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Vincent van Gogh, 1889/Oil on canvas / Oskar Reinhart Collection, Winterthur.

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CONTENT

EDITORIAL | Yeditepe Journal of Health Sciences, 13(1):1

Expanding the Scope: From Medical to Health Sciences

Dear Colleagues,

The new journal is a continuation of the Yeditepe Medical Journal, which was published until 2016. We aimed to broaden our focus to encompass all areas of health sciences, by changing the name to the Yeditepe Journal of Health Sciences. It is now published by our new publisher, DOC Design and Informatics, which also produces several other academic journals.

The concept for this journal was proposed by Prof. Bayram Yılmaz, who delegated editorial responsibilities to our team after overtaking the role of rector at another university. We would like to express our gratitude to our rector, Prof. Mehmet Durman, and Prof. Sina Ercan, Dean of the Faculty of Medicine, for their ongoing support and for facilitating the launch of our journal. We also appreciate our founding president, Mr. Bedrettin Dalan, former mayor of the beautiful city of Istanbul, for providing us with a conducive environment at Yeditepe University.

In this inaugural issue of the Yeditepe Journal of Health Sciences, we present a diverse array of articles from various disciplines. We thank our authors for choosing to publish with us. Our objective is to feature evidence-based studies, clinical and basic research, translational medicine articles, case reports, and opinion letters from different universities, countries, and health-related fields. We are committed to maintaining a swift review process, aiming for a maximum turnaround time of one month for submitted articles. The journal will be published quarterly, and we invite submissions for our upcoming August issue. We look forward to having the Yeditepe Journal of Health Sciences listed in the Emerging Sources of Citation Index, and we will work diligently to achieve this goal.

Best wishes to all our readers,

Gülderen Yanıkkaya Demirel
Editor-in-Chief

Published April 25, 2025 **Correspondence** Gülderen Yanıkkaya Demirel **DOI** 10.36519/yjhs.2025.678

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RESEARCH ARTICLE | Yeditepe Journal of Health Sciences, 13(1):2-9

Investigation of the Cytotoxicity of Arbutin Combined with Doxorubicin *In Vitro*

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Abstract

Objective: The aim of this study was to investigate the anti-cancer effects of arbutin on doxorubicin-induced cytotoxicity in the double-positive estrogen receptor +/ progesterone receptor +/ human epidermal growth factor receptor 2 negative (ER+/PR+/HER2-) breast cancer (BC) cell line MCF-7 *in vitro*.

Materials and Methods: Viability screening was performed with colorimetric MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay. Intracellular reactive oxygen species (ROS) accumulation was evaluated by dihydrorhodamine 123 (DHR123) staining. Apoptosis, necrosis and viability to arbutin, doxorubicin and their combination were assessed by Annexin V/7-AAD (7-aminoactinomycin D) staining. Cell cycle phase distribution was analyzed by DNA content analysis.

Results: Arbutin alone, at concentrations up to 500 μM, did not reduce MCF-7 cell viability over incubation periods ranging from 6 to 48 hours. Arbutin at concentrations above 20 μM transiently decreased intracellular ROS levels at 6 hours but had no significant effect at 24 and 48 hours. When combined with doxorubicin, arbutin partially reversed doxorubicin-induced reductions in cell viability, decreased late apoptosis and necrosis rates, and regulated doxorubicin-induced cell cycle disruptions.

Conclusions: These results suggest that while arbutin does not exhibit direct cytotoxicity in MCF-7 cells, it modulates doxorubicin-induced cellular responses. Future studies with arbutin at higher concentrations investigating the molecular mechanisms underlying this effect, particularly at the gene and protein expression levels, are necessary to further elucidate the potential role of arbutin in BC therapy.

Keywords: Breast cancer, arbutin, doxorubicin, apoptosis, DNA content analysis

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INTRODUCTION

Worldwide, breast cancer (BC) is the most common malignancy in women, with an estimated 2.3 million new cases and 685,000 deaths, accounting for 16% of female cancer deaths in 2020 (1). Breast cancer is a heterogeneous disease with several molecular subtypes, each associated with different prognoses. Routine evaluation of BC includes estrogen receptor alpha (ERα), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression (2); therefore, the primary subtypes are classified as hormone receptor (HR)-positive/HER2-negative, HR-positive/HER2-positive, HR-negative/HER2-positive, and triple-negative (HR-negative/HER2-negative), all of which have different survival outcomes (3). BC classification aims to provide data for oncologic decisions that would lead to the successful treatment of the disease. In this context, BC is evaluated according to its type, grade, and the stage of the tumor. The type and grade of breast tumors are based on histological subtypes and grades and are defined by the World Health Organization (WHO). BC stages are associated with tumor size, node invasion, and metastasis. These prognostic markers provide important predictive data for hormone therapies and anti-HER2-therapies (2).

Arbutin is a naturally occurring glycoside found mainly in the leaves of various plant species, most notably bearberry (*Arctostaphylos uva-ursi*) of the *Ericaceae* family, although it has also been identified in plants of the *Asteraceae*, *Proteaceae*, and *Rosaceae* families (4). Chemically, arbutin is a β-glucoside derived from hydroquinone, with the molecular formula $C_{12}H_{16}O_7$. Its structure consists of a hydroquinone molecule linked to a glucose moiety (4). Traditionally, arbutin has been used in herbal medicine, particularly in the treatment of urinary tract infections, and extracts of bearberry leaves, which are rich in arbutin, have been used for their antimicrobial properties (5). Being a competitive inhibitor of the enzyme tyrosinase, which plays a crucial role in melanin production, arbutin is widely used in cosmetic products for its skin whitening properties in addition to its medical applications (6). To date, arbutin has demonstrated potential anticancer properties in several cancer types through induction of apoptosis, inhibition of inflammatory markers, and suppression of the phosphoinositide 3-kinase/protein kinase B/mechanistic target of rapamycin (PI3K/Akt/mTOR) signaling pathway (7-9). However, to the best of our knowledge, the impact of arbutin in combination with standard chemotherapeutic agents on BC has not been reported. When considering studies suggesting enhanced therapeutic efficacy and diminished drug resistance when doxorubicin was combined with natural compounds such as resveratrol (10), we aimed to determine the efect of arbutin use on the anticancer activity of doxorubicin on MCF-7 double-positive BC cell line.

MATERIALS AND METHODS

Cell Culture Conditions

In this study, the MCF-7 double-positive BC cell line (HTB-22™, passage 14) (American Type Culture Collection - ATCC) was used. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Cat. No: DMEM-HPA; Capricorn Scientific, Germany) supplemented with 10% Fetal Bovine Serum (FBS) (Cat. No: FBS-16B; Capricorn Scientific, Germany) and 100 U/mL penicillin-streptomycin antibiotic solution (Cat. No: 15140122; Thermo Fisher Scientific, USA). Upon reaching 80% confluence, cells were detached from the flasks using trypsin-EDTA solution (Cat. No: 25300054; Thermo Fisher Scientific, USA) counted with the JuLI™ Br Cell Counting Station (NanoEnTek Inc., South Korea) and adjusted to a concentration of 1×10^6 cells per mL. For viability screening and assessment of intracellular ROS levels, 5×10^3 cells were seeded in triplicate into 96-well plates. For flow cytometric analysis of apoptosis and viability and DNA content analysis, cells were seeded at 5×10^5 cells per dish in 60 mm cell culture dishes. All experiments were performed as triplicates.

Colorimetric Evaluation of Viability

Cellular viability of MCF-7 cells after treatment with arbutin and doxorubicin was assessed with MTS assay, which is based on the reduction of the reagent to formazan salt upon reduction with cellular enzymes (11). For treatment, stock solution of arbutin was prepared by dissolving it in complete culture medium at 500 mM concentration. After seeding, the plates were incubated overnight to allow for cell attachment, and then treated with arbutin at concentrations of 0.8, 4, 20, 100, and 500 µM for 6, 12, 24, and 48 hours. At the end of the incubation, MTS reagent (Cat. No: ab197010; Abcam Limited, UK) (10% v/v) was added to the wells and the samples were incubated for 2 hours. Absorbance was read at 490 nm wavelength. Untreated cells were included as negative control, and plain culture medium was used as blank. Cytotoxicity was calculated by subtracting the blank absorbance from both the test and control groups. The corrected absorbance of the test group was then divided by the corrected absorbance of the control group and multiplied by 100 to obtain the percentage of viability after treatments.

Fluorometric Evaluation of Reactive Oxygen Species

Dihydrorhodamine 123 (DHR123) is a non-fluorescent probe commonly used to detect the production of reactive nitrogen and oxygen species in cells. Upon oxida-

tion, DHR123 is converted to fluorescent rhodamine (12). Cells were incubated with arbutin at concentrations of 0.8, 4, 20, 100, and 500 µM for 6, 12, 24, and 48 hours, after which the medium was discarded and the cells were incubated with 100 µL DHR123 solution for 40 minutes at room temperature (5 µM in DPBS). At the end of incubation, DHR123 solution was discarded, 100 µL fresh Dulbecco's Phosphate Buffered Saline (DPBS) was added to wells, and plates were read with the Varioskan LUX Multimode Microplate Reader (Thermo Fisher Scientific, USA) at an excitation/emission (ex/em) wavelength of 510/530 nm (±5 nm). Values were obtained as relative fluorescent unit (RFU).

Annexin V/7-Aminoactinomycin Staining

Annexin V/7-aminoactinomycin (7-AAD) staining is a common method used to evaluate apoptosis. In the presence of Ca^{2+} ions, annexin V binds specifically to phosphatidylserine residues, membrane phospholipids that translocate from the inner to the outer leaflet of the cell membrane during apoptosis. Meanwhile, 7-AAD, a DNA-binding dye, can only penetrate necrotic or late apoptotic cells, enabling to distinguish between different stages of cell death (13). To evaluate the apoptotic effect of arbutin, doxorubicin, and arbutin in combination with doxorubicin, cells were incubated with the compounds for 48 hours and collected by trypsinization. After centrifugation, supernatant was discarded, cells were suspended in 1 mL V Binding Buffer (Cat. No: 422201; Biolegend, USA), and labelled with Annexin V-Pacific Blue/7-AAD (Cat. No: 640926; Biolegend, USA) according to the kit instructions. After incubation at room temperature under dark for 15 minutes, 2.5×10^4 cells per tube were evaluated with DxFLEX Flow Cytometry System (Beckman Coulter, USA). Analysis was performed with Kaluza analysis software (Beckman Coulter, USA).

DNA Content Analysis

DNA content analysis is a widely used method for evaluating cell cycle distribution, identifying apoptotic cells, and determining DNA ploidy status. In this method, cellular DNA content is evaluated to distinguish the major phases of the cell cycle; G0/G1, S, and G2/M, in addition to apoptotic cells based on their fractional DNA content resulting from DNA fragmentation (14). Here, the effects of arbutin, doxorubicin, and arbutin in combination with doxorubicin on cell cycle progression were evaluated by DNA content analysis by incubating cells with the compounds, either alone or in combination, for 48 hours. At the end of the incubation, the cells were trypsinized and collected by centrifugation. The cells were fixed with 5 mL of 70% ethanol solution by incubating the tubes at 4 °C for one hour. At the end of fixation, the ethanol solution was discarded and the cells were resuspended in 500 µL of Cell Cycle Kit (Cat. No: C03551; Beckman Coulter, USA). After incubation at room temperature under dark for an hour, 5×10^4 cells per tube were evaluated with DxFLEX Flow Cytometry System (Beckman Coulter, USA). Analysis was performed with Modfit software (Verity Software House, USA).

Statistical Analysis

Statistical analysis was performed with GraphPad Prism 8.0.2 (GraphPad Software Inc, USA). One-way ANOVA followed by Dunnett's multiple comparison test was used to determine the effect of arbutin on MCF-7 cell line. For evaluating viability, apoptosis and necrosis, one-way ANOVA followed by Tukey's multiple comparison test was employed. DNA content analysis was evaluated with two-way ANOVA followed by Tukey's multiple comparison test. *P*-values less than 0.05 were considered statistically significant.

RESULTS

Arbutin Does Not Decrease Viability or ROS Accumulation in MCF-7 Cell Line

Our results indicated that within the given dose interval, arbutin did not reduce the viability of MCF-7 cells compared to the control group (Figure 1). Only a slight increase in viability was observed in the 4 µM group compared to the control group at 6 hours ($p<0.05$) (Figure 1a), although this finding was not observed at longer incubation periods (Figure 1b-d). When intracellular ROS accumulation was evaluated with DHR123 staining, even though no alterations was observed in 6 hours (Figure 1e), arbutin over 20 µM concentration led to significant decreases in ROS levels compared to the control group in 12 hours (20 µM, $p<0.05$; 100 and 500 µM, $p<0.01$) (Figure 1f). Yet, similar to 6 hours incubation duration, arbutin did not alter ROS levels in 24 (Figure 1g) and 48 hours (Figure 1h). Altogether, these results indicate that arbutin up to 500 µM concentration do not exert cytotoxicity on MCF-7 cell line but decrease ROS levels in relatively short incubation interval. Since the compound did not decrease viability at all incubation periods, further studies were conducted with 500 µM arbutin at 48 hours. The IC_{50} value of doxorubicin on 48 hours was calculated for a previous study as 2.32 µM for MCF-7 cell line (15), which was also applied either alone or in combination with arbutin for mechanistic studies.

Arbutin Partially Reverses Doxorubicin Induced Cell Death

When the effect of arbutin in combination with doxorubicin was evaluated, doxorubicin significantly decreased viability compared to the control and arbutin groups ($p<0.0001$), while when its combined with arbutin, viability was increased in comparison with the doxorubicin group ($p<0.0001$). Yet, the viability levels of

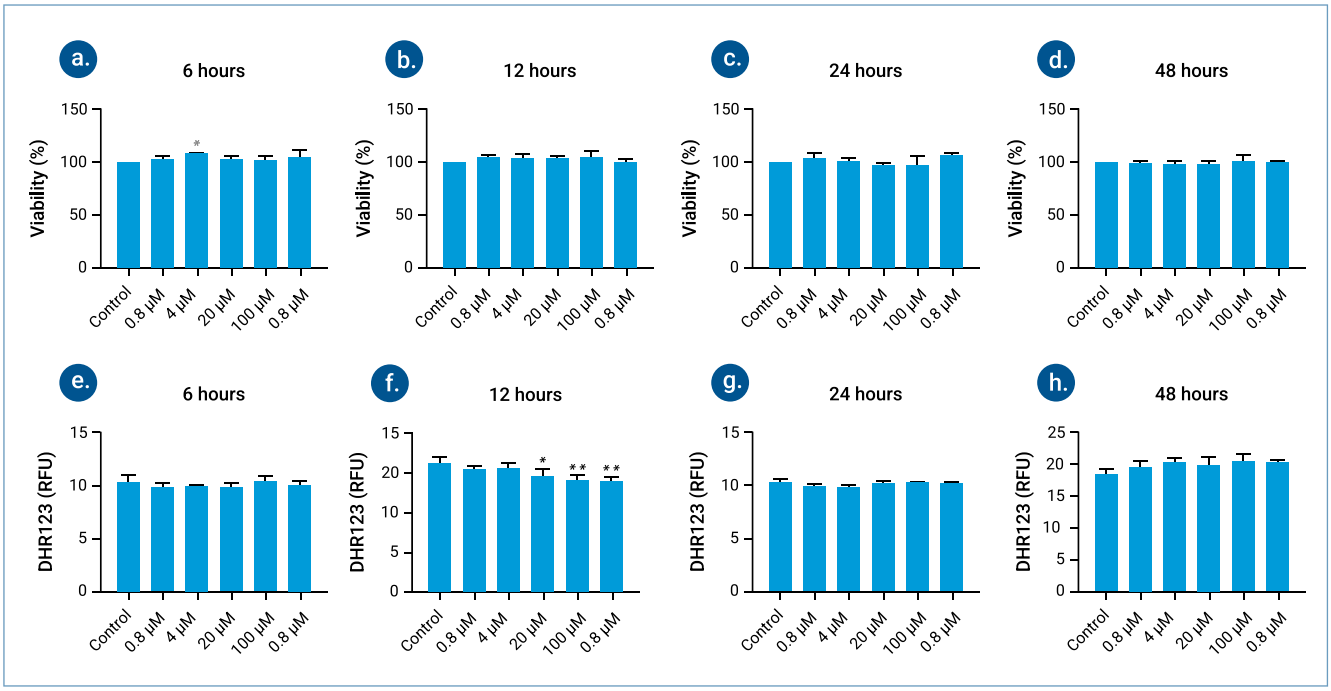

FIGURE 1. Bar graphs regarding the effects of arbutin at 0.8, 4, 20, 100 and 500 µM concentrations on MCF-7 cell line in terms of viability and intracellular reactive oxygen species at different time points. **a.** Viability upon arbutin treatment for 6 hours, **b.** Viability upon arbutin treatment for 12 hours, **c.** Viability upon arbutin treatment for 24 hours, **d**. Viability upon arbutin treatment for 48 hours, **e.** Intracellular ROS accumulation upon arbutin treatment for 6 hours, **f.** Intracellular ROS accumulation upon arbutin treatment for 12 hours, **g.** Intracellular ROS accumulation upon arbutin treatment for 24 hours, **h.** Intracellular ROS accumulation upon arbutin treatment for 48 hours. *p*-values lower than 0.05 were considered statistically significant. *p<0.05, **p<0.01.

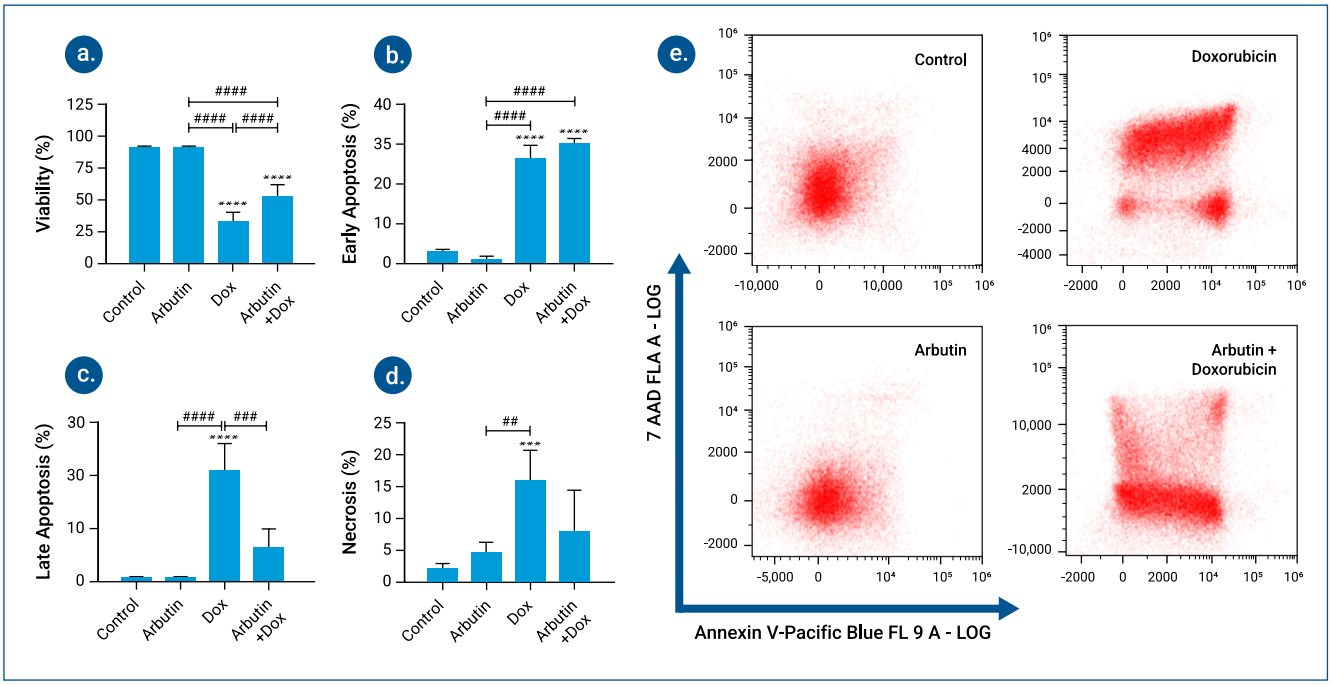

FIGURE 2. Comparisons between arbutin, doxorubicin and arbutin in combination with doxorubicin treatment in terms of viability and apoptosis evaluated by Annexin V/7-AAD staining. Bar graphic revealing **a.** Viability, **b.** Early apoptosis, **c.** Late apoptosis and d. Necrosis. **e.** Representative flow cytometry dot plots. *p*-values lower than 0.05 were considered statistically significant.

* indicates differences between the control and the treatment groups, and # indicates differences between treatment groups. ***p<0.001, ****p<0.0001; ##p<0.01, ###p<0.001, ####p<0.0001.

the arbutin+doxorubicin group were still significantly lower compared to the control and the arbutin groups (p<0.0001) (Figure 2a). On the other hand, doxorubicin increased early apoptosis levels compared to the control group (p<0.0001), but arbutin could not reverse this effect as this group also had higher early apoptosis levels when compared to both control and the arbutin groups (p<0.0001) (Figure 2b). Doxorubicin treatment significantly increased late apoptosis rates (p<0.0001), which is decreased when doxorubicin was combined with arbutin (p<0.001) (Figure 2c). Lastly, similar to late apoptosis, in the doxorubicin group, necrosis rates wereincreased compared to the control and the arbutin groups (p<0.001); though in the arbutin+doxorubicin group, no difference compared to the control or only arbutin group was observed (p>0.05) (Figure 2d). Representative flow cytometry dot plots are given in Figure 2e.

Arbutin Exhibits Regulatory Effects on Doxorubicin-Induced Cell Cycle Arrest

Evaluations regarding G0/G1 phase revealed that arbutin did not cause a significant alteration compared to the control group (p>0.05). Doxorubicin significantly decreased in this phase compared to both the control and arbutin groups (p<0.0001). The G0/G1 phase was higher in the doxorubicin+arbutin group compared to the arbutin and control groups (p<0.0001) (Figure 3a). Interestingly, arbutin decreased S phase in comparison with the control group (p<0.05). On the contrast, doxorubicin treatment increased this phase (p<0.01). In comparison with the doxorubicin group, the combination of arbutin with doxorubicin decreased the S phase (p<0.01), although this decrease did not lower its levels to the control level (p<0.01) (Figure 3a). Lastly, doxorubicin led to a significant increase in G2/M phase compared to the control group (p<0.05), and arbutin treatment did not have an impact on this accumulation as arbutin+doxorubicin group had higher G2/M phase levels compared to both control (p<0.0001) and the arbutin (p<0.01) alone group (Figure 3a). Representative flow cytometry histogram plots are provided in Figure 3b.

DISCUSSION

Arbutin, a glycosylated hydroquinone, has been extensively studied for its skin depigmenting properties due to its ability to inhibit melanin synthesis. Research indicates that arbutin effectively reduces hyperpigmentation by inhibiting melanosomal tyrosinase activity, thereby decreasing melanin production. To enhance its stability and transdermal delivery, various formulations and biotechnological methods have been developed, including enzymatic bioconversion techniques to produce α- and β-arbutin derivatives. These derivatives have demonstrated significant antimelanogenic effects, making them valuable in treating conditions characterized by hyperactive melanocyte function (16, 17).

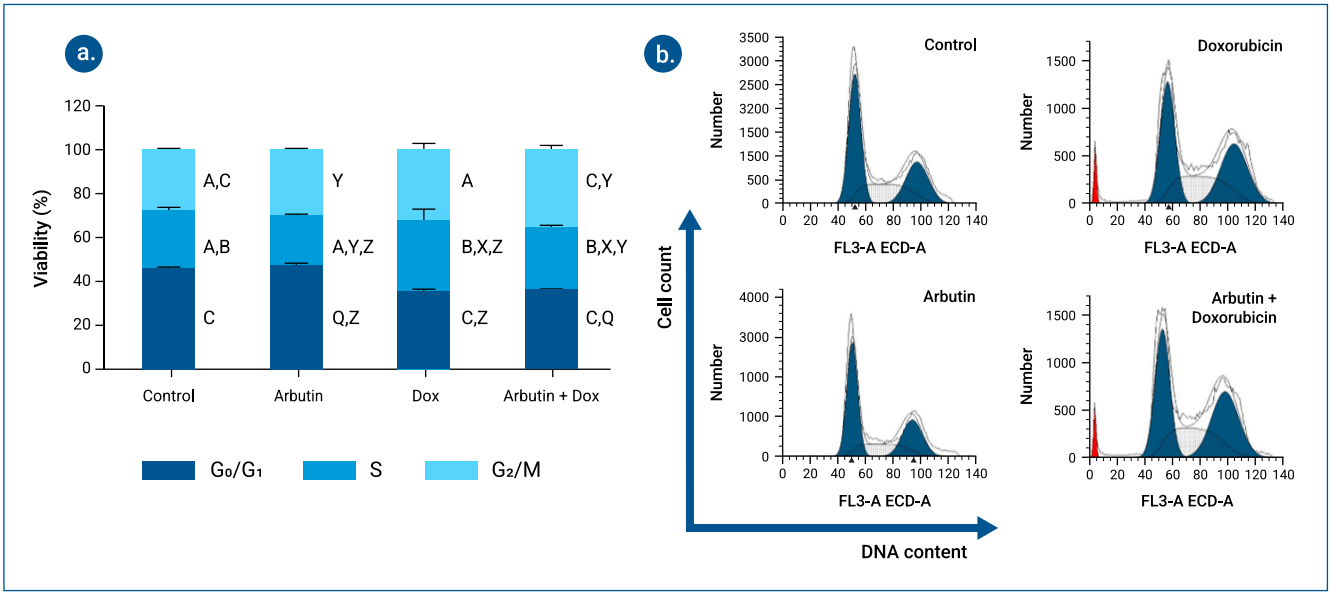

FIGURE 3. Comparisons between arbutin, doxorubicin and arbutin in combination with doxorubicin treatment in terms of cell cycle phases' distribution evaluated by DNA content analysis. **a.** Bar graphics, **b.** Representative flow cytometry histograms. p-values lower than 0.05 were considered statistically significant.

Letters A (p<0.05), B (p<0.01) and C (p<0.0001) indicate comparisons between the control and the treatment groups, letters X (p<0.05), Y (p<0.01) and Z (p<0.0001) indicate comparisons between treatment groups.

Preliminary studies investigating the anticancer activity of arbutin focused on melanoma. In a study published in 2009, Nawarak et al. aimed to investigate the anticancer effects of arbutin on A375 human malignant melanoma cells by elucidating changes in protein expression profiles following arbutin treatment (18). Proteomic analysis revealed that arbutin treatment at the concentration of 8 μg/mL resulted in significant changes in the expression of proteins associated with various cellular processes, including apoptosis, cell cycle regulation, and oxidative stress response. In particular, proteins involved in the apoptotic pathway were upregulated, suggesting that arbutin may induce programmed cell death in melanoma cells. In addition, proteins related to cell proliferation were downregulated, suggesting a potential inhibitory effect on tumor growth. These findings provide insight into the molecular mechanisms underlying the anticancer activity of arbutin against malignant melanoma cells. Also, Ma et al. used a combination of network pharmacology and experimental validation to identify compounds in *Prinsepia utilis* with potential antimelanoma activity where the authors utilized bioinformatics databases to predict compounds and their targets, followed by molecular docking to assess interactions between these compounds and key targets (19). Subsequent *in vitro* studies were performed on human melanoma A375 cells to evaluate the biological activities of the identified compounds. This study identified oleanolic acid, ursolic acid, and arbutin as active ingredients in *P. utilis*, and in vitro studies revealed that oleanolic acid and ursolic acid significantly inhibited the growth and migration of A375 melanoma cells, induced apoptosis, and reduced both tyrosinase activity and melanin synthesis yet arbutin did not exhibit significant effects. In order to enhance its efficacy, Jian et al. synthesized an acetylated derivative of arbutin to enhance its biological activity and compared its effects to those of the parent compound on B16 murine melanoma cells (8). According to this study, both arbutin and its acetylated derivative significantly reduced cell viability, promoted apoptosis, caused G1 phase cell cycle arrest, and induced mitochondrial disruption in B16 melanoma cells. These pro-apoptotic effects were associated with decreased expression of anti-apoptotic proteins B-cell lymphoma 2 (Bcl-2) and B-cell lymphoma-extra-large (Bcl-xL), indicating involvement of the mitochondrial pathway. However, it should be noted that the lowest dose of arbutin used for the mechanistic evaluations was 1.4 mM, which is approximately three times higher than the dose (500 μM) used in our current study.

Arbutin has previously been studied in the treatment of various cancers in vitro beside melanoma. In a study published in 2020, the authors aimed to investigate the antioxidant effects of arbutin on LNCaP (lymph node carcinoma of the prostate) cancer cells (20). Researchers treated the cells with arbutin up to 2 mM and evaluated intracellular ROS levels, induction of apoptosis, and expression of pro-inflammatory cytokines IL-1β and tumor necrosis factor alpha (TNF-α). The authors showed that arbutin significantly decreased intracellular ROS levels in a dose-dependent manner and significantly induced apoptosis in LNCaP cells, in addition to reducing IL-1β and TNF-α expression levels at 1 mM concentration. In a study published by Ciğerci et al., the authors investigated the anticarcinogenic potential of high concentrations of arbutin and its protective effects against cisplatin-induced toxicity at low concentrations on HepG2 human hepatocellular carcinoma cell line (21). In this study, high concentrations of arbutin exhibited anticarcinogenic effects by reducing HepG2 cell viability while low concentrations of arbutin provided protective effects against cisplatin-induced toxicity, likely through its antioxidant and anti-inflammatory properties. Similarly, our results indicate that arbutin in combination with doxorubicin reduces the apoptotic efficacy of this chemotherapeutic drug, a finding consistent with a previous report. With a similar study design, Terzi et al. evaluated the effects of arbutin, both alone and in combination with cisplatin, on HT-1376 bladder cancer cells. In this study, cell viability, apoptosis induction, and cell migration were assessed, and the IC_{50} dose for arbutin was calculated as 4317 mM on 24th hour (22). The authors revealed that arbutin, both alone and in combination with cisplatin, significantly increased apoptosis and inhibited migration in HT-1376 cells, suggesting that β-arbutin may enhance the anticancer effects of cisplatin, making it a potential therapeutic candidate for bladder cancer treatment. Both of these studies highlight the fact that arbutin exerts its anti-cancer effect over 1 mM concentration, a rather high dose compared to our study.

In 2021, Yang et al. evaluated the anticancer effect of arbutin on C6 glioma cells, where the authors treated the cells with 10-60μM arbutin for 24 hours, and analyzed cell viability, apoptosis, ROS generation, mitochondrial membrane potential disruption, and expression of inflammatory markers and components of the PI3K/Akt/mTOR signaling pathway (23). The results revealed that arbutin decreased viability in C6 cell viability in a dose-dependent manner, induced apoptosis by increasing ROS production and disrupting mitochondrial membrane potential. Additionally, arbutin inhibited the expression of inflammatory markers and downregulated the PI3K/Akt/mTOR signaling pathway, suggesting its potential as a therapeutic agent against gliomas. In contrast, although much higher concentrations of arbutin were evaluated in our study, we did not observe a decrease in viability of MCF-7 cells in response to arbutin, which may reflect the relative insensitivity of this cell line to this compound. In MCF-7 cell line, arbutin was

demonstrated to exert its effect *via* inhibition of ER-α, although much higher doses were required to reduce cell viability compared to our study (9).

In addition to studies evaluating its cytotoxic effect on cancer cells, a recent study assessed arbutin's effect on the expression of programmed cell death ligand 1 (PD-L1) on tumor cells (24). Involving B16F10 melanoma and LL2 lung cancer cell lines, the primary focus of this study was to determine whether arbutin could modulate PD-L1 expression and thereby influence tumor immune tolerance. The authors reported that arbutin treatment led to a significant reduction in PD-L1 expression on both B16F10 and LL2 tumor cells, which was associated with the inhibition of the AKT/mTOR signaling pathway. *In vivo* studies further demonstrated that arbutin reduced tumor growth and decreased PD-L1 expression in tumor tissues of mice compared to control group. Altogether, these results suggested that even if arbutin cannot exert toxicity, it can effectively diminish tumor-induced immune tolerance by targeting PD-L1 expression, offering potential therapeutic implications for enhancing antitumor immunity.

In our study, arbutin at concentrations up to 500 µM did not reduce MCF-7 cell viability across 6 to 48 hours incubation periods. Regarding intracellular ROS accumulation, arbutin concentrations above 20 µM significantly decreased ROS levels at 6 hours; though no significant changes were observed at 24 and 48 hours, indicating the compound's short-term effect on this cell line. When combined with doxorubicin, arbutin partially reversed doxorubicin-induced reductions in cell viability and decreased late apoptosis and necrosis rates, and cell cycle analysis revealed that arbutin regulated doxorubicin-induced disruptions. These findings suggest that arbutin alone does not exhibit cytotoxic effects on MCF-7 cells but can modulate doxorubicin-induced cytotoxicity and cell cycle changes. Nevertheless, given that studies in the literature suggest regulatory effects of arbutin in cancer cells even at non-cytotoxic doses, a detailed evaluation of the effects of low dose arbutin treatment on gene and protein expression levels in BC may contribute to the assessment of the anticancer potential of this compound.

Ethics Committee Approval
This *in vitro* study was conducted using a commercially available cell line; therefore,ethical approval was not required.

Informed Consent
N.A

Peer-review
Externally peer-reviewed.

Author Contributions
Concept – M.H.K., B.A.; Design – M.H.K., B.A.; Supervision – F.A., B.A.; Fundings – M.H.K.; Materials – B.A. Data Collection and/or Processing – M.H.K., F.A., B.A.; Analysis and/or Interpretation – B.A.; Literature Review – M.H.K., F.A., B.A.; Writer – M.H.K., B.A.; Critical Reviews – B.A.

Conflict of Interest
The authors declare no conflict of interest.

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Scientific Presentation
This study was presented in YÜTBAT 17th National Medical Student Congress by Mehmet Halil Kılınç.

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Artificial Intelligence as Applied to Leukemia Research: A Dual Approach of Literature Review and Bibliometric Exploration

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Abstract

Objective: The application of artificial intelligence (AI) in leukemia management is rapidly expanding, with components such as machine learning (ML), deep learning (DL), and neural networks (NNs) offering innovative solutions for diagnosis and treatment. This study aims to analyze the global impact of AI in leukemia research through bibliometric analysis, highlighting trends in scientific production, institutional contributions, and keyword evolution.

Materials and Methods: A systematic literature review was conducted using Scopus and Web of Science (WoS) databases. Inclusion criteria focused on AI applications in leukemia, incorporating research articles and conference papers while excluding reviews and non-related studies. Data were analyzed using VosViewer (Version 1.6.20) and Bibliometrix-Biblioshiny to map publication trends, co-authorship networks, and keyword co-occurrence.

Results: A total of 248 documents from Scopus and 472 from WoS were analyzed. Machine learning emerged as the most frequently studied AI tool, followed by NNs and DL. A significant increase in AI-related leukemia research has been observed since 2017. The United States and China were the most active contributors. Studies primarily focused on acute leukemia, while chronic leukemia subtypes received comparatively less attention. Institutions and journals have increasingly prioritized AI in leukemia research, indicating growing academic and clinical interest.

Conclusion: The integration of AI into leukemia research is accelerating, with ML leading the way. However, more studies are needed to explore chronic leukemia subtypes and translate AI-driven advancements into clinical practice. The increasing global interest in AI applications suggests that these technologies will play a crucial role in future leukemia management.

Keywords: Artificial intelligence, bibliometric analysis, deep learning, leukemia, machine learning, neural networks

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INTRODUCTION

Hematologic neoplasms (HNs) arise from abnormal hematopoiesis, a process involving the differentiation of hematopoietic stem cells (HSCs) into either myeloid or lymphoid progenitors, followed by further proliferation and accumulation of immune or blood cells. Each type has distinct clinical characteristics and specific classification criteria (1-3). According to the World Health Organization (WHO) (3) and an international consensus article (4), blood cancers are classified based on their immunophenotypes, morphological features, clinical presentations, and cytogenetic/molecular alterations which widened the classification of all types of HNs (5). As a part of HNs, leukemia is clustered into different subtypes based on the pace of disease progression as acute and chronic leukemia and based on the type of cell they originated lymphoid or myeloid cells (6). Leukemia ranks as the 13th most common cancer and the 11th leading cause of cancer-related deaths with a global incidence of over 400,000 and a prevalence of more than 1.3 million, according to the GLOBOCAN 2020 report. In the United States, it is estimated that around 60,000 individuals will receive a diagnosis of leukemia in 2023 (7). This includes cases of acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), and other subtypes, respectively in cases (8). The treatment modalities for leukemia include a range of chemical therapeutic agents like chemotherapy, targeted therapy, immunotherapy, and gene or cell therapy such as Chimeric antigen receptor T-cell (CAR-T), radiotherapy as well as hematopoietic stem cell transplantation (1). Current diagnostic tools for leukemia include physical exams, complete blood count, peripheral blood smears, immunophenotyping, bone marrow examinations (aspiration and biopsy), cytogenetics, immunohistochemistry, and imaging (9, 10). Early diagnosis and determining the subtype of disease are critical, and the tests that play a significant role in achieving this. However, certain challenges need to be addressed. For instance, the evaluation of these tests has inter-evaluator variation, and deviations can occur due to conducting experiments in different laboratory conditions and by different individuals. Sample processing and approval processes are also time-consuming, and clinical interventions such as bone marrow aspiration can be difficult. Therefore, it has become vital to integrate technology into the clinic, develop new methods, and reach a consensus in test evaluation (11, 12). In addition, integrating technology into clinical practice can aid treatment. This includes computer-based drug design for the disease's biological targets, monitoring technologies for dosages and remissions based on the patient's pathological history, software that integrates data from genetic, histological, microbiological, and routine tests, user-free evaluation methods by processing patient sample images, programs to aid patient-physician communication and symptom monitoring, and reducing patient hospitalization time. All these benefits pave the way for new developments that create new visions and missions both in healthcare and leukemia management. These developments will enable the effective provision of health services to meet the increasing demand for leukemia treatment (12-14). To achieve these goals, first, healthcare professionals should integrate data management tools to make the data meaningful, validated, standardized, and subjective (15).

The recent growth in humanity's cumulative knowledge has resulted in the integration of technological advancements in data management and contextualization. This includes stages such as collection, identification, classification, processing, exploration, analysis, and interpretation of large-scale data from different sources. These processes help in identifying current or prospective issues and finding solutions to them (16). However, data management enables us to better handle complex tasks, it is difficult to accomplish through human intelligence (HI) or natural intelligence (NI) alone due to the detailed nature of data and the requirement of time and effort. For these reasons, using artificial intelligence (AI) tools has extensive benefits as they offer valuable insights and even foresight, and recently gained attention from researchers or institutes globally (17). As historical background, Alan Turing proposed the Turing test to determine "if machines can think like humans". After that, the term "artificial intelligence" was first mentioned by John McCarty as a discipline that investigates the functions of machines and their integration with HI as performing or simulating cognitive activities akin to humans (18, 19). As we strive towards achieving zero-touch simulation of HI, the field of AI has expanded and evolved into six distinct yet interrelated subcategories, namely machine learning (ML), deep learning (DL), NNs (NN), natural language processing (NLP), computer vision (CV), and robotics (in this article NLP, CV, and robotics were excluded) (20). With the advancements in ML, AI has reached a level where it can perform tasks that previously required HI. It is also capable of processing large amounts of data on its own, without requiring any explicit instructions on how to carry out the tasks. Furthermore, it can generate solutions to complex problems when presented with more intricate data (21). Deep learning is another subset of AI that uses data combinations to derive meaningful results. The process involves combining data in various ways and summarizing the outcomes. These combinations are then added back to the data set as separate elements and the process repeats. In essence, this structure mimics the neural network similar to neurons in the human brain, sim-

ulating the recall of memories and making connections between phenomena (22). Although NNs form the basis of DL, the primary difference between them is the number of connections that have been made during data processing, with DL having more complex connection patterns as its name implies (23).

Throughout its evolution, AI has played a crucial role in integrating knowledge and experience in medicine and other fields it impacts. It has greatly lightened the burden of healthcare in numerous branches and aspects, both physically and virtually with all of its constituents (24). To reveal the potential of AI tools, bibliometric studies can be applied. Bibliometric studies reveal the development and accumulation of knowledge and facilitate qualitative and quantitative literature analysis to understand authors' perspectives and orientations toward topics. By virtue of these studies, the amount of scientific output that has been made in a specific discipline and information gaps can be assessed, then these assessments pave the way for future studies and for designing innovative ideas (25). Literature analysis using a literature database involves filtering data sets based on keywords and specific inclusion or exclusion criteria. This helps to define the research scope and ensure that the conclusions drawn fall within a spectrum between objective and subjective which can be thought-provoking for the researchers (26).

Accepting AI tools as current and future orchestrators in leukemia management, we aimed to describe the current state of AI, ML, DL, and NN tools applied in leukemia and its subtypes using literature, bibliometric, network, and descriptive analysis, worldwide.

MATERIALS AND METHODS

A comprehensive manual review of the literature was conducted utilizing the Scopus and Web of Science (WoS) databases separately to extract knowledge from articles and conference proceedings on the correlation between leukemia and AI tools such as ML, DL, and NNs. To ensure a detailed analysis, the keywords were divided into discrete groups, including leukemia and AI tools, all AI tools and leukemia, with each group designed with queries that do not interfere with other queries to understand the terms of AI tools and their applications in leukemia and its subtypes by using Boolean operators "AND" and "AND NOT". The conceptualization of queries is referred to in Appendix 1 (see Supplementary Materials). The publication year was not indicated in the queries; instead, the oldest publication year was confirmed as the beginning and the final published document as the end. For the Scopus database query search, publication years of documents were between 1989-2023 while for WoS, it was 1994-2023. Only research articles and conference papers that deal with the application of AI tools in leukemia were included. In contrast, research on other types of cancer or diseases, reviews, and opinions on this topic were excluded. The information contained in the exported CSV files (taken from Scopus) and plain text files (taken from WoS) were counted and checked for duplication. The final number of documents is indicated (Tables 1-2). All groups separated by keywords were combined in each Scopus and WoS results, and co-occurrence with keywords and co-authorship within countries were determined using VOSviewer (Version 1.6.20). All the keywords and countries are scaled in the time interval of 2013-2023 to show trends in the usage of keywords and current attention taken by countries in the last decade. The bibliometric analysis by using manually collected files was done by the Biblioshiny platform which is supported by R programming and within the scope of the data taken from Scopus and WoS are categorized as annual scientific publications, affiliation production over time, and the number of publications by journals in timeline.

RESULT

Overview- Scopus

We found a total of 248 distinct documents *via* a manual Scopus database search as chasing the relevance of AI tools and leukemia within the total of 3312 documents that were counted with unfiltered query search. We excluded the documents that a) do not have available abstract or comma-separated values (CSV) files, b) other document types rather than articles and conference papers, and c) studies that have not been specified in the context of leukemia and its subtypes. Out of the 248 documents analyzed, most of them (135 documents, or 54.4%) were focused on using ML algorithms to study leukemia. Following ML, AI (n=50, 20.2%), NNs (n=34, 13.7%), DL (n=19, 7.7%) and all AI tools combined (n=10, 4%) are on the list respectively (Table 1).

Overview – Web of Science

We searched the WoS database and manually screened 472 documents (selected from a total of 1952 documents that were retrieved by unfiltered query search) related to leukemia research. We excluded documents that either didn't have an abstract or plain text file, were not articles or conference proceedings or were not relevant to leukemia research. Out of the 472 documents, 249 (52.8%) were related to ML, followed by NNs (110, 23.3%), DL (57, 12%), and AI (53, 11.2%). All AI tools combined accounted for only 3 (0.7%) of the documents (Table 2).

TABLE 1. The process of studying AI tools and their application in leukemia management involved several steps, including identification, screening, exclusion, and inclusion. Scopus queries (separated as 5 identical keyword combinations – row 1) were entered to retrieve relevant documents, and the number of documents for each step was shown along with the exclusion criteria. After manually reviewing the literature, 248 reports were identified as appropriate for further bibliometric analysis.

	Identification of Studies via SCOPUS				
Identification	"Leukemia" and "Artificial Intelligence"	"Leukemia" and "Machine Learning"	"Leukemia" and "Neural Networks"	"Leukemia" and "Deep Learning"	"Leukemia" and "Artificial Intelligence" and "Machine Learning" and "Deep Learning" and "Neural Networks"
Screening	Total documents in the interval of years 1989-2023 **(n=559)**	Total documents in the interval of years 1998-2023 **(n=1265)**	Total documents in the interval of years 1992-2023 **(n=935)**	Total documents in the interval of years 2013-2023 **(n=512)**	Total documents in the interval of years 2017-2023 **(n=41)**
	Document Type: Article and Conference Paper **(n=392)**	Publication stage at Final **(n=1250)**	Document type: Article and Conference Paper **(n=799)**	Document type: Article and Conference Paper **(n=403)**	Document Type: Article and Conference Paper **(n=23)**
	Written in English **(n=386)**	Document type: Article and Conference Paper **(n=1011)**	Publication stage: Final **(n=784)**	Publication stage: Final **(n=398)**	Publication stage: Final **(n=23)**
	Publication Stage at Final **(n=380)**	Written in English **(n=1000)**	Written in English **(n=776)**	Written in English **(n=395)**	Written in English **(n=23)**
	Exclusion by "**AND NOT**" Boolean Operator ("deep learning" and "leukemia") – ("artificial intelligence and leukemia") – ("neural network and leukemia) – ("artificial intelligence" and "machine learning" and "deep learning" and "neural network" and "leukemia") **(n=82)**	Exclusion by "**AND NOT**" Boolean Operator ("deep learning" and "leukemia") – ("artificial intelligence and leukemia") – ("neural network and leukemia) – ("artificial intelligence" and "machine learning" and "deep learning" and "neural network" and "leukemia") **(n=312)**	Exclusion by "**AND NOT**" Boolean Operator ("deep learning" and "leukemia") – ("artificial intelligence and leukemia") – ("machine learning and leukemia) – ("artificial intelligence" and "machine learning" and "deep learning" and "neural network" and "leukemia") **(n=116)**	Exclusion by "**AND NOT**" Boolean Operator ("machine learning" and "leukemia") – ("artificial intelligence and leukemia") – ("neural network and leukemia) – ("artificial intelligence" and "machine learning" and "deep learning" and "neural network" and "leukemia") **(n=32)**	Not excluded by Boolean Operator to see all of the results and at least 2 AI tools usage in the paper are included **(n=23)**
Excluded	Excluded documents from 82 documents **(n=32)**	Excluded documents from 312 documents **(n=177)**	Excluded documents from 116 documents **(n=82)**	Excluded documents from 32 documents **(n=13)**	Excluded documents from 23 documents **(n=13)**
Included	**n=50**	**n=135**	**n=34**	**n=19**	**n=10**
Result	Total dataset as checking duplicates **(n= 248)**				

TABLE 2. The process of studying AI tools and their application in leukemia management involved several steps, including identification, screening, exclusion, and inclusion. Web of Science (WoS) queries (separated as 5 identical keyword combinations – row 1) were entered to retrieve relevant documents, and the number of documents for each step was shown along with the exclusion criteria. After manually reviewing the literature, 473 reports were identified as appropriate for further bibliometric analysis.

	Identification of Studies via WoS				
Identification	"Leukemia" and "Artificial Intelligence"	"Leukemia" and "Machine Learning"	"Leukemia" and "Neural Networks"	"Leukemia" and "Deep Learning"	"Leukemia" and "Artificial Intelligence" and "Machine Learning" and "Deep Learning" and "Neural Networks"
Screening	Total documents in the interval of years 1993-2023 **(n=467)**	Total documents in the interval of years 1998-2023 **(n=762)**	Total documents in the interval of years 1992-2023 **(n=435)**	Total documents in the interval of years 2013-2023 **(n=280)**	Total documents in the interval of years 2020-2023 **(n=7)**
	Document Type: Article and Proceeding Paper **(n=363)**	Document type: Article and Proceeding Paper **(n=636)**	Document type: Article and Conference Paper **(n=411)**	Document type: Article and Conference Paper **(n=255)**	Document Type: Article and Conference Paper **(n=4)**
	Written in English **(n=362)**	Written in English **(n=634)**	Written in English **(n=410)**	Written in English **(n=254)**	Written in English **(n=4)**
	Exclusion by **"AND NOT"** Boolean Operator ("deep learning" and "leukemia") – ("machine learning" and "leukemia") – ("neural network and leukemia) – ("artificial intelligence" and "machine learning" and "deep learning" and "neural network" and "leukemia") **(n=223)**	Exclusion by **"AND NOT"** Boolean Operator ("deep learning" and "leukemia") – ("artificial intelligence and leukemia") – ("neural network and leukemia) – ("artificial intelligence" and "machine learning" and "deep learning" and "neural network" and "leukemia") **(n=452)**	Exclusion by **"AND NOT"** Boolean Operator ("deep learning" and "leukemia") – ("artificial intelligence and leukemia") – ("machine learning and leukemia) – ("artificial intelligence" and "machine learning" and "deep learning" and "neural network" and "leukemia") **(n=212)**	Exclusion by **"AND NOT"** Boolean Operator ("machine learning" and "leukemia") – ("artificial intelligence and leukemia") – ("neural network and leukemia) – ("artificial intelligence" and "machine learning" and "deep learning" and "neural network" and "leukemia") **(n=97)**	Not excluded by Boolean Operator to see all of the results and at least 2 AI tools usage in the paper are included
Excluded	Excluded documents from 223 documents **(n=170)**	Excluded documents from documents **(n=203)**	Excluded documents from documents **(n=102)**	Excluded documents from documents **(n=40)**	Excluded documents from documents **(n=1)**
Included	**n=53**	**n=249**	**n=110**	**n=57**	**n=3**
Result	The total dataset is checking duplicates from a total of 473 documents **n= 473**				

Analysis of Publications that were Manually Selected *via* Scopus and WoS Databases

Artificial Intelligence Is Becoming a General Term

The definition of artificial intelligence, as a concept, has significantly evolved. Its expanded structure now includes various subheadings such as ML, NNs, and DL, respectively, which have contributed to a broader definition. The term is no longer characterized by strictly defined boundaries, but rather by its flexibility to encompass multiple subheadings. In academic settings, researchers often employ specific subheadings to describe their studies, rather than using the term "artificial intelligence". An analysis of the past 10 years also reveals that the use of the term "artificial intelligence" has been replaced by the more specific phrases "machine learning" and "neural networks". Such linguistic precision reflects the growing sophistication of research in this field and highlights the importance of staying up-to-date with the latest terminology (Figure 1 and Figure 3)

Machine Learning Is the Most Common AI Tool in Leukemia Management

According to a VOSviewer analysis of Scopus and WoS databases, researchers have been utilizing terms like ML, NNs, and DL, instead of the broader term artificial intelligence. Machine learning, in particular, has been the most frequently mentioned and recently used AI tool. This shift in terminology could be attributed to the fact that AI is comprised of overlapping subsets that are sequentially related, and the number of published documents follows this pattern and decreases as one moves to another subset. Therefore, researchers tend to begin with the general concept of AI and progress to more specific areas, such as ML, NNs, and DL. This suggests that as re-

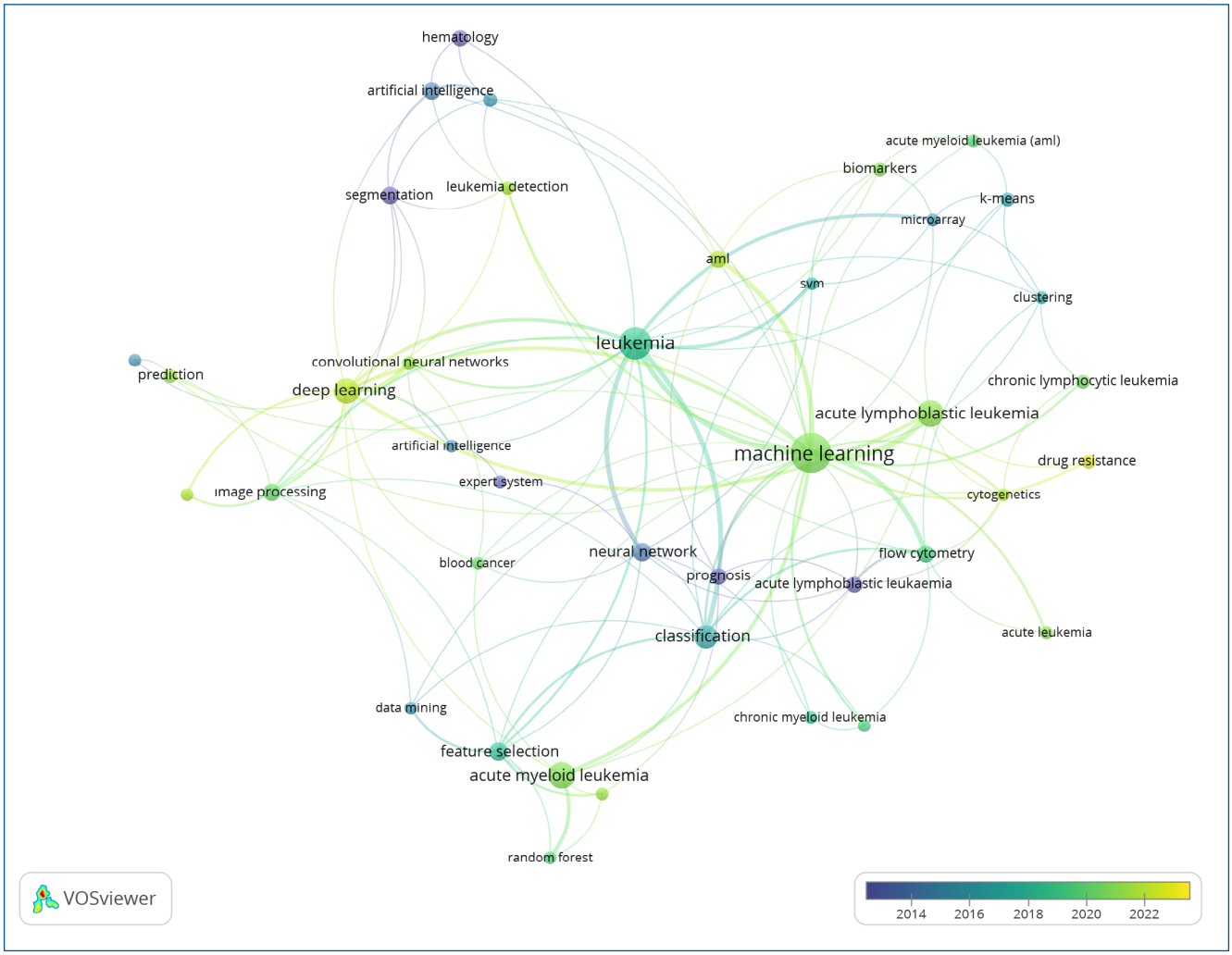

FIGURE 1. Co-occurrence analysis of author keywords from the documents that were manually selected via utilizing the Scopus database. For the colored presentation, the VOSviewer platform was used. The minimum number of occurrences of a keyword was arranged as 3 times in a total of 248 documents. Of the 671 keywords, 42 keywords met the threshold. Corresponding results were colored as in the time of last 10 years and the spectrum varies from dark purple to light yellow. This color-coded representation indicates the trend of keyword usage from 2013 to 2023 by the authors. (Keywords: "artificial intelligence" and "machine learning" and "deep learning" and "neural network" and "leukemia")

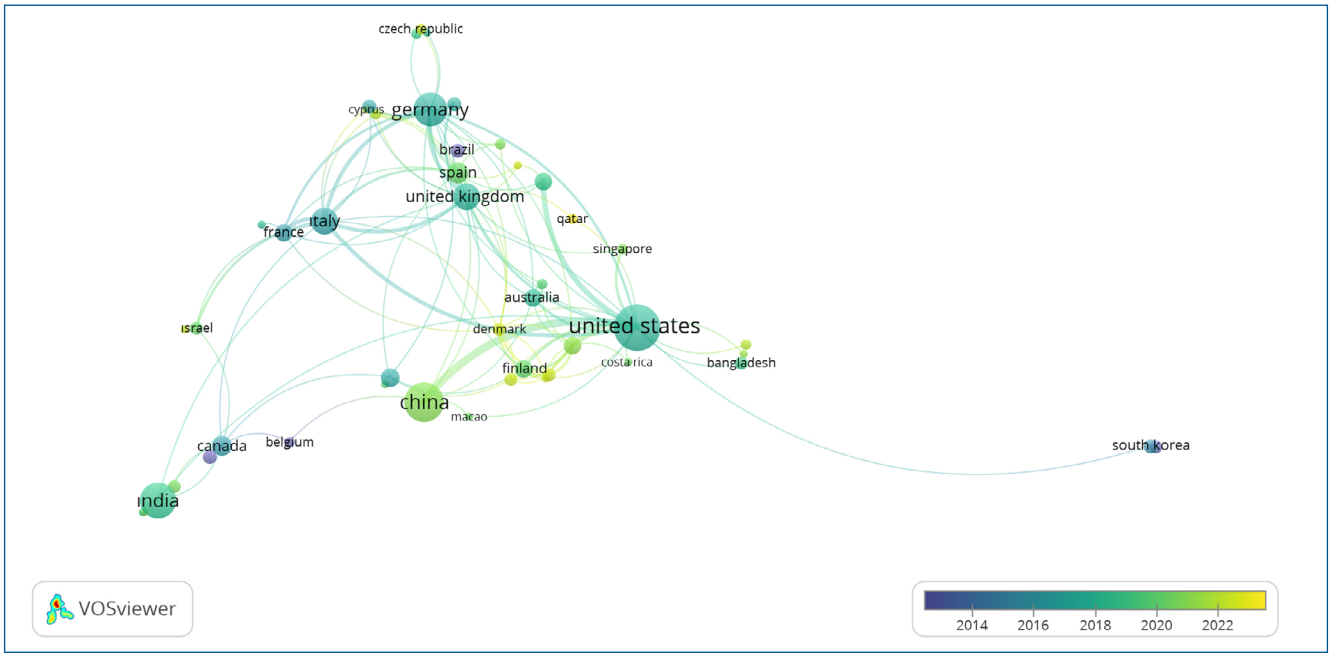

FIGURE 2. Co-authorship analysis of countries via VOSviewer, corresponding data taken by Scopus query search, and 248 documents scanned after filtering. Minimum number of documents of a country was determined as 1. Out of the 60 countries, 47 countries were found to be connected. Corresponding results were colored as in the time of last 10 years and the spectrum varies from dark purple to light yellow. This color-coded representation indicates the trend (color spectrum) country contribution (size of points) and country-based co-authorship (connected lines) between 2013 and 2023. (Keywords: "artificial intelligence" and "machine learning" and "deep learning" and "neural network" and "leukemia")

searchers gain a better understanding of the supergroup, they begin to incorporate the subgroups into their research (Table 1 – Table 2).

Limited Number of Research to Cope with Leukemia in The Era of Technology but Progress Is Promising

Our literature research was conducted by analyzing articles and conference proceedings to catch up on recent applications of AI tools in the field of leukemia management. Even though the oldest document was published in 1989 for the Scopus database in 1994 for WoS, the studies conducted in the following years were not consistent. It was only after 2017 that a steady increase was observed in the number of documents published (determined by Scopus query search) (Figure 5A). Moreover, according to WoS query search, the number of documents published has increased approximately 16-fold since 2012, with a significant increase observed in 2022 (Figure 6A).

Researchers tend to Study Leukemia with Unspecified Subtypes

In the realm of leukemia research, investigators have historically conducted studies on experimental groups with a mix of hematological cancers without any specific focus on leukemia itself. To tackle this issue, researchers have employed the use of AI and its constituents to differentiate between healthy and patient groups and to develop a precise diagnostic tool for leukemia. Initially, the study of microscopic slides of peripheral blood smears was the primary method for researchers. However, with the advent of microarrays and flow cytometry, researchers can now identify genetic landscape and immunophenotypic features, and as such, have shifted their approach to focus more on leukemia, especially AML and acute lymphocytic leukemia.

What Are the Countries' Attitudes Toward the Application of AI Tools in the Case of Leukemia?

One factor that influences the scope of topics in scientific publications is how authors approach a subject recently, and the policies followed by the countries to which they are affiliated. In this context, collaborating with authors from other countries emerges as a phenomenon that influences and enhances the research environment. The way authors from different countries collaborate and work together affects the diversity and richness of the research landscape. In our VOSviewer co-authorship analysis conducted by using Scopus and Wos databases to understand the role of countries in studies that combine leukemia and AI components, we observe that research originating from the United States and China is quite prevalent with comparable to the contribution to the field and as following the trends (Figure 2 and Figure 4). Whether they are located on different continents or

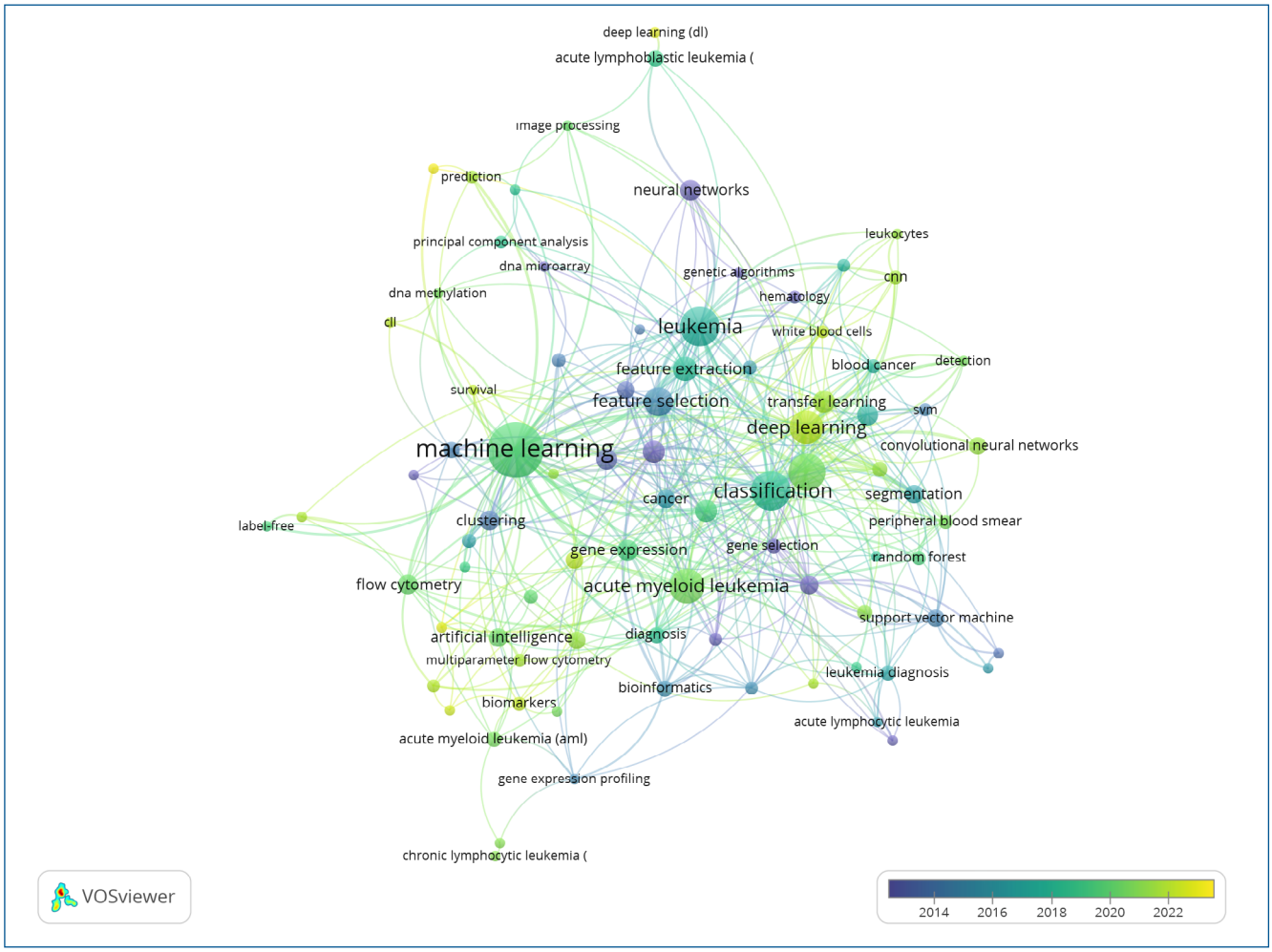

FIGURE 3. Co-occurrence analysis of author keywords from the documents that were manually selected via utilizing the Web of Science database. For the colored presentation, the VOSviewer platform was used. The minimum number of occurrences of a keyword was arranged as 3 times in a total of 473 documents. Of the 1124 keywords, 84 keywords met the threshold. Corresponding results were colored as in the time of last 10 years and the spectrum varies from dark purple to light yellow. This color-coded representation indicates the trend of keyword usage from 2013 to 2023 by the authors. (Keywords: "artificial intelligence" and "machine learning" and "deep learning" and "neural network" and "leukemia")

not, researchers are making collaborations in the way of using AI tools in the case of leukemia.

Publishers and Institutes Are Giving Attention to Study in This Field

Sharing scientific findings through journals or other scientific resources provides numerous advantages as it shapes the current and future direction of a field. Publishing has many benefits that promote scientific progress by bringing together the characteristics of scientific knowledge such as verifiability, reproducibility and sustainability and then presenting these results to the readers. We can also emphasize the importance of the author's working environment, from the production stage to the publication process, as it provides research infrastructure and collaborations. In our research, we found an increase in the number of documents published in peer-reviewed journals on the leukemia-AI tools axis (Figure 5B – Figure 6B) and an increase in the contributions of institutions across the globe which indicates that attention was drawn to this field recently (Figure 5C – Figure 6C).

DISCUSSION

This study examines the use of AI and its components—ML, NNs, and DL —in leukemia research. A search query with inclusion and exclusion criteria was designed to analyze AI applications separately. The literature review utilized Scopus and WoS databases, along with bibliometric analysis tools to identify trends based on keywords, countries, affiliations, and sources. While databases like Google Scholar and PubMed could have ex-

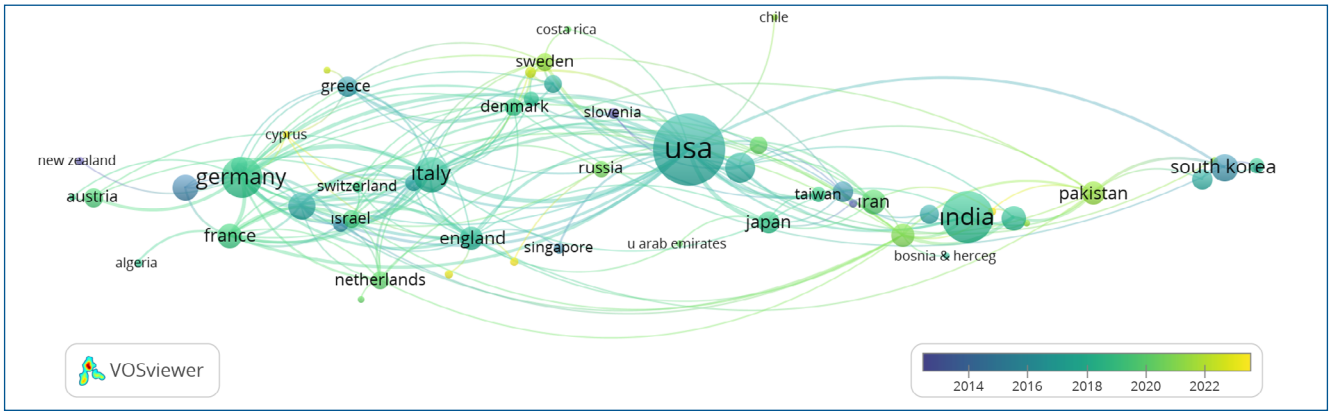

FIGURE 4. Co-authorship analysis of countries via VOSviewer, corresponding data taken by Web of Science query search, and 473 documents scanned after filtering. Minimum number of documents of a country is determined as 1. Out of the 66 countries, 60 met the threshold and 54 countries are found to be connected. Corresponding results were colored as in the time of last 10 years and the spectrum varies from dark purple to light yellow. This color-coded representation indicates the trend (color spectrum) country contribution (size of points) and country-based co-authorship (connected lines) between 2013 and 2023. (Keywords: "artificial intelligence" and "machine learning" and "deep learning" and "neural network" and "leukemia")

panded the dataset, Scopus and WoS provided the most relevant documents. The study found that ML is widely used for classifying genetic, histological, and morphological characteristics of leukemia, with a focus on myeloid and lymphocytic subtypes. However, research on chronic leukemia subgroups remains limited.

Despite differences in their advantages and the number of documents retrieved, we found no significant differences in the inferences drawn from Scopus and WoS. The country of a researcher's affiliation significantly affects research scope. Developed countries such as the United States and China play a pioneering role in AI applications for leukemia. AI is expected to be widely utilized in healthcare, but collaboration among physicians, patients, and AI developers is necessary for successful integration. Hematologists need to gain AI literacy to ensure better clinical care and decision-making.

AI's integration into hematology remains in its early stages. While AI has shown promise in diagnostic precision and efficiency, several challenges persist, including clinical validation, standardization and regulatory approvals (27, 28). AI-based models have demonstrated their ability to classify acute and chronic leukemias based on genetic and morphological patterns, yet their implementation requires physician competency in AI-driven decision-making. AI's ability to analyze high-dimensional biological data, such as next-generation sequencing (NGS) and transcriptomics, is a significant contribution to leukemia research (8). Machine learning-based classification models have enhanced early detection and risk stratification (29). However, most AI models focus on myeloid and lymphoid leukemias, while chronic leukemia subgroups remain underrepresented. Future research should emphasize AI applications for chronic leukemia progression and treatment response prediction (30).

AI implementation in healthcare raises regulatory and ethical concerns. AI models require extensive patient data, leading to issues regarding informed consent, data ownership, and cybersecurity risks. Regulatory bodies such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) emphasize transparency and explainability in AI-driven decisions. Ensuring that AI models are unbiased and validated across diverse patient populations is critical to preventing disparities in leukemia diagnosis and treatment.

CONCLUSION

In some respects, our research has limitations as the results primarily based on the findings gathered from Scopus and WoS, while these databases are comprehensive, including sources like PubMed or Google Scholar might have provided a more extensive dataset. Furthermore, the study is based on bibliometric analysis and literature review rather than direct experimental or clinical validation of AI applications in leukemia. This limits the ability to assess real-world effectiveness. While AI's potential in leukemia management is acknowledged, challenges related to data privacy, model transparency, and regulatory approvals are not deeply explored. Also, the study highlights the increasing use of AI in leukemia but does not critically compare the performance of different AI models or algorithms across studies. Additionally, the study primarily focuses on ML, DL, and NN, while other AI subfields like NLP and robotics were excluded.

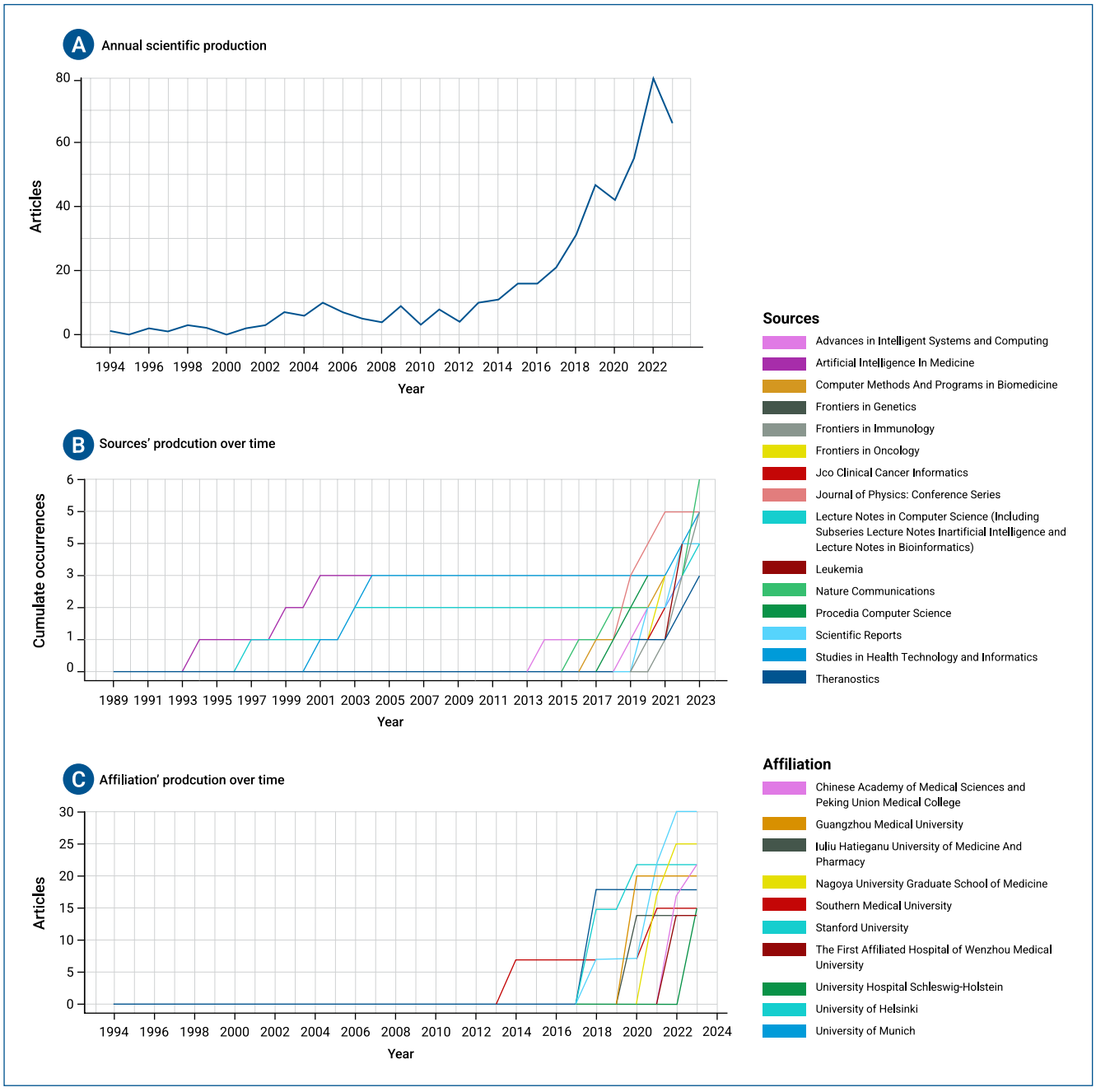

FIGURE 5A. Number of documents taken into account for the bibliometric analysis after utilizing Scopus query search and manual selection of documents. 248 documents are listed in the time interval of 1989-2023. This graph is created via the Bibliometrix-Biblioshiny platform. (Keywords: "artificial intelligence" and "machine learning" and "deep learning" and "neural network" and "leukemia")

FIGURE 5B. Scientific journals that published articles and conference proceedings on AI in leukemia between the years 1989-2023. 248 published documents were categorized according to their corresponding publisher. This graph is created via the Bibliometrix-Biblioshiny Platform. Data were taken from a Scopus query search. (Keywords: "artificial intelligence" and "machine learning" and "deep learning" and "neural network" and "leukemia")

FIGURE 5C. Number of documents retrieved from Scopus query search as indicating the affiliation's production over time (between 1994-2023). (Keywords: "artificial intelligence" and "machine learning" and "deep learning" and "neural network" and "leukemia")

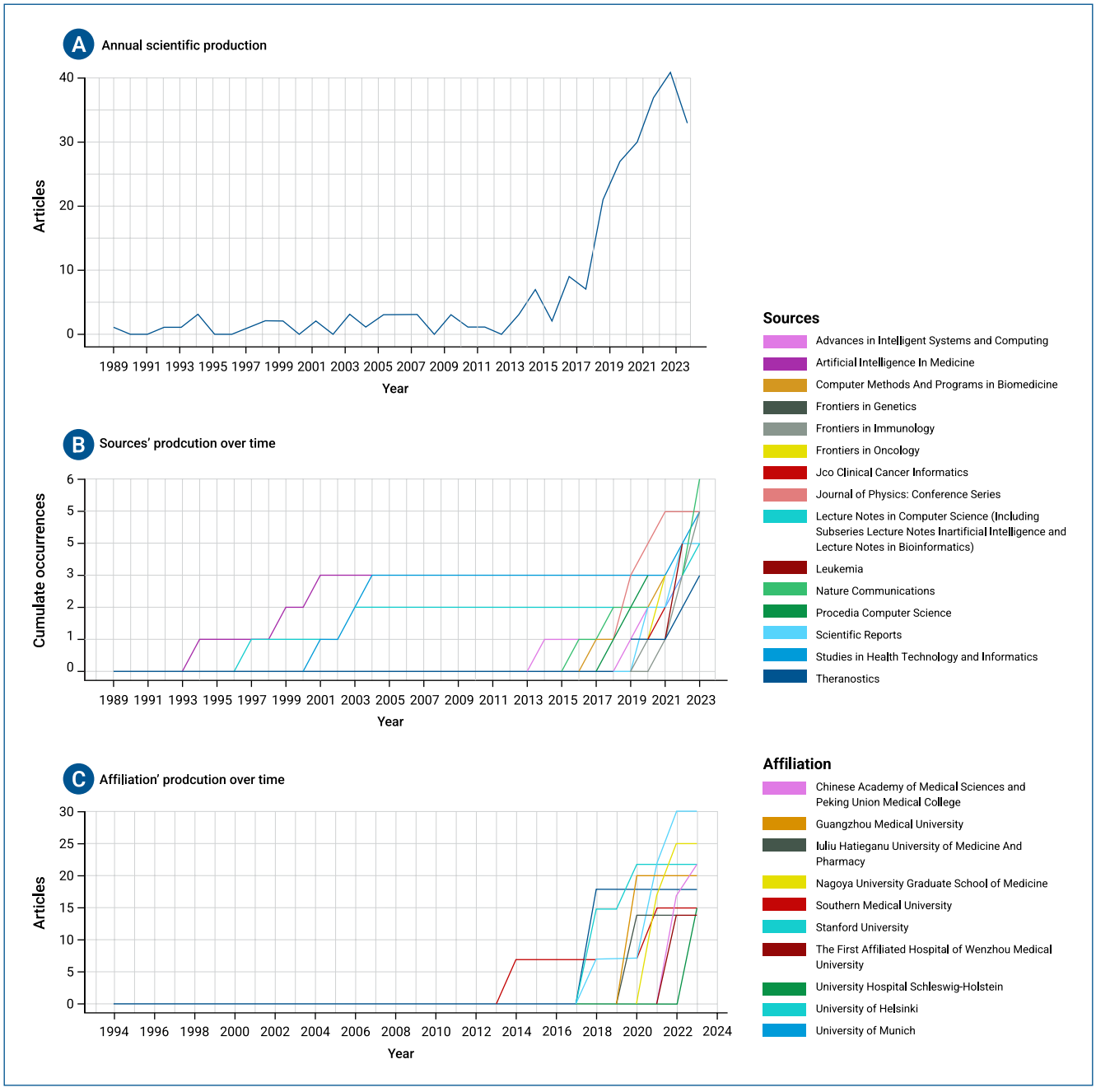

FIGURE 6A. Number of documents taken into account for the bibliometric analysis after utilizing Web of Science query search and manual selection of documents. 248 documents are listed in the time interval of 1994-2023. This graph is created via the Bibliometrix-Biblioshiny platform. (Keywords: "artificial intelligence" and "machine learning" and "deep learning" and "neural network" and "leukemia")

FIGURE 6B. Scientific journals that published articles and conference proceedings on AI in leukemia between the years 1994-2023. 248 published documents were categorized according to their corresponding publisher. This graph is created via the Bibliometrix-Biblioshiny Platform. Data were taken by Web of Science query search. (Keywords: "artificial intelligence" and "machine learning" and "deep learning" and "neural network" and "leukemia")

FIGURE 6C. Number of documents retrieved from Web of Science query search as indicating the affiliation's production over time (between 1994-2023). (Keywords: "artificial intelligence" and "machine learning" and "deep learning" and "neural network" and "leukemia")

The integration of AI in leukemia research and management has significant potential in enhancing diagnosis, treatment, and disease monitoring. Bibliometric analysis highlights the growing impact of AI—particularly ML, DL, and NN—in leukemia research. While AI applications in leukemia have gained momentum since 2017, most studies focus on acute leukemia subtypes, with relatively limited exploration of chronic leukemia.

Machine learning remains the most frequently utilized AI tool, particularly for diagnostic classification and pattern recognition. The increasing number of publications and institutional contributions demonstrates a global interest in AI-driven leukemia research, with the United States and China emerging as key contributors. Despite these advancements, challenges such as data standardization, clinical validation, and ethical considerations must be addressed to ensure AI's effective integration into routine clinical practice.

Future research should focus on expanding AI applications to chronic leukemia subtypes and improving AI-driven predictive models for personalized treatment strategies. Strengthening collaborations between clinicians, data scientists, and AI developers will be crucial in bridging the gap between AI research and real-world clinical applications. As AI continues to evolve, its potential to revolutionize leukemia management remains promising, paving the way for more precise, efficient, and personalized approaches to patient care.

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N.A

Informed Consent
N.A

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Authorship Contributions
Concept – F.A.; Design – F.A.; Supervision – F.A.; Data Collection and/or Processing – F.A.; Analysis and/or Interpretation – F.A.; Literature Review – F.A.; Writer – F.A.; Critical Reviews – F.A.

Conflict of Interest
The authors declare no conflict of interest.

Financial Disclosure
The authors declare that they have no relevant financial interest.

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Cost of Illness Analysis: Hidradenitis Suppurativa in Türkiye

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Abstract

Objective: Hidradenitis Suppurativa (HS), also known as Acne Inversa, is a chronic, inflammatory, recurring, debilitating skin disease of the hair follicles. It is most commonly present in the axillae, inguinal and anogenital regions. There are few studies available about the economic burden of HS on patients from different countries and we feel the need to develop such a project in the context of HS disease in Turkey. Therefore, the aim of this study is to estimate the yearly cost of HS disease in Turkey from the payer's perspective.

Materials and Methods: This is a prevalence-based CoI study with a focus on direct health care costs of HS from the perspective of the payer. A multipoint data collection procedure has been performed based on the literature search for HS epidemiological data, treatment choices, and direct health care costs to develop the analysis and the structure of the CoI of HS. The disease itself and treatment options have been reviewed. Assumptions and calculations were done according to the literature.

Results: The total number of Turkish people with HS has been estimated as 80.811 according to the 0.10 % prevalence rate of Garg and his colleagues' prevalence study. The 12 months costs were estimated as 21.067.174 TRY for patients on Hurley stage I, for the year 2018. We estimated the total direct cost attributable to HS as 741.615.190 TRY and revealed that the average one-year direct cost per patient was 9.177 TRY.

Conclusion: We found that the cost per patient seems similar but the proportions of the costs are different than the other published HS CoI studies from various parts of the world even though the methods differ greatly. HS is a disease which is attributed as 'rare' and 'unknown' but, surprisingly, it takes an important place in the national healthcare budget as treatment costs.

Keywords: Cost of illness, Hidradenitis Suppurativa, Acne Inversa, pharmacoeconomics, payer, Türkiye

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INTRODUCTION

Hidradenitis Suppurativa (HS) is a chronic, inflammatory, recurring, debilitating skin disease of the hair follicles that usually presents after puberty with painful, deep-seated, inflamed lesions in the apocrine gland-bearing areas of the body, most commonly the axillae, inguinal and anogenital regions and also known as Acne Inversa (Dessau definition, 1st International Conference on Hidradenitis Suppurativa/Acne Inversa, March 30–April 1, 2006, Dessau, Germany). (1) HS has an enormous burden, on patients and it is highly correlated with concomitant diseases including but not limited to: reduced quality of life, depression, stigmatization, decrease in physical activity, sexual deficiency, and several risk factors associated with cardiovascular diseases. (2)

Patients with HS deal with serious misdiagnosis issues because of the low disease awareness among health care professionals and patients. Saunte and colleagues showed that diagnostic delay for HS patients is 7.2 years from the onset of the first symptoms. (3) There is no room for biopsies and there are no validator laboratory assessments in the clinical diagnosis of HS. Despite the lack of established diagnosis criteria, diagnosis includes recurrence of lesions, chronicity, lack of clearance from antibiotics, sinus appearance and scarring, dermal contracture, multifocal lesion distribution, the existence of a variety of comedones, nodules, papules, soreness of lesions, and suppuration. (4) The primary diagnostic criteria of HS depend on the history of the patient and the clinical presentation of the disease. Symptoms include but are not limited to: involvement of axilla, genitofemoral area, perineum, gluteal area and infra-mammary area of women, the appearance of nodules (inflamed or non-inflamed), sinus tracts (inflamed or non-inflamed), abscesses, and scar formation (atrophic, mesh-like, red, hypertrophic, or linear). (5)

HS has been linked to several different adjuvants and secondary diseases, including obesity, metabolic problems, inflammatory bowel diseases (IBD) such as Crohn's disease (CD) and ulcerative colitis (UC), spondyloarthropathy, follicular occlusion syndrome, and other hyperergic diseases. (6) IBD, especially CD, is the most reported associated disease in patients with HS. Principi and his colleagues showed in their recent pooled data analyses that the prevalence of HS in IBD patients was 12.8%. 17.3% of the patients with CD and 8.5% of the patients with UC had HS as a comorbid disease. (7)

Spondylarthritis (SpA) is also linked to HS as a frequent comorbid disease. Schneider-Burrus et al showed that back pain and SpA are very common among patients with moderate/severe HS and more than 70% of HS patients were suffering from back pain. (8)

Over the last years, HS became one of the hot topic research areas and intense research are conducted to develop therapeutical strategies. (9) Although it was reported to be a rare disease, there are inconsistent prevalence data. Reported prevalence is changing from 0.053% to 4.1% depending on the methodology of the study. (10)- (11) It is good news that the misdiagnosis rate of HS was started to decline over the last decade. But revealing pharmacological characteristics and describing the HS burden on both patients and the governments became a major problem to be solved. (12)

Due to the few studies regarding the economic burden of HS on patients being conducted up to now in the US, some of the countries of Europe and Israel (13)- (14), it is inevitable to prepare such a project for Turkey. To the best of our knowledge, there has not been any HS CoI study conducted for Turkey to this study. Therefore, the aim of this study is to estimate the yearly cost of HS disease in Turkey from the perspective of the payer for the year 2018.

MATERIALS AND METHODS

This study is a prevalence-based CoI study in focus on direct health care costs from the view of the payer - the Ministry of Health. A multipoint data collection procedure has been implemented based on the literature search for HS epidemiological data, treatment choices, and direct health care costs for the structure of the CoI analysis of HS.

A literature search on studies published in English on HS was performed in PubMed with the keywords of "Hidradenitis Suppurativa", "Acne Inversa" and "Verneuil Disease" from 1949 to June 2018. All of the titles of the articles and abstracts retrieved from the database using these keywords have been systematically reviewed and analyzed. The disease itself and treatment options have been reviewed comprehensively.

Classification and Staging

Many different models have been developed to classify and stage HS and to assess the treatment success, such as qualitative models; Hurley Staging System and Refined Hurley Staging System. And there are also quantitative models; the Sartorius system, the modified Sartorius systems, the Hidradenitis Suppurativa Physician's Global Assessment (HS-PGA), and the Hidradenitis Suppurativa Clinical Response (HiSCR). Among these classification and staging tools, the most widely used scale to assess disease severity is the Hurley Staging system and HS-PGA. (2)

Hurley Staging

Hurley reported the Hurley Staging System, as a novel classification model to define HS in 1989. It classifies the disease as three different levels of severity (Table 1). Hurley staging is proposed as a tool to facilitate rational treatment decision-making for the surgical approach in a certain body location. (23)

The Hurley staging model was extensively used due to its suppleness and rapidness. However, it has some limits such as insufficient qualitativeness and its unvarying nature. Neither count of affected anatomical locations nor the count of lesions at each location was described by this model. Besides, it considers scars and fistulas as fixed or invariable characteristics which makes this tool ineffective for assessing the response of the therapy. (2)

Table 1. The definition of Hurley Staging.

Hurley Stage	Definition
I	Individual primary lesions and/or cysts without fistulae or scarring
II	Individual primary lesions and/or cysts with the presence of fistulae and scarring
III	Confluent primary and secondary lesions at the involved surface(s) with fistulae and scars

Prevalence Estimation

The prevalence of HS was reported at variance over the year. Studies reports that the prevalence is starting from 0.053% to 4.1%. It should be noted that there are many differences in the research methodologies and populations studied in. (10)- (11) Summary of prevalence studies has been sown in Table 2.

Table 2. Summary of HS prevalence studies.

Country / Reference	Number of samples	Prevalence Estimation
Denmark/ Jemec, 1988 (16)	70	4 %
Denmar k/ Jemec, 1996 (17)	599	4.1 %
France/ Revuz, 2008 (18)	10,000	1 %
United States/ Cosmatos, 2013 (19)	7,927	0.053 %
United States/ Sung, 2013 (20)	429,329	0.11 %
Denmark/ Vinding, 2014 (21)	16,404	2.1 %
United States/ Shahi, 2014 (10)	144,000	0.13 %
United States/ Garg, 2017 (11)	48 Million	0.10 %

There is no prevalence data available for Turkey. Several prevalence studies in different scopes (population-based vs. hospital), different periods (from 1988 to 2018) different diagnosis methodologies (self-reported, medically assessed, diagnosis of treatments codes) have been reviewed to estimate the prevalence for Turkey. The review showed an important variance in estimates and incertitude concerning the actual frequency of HS. According to this variety, the study with the highest number of patients with a prevalence rate of 0.10 % has been chosen for the analysis. (11) Garg and colleagues analyzed 48 million unique patients across all United States regions by using electronic health record data. Results showed 47,690 HS patients and overall HS prevalence in the United States was 0.10%. (11) When looking at the clinical characteristics of the study, HS prevalence among the white race was also determined as 0.10 % which has been assessed as similar to characteristics of Turkish people.
Turkish population information has been derived from the Turkish Statistical Institute as of Feb 2018 and used for the analysis. (26) Hurley I, Hurley II, and Hurley III variance has been calculated according to the study of Canoui-Poitrine F. et al which assessed the clinical characteristics of 302 French patients with HS. (15)

Determination of Treatment Approach

There have been several approaches to set a standard of care for the treatment of HS even though there is no widely accepted guideline available. In 2015, the European S1 guideline has been published by Zouboulis C. et al. (5) Guideline consists of a comprehensive review of the disease and the treatment options by evaluating clinical study results. The European S1 guideline is the most accepted and diverse guideline available for HS.
Italian Society of Dermatology and Venereology published a guideline for the use of a-TNFs for HS treatment in 2015 after the European S1 guideline. (24) It is mostly focused on a-TNF agents and refers to the European S1 Guideline widely.

At the beginning of 2016, the evidence-based approach has been published based on European guidelines. (25) It promotes a holistic evidence-based approach that implemented the Level of Evidence and Strength of Rec-

ommendation for the treatment of HS due to the need for evidence-based treatment guidelines. It is more like a complementary element of the European S1 guideline. Since there is no established treatment algorithm for HS, treatment methods derived from both the European S1 Guideline (5) and the Evidence-based Approach to the Treatment of HS (25) are adapted to the Turkish healthcare system within the frame of available treatment options. The costs of hospitalizations, physician office visits (physical examinations), medical and surgical treatments, and medical procedures were estimated from the literature and analysis of publicly available health databases. Costs of medical procedures were derived from the updated Social Security Institution Medical Enforcement Declaration and Republic of Turkey Social Security Institution reimbursement rates and wholesale drug costs for the year 2018. The prices of the available medical treatment options have been derived from the RxMediaPharma program.

RESULTS

Prevalence Estimation

The population of Turkey as of Feb 2018 has been announced as 80 million 810 thousand and 525 people. (26) The total number of Turkish people with HS has been estimated as 80.811 (+/- 4.041) with a 95% confidence interval (CI) according to the 0.10 % Garg and his colleagues' study. (11) The estimated patient number with HS has been shown in Table 3.

Among the patients with HS disease, Hurley classification estimation has been done and shown in Table 4. (15) According to that estimation which was calculated with the 95 % CI, the majority of the patients are in the group of Hurley stage I. The number of patients with Hurley stage I, II and III are 54.951 (+/- 2.748), 22.627 (+/- 1.131) and 3.232 (+/- 162), respectively.

Application of Treatment Approach

Tuberculosis examination has to be done for moderate and severe HS patients who are planning to have the treatment with biological agents before the initiation of the biological treatment according to the Medical Enforcement Declaration. (27) The cost of the tuberculosis examination tests is calculated as 128 TRY per patient.

The cost of doctor's office visits and hospitalization have been identified according to Medical Enforcement Declaration as both university hospitals and training hospitals. The mean price has been calculated accordingly (Table 10). Primary healthcare services have been excluded due to these healthcare services mostly serve as referral steps to the university or training hospitals and they have not taken any role in the management of the disease. Mean prices have been calculated as dermatology visits, general or plastic surgery visits, and hospitalization (standard bad tariff) 37 TRY, 50 TRY and 30 TRY respectively.

Table 3. Estimated number of patients with HS in Turkey.

Turkey Population	Prevalence (%)	Estimated Number of Patients with HS (with 95% CI)
80,810,525	0.10	80,811 (± 4041)

Table 4. Patient distribution according to Hurley staging.

Hurley Stage	Percentage (%)*	Estimated Patient Number (with 95% CI)
I	68	54,951 (±2748)
II	28	22,627 (± 1131)
III	4	3232 (±162)

*Canoui-Poitrine F, et al. (15)

Since there is no established treatment algorithm for HS as expressed in the materials and methods section, treatment methods are derived from current HS treatment guidelines and adapted to the Turkish healthcare system within the frame of available treatment options. 1st line treatment options have been used for cost calculation only. 2nd, 3rd line, and experimental treatment options have not been taken into consideration. (25) For each active substance that is present in the Turkish market, available pharmaceutical preparations have been identified from RxMediaPharma Program and the cost of unit dosage has been calculated. In this way, the mean cost of the unit dosage has been calculated for each active substance. Treatment durations have been derived from the guideline according to the unit dosage of the pharmaceutical preparations and calculated accordingly. Available 1st line treatment options and unit dosage costs have been shown in Table 5.

Medical treatment of patients with HS on Hurley stage I consists of topical clindamycin 1%, oral tetracycline 500 mg, and basic excision of the HS lesions according to the evidence-based approach treatment algorithm. (25) The recommended treatment duration of topical clindamy-

Table 5. Medical treatment options and cost of unit dosages.

Drugs	Minimum Price (TRY)	Maximum Price (TRY)	Unit Dosage Price (TRY)
Antibiotics			
Clindamycin	8.31	9.14	0.0035
Rifampicin	4.37	14.88	0.0013
Doxycycline	5.50	6.05	0.0041
Tetracycline	3.61	5.57	0.0007
Topical clindamycin	9.53	9.53	0.3177
Anti-TNFs			
Adalimumab	1186.34	1186.34	14.82

Table 6. Hurley I medical & surgical treatments and costs.

	Duration	Dosage	Unit Cost (TRY)	Total Cost (TRY)
Medical Treatment				
Topical clindamycin 1%	3 months	twice a day	0.3177 /mL	57.18
Tetracycline 500 mg	4 months	once a day	0.0007 /mg	42
Surgical Treatment				
Excision	1	NA	207.70 /session	207.70
TOTAL				**306.88**

cin is 3 months and for oral tetracycline, it is 4 months. Cost calculation has been done according to treatment duration recommendations. Surgical treatment has been calculated as 1 time in the treatment frame. The total cost has been calculated as 306 TRY for a patient on the Hurley stage I (Table 6).

With the same approach, medical and surgical treatment options have been calculated for HS patients on Hurley stage II. Medical treatment is included both topical clindamycin 1 %, oral tetracycline 500 mg, and also a combination of oral rifampicin-clindamycin 600 mg and adalimumab. (25) In addition to medical treatment, surgical treatment is recommended in a wide range if needed which are excision of the lesions, deroofing, CO2 laser excision, and primary and secondary wound closures with flap, or grafting techniques. The recommended treatment duration and cost calculation have been shown in Table 7. The total cost for a patient on the stage of Hurley II was calculated as 27.631 TRY.

For Hurley stage III patients, treatment options are much like patients on Hurley stage II except for the usage of topical clindamycin 1 %, and oral tetracycline 500 mg which these treatment options are for milder cases. (25) Surgical interventions remain the same with the Hurley II treatment scheme. Cost calculation has been done according to recommended treatment duration and again,

Table 7. Hurley II medical & surgical treatments and costs.

	Duration	Dosage	Unit Cost (TRY)	Total Cost (TRY)
Medical Treatment				
Topical clindamycin 1%	3 months	twice a day	0.3177 /ml	57.18
Tetracycline 500 mg	4 months	once a day	0.0007 /mg	42
Rifampicin 300 mg	10 weeks	twice a day	0.0013 /mg	201.60
Clindamycin 300 mg			0.0035 /mg	
Adalimumab	Continual*	160 mg at week 0, 80 mg at week 2, 40 mg weekly starting from week 4	14.82 /mg	24,897.60
Surgical Treatment				
Excision	1	NA	207.70 /session	207.70
Deroofing	1	NA	400 /session	400
CO2 laser excision	1	NA	550 /session	550
Primary wound closure	1	NA	38 /session	38
Secondary wound closure with graft	1	NA	400 /session	400
Secondary wound closure with flap	1	NA	837.20 /session	837.20
TOTAL				**27,631.28**

*According to the PIONEER clinical trial, 68% of the patients were able to apply the proposed treatment regimen in full, and in 32% of cases, the treatment was stopped at the 12th week. So the average annual dose of adalimumab has been calculated as 21 boxes with that assumption. (28)

surgical treatment has been calculated at 1 time. As a result, it appears similar to the result of Hurley stage II, the calculated cost is 27.532 TRY for a patient on Hurley stage III. (Table 8)

If the costs are grouped as medical treatment, surgical treatment, procedures/tests, and physical examinations, a total frame can be shown below, in Table 15 for patients with Hurley stages I, II, and III. For a HS patient on Hurley stage I, the yearly cost is far lower than Hurley stage II and Hurley stage III patients. Direct costs of patients according to Hurley classification are 383 TRY, 27.876 TRY, and 27.777 TRY, respectively for one patient for the year 2018. The calculation has been shown in Table 9.

As a result, the total national cost of HS to the Ministry of Health was estimated as 741.615.190 TRY (+/- 37.080.760 TRY) for the year 2018. Details of the cost calculation have been shown in Table 10. Costs have been shown with 95% CI at parenthetical in each section. It consists of direct medical costs such as medical therapy, surgical treatment, procedures, tests, and doctor's office visits (physical examinations).

The 12 months costs were estimated as 21.067.174 TRY (+/- 1.053.358 TRY) for patients on Hurley stage I, 630.760.088 TRY (+/- 31.538.004 TRY) for Hurley stage II and 89.787.927 TRY (+/- 4.489.296 TRY) for Hurley stage III for the year 2018. The largest part of the costs is attributed to medical treatment expenditures and are estimated as 654.546.051 TRY (+/- 32.727.302 TRY).

Table 8. Hurley III medical & surgical treatments and costs.

	Duration	Dosage	Unit Cost (TRY)	Total Cost (TRY)
Medical Treatment				
Rifampicin 300 mg Clindamycin 300 mg	10 weeks	twice a day	0.0013 /mg 0.0035 /mg	201.60
Adalimumab	Continual*	160 mg at week 0, 80 mg at week 2, 40 mg weekly starting from week 4	14.82 /mg	24,897.60
Surgical Treatment				
Excision	1	NA	207.70 /session	207.70
Deroofing	1	NA	400 /session	400
CO2 laser excision	1	NA	550 /session	550
Primary wound closure	1	NA	38 /session	38
Secondary wound closure with graft	1	NA	400 /session	400
Secondary wound closure with flap	1	NA	837.20 /session	837.20
TOTAL				**27,532.10**

*According to the PIONEER clinical trial, 68% of the patients were able to apply the proposed treatment regimen in full, and in 32% of cases, the treatment was stopped at the 12th week. So the average annual dose of adalimumab has been calculated as 21 boxes with that assumption. (28)

Table 9. Cost calculation.

	Cost of stages (TRY)			
	Hurley I	Hurley II	Hurley III	Total
Medical Treatment	59.18	25,198.40	25,099.20	50,356.78
Surgical Treatment	207.70	2432.90	2432.90	5073.50
Procedures & Test	0	128.70	128.70	257.40
Physical Examinations	116.50	116.50	116.50	349.50
Total cost (TRY)	383.38	27,876.50	27,777.30	

Table 10. Population adjusted cost calculation with TRY (with 95% Confidence interval).

	Hurley I	Hurley II	Hurley III	Total
Medical treatment	3,211,941.96 (±160,597.10)	563,137,972.71 (±28,256,898.64)	80,131,576.41 (±4,006,578.82)	9,298,432.47 (± 32,324,074.55)
Surgical treatment	11,272,733.12 (± 563,636.66)	54,370,847.90 (±2,718,542.40)	7,767,263.99 (±388,363.20)	3,287,095.65 (±3,650,542.25)
Procedures & test	0.00	2,876,208.69 (±143,810.43)	410,886.96 (±20,544.35)	646,481,491.09 (±164,354.78)
Physical examinations	6,322,934.08 (±316,146.70)	2,603,561.09 (±130,178.05)	371,937.30 (±18,596.86)	73,410,845.01 (±464,921.62)
Total	**20,807,609.17** (±1,040,380.46)	**622,988,590.40** (±31,149,429.52)	**88,681,664.65** (±4,434,083.23)	732,477,864.22 (±36,623,893.21)

DISCUSSION

To our knowledge, this is the first study in the context of health care utilization and cost of Illness with HS conducted for Turkey. The literature search reveals that there are less than thirty manuscripts written about HS from Turkey. The literature is mostly about case reports or series and there is no cost-related study among those. When looking at world literature, there are only a few studies that aim to find the disease-related cost. (13)- (14) Kirby and his colleagues find out in their cohort cost-identification study that the majority of the cost was the inpatient expenditures. They also compare the results with psoriasis (PsO) patients and resulted that medication costs were higher in the PsO group. The emergency department visits and inpatient care have shown as the biggest cost source in the study. (13)

Another manuscript which is a follow-through study of Kirby and his colleagues revealed almost the same results as the previous literature. Inpatient costs were the major expenditure for HS patients. The total 5-year cost for the HS patient cohort was found as 23,418,396 USD from the perspective of the payer. HS cohort was consisting of 7,901 patients and for this instance; the cost per patient could be calculated as 2.963,97 USD. (29)

Desai and Shah conducted a retrospective cohort study in England to describe the hospital resource use of patients with HS. They found out that the mean hospital resource utilization cost for a patient with HS was 2.027 GBP per patient per year. But it should be noted that the study does not include the details of medication and it is just based on outpatient, inpatient, accident, and emergency hospital attendances. (30)

Shalom and his colleagues conducted a study very recently in Israel and they compare the healthcare service utilization cost of HS patients with PsO patients and also with the general population. Community clinic visits and inpatient service utilization with drug use data have been included in the study. But biological medications were not available in Israel for the treatment of HS during the study therefore they were not included in the analysis. They found out that the burden of HS patients was greater than both PsO and the general population. There wasn't an estimate on any monetary terms in the study. (31)

The results of our analysis showed that the direct cost of patients with HS is more than that recognized in Turkey's health care system. The estimation of the total direct cost attributed to HS is 741.615.190 TRY (+/-37.080.759 TRY) and revealed that the mean one-year direct cost for one patient is 9.177 TRY (+/- 458 TRY).

Medications seem to be only the definitive important resources funded by the Turkish public health system and if we calculated the contribution margin of the medicines even by patients.

Even though the studies' methods are different, it is possible to compare the results with our study. The cost per patient seems similar between the studies but the source and the proportions of the costs were different.

This study has several limitations and they should be considered along with the results. According to current literature, there is no epidemiological data from Turkey. Accordingly, prevalence data is assumed based on the work of Garg et al. (11), and the number of existing HS

patients is hypothetically calculated by the relevant data on the population of Turkey. Likewise, the distribution of patients according to the Hurley stages was also calculated based on the study of Canoui-Poitrine et al. (15) Under or overestimation of the number of patients with HS is possible according to these calculations.

A wide variety of therapeutic options are used in the management of HS patients, as evidenced by current literature. To be able to perform an analysis on a structured system, the most ideal and optimal situation, which is based on first-line treatment options of the evidence-based treatment algorithm (25), has been considered and the calculations are made by assuming appropriate treatments are used for each Hurley stage. In reality, there might be patients that have the optimal treatments by their staging but also we know that there are patients that are not treated optimally. So it should be highlighted that the result of this analysis is the picture of an optimal situation.

For the surgical interventions, because the number and types of surgical operations that each patient need will vary, analysis is made with the minimum values and included in the result. The disease itself is unique for every patient and surgical needs will change, therefore it is not possible to reflect the real-life situation in the analysis. In addition to that, possible direct costs of HS have been discussed widely but the indirect costs are not described and not taken into account in this study. The results should be interpreted with consideration of all these limitations.

CONCLUSION

Identifying and measuring the costs of HS will let us understand the financial burden of the disease more distinctly. The resources used and the potential resources that were lost have been identified in the CoI studies. Along with the prevalence, incidence, morbidity, and mortality data, CoI studies assist to draw the frame about the effect of a disorder on the public. (32)

Determining the total CoI let us know how much society and/or payer is spending on that specific disorder and by implication the amount that would be saved if the disorder were extinguished. It may also help identify the various elements of the cost and the extent of the contribution of each sector in society. These data can help to determine research and funding priorities by highlighting areas where inefficiencies may exist and savings can be made. (33), (34)

Knowledge of the CoI can help policymakers decide which diseases need to be addressed first by health care and prevention policies. Additionally, these studies can indicate which disease cures would be valuable in reducing the burden of disease and also reducing costs. (35)
As a result, it is critical to demonstrate CoI studies to inform clinical decision-making, bring forth new policies and guidelines, and effectively allocate resources accordingly. (36)

Even though HS is a disease which attributed as ‘rare’ and ‘unknown’, it is surprising that it takes an important place in terms of treatment costs.

Here we estimated the economic burden of optimally managed HS. Intensive pharmacotherapy is required to manage symptoms, especially for the patients with Hurley II and III stages; yet, a significant proportion of patients have inadequate control with current treatment regimens according to current literature. Since there is no commonly accepted treatment guideline, physician treatment variety, patient education, and adherence to prescribed regimens remain central issues in achieving control, HS is a heterogeneous condition with variable responses to existing therapies. It is also important to take into consideration the effect of comorbidities (e.g. metabolic syndrome, obesity, etc.) on the cost of HS and outcomes.

This study has been constructed with the data available in the current literature and applied to the Turkish healthcare system. This CoI study emphasizes the value and need for longitudinal HS cohort studies and the study that evaluates how patients receive care throughout the health care system, not only of disease activity. By broadening the point of view even more widely, studies can start to take into account not only the direct costs to the whole health care system but also the indirect costs resulting from the disease's impact on the ability of the patient (and possibly caregiver person) to work, and this would address the indirect cost of the disease.

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N.A.

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Conflict of Interest
The authors declare no conflict of interest.

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RESEARCH ARTICLE | Yeditepe Journal of Health Sciences, 13(1): 34-40

Aggravating Effects of Spermine and Spermidine But not Agmatine on Pentylenetetrazole (PTZ)-Induced Seizures in Rats

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Abstract

Objective: Epilepsy, a chronic and complex disorder of the brain, is an important neurological problem worldwide. Novel drug objectives in the central nervous system (CNS) may present more effective choices in treatment of epilepsy. The present study is designed to examine the effects of certain polyamines on seizures induced by pentylenetetrazole (PTZ) in rats.

Materials and Methods: Female adult (250-300 g) Wistar albino rats were used in our study. They were randomly allocated into several groups (n=8 for each group). Spermine (1-4 mg/kg), spermidine (20-80 mg/kg), agmatine (160 mg/kg) or saline were injected to animals through intraperitoneal (IP) route 30 minutes prior to PTZ (40 mg/kg, IP) treatment. The onset time and severity of the seizures were assessed immediately after the treatment with PTZ.

Results: Spermidine treatments significantly shortened onset time of seizures, at all doses used in the study (p_s=0.0001). It significantly increased the severity of seizures at doses of 20 and 80 mg/kg (p=0.007 and p=0.03, respectively). Treatment with spermine significantly shortened onset time of seizures at dose of 4 mg/kg (p=0.002). While spermine (4 mg/kg) increased severity of seizures significantly (p=0.01; Dunnet's test), it did not cause any noteworthy alteration on the severity of seizures at other doses. Agmatine (100 mg/kg) did not have any statistically significant effect on seizures.

Conclusion: Our results suggest that spermine and spermidine but not agmatine cause some aggravating effects on the seizures induced by PTZ. The data indicate that polyamines in the CNS may be an important target for epilepsy.

Keywords: Seizure, spermine, spermidine, agmatine, pentylenetetrazole, rat(s)

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INTRODUCTION

Epilepsy, a chronic and complex disorder of the brain, is an important neurological problem worldwide. It is characterized by unpredictable seizures caused by abnormal (i.e., excessive) electrical discharges within the nerve cells in the brain, and is one of the most common disorders, influencing around 1% of the population (1, 2). There is no absolute drug-based treatment for epilepsy. Currently available drug treatments aim to prevent the occurrence of seizures with sustained and sometimes lifelong drug use. They do so by increasing the activity of inhibitory neurochemicals such as gamma-aminobutyric acid (GABA) or suppressing the activity of excitatory neurochemicals such as glutamate thereby compensating for abnormal activity in voltage-regulated ion channels (i.e. Na^+, K^+, and Ca^{2+}), which can lead to abnormal discharges in the neuronal membrane (3-5). Many studies are carried out to develop new drugs which are more effective and better tolerated (6). Further studies on the mechanisms involved in the disorder are required for a better understanding of epilepsy, and the development of targeted therapies. New drug targets in the central nervous system (CNS) may lead more effective choices for epilepsy treatment.

Polyamines are aliphatic molecules containing two or more amine groups such as putrescine, spermidine, spermine and agmatine. Along with their biosynthetic enzymes, polyamines are found in all parts of the body, including the CNS. They are involved in several cellular functions such as DNA stabilization, regulation of gene expression, ion channel function and cell proliferation (7). Recently, a polyamine, agmatine –the decarboxylated derivative of L-arginine–, has been noted as a prominent neuromodulator in mammalian brain (8, 9). Agmatine binds to α2-adrenergic and imidazoline receptors (8, 10). It inhibits the enzyme nitric oxide synthase (NOS) that contributes to nitric oxide (NO) formation and is found to antagonize glutamatergic N-methyl-D-aspartate (NMDA) receptors in rat hippocampus (11, 12). It has been shown that NOS inhibitory agents (13) and NMDA antagonists (14) prevent seizures in experimental animal models.

Conflicting results have been reported regarding the effects of polyamines on epileptic seizures. Some experimental studies indicated anticonvulsant effects of polyamines such as agmatine (15, 16) and spermine (17). However, other studies have also reported certain unfavorable effects of polyamines on epileptic seizures. For example, it has been demonstrated that spermidine induces proepileptic effects by shortening seizure latency in rats, due to increase NO production (18). Hayashi et al. also suggested that increases in the concentrations of some polyamines such as putrescine, spermidine and spermine are involved in neuronal excitability in brain during seizures (19). A study by Luszczki et al. showed that agmatine significantly reduced the anticonvulsant effects of vigabatrin against clonic seizures induced by pentylenetetrazole at a level higher than its anticonvulsant doses (20). On the other hand, stress-induced increases in polyamine levels and/or metabolism are referred to as the polyamine stress response (PSR) (21). In this context, increased polyamine levels and PSR are related to DNA fragmentation and programmed cell death (22). PSR has also been associated with some mental disorders such as suicidal behavior and schizophrenia (23). However, studies on the effects of polyamines or PSR and epileptic seizures are limited and inconclusive in terms of their findings.

Given this background, the main objective of the present study is to investigate the effects of three polyamines –spermine, spermidine and agmatine–, on the onset time and severity of seizures induced by pentylenetetrazole (PTZ), which serves as a readily available and valid experimental model in rodents (24). To this end, we recorded the onset time and severity of the seizures in rats injected with spermidine, spermine, or agmatine, 30 minutes before the administration of a subeffective dose (40 mg/kg) of PTZ in rats.

MATERIALS AND METHODS

Animals and Laboratory

Female adult (250-300 g) Wistar albino rats (Üsküdar University Experimental Research Unit – USKUDAB, Türkiye) were subjects in our study. Four animals were housed per Plexiglass cage. The rats were placed in a quiet and temperature- and humidity-controlled room (22 ± 2°C and 60 ± 5%, respectively) in which a 12/12 h light–dark cycle was maintained (light from 07:00 to 19:00). Food and water were available ad *libitum*. All processes in this study were accomplished in agreement with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (Publication No. 85-23, revised 1985). The Local Ethics Committee for Animal Experiments (HADYEK) of Üsküdar University approved the study on January 22, 2021, with the decision number 2020-14.

Drugs

PTZ, agmatine, spermine, and spermidine were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Drugs were dissolved in 0.9% saline and injected intraperitoneally (IP) to the rats in the same volume of 0.5 ml/250 g. Solutions for injection were prepared freshly before the tests.

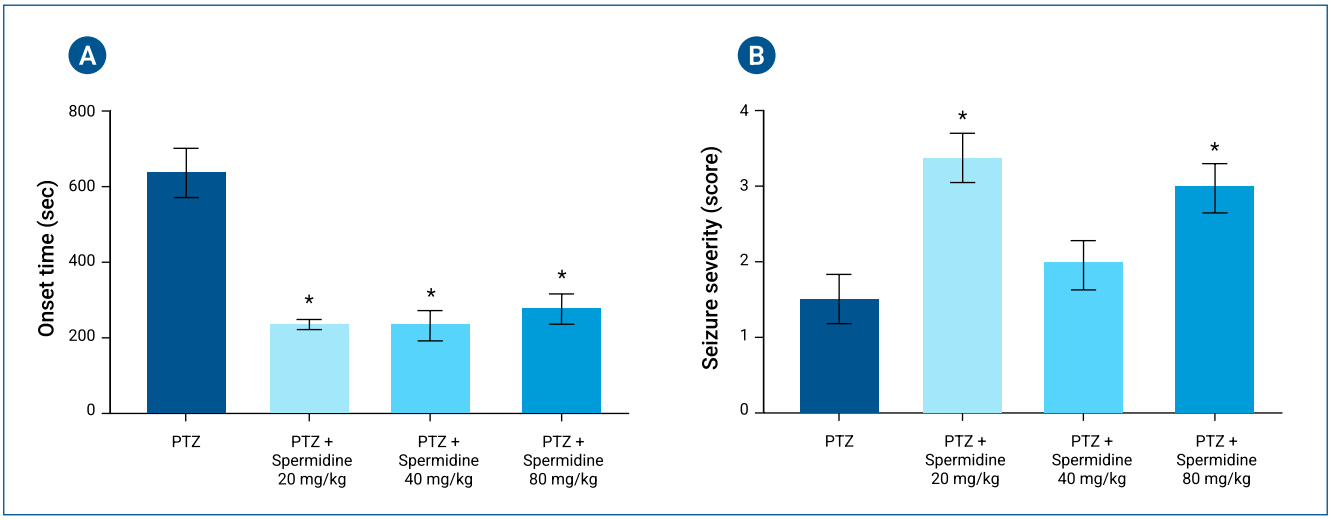

FIGURE 1. Effects of spermidine on onset time (A) and severity (B) of seizures induced by PTZ. (*p<0.05 significantly different from control; n=8 for each group).

Procedure

As the present study was based on female rats, vaginal smear screening was performed on the days on which the experiments were to be carried out, and rats in the metestrus stage were taken for experiments. Rats were then randomly assigned to specific groups (n=8 for each group).

Spermine (2, 4 and 8 mg/kg), spermidine (20, 40 and 80 mg/kg), agmatine (160 mg/kg) or saline were injected to animals by IP route 30 minutes prior to PTZ (40 mg/kg, IP) treatment. Immediately after PTZ injections, rats were placed in a plexiglas cage and recorded for onset times of "first myoclonic jerk (FMJ)", "generalized clonic seizures (GCS)" and "tonic generalized extensions (TGE)" as previously described (13). The onset times were recorded as seconds. The severity of seizures was assessed using a modified eight-point semi-quantitative scale defined previously (25). The scale can be summarized as follows: 0: no convulsion; 1: ear and facial twitching; 2: convulsive waves through the body; 3: myoclonic jerks, rearing; 4: generalized clonic seizures, turn over into side position; 5: turn over into back position, generalized clonic-tonic seizures. The observation period for seizures was restricted to 60 min. Duration of 600 sec was established as the cut-off time for computing the onset time of seizures induced by PTZ. All experimental procedures were conducted during the light period (10:30 - 12:30 am).

In our study, we used agmatine only at a high dose of 160 mg/kg. This is because agmatine has neuroprotective and anticonvulsant effects in rodents at doses ranging from 1 to 100 mg/kg. Agmatine has the potential to cause PSR at doses such as 160 mg/kg (9). We did not use higher doses because they could be toxic.

Statistical Analysis

The data were presented as the means ± standard error of the mean (SEM) and were evaluated using the Statistical Package (SPSS Version 20.0) software. The effects of spermine and spermidine on the onset time of seizures were evaluated by one-way analysis of variance (ANOVA) test followed by Dunnett test for *post-hoc* comparisons. In addition, the effects of spermine and spermidine on seizure severity were also evaluated by the Kruskal-Wallis (KW) test followed by Dunnett's T3 test for *post-hoc* analyses. Student's t and Mann-Whitney U tests were applied to evaluate the effects of agmatine on onset time and severity of seizures, respectively. The statistical significance was accepted at the level of p<0.05.

RESULTS

The effects of spermidine on onset time and severity of PTZ-induced seizures in rats

The effects of spermidine on onset time and severity of the seizures have been shown in Figure 1 A and B, respectively. One-way ANOVA and KW tests revealed some significant changes in onset time and severity of seizures induced by PTZ when the rats were subjected to spermidine treatments [F(3,28)=19.409, p=0.000 and KW=13.756, p=0.003, respectively].

Administration of spermidine shortened significantly onset time of the seizures at all doses applied in our study (p_s<0.0001; Dunnett's T3 test, Figure 1A).

Spermidine treatments also increased significantly the severity of seizures at doses of 20 and 80 mg/kg (p=0.007 and p=0.03, respectively; Dunnett's T3 test). The increase

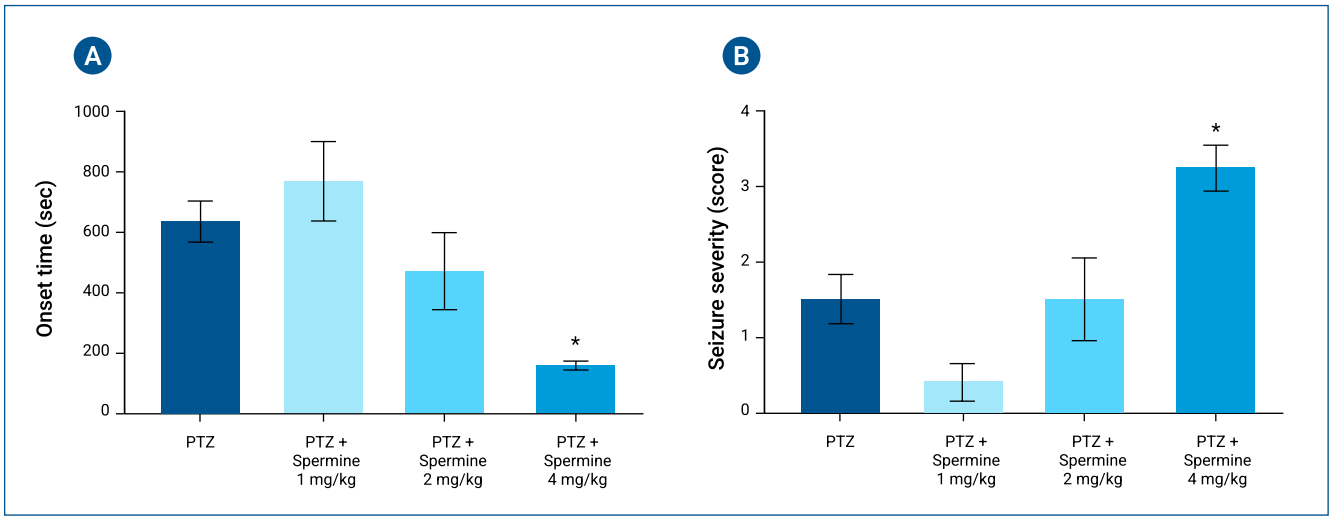

FIGURE 2. Effects of spermine on onset time (A) and severity (B) of seizures induced by PTZ. (*p<0.05 significantly different from control; n=8 for each group).

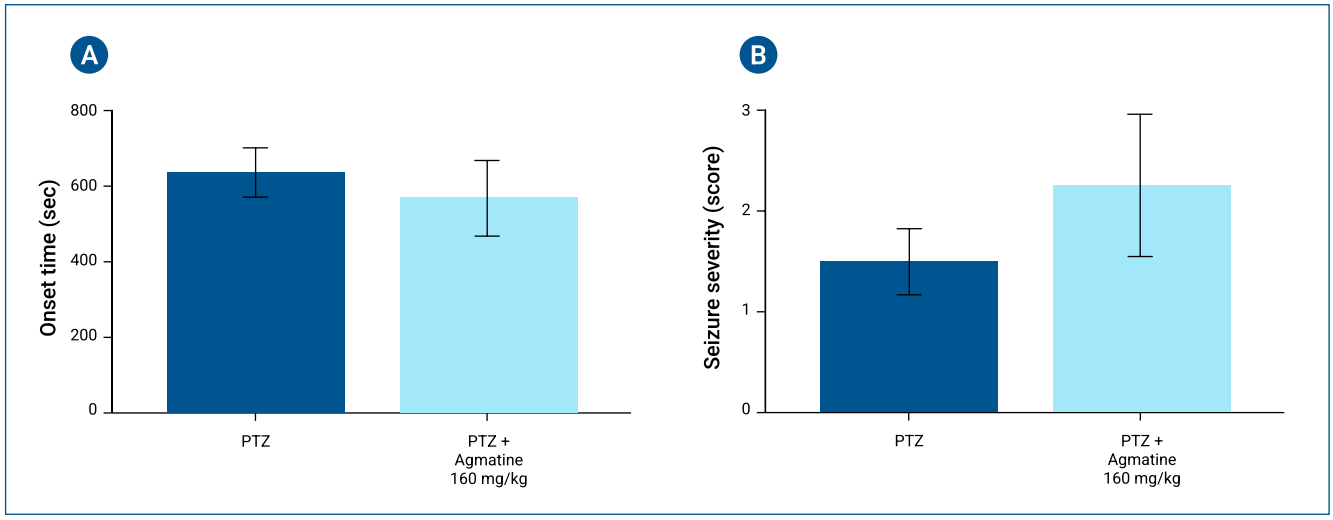

FIGURE 3. Effects of agmatine on onset time (A) and severity (B) of seizures induced by PTZ. (*p<0.05 significantly different from control; n=8 for each group).

observed in the severity of seizures at dose of 40 mg/kg was not statistically significant (p=0.851; Dunnett's T3 test; Figure 1B).

The effects of spermine on onset time and severity of PTZ-induced seizures in rats

The effects of spermine on onset time and severity of the seizures have been shown in Figure 2A and B, respectively. One-way ANOVA and KW tests revealed some remarkable changes in onset time and severity of seizures when the rats were subjected to spermidine treatments [F(3,28)=7.811, p=0.001 and KW=13.278, p=0.004, respectively].

Spermine shortened significantly onset time of the seizures at 4 mg/kg (p=0.002; Dunnett's T3 test). It also shortened onset time of the seizures at doses of 1 and 2 mg/kg but these effects did not reach a statistically significant level (p=0.694 and p=0.427, respectively; Dunnett's T3 test, Figure 2A). In addition, while spermine treatment (4 mg/kg) increased the severity of seizures significantly (p=0.01; Dunnett's T3 test), we did not observe any statistically significant difference on the severity of seizures at doses of 1 and 2 mg/kg (p=0.107 and p=1.0, respectively; Dunnett's T3 test; Figure 2B).

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(4 mg/kg) increased the severity of seizures significantly (p=0.01; Dunnett's T3 test), we did not observe any statistically significant difference on the severity of seizures at doses of 1 and 2 mg/kg (p=0.107 and p=1.0, respectively; Dunnett's T3 test; Figure 2B).

The effects of agmatine on onset time and severity of PTZ-induced seizures in rats

The effects of agmatine (160 mg/kg) on onset time and severity of the seizures have been shown in Figure 3A and B, respectively. Agmatine produced no significant change in onset time (p=0.593, Student's t test) or severity (p=0.369, Mann-Whitney U test) of the seizures.

DISCUSSION

We investigated the effect of three polyamines –spermine, spermidine, and agmatine– on epileptic seizure threshold and severity in an experimental model. Because we predicted that the PSR would cause a lower seizure threshold and exacerbate the severity of seizures, we used a relatively low dose (40 mg/kg) of PTZ for seizure induction. PTZ is generally used at higher doses (i.e. 60 and 80 mg/kg) to induce severe seizures in rats (26-28). Our results clearly demonstrated that while spermine and spermidine potentiated PTZ-induced seizures, agmatine did not cause any statistically significant difference on onset time and the severity of seizures. Our findings regarding spermine and spermidine are in line with those of previous reports indicating neuronal excitability in the epileptic brain with increased polyamine concentrations (19) and spermidine-induced proepileptic effects in rats (18).

Kumar and Kumar reported that at doses of 5 and 10 mg/kg, spermine helped prevent PTZ-induced seizures in mice (17). Kaasinen et al. also suggested that the over expression of spermidine/spermine N1-acetyltransferase, an enzyme in the catabolic pathway of polyamine metabolism, elevated the threshold for PTZ-induced seizure activity in transgenic mice (29). However, in our study, while lower doses of spermine (1 and 2 mg/kg) were ineffective on the onset time and the severity of seizures, at 4 mg/kg, it shortened the onset time and caused a significant increase in seizure severity. Such a conflict between the results of different studies may be related to the use of different animal species. Mice and rats may have different sensitivity levels to the effects of spermine. Nevertheless, while spermine was found to be ineffective at low doses, the shortened latency and increased severity of epileptic seizures at higher doses may be related to PSR. In addition, aggravating effects of spermine and spermidine on seizures support the hypotheses suggesting a relationship between PSR and neuropsychiatric disorders.

It is not known where in the CNS the effects of spermine and spermidine may be important in aggravating PTZ-induced seizures. At the molecular level, there is some evidence that these polyamines interact with glutamatergic receptors such as NMDA, AMPA and kainite (30-32). In addition, Masuko et al. reported that spermine enhances NMDA receptor activation at depolarized membrane potential and increases NMDA receptor currents in the presence of glutamate and glycine (33). Moreover, it has been shown that spermine induces convulsions by CNS excitation via NMDA receptors in mice (34, 35). The role of ionotropic glutamate receptors, especially in NMDA type receptor activation, in pathogenesis of epileptic seizures is well known (36). Thus, glutamatergic activation via NMDA receptor activation may be responsible for the worsening effects of spermine and spermidine on PTZ-induced seizures. However, this hypothesis needs to be confirmed by further studies.

An interaction with GABA, the major inhibitory neurotransmitter in CNS, could also explain the aggravating effects on seizures. Several reports suggesting a relationship between GABA inhibition and PTZ-induced epileptic seizures have been published (37-39). Interestingly, PTZ also stimulates polyamine catabolism in rat brain (40). Polyamines' effects on epileptic seizures via GABA also merit further studies.

In contrast to prospects, in the present study, 160 mg/kg agmatine (a higher dosage level than its anticonvulsant doses) was found to be ineffective on seizures. Although it reduced the onset time and increased the severity of PTZ-induced seizures, the effects were not statistically significant, nonetheless. Several studies have previously shown that intraperitoneal administration of agmatine has anticonvulsant activity in rodents at doses ranging from 5 mg/kg to 100 mg/kg (15, 41, 42). Thus, we used a much higher dose of agmatine (160 mg/kg) expecting it to produce PSR, which could induce epileptic activity. We also previously observed that this dose of agmatine induced a schizophrenia-like model in rats by disrupting prepulse inhibition of the acoustic startle reflex (43). In addition, in a population-based retrospective cohort study, Chang et al. found a strong bidirectional relationship between schizophrenia and epilepsy (44). The sex-associated variations in the effects of agmatine may be related to this ineffectiveness. Agmatine may cause some sex-related effects in mice. For example, it has been shown that agmatine antagonized the caffeine-induced open-field locomotor hyperactivity in male but not in female mice (45). As the present study was carried out with female rats, the effect or lack thereof, may be related to the gender of the animals. We did not prefer higher doses of agmatine since in preliminary studies some toxic effects such as excessive sedation was observed and the use of another administration route such as subcutane-

ous administration, produces toxic effects such as ulcerative necrotic cutaneous lesions in rats (46).

The interaction between polyamines, PSR and neuropsychiatric disorders has been increasingly debated in scientific community (9, 23, 27). Although some remarkable results were obtained in the present study, detailed laboratory or clinical studies are required to confirm the findings, given the presence of conflicting results in the literature. The effects of polyamines merit in-depth investigation as some of them such as agmatine are used as food supplements.

In conclusion, our results suggest that significant changes in epileptic seizures could be produced by spermidine and spermine but not agmatine. Agmatine seems to be potentially less dangerous than spermine and spermidine, in terms of seizures. All these observations and scientific data point out that the polyamine pathway in the CNS is a novel, important, and worthwhile area for the pathogenesis, diagnosis, and treatment of epilepsy. New data to be obtained from follow-up studies will contribute to clarifying the role of polyamines in this context, and to offering new and more effective treatment options.

Ethics Committee Approval
The Local Ethics Committee for Animal Experiments (HADYEK) of Üsküdar University approved the study on January 22, 2021, with the decision number 2020-14.

Informed Consent
N.A

Peer-review
Externally peer-reviewed.

Author Contributions
Concept – D.İ.K., T.U.; Design – T.U., T.G., A.Ö.Ş., B.Ç., D.İ.K.; Supervision – T.U.E., D.İ.K., T.U., B.Ç.; Data Collection and/or Processing – D.İ.K., B.Ç., A.Ö.Ş., T.U.E.; Analysis and/or Interpretation – D.İ.K., A.Ö.Ş., T.U.; Literature Review – D.İ.K. T.U.; Writer – T.U., D.İ.K.; Critical Reviews – T.U.

Conflict of Interest
The authors declare no conflict of interest.s.

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RESEARCH ARTICLE | Yeditepe Journal of Health Sciences, 13(1): 41-58

Evaluation of Adverse Effect Reports of Hyaluronic Acid Based Medical Device Applications in the Last Ten Years (from 2013 to 2023)

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Abstract

Objective: Hyaluronic acid (HA) is a naturally occurring polymer, present in different tissues of human cells which is a crucial component of the extracellular matrix. Its exceptional properties—including biocompatibility, non-immunogenicity, biodegradability, rheological flexibility, high hydrophilicity, ease of chemical modification, and viscoelasticity—enable a wide range of therapeutic applications. However, HA injections/ applications can lead to both early and delayed complications, ranging in severity from mild to serious. Additionally, the risk profile may evolve as therapeutic approaches and technologies continue to advance.

Materials and Methods: In this study, a systematic literature search was achieved on PubMed on January 5, 2023 and only case reports conducted in humans in last ten years (from 2013 to 2023) were considered. Literature reviews, clinical trials, technical notes, recommendations, and instructional course were excluded. Reviewed case studies that did not match the determined criteria were removed.

Results: 109 case studies representing 154 patients with severe complications were examined. The year of publication, total number of patients, age and gender of patients, adverse effects/ symptoms, and the reason of adverse effects of each case was listed. The case studies were classified according to the reason of adverse effects after HA application as intravascular injection and vascular obstruction, hypersensitive/ allergic affect, delayed inflammatory reaction (DIR), virus infection, bacterial infections, and others. Reactions to HA were reported as frequent redness, swelling, bruising, nodules, erythema, edema, tenderness, blindness, pain, and rarely death.

Conclusion: To conclude, adverse effects after HA application were found to be of high incidence in the population due to its widespread use. Even though adverse reactions are declared, these findings do not increase safety concerns related to the use of HA compared to the beneficial effects of it. The majority of negative situations may be avoided with improved application procedures.

Keywords: Adverse effect, hyaluronic acid, medical device, toxic effect

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INTRODUCTION

Hyaluronic acid (HA), also known as hyaluranate or hyaluronan as a hyaluronan family member, is a linear high molecular mass polysaccharide that belongs to the glycosaminoglycans (GAGs) (1, 2). It is a crucial constituent of the extracellular matrix. The total HA amount in human is approximately15 g for an adult person at 70 kg body weight, and it is distributed in diverse tissues including synovial fluid, umbilical cord, kidney, blood vessels, vitreous body of eye, skin, cartilage, lung, brain, serum, heart valves, and muscle (2-6). The size and concentration distribution of HA varies with age, tissue type, and the severity of disease (7). The molecule mechanically holds water as the chain lengthens and coils into spherical form, allowing the passage of small molecules while excluding or retarding larger ones (8). Adjacent chains may communicate with each other to create a connection. The network-forming, viscoelastic and charge properties of living tissues are crucial for biochemical/ metabolic properties (9). The molecule has diverse biological functions because of these properties.

The crucial biological properties of HA result from its spatial and chemical structure, elasticity, high molecular mass, high viscosity, hydrophilic characters, and highly negative charge. The molecule has diverse biological functions because of these properties. Biological functions of HA can be listed as retention of water in the matrix, hydration of tissue, lubrication, water homeostasis, transportation, adhesion, proliferation and differentiation, neutrophil adhesion, cellular interaction/ division/ migration, fetal wound healing, bone resorption, development/ aggregation/ adhesion of red blood cell leading to formation of bone (8, 10). The average molecular mass of HA has an impact on its physico-chemical properties (7). The increase of the molecular concentration/weight enhances stability, viscosity and viscoelasticity (8).

HA can be derived from skin, synovial fluid, umbilical cord, and rooster comb of animals. It can also be isolated from ***bacteria*** through direct isolation or a biotechnological process named as fermentation (8). Most of commercially available HA is derived from bacterial fermentation for a few key reasons—mostly revolving around safety, scalability, sustainability, and purity. For example, animal-derived HA carries risks of allergic reactions and disease transmission (e.g., prions or viruses). It also raises ethical concerns regarding animal use. However, bacterial fermentation avoids these issues entirely. It uses non-pathogenic strains like *Streptococcus zooepidemicus, Streptococcus equi* or genetically engineered *Escherichia coli* to produce HA in a lab setting. Fermentation methods allow for more controlled environments, which means fewer impurities and easier standardization of molecular weight. For instance, bacterial sourced HA should be free of pyrogens (8). Bacterial fermentation is renewable, non-animal-based, and scalable, industrial fermentation tanks can be optimized for consistent, large-scale production, making it cost-effective and eco-friendly (8).

According to recent studies, HA is almost fully metabolized and reabsorbed within six months, without causing any type of fibrosis or implant waste on the applied area (11). Due to its unique properties, it became a great biomaterial for drug administration, medical, food and cosmetic applications (12-14). It has been used in different medical application including ophthalmology, rheumatology, orthopedics, dentistry, and dermatology (12). The increasing clinical usage of HA has stimulated the interest of industry leading to the development of many derivatives with enhanced residence time in the joint cavity. HA fillers can be applied safely and effectively with a needle or cannula if performed correctly. HA fillers are the well- known and preferable products for nonsurgical rejuvenation of face and discreet tissue augmentation. These activities have presented encouraging results, paving the way for future biological uses of HA-derivatives.

HA is biocompatible, biodegradable and also non-immunogenic which made it a useful material for many different applications (4). In literature, biocompatibility tests of HA cover various sets of test methods including reproductive and developmental toxicity studies, repeated dose oral toxicity test, mutagenicity tests, acute/ chronic/ subacute toxicity tests, antigenicity tests and micronucleus assays. However, the toxic effect of HA application is observed. Injecting larger volumes of hyaluronic acid may increase the risk of foreign-body reactions. Moreover, breakdown products of HA products could induce hypersensitivity, and also variations in H_2O-binding capacity. These products could lead to localized responses including pain and swelling. Native form of HA has a very long polymer (high-molecular-weight hyaluronic acid - HMWHA) which is present in the majority of tissues as a primary component of the extracellular matrix (6) and the molecule may be cut down into smaller components, referred as low-molecular-weight hyaluronic acid (LMWHA) in some conditions (6, 13). While HMWHA has an effect on cancer resistance ***via*** inhibiting cellular motility, LMWHA shows a tumor progression effect by cell movement (13). LMWHA promotes angiogenetic activity (a potent stimulator of blood vessel growth) and can present pro-inflammatory activity or promote tumor progression (6, 8, 13).

There have been few instances of adverse hypersensitivity responses including the emergence of hematoma, asymmetry, over-correction, under-correction, and tissue necrosis caused by HA injection experience (15).

This study aims to evaluate published adverse effects/ reactions of HA based medical devices applications in the last ten years (from 2013 to 2023).

MATERIALS AND METHODS

Data collection and search strategy

A systematic case study search was carried out on PubMed up to January 5, 2023. The search was limited to case reports/ studies conducted in humans in the last ten years (from 2013 to 2023) and studies were selected based on inclusion *criteria*. The following key words were used: ("hyaluronic acid" and "adverse effect"), ("hyaluronic acid" and "adverse reactions"), ("hyaluronic acid" and "toxic effect"), ("hyaluronic acid" and "toxicity"), ("sodium hyaluronate" and "adverse effect"), ("sodium hyaluronate" and "toxic effect"), ("sodium hyaluronate" and "toxicity") and last ten years (from 2013 to 2023) limit was chosen for the years of publication.

Only studies in English were considered. Literature reviews, clinical trials, technical notes, recommendations, and instructional course were excluded.

During the research phase, case studies that were strictly relevant to the issue were discovered initially in each of the journals, with studies on animal models and in vitro studies being discarded following the main selection. The full text of the remaining accessible articles *via* Yeditepe University Information Center Electronic Resources Library were retrieved and reviewed to determine if they met the inclusion *criteria*. Each case study was reviewed for its year of publication, total number of patients, age and gender of patients, adverse effects/ symptoms, and reason of adverse effects.

Results and Discussion

In total, 588 case studies presenting the forementioned key words were identified through the initial search on PubMed database for the last ten years (from 2013 to 2023). Following de-duplication, the title and abstracts of the case studies were examined. Reviewed studies that did not match the determined inclusion ***criteria*** were not included. The full text of the remaining accessible case studies ***via*** Yeditepe University Information Center Electronic Resources Library were retrieved and reviewed to specify whether they met the inclusion ***criteria***. Finally, 109 case studies representing 154 patients with severe complications were included. Then, the case studies were categorized according to reason of adverse effects.

Fifty-one case studies representing 69 patients were found to be related to intravascular injection and vascular obstruction caused by HA soft tissue fillers (Table 1). Dermal vascular obstruction due to accidentally intravascular injection of filler was found to be rare but caused serious adverse effects. Dermal ischemia can contain skin necrosis, livedo reticularis, erythromelalgia, ulceration, or dermal infarct (16). The severity of the consequences related with the nature and amount of the filler (17). The nature of HA fillers vary in terms of viscosity (high density), particle size and crosslinking density (16). For example, increasing density and/or particle size may cause fillers to more easily block blood vessels and become more difficult to disperse after injection. Cross-linking makes fillers more durable, but they are more difficult to dissolve with hyaluronidase, the enzyme used to reverse filler complications (16). Therefore, a high-viscosity, large-particle filler used inappropriately (wrong depth or wrong area) increases the chance of serious vascular events. The injected amount is also critical as higher volume increases pressure within tissues, making it easier for the filler to enter into a blood vessel if the needle or cannula is improperly placed, which obstructs blood flow, and a larger embolus means a higher risk of ischemia and necrosis (16-18). HA products may cause erythema, swelling, bruising, pain, blindness and pruritus because of deterioration of the vascular and dermal structures (Table 1).

Injectable fillers are used in many different anatomic locations; however, some areas show increased risk of complications. Due to their rich terminal blood supply, the glabella, the nose, forehead, and periorbital region are particularly prone to visual complications. The injection technique and slow injections of small volume of HA filler may cause vascular damage during removal of the needle or cannula (17, 18). Therefore, the needles must be used with caution in highly risky areas to avoid vascular complications. Moreover, the technique of the operator is an important issue for the safety of the patient. Performing of hyaluronidase injection is the most effective and immediate way for the treatment of HA associated cases (16).

In theory, non-animal stabilized HA does not have any risk of allergic reactions due to its unique characteristic of biocompatibility. However, Table 2 represents case studies where hypersensitive/ allergic effect of HA for 18 case studies and 27 patients in last ten years (from 2013 to 2023) were observed. Bacterial derivative HA is most popularly used as facial filler because of its very low incidence of hypersensitivity/allergic activity.

Depending on the time of onset, hypersensitive activity may be classed as acute/ early or delayed/ late. Type I hypersensitive activity occur within minutes or hours after HA application as a result of an immunoglobulin-mediated immunological response (67). The most typically reported adverse effects with HA usage as dermal face

Table 1. Information of case studies with vascular injection and obstruction after hyaluronic acid application..

References	Age/ Gender	Application Side	Adverse Effects	Reason
Kim et al., 2015 (19)	34/F	Eyelid	Upper eyelid retraction, blunt pain, swelling, and heaviness of the affected eye	Intravascular injection
Kim & Alhusayen, 2015 (20)	64/M	Knee	Painful skin eruption over the right knee	Intravascular injection, vascular obstruction
Choi et al., 2016 (21)	29/F	Lower eyelids	Nodules	Inadequate injection (depth)
Li et al., 2016 (22)	25/F	Nose (non-surgical rhinoplasty)	Right eye blindness, limb weakness	Intravascular injection, vascular obstruction
Hu et al., 2016 (18)	41/F	Forehead	Blindness, edema, skin discoloration	Intravascular injection, vascular obstruction
Kang et al., 2016 (23)	46/F	Glabella, forehead, and nose	Redness, swelling, numerous pustules, and dark regional necrosis	Intravascular injection, vascular obstruction
Andre & Haneke, 2016 (24)	46/F	Nasolabial folds	Sensitivity and redness of right nasolabial crease and nose, Nicolau syndrome	Intravascular injection, vascular obstruction
	35/F	Nasolabial folds	Pain and redness	
	32/F	Nasolabial folds	Pain and redness	
	40/F	Nose tip	Livedoid pattern	
	30/F	Lips	Pain, livedoid pattern	
	42/F	Nasolabial folds	Pain, swelling, redness, livedoid pattern	
Bertl et al., 2017 (25)	28/F	Dental tissue (intra oral)	Swelling on lip, pain	Inflammatory reaction, intravascular injection
	30/F	Dental tissue (intra oral)	Swelling, pain, discoloration (livedo reticularis)	
Yang et al., 2017 (26)	27/F	Bilateral temple augmentation	Swelling and burning pain, alopecia	Intravascular injection, vascular obstruction
Maruyama, 2017 (27)	57/F	Glabella, eyebrow	Erythema, purple discoloration, and severe pain	Vascular obstruction
Salval et al., 2017 (28)	22/F	Nose tip, forehead	Pain, swelling, discoloration, and edema	Infection, intravascular injection, vascular obstruction
Bae et al., 2018 (29)	29/F	Nasal tip	Painful erythematous swelling, ain and dizziness, and her vision became blurred	Intravascular injection, vascular obstruction

Table 1. (Continued)

References	Age/ Gender	Application Side	Adverse Effects	Reason
Wang et al., 2018 (30)	24/F	Mentum	Soreness and swelling, the anterior region of chin became pale	Intravascular injection, vascular obstruction
	42/F	Chin	Pain, headache and discomfort, swelling	
Hao et al., 2018 (31)	20/F	Right forehead	Blindness, erythema, cherry-red spots	Emboli, pressure, thrombosis
Thanasarnaksorn et al., 2018 (32)	36/F	Nose (non-surgical rhinoplasty)	Nausea and pain in the left eye, vision loss, phthalmoplegia, ptosis, dizzines	Intravascular injection, vascular obstruction
	36/F	Nose (non-surgical rhinoplasty)	Pain and vision loss	
	26/F	Nose (non-surgical rhinoplasty)	Blindness	
	23/M	Nose (non-surgical rhinoplasty)	Blindness, pain, headache, nausea, and vomiting	
	61/F	Forehead	Severe headache, skin blanching and blurred vision	
	31/F	Temporal area	Blurred vision	
Vidič & Bartenjev, 2018 (33)	50/F	Glabellar region, upper lip, nasal root	Erythematous, livedoid rash, swollen of eyelids	Infection, blood vessel damage on the application site
Jeong et al., 2018 (34)	37/F	Nose (non-surgical rhinoplasty)	Purpuric discoloration, swelling, and pain	Intravascular injection, vascular obstruction
Loh et al., 2018 (35)	50/F	Face	Painful skin eruption on the left knee, erythematous reticulate, livedo reticularis	Intravascular injection, vascular obstruction
Wibowo et al., 2019 (36)	40/F	Nasal dorsum	Upper eyelid ptosis, blindness, deep pain, skin color change	Intravascular injection, vascular obstruction
Park et al., 2019 (37)	58/F	Face	Alopecia on the ipsilateral scalp	Intravascular injection, vascular obstruction
Yao et al., 2019 (38)	21/F	Forehead	Blindness, severe pain in eye, headache	Vascular obstruction
Shoughy, 2019 (39)	36/F	Glabeller region	Blindness, weakness of the left arm, dark pigmented lesions over the eyelid	Intravascular injection, vascular obstruction
Ansari et al., 2019 (40)	20/F	Glabellar region	Blindness, iolaceous pigmentation	Vascular obstruction
Lima et al., 2019 (41)	42/F	Cheeks, nasolabial folds and the chin	Yellowish spot, small lump	Intravascular injection, vascular obstruction

Table 1. (Continued)

References	Age/ Gender	Application Side	Adverse Effects	Reason
Hu et al., 2019 (42)	22/F	Eyebrow	Orbital pain on the right side, blurry vision, perineuritis (OPN)	Obstruct blood flow
Khalil et al., 2020 (43)	54/F	Eyebrow and glabellar crease	Edema, xerophthalmia, and dryness	Wrong application
Cassiano et al., 2020 (44)	57/F	Rejuvenation of the frontal region	Pain, erythema, and edema in the frontal region	Intravascular injection, vascular obstruction
Downie et al., 2020 (45)	54/F	Temples and periorbital region	Severe frontal headache and nausea, dizziness, binocular diplopia, and vomiting, blindness	Intravascular injection, vascular obstruction
	37/F	Nasal bridge and periorbital region	Binocular diplopia, blindness	
Desmottes et al., 2020 (46)	63/M	Knee	Painful livedoid patch	Excessive pressure and vascular compression
Kim et al., 2020 (47)	23/F	Nasal dorsum	Dizziness, headache, horizontal diplopia, oculodynia, blurred vision, and lateral deviation	Vascular occlusion
Hirsch et al., 2020 (48)	19/F	Upper lip	Instant burning pain	Vascular occlusion
Akoglu et al., 2020 (49)	37/F	Cheekbone	Pain, stinging sensation on eye,	Vascular obstruction
Uz et al., 2020 (50)	46/F	Buttocks	Agitation, altered mental status, and drowsiness	Intravascular injection, vascular obstruction
Yang et al., 2020 (51)	40/F	Nose	Periocular pain and blindness, nausea, vomiting, and headache, and lost consciousness	Intravascular injection, vascular obstruction
Zeltzer et al., 2020 (52)	21/F	Upper lip	Pain, ischemia	Intravascular injection, vascular obstruction
Yang et al., 2020 (53)	33/F	Vagina	Dyspnea, cough, dizziness, fatigue, and cyanosis of the lips, vaginal infection, pulmonary vascular embolism, death	Intravascular injection, vascular obstruction
Toussi et al., 2020 (54)	72/F	Right ankle (long-standing osteoarthritis)	Worsening, burning, painful rash on right foot	Tissue necrosis and arterial injury
Berríos-Hernández et al., 2020 (55)	72/F	Knee	Pain, extensive, livedoid, erythematous lesion	Intravascular injection, vascular obstruction, incorrect injection
Sud et al., 2021 (56)	37/F	Lip and chin	Lip pain, swelling, and rash	Intravascular injection, vascular obstruction

Table 1. (Continued)

References	Age/ Gender	Application Side	Adverse Effects	Reason
Aaron et al., 2021 (57)	66/F	Knee	Cutaneous necrosis of right ankle and foot	Intravascular injection, vascular obstruction
Chen et al., 2021 (58)	22/F	Nasal dorsum	Diplopia and orbital pain, erythematous, pupil dilation, exotropia, visual field defect, and limitation of extraocular movements	Intravascular injection, vascular obstruction
Master et al., 2021 (59)	30/F	Tear trough	Lumpiness and significant malar edema	Inadvertent placement
	64/F	Tear trough	Longstanding puffiness under the left eye	
	58/F	Cheeks and tear toughs	-	
Tao et al., 2021 (60)	22/F	Left temporal and forehead	Headache, vertigo, nausea, and blindness of left eye, hemiplegia, sensory disturbance, and petechiae	Intravascular injection, vascular obstruction
Moore et al., 2021 (61)	59/F	Glabella and nasal dorsum	Right eye blindness, dizziness, nausea, and a right frontal headache, stroke	Intravascular injection, vascular obstruction
Sasongko et al., 2022 (62)	55/F	Face	Blindness	Vascular obstruction
Danks et al., 2022 (63)	38/F	Nose (non-surgical rhinoplasty)	Blindness	Intravascular injection, vascular obstruction
Yang et al., 2022 (64)	18/F	Chin augmentation.	Numbness and intense pain, dysphonia, limited mouth opening, and paleness	Intravascular injection, vascular obstruction
Davidova et al., 2022 (65)	43/F	Glabella	Blindness, swelling, no light perception on left eye, pupillary defect, cherry-red spots	Intravascular injection, vascular obstruction
Nguyen et al., 2022 (66)	27/F	Nasal augmentation	Nasal pain, headache, and blindness	Intravascular injection, vascular obstruction

filler are hypersensitive/ allergic action and inflammation at the application area (5, 16, 68). Injection area inflammations include redness, swelling/ nodules, erythema, edema and tenderness (17, 69, 70). Same symptoms were also determined in this case study research project.

The minor inflammatory responses are considered as normal, caused by resident macrophage infiltration and fibroblast activation. These immunologic activities lead to collagen synthesis which attaches the HA filler to the tissue. These symptoms usually resolve within a few days, and biodegradable HA dermal fillers are reabsorbed (70).

Table 3 presents the case studies with delayed inflammatory reaction (DIR) after HA injection. 21 case studies and 31 patients meeting the inclusion *criteria* were determined in the literature for the last ten years (from 2013 to 2023). DIR (Type IV reactions) can be seen from one month to years after injection of HA (70). Various mechanisms can be involved during DIRs including systemic infections, trauma, injection technique (for example: intramuscular implantation), circulating anti-HA antibodies, volume of filler, repeated application, vaccines, and immunogenic reactions to the cross-linking agents produced during product degradation (87, 88). Delayed type IV hypersensitivity reactions are mostly triggered

Table 2. Information of case studies with hypersensitivity/ allergy reaction after hyaluronic acid application.

References	Age/ Gender	Application Side	Adverse Effects	Reason
Colbert et al., 2013 (71)	65/F	Nasiolabial folds	Perioral augmentation, granulomatous inflammation	Properties of the filler, the volume injected and previous infection or trauma, hypersensitivity reaction
Hatcher & Goldman, 2014 (72)	46/F	Nasolabial fold	Swelling and redness, erythema	Inflammatory reaction
Kim et al., 2015 (73)	41/F	Nasolabial folds	Erythematous plaque	Allergic reaction
Bertl et al., 2017 (25)	28/F	Dental tissue (intra oral)	Swelling on lip, pain	Inflammatory reaction, intravascular injection
	30/F	Dental tissue (intra oral)	Swelling on lip, pain, discoloration (livedo reticularis)	
Dominguez et al., 2017 (74)	57/F	Vocal fold	Dysphonia, odynophagia, and dyspne	Hypersensitivity reaction
	28/M	Vocal fold	Odynophagia and rough voice quality, erythematous, edematous	
	51/F	Vocal fold	Odynophagia, ipsilateral otalgia, and dyspnea	
	65/F	Vocal fold	Dysphonia, edema, decreased amplitude, and wave	
	57/F	Vocal fold	Severe pain, edematous and erythematous	
	66/M	Vocal fold	Dyspnea, rough voice quality, and odynophagia	
	58/F	Vocal fold	Rough voice and odynophagia, dyspnea	
Kocak et al., 2017 (75)	77/M	Knee	Pain, swelling	Inflammatory reaction
Mitsuyama et al., 2017 (76)	78/F	Eye (dry eye treatment)	Bilateral periocular pruritic erythema and oedema, allergic contact dermatitis	Immune mediated adverse effects
Paolino et al., 2017 (77)	41/F	Lip	Pigmented lesion of the lower lip (melanosis)	Immune mediated adverse effects
Traboulsi et al., 2017 (78)	23/F	Laryngeal (laryngoscopy using the trans nasal route)	Globus sensation, dysphagia, and shortness of breath	Allergic and hypersensitivity
Alcântara et al., 2017 (79)	54/F	Dental gel application	Asymptomatic firm nodularities in the lower and upper lips, granulomatous reaction	Foreign body reactions
Gandy et al., 2017	55/F	Perioral area	Swelling and redness, erythematous, granulomatous plaque, nodules	Immune mediated adverse effects

Table 2. (Continued)

References	Age/ Gender	Application Side	Adverse Effects	Reason
Wu et al., 2018 (81)	39/F	Full face	Facial nodules, redness, and swelling	Hypersensitivity reaction, allergy
Sarigul Guduk, 2021 (82)	41/F	Tear trough	Eyelid swelling	Allergic reaction
	40/F	Midface and lower eyelids	Eyelid swelling	
	43/F	Midface and tear trough, periorbital area	Eyelid swelling	
Schattner & Haj-Yahya, 2018 (83)	100/F	Inner-lower-lip and gums to treat gingival sores	Swelling of the tongue and inability to swallow, angioneurotic edema	Allergic reaction
Decates et al. 2020 (68)	32/F	Tear trough	Swelling of the left cheek, edema	Hypersensitivity
Chen et al., 2020 (84)	21/F	Lip, nose tip, chin, and the right nasal root	Redness and pain, swelling, periorbital edema, and conjunctival congestion, adache and fever, with gradual hyper myotonia.	The ingredients of the filler essence, hypersensitivity, and inflammatory reaction by infection
Wege et al., 2021 (85)	27/F	Lips	Lumps on upper lip, swelling	Immune mediated adverse effects
Hayat et al., 2022 (86)	41/F	Cheek	A mass on left eye	Inflammatory reaction

by $CD4^+$ cells and T lymphocytes (67). It may manifest as inflammatory nodules, induration, erythema, swelling and edema (15, 67, 87, 89).

Type IV hypersensitivity after HA application due to virus infection (like *influenza*, COVID-19 and *Herpes simplex*) seems to be the most common cause for delayed hypersensitivity reaction in recent years. Table 4 presents the delayed hypersensitivity case reports related to viral infection in the last ten years. Some cases reported that a hypersensitivity reaction was seen more than 24 hours after a vaccination for COVID-19 (108-111). Currently, it remains unclear whether there is a specific progression to delayed hypersensitivity reactions following COVID-19 vaccination, how long adverse effects are most likely to occur after hyaluronic acid (HA) injection, and what the optimal waiting period is for HA treatment after receiving the COVID-19 vaccine. Treatment with HA changed from weeks to months before vaccination, and while the responses resolved within a few days, others remain refractory, with phases of worsening, improvement, and recurrence months following vaccination according to case studies presented in Table 4.

Table 5 represents the case studies related to bacterial infections after HA application in poor conditions. Infection risk may be increased with filler injection as a result of skin barrier damage. Therefore, the use of aseptic techniques is very crucial during the application. In recent years, the fact that dermal fillers purchased from the electronic trade web sites has increased the rate of the self-injection of the formulations under unsuitable environmental conditions, and this has caused an increase in infections (117-119). Cellulitis, abscess formation, nodules or granulomatous inflammation are the most common complications after skin disruption during HA filler injection (33, 117, 120). In this research, similar symptoms were summarized in Table 5.

In this study, the reasons of adverse reactions after HA application were categorized as intravascular injection and vascular obstruction, hypersensitivity/ allergic effect, DIR, viral and bacterial infections. The rest of the

Table 3. Information of cases with delayed inflammatory reaction (DIR) after hyaluronic acid injection.

References	Age/ Gender	Application Side	Adverse Effects	Reason
D'Acunto et al., 2013 (90)	49/F	Eyelid	Anthelasma palpebrarum on right lower eyelid	Delayed inflammatory reaction (DIR)
	67/F	Eyelid	Xanthelasma, ellow-colouredplaque	
Novoa et al., 2013 (91)	54/F	Interciliary sulci and nasolabial folds	Erythematous-violaceous nodules on face, brownish papules on shoulder, elbowa, knees	DIR
Rongioletti et al., 2015 (92)	59/F	Glabella, cheeks, nasolabial and perioral areas	Firm, nodular swellings	DIR
	53/F	Face	Multiple- painful- nonulcerated, hard nodules, deep granulomatous giant cell reaction	
	72/F	Lip	Nodular swelling, tender, non-painful,	
Goodman, 2015 (93)	49/F	Prejowl sulci, marionette regions, and nasolabial grooves	Swelling	DIR
Bitterman-Deutsch et al., 2015 (94)	29-56/F (5 patients)	Glabella area, lips, and the back of the hands (for 2 patients) Nasolabial folds Matriderm / matridur to nasolabial folds and zygomas Glabella and eyes	Asymmetrical edema of the face	Delayed side effects
Iverson & Patel, 2017 (95)	72/F	Tear trough	Bilateral lower eyelid "fluid filled" bags, late-onset edema	Delayed side effects
Hibler et al., 2019 (96)	50/F	Face	Facial swelling	DIR
Boger et al., 2019 (97)	68/F	Midface	Swelling around eyes, oedema	Delayed migration
Capodiferro et al., 2019 (98)	50/F	Lip	Nodular lesion	DIR
Parulan et al., 2019 (99)	49/F	Lateral zygomatic regions	Swelling on eye area	DIR
Choi et al., 2019 (100)	69/F	Forehead	Erythematous nodules on the forehead and scalp	Delayed side effects
Pathmanathan & Dzienis, 2019 (101)	52/M	Cheeks	Nodules and oedema	Cetuximab-related dermal filler reaction, DIR

Table 3. (Continued)

References	Age/ Gender	Application Side	Adverse Effects	Reason
Caldas Pozuelo et al., 2020 (102)	74/F	Lips	Nodules in both lips and perioral region	Delayed side effects
Horriat et al., 2020 (103)	48/F	Glabella, nasolabial folds, and marionette line	Facial edema, erythema, itchiness, and mild fever	DIR
Tonin et al., 2020 (104)	70/F	Upper arm skin	Multiple subcutaneous nodules on both arms	Delayed side effects
Alawami & Tannous, 2021 (105)	47/F	Different sites of face	Episodic abdominal pain, facial and lip swelling	Delayed type IV hypersensitivity
Sarigul Guduk, 2021 (82)	43/F	Temples and lips	Fever, swelling and bruising in the lips, headache, and malaise	DIR, Viral infection
Sullivan & Hawkes, 2021 (106)	71/M	Knee	Pain and swelling, erythema, inflammatory reactions associated with chills, erythematous- pruritic- scaly papules and plaques	DIR
	65/F	Knee	Erythematous macules and papules	
Grillo, 2022 (107)	45/F	Lip, full face	Edema,	DIR
Alli et al., 2022 (89)	66/F	Midface and peri-oral areas	Firm- slightly warm peri-oral swellings,	DIR
Dua & Bhardwaj, 2022 (67)	61/F	Cheeks and sunken eyes	Nodules in the subalar area on both cheeks	DIR
	44/F	Tear trough	Non-tender nodules	
	36/F	Lip, cheek	Edema and tender nodules	

case studies were presented as “other” in Table 6. Factors affecting adverse reactions after HA injection may also include the amount and specifications of product, and previous infection or trauma (71). On the other hand, although the results of cosmetic face injections are typically positive and problems are rare, some patients have experienced extreme anxiety or depression as a result of the application. These individuals have serious emotional issues, which reduce satisfaction with cosmetic operations and may result in lawsuits and disagreements. These cases are not uncommon in the clinic. In this study, only one case related to an emotional disorder was identified (122).

Another reason of adverse reaction that was reported in the literature is the presence of other filler components such as crosslinkers (like 1,4-butanediol diglycidyl ether (BDDE) which has the most popular use in the market, polyethyleneglycol diglycidyl ether (PEGDE), divinylsulfone (DVS)) or the physiologic buffer solution (PBS) within the HA products (84, 123). When these filler components were considered, safety data sheets for BDDE includes that exposure at concentrations exceeding 2 ppm may result in skin irritation or allergic sensitization. Consequently, the residual BDDE content in commercially approved HA-based fillers is expected to be either non-detectable or maintained well below this toxicity threshold, typically <2 ppm, to ensure biocompatibility and patient safety. PEGDE exhibits chemical properties and reactivity due to the presence of epoxide groups which is also seen in BDDE structure. These groups enable both compounds to form covalent cross-links with hydroxyl groups on HA chains, enhancing the mechanical stability and longevity of the resulting hydrogels (124). Especially, the purchased products via e-trade platforms usually have a low amount of HA (non-animal

Table 4. Information of case studies with viral infection after hyaluronic acid application.

References	Age/ Gender	Application Side	Adverse Effects	Reason
Wang et al., 2020 (112)	24/F	Nose (non-surgical rhinoplasty)	Crusted papules, erythema, pain and swelling	Exposure to viral infection (Herpes simplex)
Munavalli et al., 2022 (110)	50/F	Cheels, lips, tear toughs over	Lips burning, swelling of face, edema, erythema, and tenderness	Exposure to COVID-19 spike protein (DIR)
	51/F	Nasolabial folds, tear troughs, malar, and mid cheeks and upper/ lower lips, earlobes,	Injection site pain and irritation, edema, erythema, and tenderness	
	36/F	Bilateral tear troughs and upper and lower lip	Bilateral infraorbital perioral edema, infraorbital swelling and perioral angioedema	
	43/F	Tear trough	A mild tenderness underneath the right eye, swelling under the left eye	
Michon, 2021 (109)	39/F	Tear trough	Tender, erythematous, swelling, swollen, tender to touch	Exposure to COVID-19 spike protein (DIR)
	61/F	Chin and jawline, palpebromalar groove, tear trough	Facial swelling	
Savva et al., 2021 (113)	38/F	Lip	Small erythematous nodules on both the upper and lower lip, mild pain, mild tenderness	Exposure to COVID-19 spike protein (DIR)
Rowland-Warmann, 2021 (87)	22/F	Nose (non-surgical rhinoplasty)	Erythema, edema, induration, mild associated tenderness, and a tight feeling	Exposure to COVID-19 spike protein (DIR)
Beamish et al., 2022 (108)	23/F	Malar eminences, lips, jaw and chin	Painful asymmetric swelling over maxilla, lips and lower jaw	Exposure to COVID-19 spike protein (DIR)
Calvisi, 2022 (114)	60/F	Lip	Swelling in the upper lip	Exposure to COVID-19 spike protein (DIR)
	45/F	Lip	Angioedema in the upper lip	
	40/F	Nasolabial fold	Erythema and edema	
Liu & Ledinh, 2022 (115)	58/F	Facial rejuvenation, abiomental folds	Nodule in right perioral area	Exposure to COVID-19 spike protein (DIR)
Bulatova et al., 2022 (116)	58/F	Nasolabial area	Chills, myalgia, dysphagia, sore throat, dry cough, fatigue, and intermittent fever, Painful subacute thyroiditis	Immune mediated adverse effects, influenza vaccine

Table 4. (Continued)

References	Age/ Gender	Application Side	Adverse Effects	Reason
Ortigosa et al., 2022 (111)	35/F	Lips, nasojugal furrow, malar, and chin regions	Delayed swelling, induration, and edema in lips and chin	Exposure to COVID-19 spike protein (DIR)
	47/F	Around eye	Edema in the lower eyelids	
	34/F	Superior and inferior lips	Pain and mild edema in the lips	
	56/F	Lip and chin	Induration and edema in the mandible and chin	
	43/F	Nasolabial folds and lips	Edema, erythema, increase in the temperature of lips, fever, sensation of fatigue, and purpuric lesions of the extremities.	

Table 5. Information of case studies with bacterial infection after hyaluronic acid application.

References	Age/ Gender	Application Side	Adverse Effects	Reason
Park & Seo, 2013 (120)	62/F	Glabella, forehead	Inflamed nodule on the glabellar, swelling	Bacterial Infection
Salval et al., 2017 (28)	22/F	Nose tip, forehead	Pain in the glabella and forehead region, swelling, reticulated nasal skin discoloration and edema	Infection, intravascular injection, vascular obstruction
Vidič M & Bartenjev, 2019 (33)	50/F	Glabellar region, upper lip, nasal root	Erythematous, livedoid rash, swollen of eyelids	Infection, Blood vessel damage on the application site
Chen et al., 2020 (84)	21/F	Lip, tip of the nose, chin, and the right nasal root	Redness and pain, swelling, periorbital edema and conjunctival congestion, adache and fever, with gradual hypermyotonia	The ingredients of the filler essence, Hypersensitivity and inflammatory reaction by infection
Matsuki et al., 2021 (121)	73/F	Left shoulder and knee	Dead, bullae, and erythema	Infection (streptococcal toxic shock syndrome - STSS)
Allepot et al., 2021 (117)	45/F	Breast	Bilateral breast infection, fewer, nodules	Not sterile product, infection
Khor et al., 2021 (119)	31/M	Penile girth	Pain and swelling of the penile shaft	Bacterial Infection

Table 6. Information of case studies with other complications after hyaluronic acid application.

Authors	Age/ Gender	Application Side	Adverse Effects	Reason
Colbert et al., 2013 (71)	65/F	Nasolabial folds	Perioral augmentation, granulomatous inflammation	Filler properties, injected volume, previous infections, trauma, hypersensitivity
Vasquez el al., 2019 (126)2B	61/F	Tear trough and nasojugal groove	Infraorbital edema	Particulate, hydrophilic nature of the selected filler
Wang et al., 2020 (122)	32/F	Forehead and glabella	Panic, headache and insomnia, tension headache, tachycardia, breath shortness, sleep disorders	Emotional disorder syndrome
Chen et al., 2020 (84)	21/F	Lip, chin, tip of the nose, and the right nasal root	Redness and pain, swelling, periorbital edema and conjunctival congestion, headache and fever, with gradual hypermyotonia.	The ingredients of the filler essence, hypersensitivity and inflammatory reaction by infection
Simões Pires, et al., 2021 (127)	38/F	Over eyelid	Edema and erythema, a yellowish plaque, Development of xanthelasma	Unknown
Liu et al., 2021 (128)	43/F	Tear trough	Thin, soft, and yellow papules, development of xanthelasma	Unknown
Kato & Inoue, 2022 (123)	63/F	Rejuvenation of entire face	Severe pain, minimal lacrimation, abdominal colic and nausea, facial redness and swelling, urticaria all over body, abdominal colic	Other filler components (e.g., crosslinker or Phosphate Buffer Saline" (PBS) within the filler products)

based), herbal extracts (poorly identified or non-standardized), and additional ingredients (such as preservatives, stabilizers, colorants or fragrances) which are produced in poor safety conditions (125).

CONCLUSION

HA/ sodium hyaluronate is a very well-known and widely used commercial product due to its unique properties of biocompatibility, non-immunogenicity, biodegradability, rheological behavior, high hydrophilicity, ease of chemical modification and viscoelasticity. These unique properties have rendered it as a great biomaterial for drug administration, medical, food and cosmetic applications. Moreover, HA fillers become one of the most common temporary fillers on market due to their low immunogenicity and ability to be enzymatically destroyed by hyaluronidase.

HA including products are now widely accepted as a material of choice for the relatively less invasive medical applications. However, HA injection/ application can cause acute and/or delayed adverse effects ranging from mild to severe degree, and the profile of risk may change as the therapeutic landscape advances.

Adverse effects after HA application are prevalent in the population because HA containing products are used so often. Even though adverse reactions are reported after HA application, these data do not increase safety concerns regarding the use of HA compared to the beneficial effects of it. The majority of negative situations are avoided with adequate preparation and approach. Therefore, to decrease its adverse reactions, the physician who applies the HA should have enough experience to decide, to select and to apply HA. HA containing product application should be carried out in a registered health care facilities having all the necessary equipment to combat any adverse reactions during the application.

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CASE STUDY | Yeditepe Journal of Health Sciences, 13(1): 59-63

The Management of Autism by a Preventive and Pathophysiological Approach: A Case Report

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Abstract

The aim of this case report is the evaluation of the follow-up and treatment process in a patient with the diagnosis of autism spectrum disorder (ASD). A patient with ASD had social regression, limited eye contact, abdominal pain, and constipation. Urine lead, mercury and aluminum levels were above the normal range. Intestinal *flora* analysis found severe dysbiosis and intestinal inflammation. A combined preparation (*Chlorella vulgaris*, *Coriandrum sativum*, and probiotics) and a sugar, casein, and lactose-free diet were continued for one year. After 1 year; mercury level was normal and lead level was decreased. Constipation and pain were also relieved.

Keywords: Autism spectrum disorder, pathophysiology, heavy metals, phytotherapy, probiotics, diet

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental condition that is usually diagnosed in early childhood. The rapid increase witnessed in recent years compared to earlier dates has reinforced the hypothesis that environmental factors have a role in disease development. In several studies, exposure to environmental pollutants containing various toxic metals such as lead (Pb), mercury (Hg), aluminum (Al), and metallic arsenic (As) was blamed as an important risk factor in the etiology of ASD (1, 2). It was shown that exposure to toxic metals may cause neuroinflammation, particularly in the developing brain, and an increase in inflammatory cytokines. Within the neuronal cells, toxic metals may increase oxidative stress, may cause endoplasmic reticulum stress and essential metalloprotein degradation, and thus may lead to severe neuroinflammation, excitotoxicity and apoptosis (3). Observations in children with ASD have revealed

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that there is at least one symptom associated with digestive systems in half of these patients (4). In recent years, scientists have suggested that intestinal dysbiosis may be one of the most important mechanisms in ASD pathophysiology. We have taken the etiologic mechanism of ASD and clinical signs of the patient into consideration, while planning the treatment of this patient.

CASE REPORT

Before the child was referred to us, a pediatric neurologist initially diagnosed him as being on the autism spectrum based on the criteria of Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition (DSM-IV). According to the results of the Vineland Adaptive Behavior Scale (VABS), the child was behind his peers regarding communication skills and socialization areas and was referred to an ASD center for special education.

When he was referred to our clinic, he was two years old. In the first assessment he was socially regressed compared to his peers, he had repetitive behaviors and his eye contact was limited and he did not respond to verbal communication. Moreover, he frequently had recurring abdominal pain, constipation, and stool with a foul odor. The family was in search of preventive measures for his future life.

Toxic metal and intestinal microbiota analysis using the latest scientific information was planned for this patient to establish a firm diagnosis. Stool was collected for intestinal microbiota assessment and Stool Flora Scan (SFS) Plus® test (Laben Laboratories, Türkiye) was performed. The patient's flora index was measured as 13, indicating a severe imbalance in intestinal microbiota. According to the classification system used, scores between 8 and 14 reflect severe dysbiosis, while 0–3 is considered normal, 4–7 indicates moderate dysbiosis, and values above 14 represent very severe dysbiosis. Moreover, the calprotectin level — a biomarker of intestinal inflammation — was elevated 73 mg/kg (reference range: 0-50 mg/kg).

Table 1. Urine mercury, lead, and aluminum levels in 2009 and 2010.

	March 02, 2009	March 29, 2010	Reference Range (mcg/g)
Mercury	14.1	3	<5
Lead	25.9	19.5	<5
Aluminum	63	-	<60

The patient was sent to the Forensic Medicine Institute for toxic metal and mineral analysis. According to the institute's CDC-based protocol, urine was collected for eight hours after six tablets of DMSA 100 mg was given to the patient. The lead level in the urine was five times higher than the upper limit of normal (ULN) (25.9 µg/g), mercury level was three times higher than the ULN (14.1 µg/g), aluminum was a little higher than the ULN (63.2 µg/g).

We have planned to fulfill three treatment targets: detoxification by heavy metal chelation, regulation of intestinal flora and diet, and training the patient and his parents. For metal chelation, phytochelators (chlorella, coriander) were preferred. As phytochelators, a combination preparation containing both probiotic bacteria and *Chlorella vulgaris* and *Coriandrum sativum* was used as drop formulation. The treatment was started with one drop twice a day. With weekly increments, the dose was increased to daily twenty-six drops and the treatment continued for one year. After the treatment, from 2009 until the beginning of 2020 follow-up interviews were done for ten years, the frequency of interviews was two times yearly in the first three years and then annually. During this time, *Chlorella*, *C. sativum*, *Allium ursinum*, probiotics and prebiotics were used alternatively (5-7). To assist in microbiota regulation, a diet without refined sugar and carbohydrates was planned for the patient. There are several research showing the benefits of diet regulation for the treatment of children with ASD (8).

To keep the child away from toxic material the family preferred consumption of organic food, wearing organic clothing and using natural furniture without dye. The child's education was continued by instructions of a child development specialist between two to four years of age. By the inductively coupled plasma – mass spectrometry (ICP-MS) method, pre-treatment on March 2, 2009 and one year later post-treatment on March 29, 2010 values and reference range of heavy metals in the urine was listed, respectively. Mercury was normalized (from 14.1 mcg/g to 3 mcg/g; reference: <5), lead was decreased (from 25.9 mcg/g to 19.5 mcg/g; reference: <5) aluminum was slightly high in the first test, but there is no data about aluminum in the second test because of technical reasons. (Table 1).

After ten years, on July 19, 2019, toxic metal and mineral tests were repeated using ICP-MS/MS in blood samples in another laboratory. All the measured sixteen toxic heavy metal levels in the blood sample of the patient were found to be normal according to the laboratory's reference ranges (Table 2). SFS Plus® test could not be repeated due to financial reasons thus, flora index and calprotectin levels cannot be measured. However, clinical observations including stooling with foul odor, de-

creased constipation attack, and absence of abdominal pain showed that intestinal flora imbalance changed positively. There is accumulating evidence about correlation between ASD and environmental factors and gastrointestinal system (GIS) disorders. The best therapeutic approach seems to be early started preventive strategies.

Exposure to lead in earlier times of infancy may lead to neurochemical alterations, growth retardation neurotoxicity, decreased cognitive development, and a decrease in attention and executive brain functions (3). Although the accepted serum lead level in children is lower than 10 µg/dL, some studies have shown that even lower levels can cause neurotoxic symptoms (9).

It was shown that mercury exposure may lead to damage in organelles such as endoplasmic reticulum. The main target in mercury toxicity is the central nervous system. Both metallic and organic mercury may easily pass the blood-brain barrier and placenta and thus may be observed in breast milk and may be transferred to the fetus (3).

It is shown that compared with children without ASD, heavy metals such as mercury and lead were found to be significantly higher in children with ASD (10). In a meta-analysis of 48 studies, toxic metal levels of children with ASD were measured in various samples such as whole blood, red blood cells, serum, plasma, urine, and hair. Specifically, mercury and lead were higher in peripheral blood and red blood cells, and the authors reported the role of environmental factors in the etiology of ASD (11). Exposure to aluminum may suppress cellular energy synthesis by affecting several glycolytic enzyme pathways and may lead to neurotoxicity (3).

In children diagnosed with ASD it is assumed that there is a disruption in toxic metal elimination rather than exposure to toxic elements. The most preferred therapeutic approaches are avoiding exposure to toxic metals, elimination by metal chelation, supplementation of vitamin-mineral deficiencies, regulation of diet and treatment with antioxidants and anti-inflammatory drugs (12).

Since the tests indicated higher levels of these heavy metals in the patient, we aimed to eliminate lead, mercury, and aluminum heavy metals from the body of our patient by chelation therapy. Elimination of metal toxicity to relieve neuroinflammation and to correct intestinal dysbiosis was the mainstay of our treatment.

The use of chemical chelators which are rather used in acute toxicities such as DMSA, EDTA is limited due to their adverse effects that impede compliance. (13). We

Table 2. Blood mercury, lead, and aluminum levels in 2019.

	July 19, 2019	Reference Range (µg/L)
Mercury	0.2	<0.2
Lead	17.1	<28
Aluminum	<10	<11.4

preferred phytotherapeutic preparations. It is known that extract of *C. vulgaris* has a potent chelating effect on heavy metals such as lead, mercury, arsenic, and cadmium (14,15). *Coriandrum sativum* can mobilize heavy metals particularly mercury and lead from the tissues (6, 5). *Allium ursinum* contains numerous sulfur compounds including valuable sulfhydryl groups oxidizing mercury, cadmium, and lead and increasing their water solubility (7). Thus, *Chlorella*, *Coriandrum*, and *Allium* preparations that were used in our patients were produced from cultured plants and given in standard doses as drops or capsules that are chemically pure. After the use of phytochelators, heavy metal levels in blood were within normal levels in our patient. We also did not experience any side effects after phytochelation therapy. Gut microbiota affects brain development and behaviors *via* neuroendocrine, neuro-immune, and autonomous nervous systems (4). Alimentary tract symptoms observed in ASD may stem from altered intestinal microbiota. In ASD, increasing intestinal permeability and easy leakage of bacterial metabolites from the intestinal barrier is associated with a "leaky gut" and affects neurodevelopmental mechanisms in early childhood (16). In children with ASD who have taken probiotics, significant improvements in behavioral symptoms have been observed. Their bowel habits and behavior improved significantly when treated with probiotics containing five specific probiotic strains. These five probiotic strains are *Lactobacillus acidophilus* and *casei*, *Bifidobacterium bifidum* and *longum*, and *Lactobacillus delbrueckii* (17).

In children with ASD, there are some observations indicating the role of gut microbiota in occurrence of ASD symptoms and their severity such as correlations between severity of ASD symptoms and GI symptoms, definitive profiles of gut microorganisms and metabolites and numerous neurologic disorders stemming from the association between gut and brain (4,18). Many researchers have suggested that the increase of *Candida* spp. in gut flora and particularly *Candida albicans* may decrease carbohydrate and mineral absorption and increase toxin levels and thus may contribute to autistic behaviors (19). Increased calprotectin level in stool is considered as a

biomarker for inflammation. Calprotectin is a calcium and zinc binding protein released by neutrophils. In case of an inflammation in the GIS neutrophils migrate to the area and release calprotectin and thus calprotectin level increases in the stool (20). The patient had high calprotectin levels when he first came to our clinic. Although calprotectin levels cannot be measured after treatment, due to clinical improvement in GIS symptoms, we can argue that intestinal inflammation was recovered.

Families of children with ASD often refer to complementary and alternative medicine (CAM) practices. CAM practices are primarily preferred for improving health, treating specific symptoms, avoiding the adverse effects of conventional medicine, or relieving the main symptoms of ASD. Prevalent CAM categories are natural products, special diets, mind, and body therapies including acupuncture and chiropractic manipulations and other biomedical treatments such as probiotics, vitamins. Conventional studies have shown that some CAM therapies are ineffective, and some require more studies (21).

Training the parents is also important. Parent training is carried out by professionals of the subject by using various methods including didactic, role play, discussions, video guidance and thus information and skills are transferred to patients/caregivers.

We had a multidisciplinary approach in this case. While the patient was getting special education, we used a treatment using CAM practices. We chose phytochelators for heavy metal chelation, also supported this treatment with probiotics, vitamin supplementation and special diets.

CONCLUSION

This patient is now 13 years old with the help of family support, special education and the therapies mentioned above, he can build social communication. His academic success continues with minimal support, and he became an individual continuing his life with achievements in painting and music.

As it is obviously seen, a holistic therapeutic approach may be beneficial in ASD. After treatment by functional therapies and diet regulation and having support from a very considered family, it seems that it is possible for autistic individuals to participate in social life and continue their academic success by eliminating accumulated neurotoxic metals in children with a tendency to toxicity, correcting gut microbiota dysbiosis and perpetuating lifelong preventive therapies. However, more studies are needed in this area to optimize the therapy and establish a standard.

Ethics Committee Approval :N.A.

Informed Consent: Written informed consent was obtained from the patient's legal guardian to participate in the study.

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