2017 Dec;43:143–148. doi: 10.1016/j.iccn.2017.06.003.
27. Mittal M, Kumar A, Srivastava D, Sharma P, and Sharma S, "Computerized versus traditional local anesthesia in pediatric patients," *Journal of Clinical Pediatric Dentistry*. 2015 Nov;39(5):470–474. doi: 10.17796/1053-4628-39.5.470

28. Topaloğlu Ak A, Coğulu D, Ergin E, and Atilla E, “Çocuklarda Süt Dişi Tedavileri Sirasında Uygulanan Bilgisayar Kontrollü Anestezi Sistemi İle Konvansiyonel İnfiltratif Enjeksiyon Anestezi Yönteminin Karşılaştırılması,” *Aydın Dental Journal*. 2018;4(1):1-8.