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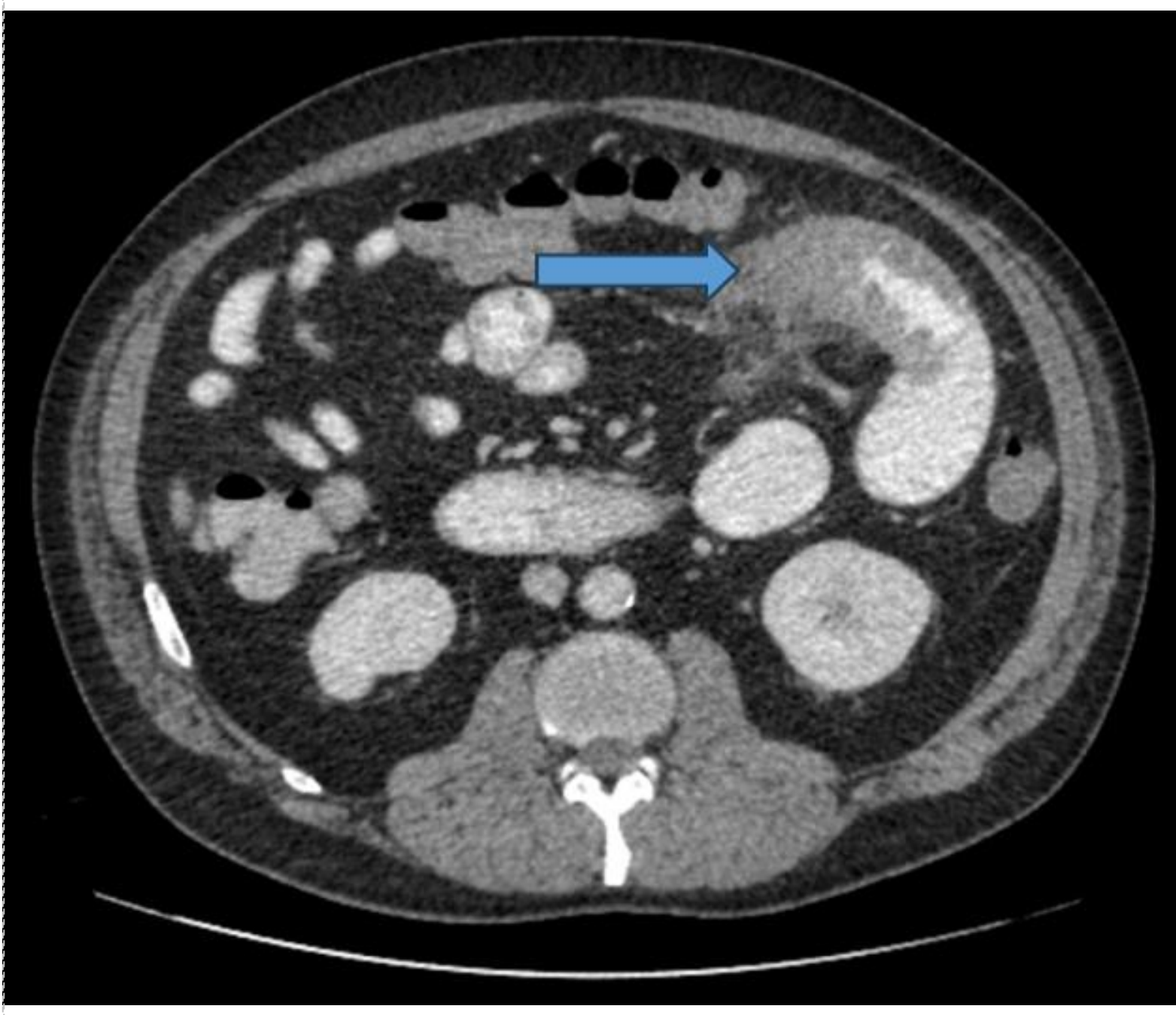

International Journal of Medical Science and Discovery
Open Access Scientific Journal
www.medscidiscovery.com,
Lycia Press London UK ISSN: 2148-6832

March, 2024, Vol .11 No .3

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Indexed Databases: Chemical Abstracts (CAS), Index Copernicus, Open Air, ULRICHS Database, ProQuest, Advanced Science Index, Research Bible, Scholar Google, NLM Catalog

Medical Science and Discovery is an international open access, peer-reviewed scientific research journal.

ISSN: 2148-6832 (Print)

E-ISSN: 2148-6832 (Online)

Category: Multi Disciplinary Health Science Journal

Abbreviated key title: Med. Sci. Discov.

Frequency: Monthly

Review System: Double Blind Peer Review

Circulation: Globally, Online, Printed

Article Processing Charge (APC): Free

Licensing: CC-BY-NC 4.0 International License Environmental

Editor-in-Chief: Assoc. Prof. Dr. Asghar Rajabzadeh, Anatomical Department of lorestan, University of Medical Sciences, Tabriz, Iran

Established: 30.04.2014

Web address: www.medscidiscovery.com

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Phone : +44 776 090 2125

Design and preparation of PDFs, Language editing, Web site design, Graphical design Services of international Journal of Medical Science and Discovery has been contracted with Lycia Press LONDON, UK (as Publisher), by the MSD Board of Directors

Publisher: Lycia Press London UK.

Address: 3rd Floor 86 - 90 Paul Street, EC2A 4NE, London, UK

Web address: www.lycians.com

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MSD

Medical Science and Discovery
ISSN: 2148-6832

Investigation of Vascular Endothelial Growth Factor and Endostatin Levels in Some Rat Tissues in Response to Cold Stress and Diet

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ABSTRACT

Objective: Obesity, the disease of our age, is a condition that occurs when there is an excess of fat tissue in the body. It is not merely a concern about weight gain, but rather a medical issue that elevates the risk of various diseases including heart disease, diabetes, high blood pressure, and certain cancers. This study aimed to explore the impact of a high-fat diet under normal conditions and cold stress, as well as the influence of propolis as a dietary supplement, on vascular endothelial growth factor (VEGF) and endostatin levels in rats fed with propolis.

Material and Methods: Thirty-six 3-month-old female Wistar rats (6 rats in each group) sourced from Inonu University Experimental Animal Production and Research Center were utilized for the study. Propolis was administered by gavage, dissolved in water, at a dosage of 2 mL per day for two weeks.

Results: The group exhibiting at least a 20% increase in weight due to high-fat diet consumption was categorized as the obese group. Tissues including heart, liver, lung, brown adipose, and white adipose tissues were procured from the obese, propolis-treated, and control groups. Endostatin and vascular endothelial growth factor levels were assessed in the tissues using the ELISA method. The study revealed an elevation in VEGF levels in brown adipose tissue in both cold stress and propolis treatment groups, accompanied by a reduction in white adipose tissue compared to the control group. Additionally, VEGF levels displayed a general increase in lung, liver, and heart tissues. Conversely, endostatin levels, an antiangiogenic factor, decreased in brown adipose tissue while increasing in white adipose tissue. In liver, lung, and heart tissues, endostatin levels exhibited a general decrease.

Conclusion: The findings suggest that both cold stress and propolis treatment influence VEGF and endostatin levels in various rat tissues, indicating potential implications for obesity-related conditions and angiogenesis regulation.

Keywords: VEGF, Obesity, Endostatin, Propolis

Research Article

Received 10-01-2024

Accepted 28-02-2024

E-Pub: 01-03-2024

Issue Publication: 30-03-2024

INTRODUCTION

Adipose tissue is an important metabolic organ and plays a key role in energy homeostasis. Depending on cell morphology and tissue function, mammals have two types of adipose tissue: white and brown. These adipose tissues have different physiological roles: White adipose tissue (WAT) is highly adapted to store excess energy in the form of triglycerides, while Brown adipose tissue (BAT) radiates energy to generate heat by converting glucose and fatty acids into the resulting proton-movement force. Brown adipose tissue thermogenesis depends on uncoupling protein 1 (UCP1), specifically expressed in brown fat mitochondria and responsible for the unique metabolic function of brown adipose tissue. UCP1 is known to distribute the proton gradient across the inner mitochondrial membrane, thereby uncoupling the electron transfer system from adenosine triphosphate synthesis, resulting in energy dissipation as heat. Brown adipose tissue plays a crucial role in regulating energy expenditure to adapt to cold environments (1).

http://dx.doi.org/10.36472/msd.v11i3.1117

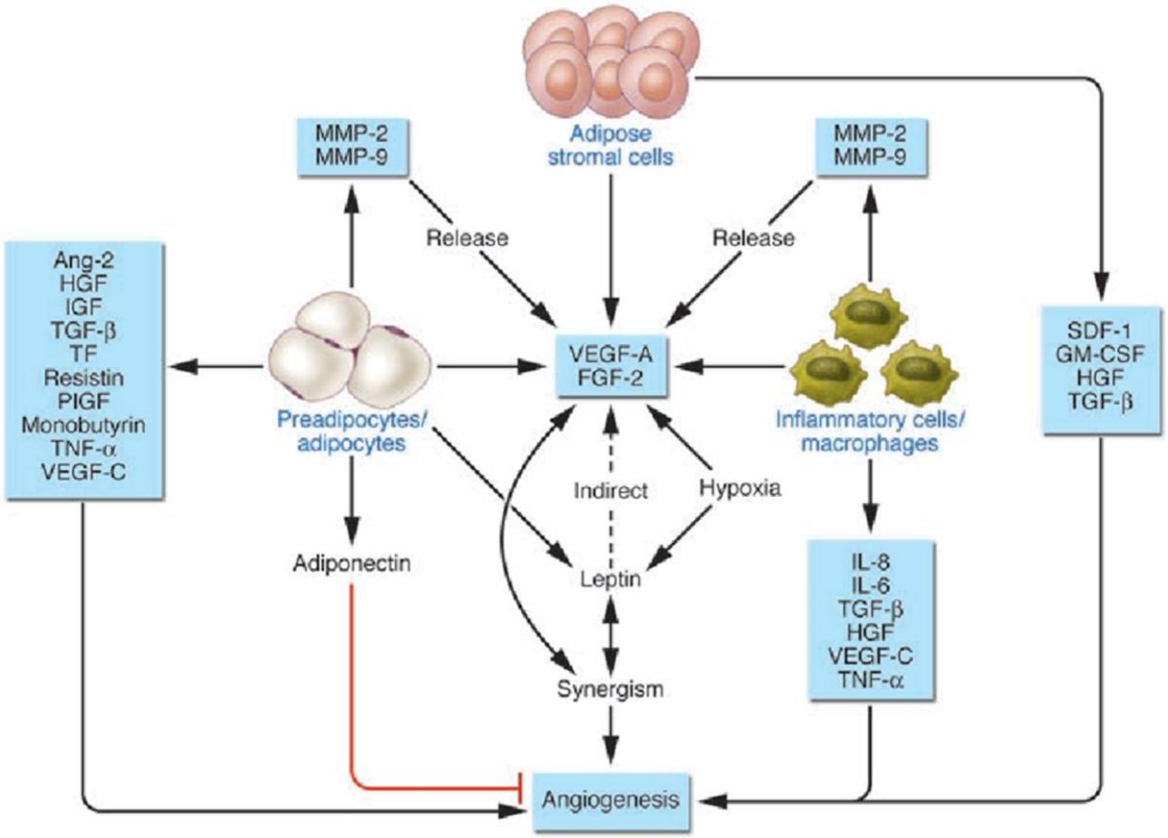

Figure 1. Regulation of adipose tissue angiogenesis by multiple factors (22).

Table 1. Angiogenic and antiangiogenic factors involved in angiogenesis

Angiogenesis inducers	Angiogenesis inhibitors
VEGF (Vascular endothelial growth factor)	Thrombospondin- 1
PGF (Placental growth factor)	Endostatin
FGF (Acidic, basic fibroblast growth factor)	Vasostatin
TGF-α (Transforming growth factor-α)	Vascular eldothelial growth factor inhibitor
TGF-β (Transforming growth factor-β)	Platelet factor-4 fragment
EGF (Epidermal growth factor)	Prolactin derivative
HGF (Hepatocyte growth factor)	Restin
TNF-α (Tumor necrosis factor-α)	Interferon-α-β
PDGF (Platelet-derived growth factor)	Angiopoetin-2
GCSF (Granulocyte colony stimulating factor)	Antithrombin-3 fragment
IL-8 (Interleukin-8)	Interferon-inducible protein- 10
Angiogenin	
Proliferin	

Adipose tissue is highly vascularized and each adipocyte is supplied by an extensive capillary network [2, 3, 4]. Adipose tissue growth is dependent on angiogenesis and can be inhibited by angiogenesis inhibitors. Treatment with angiogenesis inhibitors leads to weight loss and adipose tissue loss, adipose tissue mass is sensitive to angiogenesis inhibitors, and its vascularization can be regulated [5, 6, 7]. Angiogenesis, the formation of new capillaries from existing blood vessels, is a complex, multi-stage process involving a series of cellular events leading to new vascularization [8, 9]. Angiogenesis plays a central role in various physiological processes in the human body during fetal development and tissue repair after surgery or trauma. Angiogenesis is a hallmark of wound healing, the menstrual cycle, cancer and various ischemic and inflammatory diseases [10, 11].

The realization that tumor growth is associated with new blood vessels has led to the search for biochemical factors mediating angiogenesis, expanding knowledge of pathological processes and opening new possibilities for diagnosing and treating diseases [12]. The basic process of angiogenesis can be simply described as multiple stages. First, the stimuli of angiogenesis cause increased endothelial cell permeability and cell proliferation or cell division as the newly formed capillaries elongate (13). The second is the degradation of basement membrane components by proteolysis, which promotes the invasion of endothelial cells into the stroma of adjacent tissue, where the joint activity of the plasminogen activator (PA) system and matrix metalloproteinases (MMPs) is required (14). Third, since the newly formed capillaries form a multicellular structure,

migrating endothelial cells trigger lumen formation, forming a new capillary channel. Finally, the capillary membrane, adhesive junction and endothelial cells are formed.

Adipose Tissue-derived Angiogenesis Factors

Growing adipose tissue contains a variety of cell types, including adipocytes, adipose stromal cells, endothelial cells and inflammatory cells (15). The diversity of heterogeneous cell populations determines the expression of multiple growth factors and cytokines that individually or co-regulate vessel growth, but little is known about the functional interaction between these factors (16). Growing adipocytes contain angiogenic factors such as leptin, VEGF, FGF-2, HGF, IGF, TNF-α, TGF-β, placental growth factor (PlGF), VEGF-C, resistin, tissue factor (TF), neuropeptide Y (NPY), heparin-binding epidermal growth factor (17-19). Preadipocytes and adipocytes also produce small non-protein lipid molecules such as monobutyrin, stimulating angiogenesis in adipose tissue (20, 21). The regulation of adipose tissue angiogenesis by multiple factors is shown in Figure 1 (22).

VEGF plays a central role in the growth or development of most healthy and pathological tissues. The omentum expresses the highest level of VEGF among all adipose tissues studied in the body (23). Localization studies have shown that adipocytes are the primary source of VEGF, which can function as a factor of angiogenesis and vascular survival for the omental vasculature. In addition, adipose-infiltrating inflammatory cells and adipose stromal cells also contribute significantly to VEGF production (15, 24, 25).

Inactivation of PlGF (placental growth factor) function in mice results in impaired adipose tissue development due to defective angiogenesis, suggesting that other VEGF members also modulate adipogenesis through the vascular system (26). Gene expression profiling analysis in rat WAT shows a distinct pathway of adipokines including leptin, adiponectin and resistin growing against stagnant adipose tissues (17). Resistin, a specific adipokine, is a novel angiogenesis factor that directly promotes endothelial cell proliferation, migration and tube formation (22). IGF-1 and TNF-α are two other angiogenic factors increased in the expansion of adipose tissues (27). IGF-1 serves as a survival factor for various cell types and assumes a critical role in preserving vascular integrity within adipose tissue (28). In addition to its direct angiogenesis activity, TNF-α is a potent inflammatory cytokine that links inflammation, angiogenesis and adipogenesis (29). IL-8 becomes a survival factor for adipocytes in vivo, possibly through stimulation of angiogenesis (30). Thus, inflammatory cells in growing adipose tissues are coordinators for coupling the simultaneous events of adipogenesis and angiogenesis (Table 1) (22).

Endostatin, a naturally occurring 20-kDa C-terminal fragment, is derived from collagen XVIII through proteolytic cleavage at its carboxylic terminus. Endostatin administration has been reported to reduce adipose tissue expansion, inhibit the regulation of angiogenesis, and suppress tumor activity by interfering with the action of angiogenic factors such as vascular endothelial growth factor (VEGF) (218). Endostatin is an endogenous inhibitor of angiogenesis. It interferes with TNF a activation of C-Jun N-terminal Kinase, and blocks pro-angiogenic factors required for angiogenesis (5).

Propolis extracts are considered as nutritional products with potential for managing obesity and comorbidity. However, the composition of propolis extracts is highly variable and depends on the botanical origin of the plants used by bees to produce propolis. Propolis is a resinous substance collected by bees from buds, trees or shrubs in the plant ecosystem close to the hive. Propolis is used as a folk medicine in Europe, Asia and South America. The various biological and pharmacological effects reported for propolis activity are related to phenolic compounds, which can vary widely in quality and quantity depending on the plant source and place of collection. Three types of propolis, namely brown propolis from Populus sp., have been subjected to in-depth investigations in terms of identification, characterization, and biological activities. It is characterized by the presence of polyphenolic taxonomic markers such as pinocembrin, chrysin and galangin, and substituted cinnamic acids, especially phenylethyl caffeate (CAPE) esters. Green propolis from Baccharis dracunculifolia from the Minas Gerais region in Brazil is characterized by the presence of artepellin C, p-coumaric and drupanin as taxonomic markers and red propolis from Dalbergia ecastophyllum from the Algolas region. Brazilian is characterized by the specific presence of isoflavans, isoflavones or pterocarpan class represented by vestitol, formononetin and medicarpin (31).

MATERIAL and METHODs

- Experimental Animals Used in the Study

Three-month-old female Wistar rats, bred by the İnönü University Experimental Animal Production and Research Center, were utilized in this study. The rats were housed in specialized cages under controlled conditions at 24°C, with a 12-hour light/dark cycle, until the commencement of the experiment. Rats weighing between 220-250 g were included in the study. The animals were divided into six groups:

Group 1 (Normal weight- Normal feeding): Fed with pellet feed without additives.

Group 2 (Obese group - High-fat diet): Obesity was achieved by high-fat diet (HFD).

Group 3 (Normal weight- Cold stress): They were fed with pellet feed without additives and cold stress was applied.

Group 4 (Obese group - Cold stress): They were fed a high-fat diet (HFD), and cold stress was applied.

Group 5 (Normal weight - Propolis treatment): They were fed with pellet feed without additives and propolis was applied.

Group 6 (Obese group - Propolis application - Cold stress): Rats in this group were fed a high-fat diet (HFD) and subjected to both propolis administration and cold stress.

Rats were weighed at the beginning of the experiment, and their weights were recorded. Afterwards, the rats were weighed every week until the day of slaughter and their weights were recorded.

- Normal Nutrition

Rats in the caloric fed groups were fed standard chow and water was given ad libitum. The feeds were placed on the cage.

http://dx.doi.org/10.36472/msd.v11i3.1117

-High Fat Diet

Rats in the high-fat diet groups were fed with DIO Rodent Purified Diet w/60% Energy From Fat- Blue special rat chow and given as much water as they could drink.

-Propolis Application

In our study, we utilized liquid, alcohol-free commercial propolis produced in Turkey, sourced from Asitane company. Propolis was administered via gavage every day for a duration of 2 weeks at a dosage of 100 mg/kg/day, diluted in drinking water.

-Cold Stress Practice

The cages for cold stress application were kept at 12±2o C for 48 hours. Rats were controlled by observation during cold stress application.

-Tissue Harvesting

Firstly, 1500 µL/kg ketamine and 500 µL/kg xylazine were administered intramuscularly for anesthesia. The unconsciousness of the rats was controlled by pinching their hind legs with pliers. An incision was made in the abdomen of anesthetized rats and cut up to the rib cage. The rib cages were also opened, and the vena cava was cut. Perfusion was completed by injecting 5 ml of saline into the right and left ventricles of the heart.

-Collection and Homogenization of Liver, Lung, Heart, Brown and White Fat Tissues

After perfusion, the rat was euthanized by removing the heart and the tissues were removed one by one. Lung tissue and then some of the liver tissue were removed. They were re-perfused in saline and wrapped in labelled aluminum foils. Intestines were removed, and retroperitoneal white adipose tissue was removed. The dorsal part of the rat was opened and interscapular brown adipose tissue was removed. Each tissue was placed in liquid nitrogen without waiting.

After dissection, the tissues were removed from liquid nitrogen and stored at -40 °C until analysis. For homogenization, tissues were placed in pre-weighed microcentrifuge tubes, then weighed again. Subsequently, 20 µl/mg of PBS buffer (2 mM, pH 7.3) was added. Tissues were cut into smaller pieces with tissue scissors in the microcentrifuge tubes. Subsequently, tissues were sonicated using an ultrasonifier (PVC Kinematica Status) for 20 seconds on ice. The sonicated tissues were then stored at -40°C.

-Vascular Endothelial Growth Factor and Endostatin Measurement

The amount of VEGF and endostatin in liver, lung, heart, white and brown adipose tissue homogenate samples were determined with the Rat VEGF ELISA Kit (EK0540) and Rat Endostatin ELISA Kit (EK1377) manufactured by Boster Biological Technology Ltd. using a microplate reader (BioTek® Instruments, Inc., Eon) at 450 nm. As a result of the measurements, VEGF and Endostatin amounts were calculated using the standard graph according to the protocol given in the kit. GraphPad program was used for calculations.

-Statistical Method

Statistical evaluations were performed with SPSS for Windows Version 15.0 package program. Data related to measurable variables were given as mean ± standard error. The t-test method was used to determine the differences between the groups. The value found was evaluated at 5% significance level (95% confidence interval, $p<0.05$).

RESULTs

Weight (normal weight and obese), temperature (room temperature and cold stress treatment), nutrition (normal diet, high fat diet and propolis treatment) were taken into consideration in the evaluation of the findings. Among the groups formed according to these three factors, the "Normal weight and room temperature" treatment group was considered as the control group. **Table 2** shows the vascular endothelial growth factor (VEGF) levels according to the groups. VEGF levels in brown adipose tissue increased in the obese- room temperature group compared to the normal weight- room temperature group and this increase was found to be statistically significant ($p<0.05$). The highest increase was found in the obese-room temperature group (532.03±8.97ng/L). In the obese-room temperature group, there was a statistically significant increase compared to the normal weight propolis-fed room temperature group ($p<0.05$). Similarly, in the normal weight-cold stress group, a statistically significant increase was observed compared to the room temperature group ($p<0.05$). Furthermore, in the normal weight-propolis nutrition-room temperature group, there was a statistically significant increase compared to the room temperature group ($p<0.05$). In the obese-cold stress group, obese propolis nutrition showed an increase compared to the cold stress group and this increase was found to be statistically significant ($p<0.05$).

Table 2. Vascular endothelial growth factor (VEGF) levels in brown adipose tissue in relation to obesity, cold stress and propolis treatment.

Group	VEGF (ng/L)
Normal Weight - Room Temperature	244,40±23,43[a]
Obese - Room Temperature	532,03±8,97[b]
Normal Weight - Cold Stress	393,61±21,91[c]
Obese - Cold Stress	319,03±22,91[d]
Normal Weight - Propolis - Room Temperature	439,43±15,60[e]
Obese - Propolis - Cold Stress	257,46±14,47[a]

The difference between the groups indicated by different letters has been found to be statistically significant.

Table 3 shows the vascular endothelial growth factor (VEGF) levels in white adipose tissue. VEGF levels in white adipose tissue increased in the normal weight - room temperature group compared to the obese group - room temperature group and this increase was found to be statistically significant ($p<0.05$). The highest increase was found in the normal weight-room temperature group (394.36±21.53 ng/L). There was an increase in the obese group - room temperature group compared to the obese group - propolis nutrition - cold stress group, and this increase was found to be statistically significant ($p<0.05$). Obese group - cold stress group showed an increase compared to normal weight-cold stress group, which was found to be statistically significant ($p<0.05$).

http://dx.doi.org/10.36472/msd.v11i3.1117

There was an increase in the obese group - propolis nutrition - cold stress group compared to the normal weight - cold stress group, and this increase was found to be statistically significant ($p<0.05$).

Table 3. Vascular endothelial growth factor (VEGF) levels in white adipose tissue in relation to obesity, cold stress and propolis treatment.

Group	VEGF (ng/L)
Normal Weight - Room Temperature	394,36±21,53[a]
Obese - Room Temperature	273,85±12,83[b]
Normal Weight - Cold Stress	63,99±4,88[c]
Obese - Cold Stress	149,80±11,52[g]
Normal Weight - Propolis - Room Temperature	82,36±4,35[e]
Obese - Propolis - Cold Stress	132,35±13,73[f]

The difference between the groups indicated by different letters has been found to be statistically significant.

Table 4 shows vascular endothelial growth factor (VEGF) levels according to the groups. VEGF levels in cardiac tissue increased in the obese-over-ambient temperature group compared to the normal weight-over-ambient temperature group and this increase was found to be statistically significant ($p<0.05$). The highest increase was observed in the obese-room temperature group (228.62±9.11 ng/L). Additionally, there was a statistically significant increase in the obese-cold stress group compared to the obese-propolis nutrition-cold stress group ($p<0.05$). Moreover, there was a statistically significant increase in the obese-cold stress group compared to the normal weight-cold stress group ($p<0.05$). There was an increase in the obese-propolis nutrition-cold stress group compared to the normal weight-cold stress group and this increase was found to be statistically significant ($p<0.05$).

Table 4. Vascular endothelial growth factor (VEGF) levels in heart tissue in relation to obesity, cold stress and propolis treatment.

Group	VEGF (ng/L)
Normal Weight - Room Temperature	147,70±15,08[a]
Obese - Room Temperature	228,62±9,11[b]
Normal Weight - Cold Stress	74,52±4,37[c]
Obese - Cold Stress	203,72±13,50[d]
Normal Weight - Propolis - Room Temperature	156,56±11,86[a]
Obese - Propolis - Cold Stress	163,20±9,55[a]

The difference between the groups indicated by different letters has been found to be statistically significant.

Table 5 shows vascular endothelial growth factor (VEGF) levels according to the groups. VEGF levels in lung tissue increased in the obese-chamber temperature group compared to the normal weight-chamber temperature group and this increase was found to be statistically significant ($p<0.05$).

A statistically significant increase was observed in the obese-cold stress group compared to the obese-propolis nutrition-cold stress group ($p<0.05$).

The highest increase was recorded in the obese-cold stress group (236.50±12.29 ng/L). Furthermore, there was a statistically significant increase in the obese-cold stress group compared to the normal weight-cold stress group ($p<0.05$).

Table 5. Vascular endothelial growth factor (VEGF) levels in lung tissue concerning obesity, cold stress and propolis treatment.

Group	VEGF (ng/L)
Normal Weight - Room Temperature	122,66±8,39[a]
Obese - Room Temperature	166,98±8,43[b]
Normal Weight - Cold Stress	139,16±10,97[a]
Obese - Cold Stress	236,50±12,29[c]
Normal Weight - Propolis - Room Temperature	36,42±2,86[d]
Obese - Propolis - Cold Stress	193,62±8,77[e]

Statistically significant differences between groups, denoted by different letters, have been observed.

Table 6 shows vascular endothelial growth factor (VEGF) levels according to the groups. VEGF levels in the liver tissue increased in the obese-over-ambient temperature group compared to the normal weight-over-ambient temperature group, and this increase was found to be statistically significant ($p<0.05$). The obese-room temperature group had the highest increase (189.52±7.04 ng/L). There was an increase in the normal weight-propolis nutrition- room temperature group compared to the normal weight- room temperature group, and this increase was found to be statistically significant ($p<0.05$). A statistically significant increase was observed in the normal weight-cold stress group compared to the normal weight-room temperature group ($p<0.05$).

Table 6. Vascular endothelial growth factor (VEGF) levels in liver tissue concerning obesity, cold stress and propolis treatment.

Group	**VEGF (ng/L)**
Normal Weight - Room Temperature	88,32±5,43[a]
Obese - Room Temperature	189,52±7,04[b]
Normal Weight - Cold Stress	137,12±10,85[c]
Obese - Cold Stress	143,34±12,84[d]
Normal Weight - Propolis - Room Temperature	144,70±13,39[d]
Obese - Propolis - Cold Stress	138,12±14,70[d]

The difference between the groups indicated by different letters has been found to be statistically significant.

Table 7 shows vascular endostatin (ES) levels according to the treatment groups. ES levels in brown adipose tissue decreased in the normal-cold stress group compared to the obese-cold stress group and this decrease was found to be statistically significant in the obese cold stress group ($p<0.05$). The most significant decrease was found in the obese-cold stress group (1.77±0.006 ng/L). A statistically significant increase was observed in the obese-propolis nutrition-cold stress group compared to the obese-cold stress group ($p<0.05$). There was an insignificant increase observed in the normal weight propolis nutrition room temperature group compared to the normal weight room temperature group ($p>0.05$).

http://dx.doi.org/10.36472/msd.v11i3.1117

Table 7. Endostatin (ES) levels in brown adipose tissue in relation to obesity, cold stress and propolis treatment.

Group	ES (ng/L)
Normal Weight - Room Temperature	2,36±0,11[a]
Obese - Room Temperature	2,02±0,07[a]
Normal Weight - Cold Stress	2,28±0,18[a]
Obese - Cold Stress	1,77±0,06[b]
Normal Weight - Propolis - Room Temperature	2,12±0,08[a]
Obese - Propolis - Cold Stress	2,26±0,16[a]

The difference between the groups indicated by different letters has been found to be statistically significant.

Table 8 shows the treatment groups' vascular endostatin (ES) levels. ES levels in white adipose tissue increased in the obese - room temperature group compared to the normal - room temperature group and this increase was found to be statistically significant ($p<0.05$). The highest increase was found in the obese - room temperature group (3.00±0.14 ng/L). There was an increase in the obese - cold stress group compared to the normal - cold stress group, which was found to be statistically significant ($p<0.05$). A statistically significant increase was observed in the obese propolis nutrition group compared to the normal weight propolis nutrition room temperature group ($p<0.05$).

Table 8. Endostatin (ES) levels in white adipose tissue in relation to obesity, cold stress and propolis treatment.

Group	ES (ng/L)
Normal Weight - Room Temperature	1,55±0,18[a]
Obese - Room Temperature	3,00±0,14[b]
Normal Weight - Cold Stress	1,33±0,14[a]
Obese - Cold Stress	2,59±0,14[c]
Normal Weight - Propolis - Room Temperature	1,96±0,14d
Obese - Propolis - Cold Stress	2,59±,021[c]

The difference between the groups indicated by different letters has been found to be statistically significant.

Table 9 shows vascular endostatin (ES) levels according to the groups. ES levels in cardiac tissue increased in the obese - room temperature group compared to the normal - room temperature group and this increase was found to be statistically significant ($p<0.05$). The highest increase was found in the obese - room temperature group (2.34±0.18 ng/L). In the normal weight-cold stress group, there was a statistically significant increase compared to the obese-cold stress group ($p<0.05$). Additionally, in the obese room temperature group, there was a statistically significant increase compared to the obese-cold stress group ($p<0.05$). A statistically significant increase was observed in the normal weight propolis nutrition room temperature group compared to the obese propolis nutrition cold stress group ($p<0.05$).

Table 9. Vascular endostatin (ES) levels in heart tissue in response to obesity, cold stress and propolis treatment.

Group	ES (ng/L)
Normal Weight - Room Temperature	1,68±,12[a]
Obese - Room Temperature	2,34±0,18[b]
Normal Weight - Cold Stress	1,69±0,08[a]
Obese - Cold Stress	1,22±0,14[c]
Normal Weight - Propolis - Room Temperature	1,79±0,04[d]
Obese - Propolis - Cold Stress	1,41±0,08[e]

The difference between the groups indicated by different letters has been found to be statistically significant.

Table 10 shows vascular endostatin (ES) levels according to the treatment groups. ES levels in lung tissue increased in the normal - room temperature group compared to the normal - propolis feeding - room temperature group, and this increase was found to be statistically significant ($p<0.05$). The highest increase was found in the normal - room temperature group (1.77±0.06 ng/L).

Table 10. Vascular endostatin (ES) levels in lung tissue in response to obesity, cold stress and propolis treatment.

Group	ES (ng/L)
Normal Weight - Room Temperature	1,77±0,06[a]
Obese - Room Temperature	1,56±0,12[b]
Normal Weight - Cold Stress	1,14±0,05[c]
Obese - Cold Stress	1,34±0,13[d]
Normal Weight - Propolis - Room Temperature	1,40±0,11[b]
Obese - Propolis - Cold Stress	1,32±0,11[d]

The difference between the groups indicated by different letters has been found to be statistically significant.

Table 11 shows vascular endostatin (ES) levels according to the treatment groups. ES levels in liver tissue increased in the normal - cold stress group compared to the obese - cold stress group and this increase was found to be statistically significant ($P<0.05$). The highest increase was found in the normal - cold stress group (2.47±0.15 ng/L). In the normal room temperature group, there was a statistically significant increase compared to the obese room temperature group ($p<0.05$). Additionally, there was a statistically significant increase observed in the normal room temperature group compared to the normal propolis nutrition room temperature group ($p<0.05$).

Table 11. Endostatin (ES) levels in liver tissue in relation to obesity, cold stress and propolis treatment.

Group	ES (ng/L)
Normal Weight - Room Temperature	2,22±0,20[a]
Obese - Room Temperature	1,39±0,12[b]
Normal Weight - Cold Stress	2,47±0,15[a]
Obese - Cold Stress	0,89±0,07[c]
Normal Weight - Propolis - Room Temperature	0,99±0,06[c]
Obese - Propolis - Cold Stress	0,71±0,03[c]

The difference between the groups indicated by different letters has been found to be statistically significant.

DISCUSSION

This study aimed to investigate the levels of VEGF, an angiogenic factor, and endostatin, an antiangiogenic factor, in liver, lung, heart tissue, white adipose tissue, and brown adipose tissue in cases of obesity induced by cold stress and propolis feeding. Upon evaluation of the findings, it was observed that VEGF levels increased in brown adipose tissue in both cold stress and propolis treatment groups, whereas they decreased in white adipose tissue. Similarly, VEGF levels showed a general increase in lung, liver, and heart tissues. Conversely, endostatin levels, as an antiangiogenic factor, decreased in brown adipose tissue but exhibited a general increase in white adipose tissue.

In liver, lung, and heart tissues, endostatin levels generally decreased.

When white adipose tissue and brown adipose tissue are evaluated in terms of energy and heat extraction, it is thought that there may be increases, while differences in other tissues may vary depending on the function of the tissues.

In a study on cold stress, it was reported that cold stress caused an increase in VEGF levels in brown adipose tissue of rats (32). The study we conducted is similar to the increase in VEGF level in brown adipose tissue due to cold stress application. In addition, a general increase in VEGF levels was found in white adipose tissue, lung, liver and heart tissue due to cold stress application. There is no study on endostatin, a proteolytic fragment of collagen XVIII, a potent angiogenesis inhibitor, in relation to exposure to cold stress. Our study detected a decrease in endostatin levels in rats exposed to cold stress for the first time. However, a study on diet-dependent endostatin was conducted by Wang et al. (2015) (33). Wang et al. (2015) reported that there was no change in the weight of the heart, liver, lungs and kidneys, while endostatin administration to mice fed a high-fat diet reduced the weight of white tissue. It was also reported that endostatin was effective in the inhibition of obesity development in mice fed a high-fat diet, with a detailed histological analysis of white adipose tissue revealing that the size of adipocytes in mice fed a high-fat diet given endostatin was smaller than in animals fed an endostatin high-fat diet.

Propolis is a natural product obtained by mixing bee secretions with plant exudates. Since propolis is rich in flavonoids and cinnamic acid derivatives, the application of propolis extracts in treatments against cancer, inflammation and metabolic diseases has been investigated. Accumulating evidence utilizing animal and cellular models suggests that propolis extracts have therapeutic effects on obesity by controlling adipogenesis, adipokine secretion, food intake and energy expenditure. Research in animal and cellular models has shown that propolis modulates oxidative stress, accumulation of advanced glycation end products and adipose tissue inflammation, all of which contribute to insulin resistance or impairments in insulin secretion (34). In the study performed by Culum and Yurekli (2020), the effects of resveratrol, which has anti-inflammatory, anti-oxidant and anticancer effects, on angiogenesis, white adipose tissue and brown adipose tissue, and its effects on vascular endothelial growth factor were investigated. It has been stated that resveratrol increases the level of VEGF in brown adipose tissue and that resveratrol may possibly have proangiogenic activity. Our study determined that rats given propolis, which has antibacterial, antitumor and antioxidant properties, generally caused an increase in VEGF levels (35). Propolis, produced both in our country and around the world and known to have various effects on human health, has antibacterial, cytostatic, free radical protective activity, while bactericidal, antifungal and antiparasitic activity, antioxidant, anticarcinogenic, wound healing, cell regenerative and aniogenic properties have also been researched (36). Studies have shown that propolis suppresses angiogenesis. In the study conducted by us, in addition to the features mentioned above, a study was conducted on the levels of angiogenic VEGF and antianiogenic endostatin in brown adipose tissue and white adipose tissue due to the application of cold stress to obesity. Especially considering obesity and cold stress, it is thought that propolis will generally increase VEGF levels in brown adipose tissue compared to control groups and that propolis will have beneficial effects in terms of energy efficiency/usage. When we look at endostatin data, it is important to investigate more aniogenic and antioaniogenic factors, since very clear data are not obtained as in VEGF. According to the literature, no study investigates the levels of VEGF and endostatin, which are angiogenic and antiangiogenic factors, due to propolis diet and cold stress application.

CONCLUSION

In our study, it was found that endostatin levels were generally increased in brown and white adipose tissue due to cold stress and obesity, while endostatin levels decreased in heart, lung and liver tissue. Our study studied the effects of cold stress application and propolis nutrition on angiogenic and antiangiogenic factors. According to literature data, it is known that propolis has angiogenic properties. Although the results obtained in our study are similar to the literature data, it is obvious that more studies are needed in the future, considering that there are many angiogenic-antiangiogenic factors. When propolis and cold stress were evaluated together, it was determined in our study that they may play a role in regulating energy efficiency in brown adipose tissue.

Acknowledgements: This study was supported by Inonu University, Scientific Research Projects Unit with the project number FYL-2020-2078.

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author Contributions: FÇ, MY: Designed and directed the study, Literature search, Data collection, Statistics **FÇ, MY:** Article writing, Final revisions. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval: The present study was conducted in strict accordance with the principles outlined in the Declaration of Helsinki. Ethical approval for the study was obtained from the appropriate ethics committee.

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Medical Science and Discovery
ISSN: 2148-6832

Single Center Evaluation of Long-Term Results of Glargin U-300 in Insulin Naive Patients In a Real-World Setting

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ABSTRACT

Objective: Insulin therapy stands as one of the most effective and well-established therapeutic options for managing glycemic control in Diabetes Mellitus (DM). Glargine 300 U/mL (Gla-300) represents a new long-acting insulin analog, which has demonstrated a decrease in the risk of hypoglycemia and a reduction in the total number of injections due to prolonged insulin absorption. In this study, we investigated the long-term effects of Gla-300 on Fasting Plasma Glucose (FPG) and HbA1c levels, as well as the incidence of hypoglycemia in insulin-naive patients admitted to the Internal Medicine outpatient clinic, over a period of 0, 3, 6, 12, and 24 months..

Material and Methods: Between January 2018 and June 2022, insulin-naive patients diagnosed with Type 2 Diabetes Mellitus (T2DM) who initiated treatment with Gla-300 and sought care at the Internal Medicine outpatient clinic were subjected to retrospective analysis.

Results: The study included 49 insulin-naive patients. A statistically significant decrease was observed in Fasting Plasma Glucose (FPG) ($p = 0.03$) and HbA1c ($p = 0.02$) levels during the 24-month follow-up period of Glargine U-300. Additionally, a significant reduction in both FPG ($p < 0.01$) and HbA1c ($p < 0.01$) values was achieved at the time of diagnosis and at 3 months. Hypoglycemia was reported in only 1 patient (2%) during our study, indicating a very low hypoglycemia rate.

Conclusion: Diabetes mellitus (DM) poses a significant public health challenge, resulting in economic burden and diminished quality of life. Developed to address these challenges, Gla-300 serves as a long-acting basal insulin that effectively reduces the risk of hypoglycemia while offering targeted glycemic control, as evidenced by our study findings. In Turkey, there is a pressing need for multicenter, prospective real-world studies that incorporate parameters such as insulin dosage and weight monitoring.

Keywords: Glargine 300 U, Long acting Insulin, Diabetes Mellitus, Hypoglycemia

INTRODUCTION

Diabetes Mellitus (DM) represents a significant public health challenge with a rapidly increasing prevalence worldwide (1). The primary objective in managing DM is to achieve glycemic control, which serves as the cornerstone in preventing long-term macro and micro complications such as retinopathy, neuropathy, nephropathy, myocardial infarction, and stroke (2). Insulin therapy stands as one of the most effective and well-established therapeutic options for managing glycemic control. While basal and rapid-acting insulin therapy combinations are preferred for patients with Type 1 DM, insulin therapy is considered as a second-line treatment for patients with Type 2 DM who do not achieve adequate glycemic control with oral antidiabetic medications (3). Hypoglycemia, the most common adverse effect of insulin therapy, is a significant concern for patients, often leading to reduced insulin utilization (3,4). However, the development of long-acting basal insulins has contributed to a decrease in the risk of hypoglycemia, along with a reduction in the total number of injections due to prolonged insulin absorption (5,6). Glargine 300 U/mL (Gla-300) emerges as a novel long-acting insulin analog (7).

In this study, we examined the long-term effects of Gla-300 on Fasting Plasma Glucose (FPG) and HbA1c levels, as well as the incidence of hypoglycemia, in insulin-naive patients attending the Internal Medicine outpatient clinic at Mersin City Training and Research Hospital. Data was collected at intervals of 0, 3, 6, 12, and 24 months between January 2018 and June 2022.

Research Article

Received 21-02-2024

Accepted 05-03-2024

E-Pub: 07-03-2024

Issue Publication: 30-03-2024

http://dx.doi.org/10.36472/msd.v11i3.1133

MATERIAL and METHODs

Patients diagnosed with Diabetes Mellitus (DM), aged >18 years, and admitted to the Internal Medicine outpatient clinic between January 2018 and June 2022 were screened. Among 1200 patients receiving Gla-300 for DM, those with type 1 DM, previous insulin therapy, or inadequate follow-up were excluded. A total of 49 insulin-naive patients were enrolled in the study. Data on age, sex, DM history, comorbidities, complications, medications, additional treatments post-Gla-300 initiation, and FPG and HbA1c values at diagnosis and at 3, 6, 12, and 24 months were retrospectively extracted from electronic medical records.

Statistical Analysis

Data were analyzed using SPSS 21.0 (IBM Corp., Armonk, NY, USA). Normal distribution of study variables was assessed using the Kolmogorov–Smirnov test. Numerical variables were expressed as mean ± SD (standard deviation), while categorical variables were presented as numbers and percentages. Paired samples t-test was used to compare means between two related groups, and ANOVA test was employed for repeated measurements involving three or more groups. Ethical approval for non-invasive clinical research was obtained from the Mersin University Ethics Committee (2022/602).

RESULTs

Between January 2018 and June 2022, a total of 1200 patients with DM receiving Gla-300 were identified. Patients with type 1 DM, previous insulin therapy, or those with no follow-up were excluded from the study, resulting in 49 insulin-naive patients being included. Among the participants, 28 (57.1%) were women and 21 (42.9%) were men, with a mean age of 56.2 ± 6.9 years. The most common comorbidity was hypertension, present in 18 patients (36.3%), and the most prevalent complication was neuropathy, observed in 19 patients (38.8%). Additionally, 11 patients (22.4%) had not received any previous therapy. Demographic characteristics of the patients are summarized in **Table 1**.

During the 24-month follow-up, hypoglycemia was observed in 1 patient (2.0%). In addition to Glargine U-300, 7 patients (14.3%) were administered insulin aspart, 5 (10.2%) received insulin glulisine, and 2 (4.1%) were treated with insulin lispro. Overall, 14 patients (28.6%) received rapid-acting insulin analogs during the follow-up period. The changes in FPG and HbA1c values over time are illustrated in **Figure 1**.

A statistically significant decrease was observed in FPG ($p = 0.03$) and HbA1c ($p = 0.02$) during the 24-month follow-up of Glargine U-300. Significant decreases were also noted in FPG ($p < 0.01$) and HbA1c ($p < 0.01$) values at the time of diagnosis and at 3 months. However, there were no significant changes in FPG ($p = 0.6$) and HbA1c ($p = 0.3$) values at 3 months and 6 months, nor in FPG ($p = 0.7$) and HbA1c ($p = 0.7$) values at 6 months and 12 months, or at 12 months and 24 months (FPG: $p = 0.6$, HbA1c: $p = 0.7$). Additionally, these values remained stable over time. The FPG and HbA1c levels of patients using Glargine U-300 are summarized in **Table 2**.

Tablo 1. Demographic data of the patients

Features	n (%)
Female	28 (57.1)
Age mean± SD	56.2 ± 6.9
Diabetes mellitusyears mean± SD	12.9 ± 6.9
Comorbidity	
Hypertension	18 (36.7)
Hyperlipidemia	2 (4.1)
Coronary Artery Disease	2 (4.1)
Ankylosing Spondylitis	1 (2.1)
Ulcerative Colitis	2 (4.1)
Multiple Diseases	11 (22.4)
No diseases	13 (26,5)
Complications	
Neuropathy	19 (38.8)
Neuropathy+Retinopathy	5 (10.2)
Diabetic Foot	3 (6.1)
Chronic Renal Failure	3 (6.1)
Coronary Artery Disease	2 (4.1)
Oral Anti Diabetic Drugs	
Biguanide	4 (8.2)
Biguanide +Sulfonylurea	11 (22.4)
Thiazolidinedione+ Biguanide	2 (4.1)
Thiazolidinedione+ Biguanide+Sulfonylurea	7 (14.3)
Thiazolidinedione+ Biguanide+DPP-4	9 (18.4)
Thiazolidinedione+ Biguanide +SGLT-2	3 (6.1)
DPP4+SGLT-2	2 (4.1)
No	11 (22.4)

http://dx.doi.org/10.36472/msd.v11i3.1133

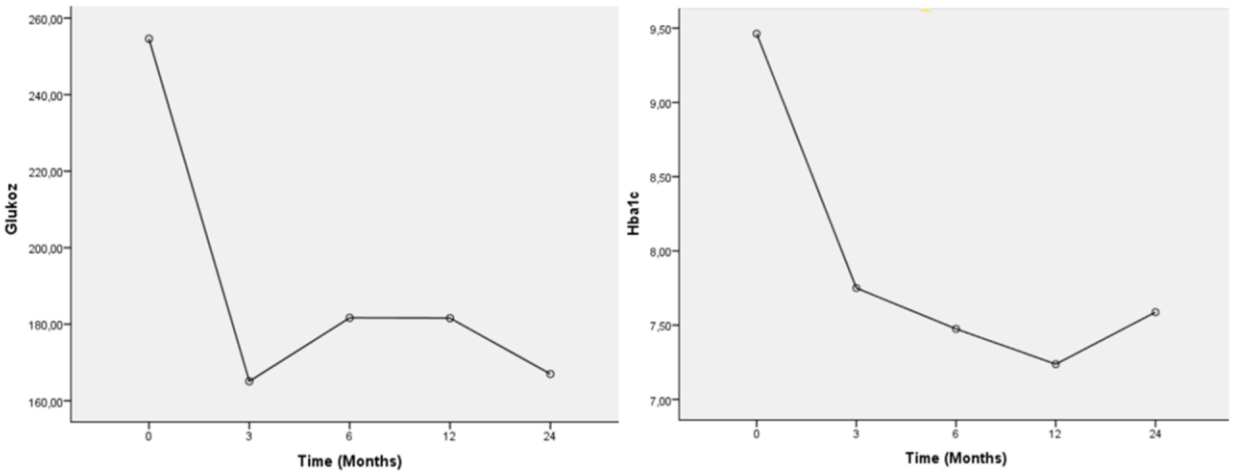

Figure 1. The change in Fast Plazma Glukoz and Hba1c values over time

Table 2. Fasting Plazma Glukoz and Hba1c levels in patients using Glargin U-300

Features	Months					*p*
	0	3	6	12	24	
Fasting Plazma Glucose mg/dl mean± SD	252±126.7	135.1±48,3	142.6±40.7	130.5±46.9	135.4±41.5	**0.03**
Hba1c mean± SD	9.4±1.9	7.8±1.5	7.5±0.9	7.2±1.3	7.5±1.2	**0.02**

DISCUSSION

The World Health Organization (WHO) has reported that type 2 diabetes mellitus (DM) is most prevalent in individuals aged 65 years or older in developed countries and in the middle-aged population in developing countries (8). In our study, the mean age was 56.2 ± 6.9 years, which aligns with the WHO data. While DM was traditionally assumed to be equally distributed between sexes, the NAHES-II study indicated that 55% of newly diagnosed DM cases are women (9,10), and it is predicted that DM will be 10% more common in women than men by 2025 (11). In our study, 57.1% of the patients were women, suggesting an increasing trend in women with DM.

Hypertension is observed 1.5–2 times more frequently in patients with diabetes mellitus (DM) compared to the general population and affects 30%–50% of individuals with Type 2 DM (12). Furthermore, neuropathy is the most common complication associated with DM (13). In our study, neuropathy emerged as the most prevalent complication with a frequency of 38.8%, while hypertension was identified as the most common comorbid condition at 36.3%, aligning with findings from the literature.

Cetinarslan et al. investigated 108 insulin-naive patients initiating insulin glargine U300 and found a statistically significant decrease in mean HbA1c levels from 9.4% at diagnosis to 7.5% and 7.3% at 12 and 24 weeks, respectively. Mean fasting plasma glucose (FPG) levels decreased from 194.7 mg/dl to 126.5 mg/dl at week 12 and 131 mg/dl at week 24 (14).

In a multicenter, prospective study, Galstyan et al. evaluated 4442 insulin-naive patients initiating insulin glargine U300. They reported HbA1c levels of 9.28 ± 1.0, 8.19 ± 1.04, 7.77 ± 1.06, and 7.38 ± 0.97 at 0, 3, 6, and 12 months, respectively (15). Another study showed an HbA1c level of 7 at 6 months (16).

In our study, HbA1c values were 9.4 ± 1.9, 7.8 ± 1.5, 7.5 ± 0.9, 7.2 ± 1.3, and 7.5 ± 1.2 at 0, 3, 6, 12, and 24 months, respectively, and targeted decreases in FPG were achieved during the 24-month follow-up.

As the risk of hypoglycemia associated with basal insulin therapies is low, they are considered cost-effective both in the short and long terms. Short-term health expenditures primarily result from a reduction in hypoglycemia symptoms (17). Decreased anxiety about experiencing hypoglycemia reduces the likelihood of treatment discontinuation, leading to fewer long-term complications of diabetes mellitus (DM). Consequently, improved patient comfort and a general reduction in health expenditures are achieved both in the short and long terms (18).

Galstyan et al. reported a hypoglycemia incidence of 1.99% with Gla-300 over a 12-month period (15), noting that their findings were consistent with other studies demonstrating low rates of hypoglycemia associated with Gla-300 use (19,20). In our study, hypoglycemia was observed in only 1 patient (2%), indicating a very low hypoglycemia rate similar to that reported in other studies. This suggests that Gla-300 is a cost-effective insulin analog.

The limitations of our study include the absence of pre- and post-treatment weight and insulin doses due to the retrospective design, as well as the inability to generalize the results to the broader population given the study's single-center cross-sectional nature and small sample size. However, our study's strength lies in being the first real-world investigation conducted in our region among insulin-naive patients, spanning a lengthy period of 24 months.

CONCLUSION

Diabetes mellitus (DM) represents a significant public health concern, contributing to economic burdens and diminished quality of life. In response, various therapeutic interventions are being developed to enhance quality of life and mitigate complications. Among these developments, Gla-300 has emerged as a long-acting basal insulin capable of reducing the risk of hypoglycemia while offering precise glycemic control, as evidenced by our study's findings. In Turkey, there is a pressing need for multicenter, prospective real-world studies incorporating parameters such as insulin dosage and weight monitoring.

Acknowledgements: Informed consent was not obtained because our study was a retrospective study.

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author Contributions: DG, SME: Designed and directed the study, Literature search, Data collection, Statistics **DG, SME:** Article writing, Final revisions. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval: The present study was conducted in strict accordance with the principles outlined in the Declaration of Helsinki. Ethical approval for the study was obtained from the appropriate ethics committee.

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MSD

Medical Science and Discovery
ISSN: 2148-6832

Evaluation of Perfusion and Function in Cardiac Radionuclide Imaging in Cases of Heart Failure with Mid-Range Ejection Fraction

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ABSTRACT

Objective: Heart failure with mid-range ejection fraction (HFmrEF) poses a significant clinical challenge due to its diverse etiology and variable prognosis. Patients with HFmrEF exhibit an intermediate level of left ventricular dysfunction, making their management and prognosis less well-defined compared to those with heart failure with reduced ejection fraction (HFrEF) or preserved ejection fraction (HFpEF). Coronary artery disease (CAD) is a common underlying cause of HFmrEF and can further exacerbate myocardial dysfunction under stress conditions. In this study, we aimed to evaluate the change in left ventricular ejection fraction with stress in the presence of coronary artery disease in cases of heart failure with mid-range ejection fraction.

Material and Methods: In this retrospective study, we included 507 patients diagnosed with coronary artery disease and an left ventricular ejection fraction (LVEF) of 41-49% measured by echocardiography. All patients underwent a treadmill exercise test using the Bruce protocol, with progressively increasing speed and incline. Myocardial perfusion was assessed using stress gated myocardial perfusion scintigraphy (MPS), and fixed and reversible defects were identified in cases of coronary artery disease. Cardiac scintigraphic images were acquired from the right anterior oblique to the left posterior oblique. We calculated post-stress LVEF and the percentage decrease in LVEF to evaluate cardiac function.

Results: Resting LVEF was measured as 46 (43-50), while post-stress LVEF was 35 (25-47) in all patients. Myocardial perfusion was evaluated using stress gated MPS in all patients, with 200 (39.5%) patients showing both fixed and reversible perfusion defects. The rate of decrease in LVEF due to stress was significantly higher in patients with reversible perfusion defects (15.90 (6-30.43) vs. 28.26 (24-43.18), p: 0.0005). Post-stress LVEF was lower in patients with reversible perfusion defects (40 (31-47) vs. 33 (25-38), p: 0.0005).

Conclusion: In cases of impaired left ventricular perfusion, quantitative calculations of LVEF may vary, and their reliability may decrease as the ejection fraction decreases under stress conditions. Clinicians should consider this variability in the follow-up of patients with heart failure and mid-range ejection fraction.

Keywords: radionuclid imaging, HFmrEF, ejection fraction, myocardial perfusion

INTRODUCTION

Heart failure (HF) is a complex clinical syndrome characterized by impaired ventricular filling or ejection, leading to inadequate tissue perfusion and systemic congestion (1). The severity and management of HF often depend on the LVEF, which categorizes HF into three main subtypes. Preserved EF, defined as LVEF ≥ 50%, typically involves impaired diastolic function and is associated with HF symptoms despite normal systolic function. Reduced EF, with LVEF ≤ 40%, reflects systolic dysfunction and is commonly observed in patients with HF. HFmrEF is diagnosed when LVEF falls between 41% and 49% in the presence of HF.

Following myocardial infarction (MI), the heart undergoes a remodelling process characterized by changes in ventricular wall thickness due to mechanisms such as myocyte hypertrophy and fibrosis (1-3). Myocardial perfusion imaging using cardiac radionuclide imaging techniques allows for the assessment of myocardial perfusion defects, providing valuable insights into the extent and reversibility of myocardial ischemia.

Research Article

Received 23-02-2023

Accepted 17-03-2024

E-Pub: 19-03-2024

Issue Publication: 30-03-2024

In this study, we aimed to evaluate the impact of reversible myocardial perfusion defects, as detected by stress gated myocardial perfusion scintigraphy (GMPS), on ventricular function in patients diagnosed with HFmrEF and CAD. By examining changes in ventricular function associated with reversible perfusion defects, we seek to contribute to the existing literature and enhance our understanding of the pathophysiology and clinical implications of myocardial ischemia in patients with HFmrEF.

MATERIAL and METHODs

In our retrospective study, we included 507 patients diagnosed with CAD and an LVEF value of 41-49%, determined by echocardiography (Echo). Each patient underwent both left ventricular echo (Echo-LV) and gated myocardial perfusion scintigraphy (GMPS) evaluations. Echo examinations were conducted within two weeks prior to the GMPS examination. Post-stress LVEF was calculated in all patients using quantitative calculations with MPS following stress induction.

During the GMPS evaluation, all patients exhibited a fixed defect in left ventricular perfusion, with some also presenting a reversible defect. Treadmill exercise testing was performed using the Bruce protocol, with patients reaching at least 85% of their maximum heart rate and exercising for a minimum of 6 minutes before cessation due to fatigue. Patients fasted for a minimum of 3-4 hours prior to testing.

For stress imaging, 9-11 mCi of Tc-99m-sestamibi was administered intravenously, while rest imaging utilized 27-33 mCi of Tc-99m-sestamibi. Wall movements and thickness were evaluated using an automatic analysis program during the MPS examination. SPECT images were acquired from the right anterior oblique to the left posterior oblique using a Siemens-Ecam gamma camera.

Additionally, LVEF was calculated via transthoracic 2D echocardiography. MPS SPECT studies were synchronized with the ECG, allowing determination of end-systolic and end-diastolic volumes. Imaging protocols adhered to established guidelines.

Statistics: SPSS-21 software was used for statistical analysis. The number and percentage of cases were given in categorical variables. Continuous variables are expressed as median (minimum-maximum). Mann-Witney U test was used for continuous variables that did not show normal distribution. Chi-square test was used for categorical variables. $p<0.05$ was considered statistically significant.

RESULTs

The median age of the patients included in our study was 63 years (range: 52-78). Among the total 507 patients, 323 (63.7%) were male. The average left ventricular ejection fraction value calculated by echocardiography (Echo-LVEF) was 44 (range: 41-47).

In all patients, both post-stress and rest LVEF values were assessed following myocardial perfusion scintigraphy (MPS) examination. Resting LVEF was determined to be 46 (range: 43-50), while post-stress LVEF was measured at 35 (range: 25-47).

Myocardial perfusion was evaluated using exercise gated MPS in all patients. Among them, 200 (39.5%) exhibited a reversible perfusion defect in addition to a fixed defect.

Patients with a reversible perfusion defect demonstrated a significantly higher rate of decrease in LVEF due to stress compared to those without (15.90 vs. 28.26, p: 0.0005). Furthermore, post-stress LVEF was notably lower in patients with a reversible perfusion defect compared to those without (40 vs. 33, p: 0.0005).

The summarized results are presented in **Table 1-2** and **Figure 1**.

Table 1: Data on left ventricular wall perfusion in cases with Heart Failure with Mid-Range Ejection Fraction

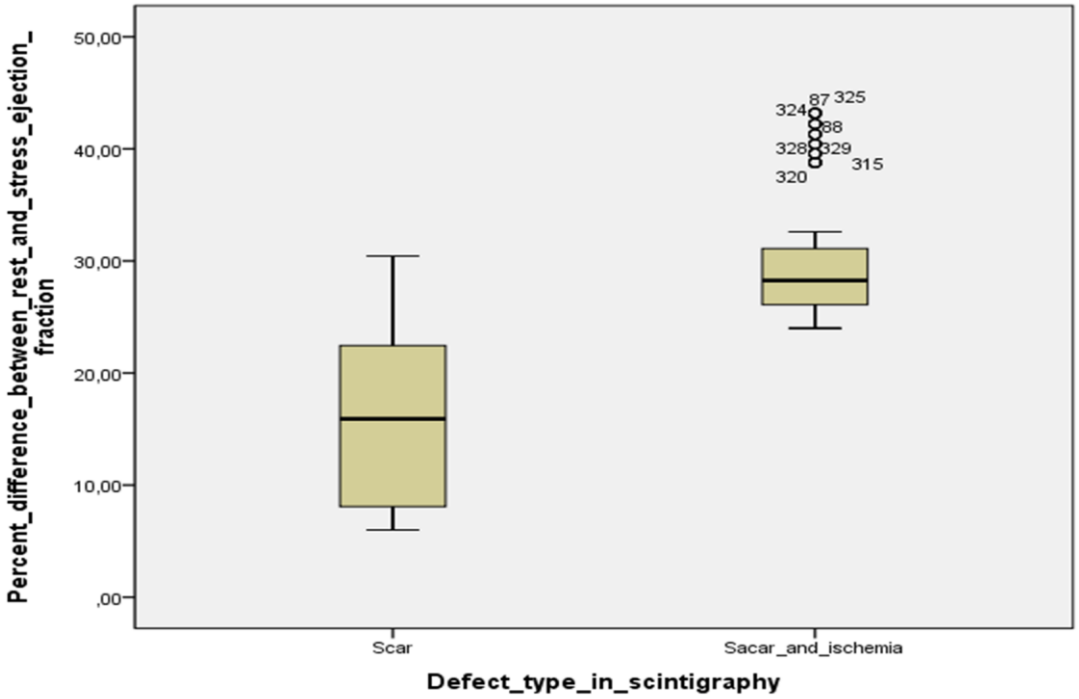
	Patients without Reversible Perfusion Defect (n:307)	Patients with Reversible Perfusion Defect (n:200)	p
Age	63(53-77)	64 (52-78)	0.417
Gender(female/male)	100(32.6%)/207(67.4%)	84(42%)/116(58%)	0.031
EF calculated by echocardiography	44 (41-47)	44 (41-47)	0.859
Radionuclide imaging-rest EF	46 (43-50)	46 (43-50)	0.817
Radionuclide imaging-post-stress EF	40 (31-47)	33 (25-38)	0.0005
Decrease in EF due to stress in radionuclide imaging (rest EF-Stress EF)(%)	15.90 (6-30.43)	28.26 (24- 43.18)	0.0005

Table 2: Significance of gender and percentage reduction in left ventricular ejection fraction in response to effort in patients with Heart Failure with Mid-Range Ejection Fraction

	Confidence Interval	Odds	p
Gender	0.012-0.177	0.46	0.0005
Decrease in EF due to stress in radionuclide imaging (rest EF-Stress EF)(%)	1.683-2.239	1.942	0.0005

 http://dx.doi.org/10.36472/msd.v11i3.1135

Figure 1: Display of the percentage change in rest and post-stress ejection fraction according to perfusion defect type



DISCUSSION

In 10-24% of heart failure patients, left ventricular ejection fraction falls within the range of 41-49%, leading to the classification of mid-range ejection fraction (mrEF) (2-5). Approximately half of the remaining patients exhibit LVEF ≤ 40%. Decreased LVEF serves as an adverse prognostic indicator in HF patients. However, it's important to note that LVEF calculated by echocardiography (Echo-LVEF) may not always reflect contractility accurately, and patients with similar LVEF values may have different underlying pathophysiology, resulting in varying prognoses (6).

While LVEF is commonly regarded as a measure of cardiac function, structural changes also significantly impact function. In hearts unaffected by structural changes from CAD, stroke volume experiences minimal alterations in response to changes in afterload. However, in cases of CAD, such as those included in our study, the decrease in LVEF during exertion is more pronounced, especially in the presence of reversible perfusion defects. Our findings suggest that in patients with heart failure and mid-range ejection fraction, the presence of reversible perfusion defects exacerbates the decline in cardiac function during exertion.

This decline in function during exertion may stem from the heart reaching its maximum capacity to increase contraction in response to increased stretch. Factors such as decreased calcium affinity for Troponin C and reduced calcium availability in myocardial cells may contribute to this phenomenon (7). Structural changes following myocardial infarction, including stretched and infarcted tissue, lead to increased left ventricular volume, hypertrophy in non-infarcted areas, and alterations in heart shape from elliptical to spherical, negatively impacting heart function (9-11).

Cardiac remodelling, characterized by adaptive and maladaptive processes, further influences heart function. Adaptive remodelling initially maintains heart function in response to acute cardiac damage, while progressive remodelling is associated with a poor prognosis (12). The transition from adaptive to maladaptive remodelling remains poorly understood, but it contributes to the progression of HF (13). Post-MI cellular changes, such as myocyte hypertrophy, loss, and fibrosis, along with mechanical tension on myocytes, trigger signalling pathways and lead to increased wall stress and thickness, further exacerbating energy imbalance and ischemia (14-16).

Collagen synthesis and degradation, influenced by hemodynamic and neuro-hormonal factors, play critical roles in HF due to cardiac remodelling (17, 18). Additionally, inflammatory cytokines contribute to pathological remodelling and HF pathophysiology (21). Angiotensin-II and ACE inhibitors may modulate this process, while inflammatory cytokines potentially exacerbate heart dysfunction (20,21).

CONCLUSION

In conclusion, our study highlights the impact of impaired left ventricular perfusion on ejection fraction in patients with heart failure, particularly those with mid-range ejection fraction. We observed that as the ejection fraction decreases, especially in the presence of reversible perfusion defects, quantitative calculations may become variable, leading to decreased reliability in assessing cardiac function.

These findings underscore the importance of careful consideration and monitoring in the follow-up of patients with impaired left ventricular perfusion.

Healthcare professionals should be aware of the potential variability in quantitative measurements and exercise caution when interpreting these results in clinical practice. Additionally, further research is warranted to elucidate the underlying mechanisms contributing to this variability and to develop more accurate methods for assessing cardiac function in these patients. Such efforts will ultimately improve patient care and outcomes in the management of heart failure with impaired left ventricular perfusion.

Study Limitations:

The weakness of our study is that it is retrospective.

Abbreviations:

HFmrEF: Heart Failure with Mid-Range Ejection Fraction
MPS: myocardial perfusion scintigraphy
ME: Myocardial Enfarction
SPECT: single photon emission computerized tomography
BP: Bruce protocol
LVEF: left ventricular ejection fraction
EDV: end-diastolic volume
ESV: end-systolic volume
CAD: coronary artery disease
Echo: echocardiography

Acknowledgements: None

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author Contributions: SC: Designed and directed the study, Literature search, Data collection, Statistics **SC:** Article writing, Final revisions. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval: The present study was conducted in strict accordance with the principles outlined in the Declaration of Helsinki. Ethical approval for the study was obtained from the appropriate ethics committee. Informed consent was obtained from all participants of this study. Our retrospective study was approved by Gaziosmanpaşa Training and Research Hospital Ethics Committee with 70 numbers on 08.06.2022.

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Medical Science and Discovery
ISSN: 2148-6832

Bibliometric Analysis of The Top 100 Most Cited Articles on The Thalamus Anatomy

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ABSTRACT

Objective: The thalamus regulates complex tasks like motor function and executive control while transmitting sensory information to higher centers. Bibliometric analysis analyzes studies in a research area and guides planning studies in that area. Despite bibliometric analysis in anatomy, there is no study on the thalamus' anatomy. This study aims to perform a bibliometric analysis of the 100 most cited articles on the anatomy of the thalamus, a clinically important region, to guide research in this area, as there is no study on this topic in anatomy.

Material and Methods: Bibliometric analysis was used to evaluate human studies on the anatomy of the thalamus that were published in the Web of Science database between 2004 and 2023. As a result of the analysis, 1704 documents from the last twenty years were found. The data of the first 100 most cited articles were obtained.

Results: The average number of citations of the articles was 229.14. The publication years of the first 5 most cited studies were 2006-2010. The most cited study was by Heckemann et al. (2006). Articles were mostly published in NeuroImage. The United States has the strongest bibliographic link, publishes the most articles and is the most cited. Snyder Abraham Z. is the author with the most articles on this topic. Keyword co-occurrence analysis revealed 4 different clusters: the thalamus and its relationship to related anatomical structures, the connection between the thalamus and psychiatric and mood disorders, the relationship of the thalamus to the cerebral cortex, and the function of the thalamus.

Conclusion: Researchers show high interest in studies on the anatomy of the thalamus. The fact that the studies to be planned on the anatomy of the thalamus have to do with neuroimaging is one of the factors that may increase the number of citations.

Keywords: Thalamus, anatomy, bibliometric analysis, neuroimaging

Research Article

Received 09-03-2023

Accepted 21-03-2024

E-Pub: 22-03-2024

Issue Publication: 30-03-2024

INTRODUCTION

The thalamus is a nuclear complex that borders the upper part of the 3rd ventricle from both sides (1), and is located in the diencephalon (2). It plays an important role in many sensory and motor functions such as arousal, cortical synchronization, emotion, cognition and memory (3, 4). The thalamus is not only a relay, but also plays a role in controlling executive networks and regulating complex behaviours such as behavioural flexibility and reward-directed behaviour (5, 6).

The thalamic nuclei are roughly divided anatomically into anterior, medial and lateral nuclei of the thalamus (7) by a white matter called the internal medullary lamina of the thalamus (8) , which divides the internal structure of the thalamus in the shape of a "Y". In the posteroinferior region, the thalamic nuclei are also divided into core groups, which include the medial and lateral geniculate bodies (7). In the clinic, it is very important to know the anatomy of the thalamic nuclei and the vascular system that supplies them. This is because vascular dysfunction in one of the thalamic nuclei can lead to infarcts and loss of function in certain areas (9).

The thalamic vascular areas are divided into 7 regions: anterior, inferolateral, paramedian, posterior, anteromedian, central and posterolateral (9, 10). Memory loss, hemiparesis and emotional disturbances are located in the anterior region; contralateral hemianesthesia, hemibody pain and movement disorders are located in the inferolateral region; visual disturbances, hemiparesis, memory loss and akinetic mutism are located in the paramedian region; hemianopsia, contralateral hemianesthesia, hemibody pain in the posterior region; vertical gaze paresis, amnesia, aphasia and abnormalities in the anteromedian region; cognitive loss, vertical gaze paresis, loss of arousal and ataxia in the central region; aphasia, ataxia and loss of executive function in the posterolateral region (9).

In addition, thalamic nuclei are associated with many diseases such as attention deficit hyperactivity disorder (11), Parkinson's disease (12), multiple sclerosis (13), cognitive disorders and dementia (14) and autism (15). The insufficient evidence of the pathways associated with the connection of thalamic nuclei to disease (13, 16) increases researchers' interest in anatomical studies of the thalamus.

The synthesis of previous studies in the field is an important component for organizing new research in the future (17). Bibliometric analyses provide cumulative information by mapping and visualizing the publications published and indexed in a scientific field, their characteristics, and their literature status (18, 19). In bibliometric studies, the publications in the literature are analyzed quantitatively using statistical methods. The bibliometric study aims to determine which topics current research focuses on (20, 21). In this way, it helps researchers plan future research using the information from past research. Bibliometric studies on anatomy, one of the basic medical fields, have already been conducted (21, 22). However, no bibliometric study on the anatomy of the thalamus was found. Considering the clinical importance of the thalamus, a bibliometric analysis of the 100 most cited articles on the anatomy of the thalamus could be useful to guide future studies. Therefore, this study aims to perform a bibliometric analysis of the 100 most cited articles on the anatomy of the thalamus.

MATERIAL and METHODs

In this study, studies on the anatomy of the thalamus in the Web of Science database from the last 20 years were analyzed. The date of data collection was 13.02.2024. Since the year 2024 has not yet ended, publications in 2024 were not included in the study to prevent them from influencing the results of the study. No language restrictions were applied for this bibliometric analysis. Human anatomy studies, research articles, articles indexed in the Science Citiation Index Expanded, the Social Sciences Citiation Index and the Emerging Sources Citiation Index, and articles from the last 20 years (2004-2023) were included in this study. Exclusion criteria were animal studies (studies with mice, rabbits, rats, zebrafish, monkeys, gerbils or primates), proceeding papers, book chapters, early access papers, reviews and/or meta-analyses. In view of all this information, our search query is ((TS=(“thalamus" AND "anatomy”)) OR TS=(“thalamic anatomy”)) OR TS=(“thalamus" AND "anatomical”)) OR TS=(“thalamus" AND "neuroanatomy”)) OR TS=(“thalamic" AND "nuclei" AND "anatomy”)) OR TS=(“thalamic" AND "nucleus" AND "anatomy”)) OR TS=(“thalamocortical" AND "anatomy”)) OR TS=(“thalamo-cortical"AND "anatomy”)) OR TS=(“corticothalamic" AND "anatomy”)) OR TS=(“cortico-thalamic" AND "anatomy”)) NOT TS=("mouse")) NOT TS=("mice")) NOT TS=("rabbit")) NOT TS=("rat")) NOT TS=("monkey")) NOT TS=("zebra fish")) NOT TS=("zebrafish")) NOT TS=("primate")) NOT TS=(gerbil) (https://www.webofscience.com/wos/woscc/summary/2b264d9e-d067-4380-b704-38162e88c68b-cbe909e6/times-cited-descending/1).

Initially, 2648 documents were retrieved with our research request. When the document type (research article) and the Web of Science Index were taken into account, the number of documents decreased to 2224 and 2195 respectively. After removing "zoology" and "veterinary science" categories, which we assumed to include possible animal studies from the WOS categories, 2122 documents were obtained. Finally, when the studies from the last 20 years were considered, the total of 1704 documents were reached. The titles, abstracts and/or full texts of these studies were checked for compliance with the possible inclusion criteria, and the data of the first 100 most cited articles were extracted from the Web of Science database and transferred to an Excel file.

Statistical Analysis

In this study, the descriptive data of the studies on the anatomy of the thalamus in the Web of Science database for the last 20 years were presented as tables or graphs in the form of numbers and percentages. In addition, a bibliometric analysis of the reviewed studies was performed using the VOSviewer software (version 1.6.17 of VOSviewer). VOSviewer is a free program developed for the creation and display of bibliometric maps (23). In bibliometric maps, items are represented by circles. These circles are labeled, and the size of each circle indicates the weight of the corresponding item (24). In other words, the more important an item is, the larger its label and the larger the area of the circle. The lines between the circles on the map show the total link strength; higher total link strength indicates a stronger relationship between items (23).

RESULTs

Descriptive Information about the top 100 most cited articles

The average citation number of the top 100 most cited studies on the anatomy of the thalamus in the Web of Science database published between 2004 and 2023 is 229.14 (range: 125-670). The most cited study is Heckemann's "Automatic anatomical brain MRI segmentation combining label propagation and decision fusion" published in 2006. The top 5 most cited studies on the anatomy of the thalamus between 2004 and 2023 were mostly published between 2006 and 2010. Only one study (Automated anatomical labeling atlas 3) was published in 2020. All articles are written in English. Authors with more than one publication as first author are Zhang S (n=2), Butson (n=2), Zang DY (n=2), Nosarti (n=2), Haznedar (n=2) and Rose (n=2). The 100 most cited articles are listed in Appendix 1. Most of these articles were published in NeuroImage (n=27) (**Figure 1**).

http://dx.doi.org/10.36472/msd.v11i3.1141

Bibliographic Coupling Analysis of the Countries

Bibliographic coupling evaluates the similarity between documents based on the number of shared references (25). Martyn introduced the term as "two papers that share one reference contain one unit of coupling, and the value of a relationship between two papers having one or more references in common is stated as being of strength one, two, etc., depending on the number of shared references." (26). Kessler confirmed the existence of a subject relationship between bibliographically matched documents (27).

A bibliographic coupling of countries occurs when documents from two countries cite documents from a third country (28). When we review the most cited studies in the field of anatomy related to the thalamus, the bibliographic matching of countries is shown in Figure 2. The scale in Figure 2 shows the relationship between the colors and the number of citations in each country. The figure shows that the country with the strongest connection is the United States of America (USA) (TLS=4532). After the USA, the countries with the highest number of similar studies to other countries are the United Kingdom (TLS=4317), France (TLS=2788), and Germany (TLS=2056).

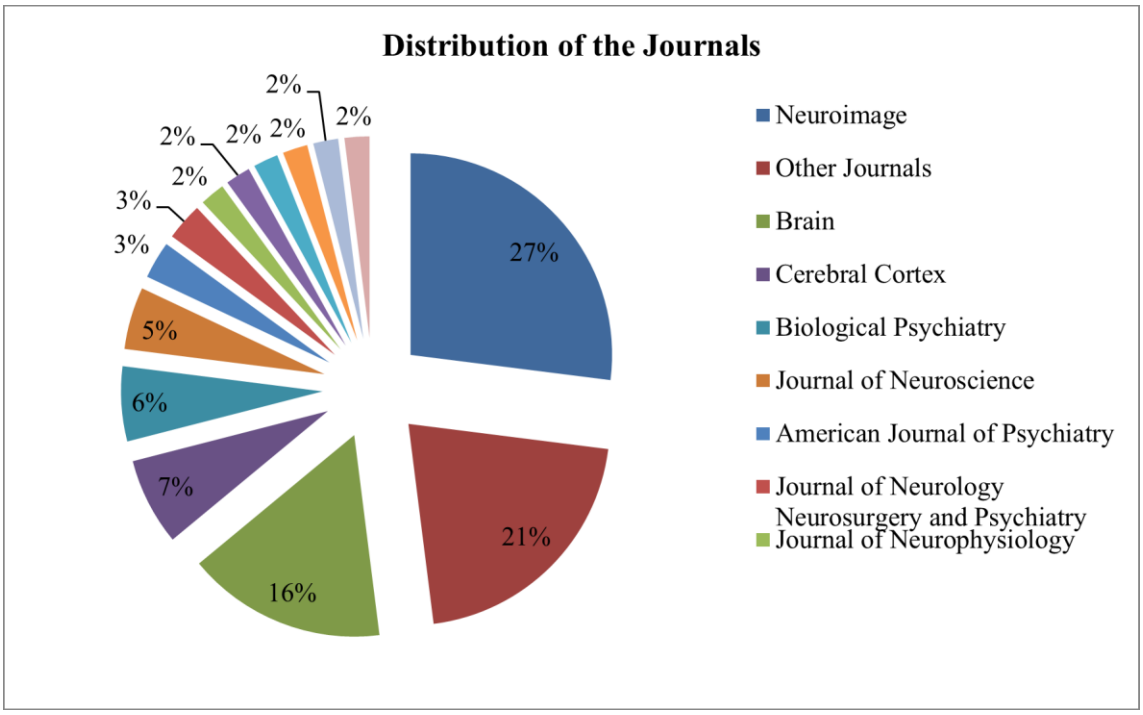

Figure 1. Distributions of the journals

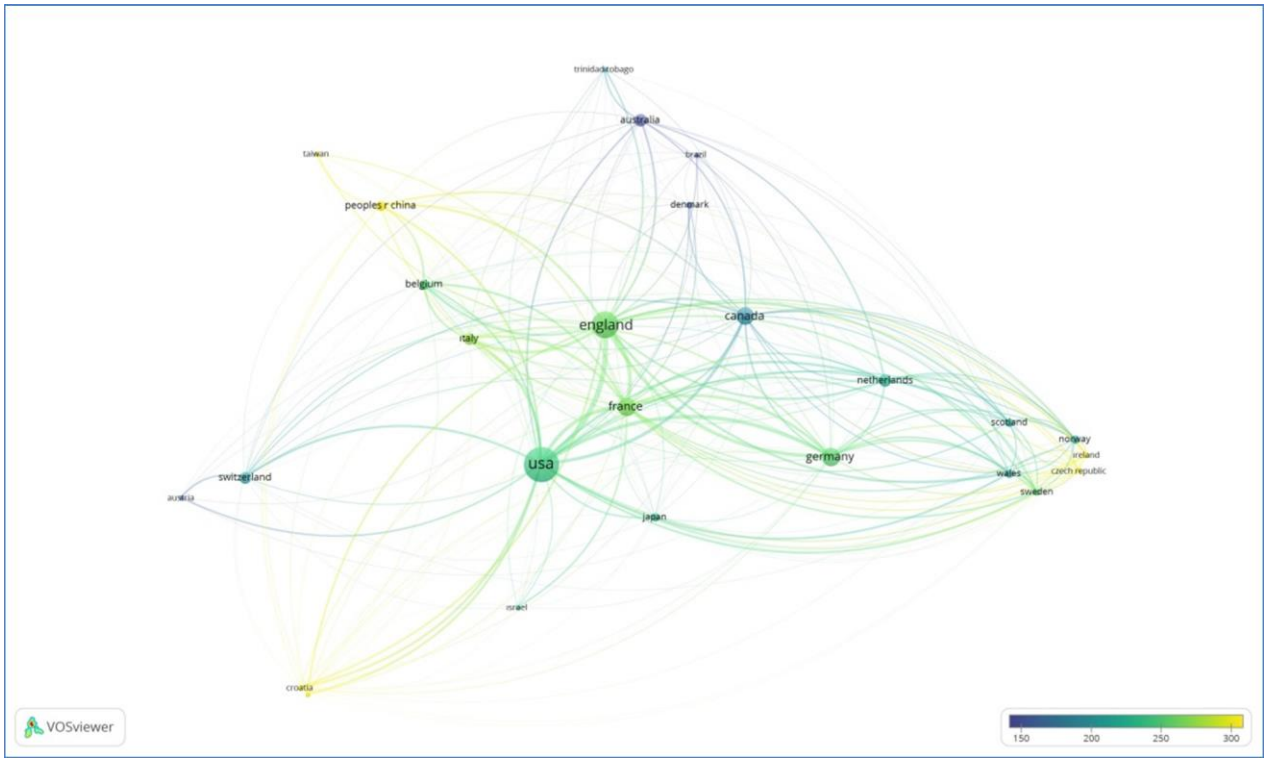

Figure 2. Bibliographic coupling analysis of the countries

http://dx.doi.org/10.36472/msd.v11i3.1141

The number of articles published, the number of citations and the total link strength values of the countries are shown in Table 1. The top three countries with the highest number of publications (taking into account the country of affiliation of all authors) are the USA, England and France. These countries are also the three most cited countries. Taiwan has the highest average number of citations per article (n=573).

Co-authorship Network Analysis

Reporting co-authorships, author citations, and identifying authors who have contributed most to studies on the thalamus aid researchers in staying updated on current developments and understanding the dynamics of the topic.

In this analysis, we excluded studies with more than 25 authors due to the high number of authors and author collaborations. The links in Figure 3 indicate co-authorships or collaborations between authors. A high total link strength indicates a high number of documents that the author has co-authored with other authors. The size of the circles labeled with the authors' names is related to the number of documents of the author. A larger circle indicates that the author is more productive in the related field (23). The top 3 authors with the highest number of collaborations with other authors in the related field are Williams Steven C. R. (TLS=22), Lu Guangming (TLS=20) and Snyder Abraham Z. (TLS=19). Snyder Abraham Z. is also the author with the most documents and the most citations in the related field (Table 2). Table 2 lists the authors with 2 or more documents in the related field in descending order by number of citations.

Tablo 1. Number of documents and citations, and bibliographic coupling details according to the countries

Ranking	Countries	Documents (taking into account the country of affiliation of all authors), n (%)	Documents (taking into account the country of affiliation of all authors), n (%)	Citiation number	The average number of citations per article	TLS
1	USA	46 (27.71)	39 (39)	11110	241,52	4532
2	England	27 (16.27)	18 (18)	6931	256,70	4317
3	France	13 (7.83)	5 (5)	3408	262,15	2788
4	Germany	13 (7.83)	8 (8)	3269	251,46	2056
5	Canada	12 (7.23)	5 (5)	2262	188,50	1591
6	Italy	5 (3.01)	1 (1)	1354	270,80	1231
7	Netherlands	6 (3.61)	2 (2)	1335	222,50	1135
8	Wales	3 (1.81)	-	630	210,00	1017
9	Sweden	2 (1.20)	-	490	245,00	935
10	Belgium	4 (2.41)	2 (2)	986	246,50	855
11	People's Republic of China	4 (2.41)	4 (4)	1363	340,75	761
12	Scotland	3 (1.81)	1 (1)	635	211,67	754
13	Norway	3 (1.81)	1 (1)	627	209,00	602
14	Australia	6 (3.61)	5 (5)	935	155,83	591
15	Czech Republic	1 (0.60)	-	308	308,00	570
16	Ireland	1 (0.60)	-	308	308,00	570
17	Croatia	1 (0.60)	-	320	320,00	468
18	Slovenia	1 (0.60)	-	320	320,00	468
19	Switzerland	5 (3.01)	4 (4)	1010	202,00	467
20	Austria	1 (0.60)	5 (5)	165	165,00	216
21	Trinidad Tobago	1 (0.60)	-	196	196,00	207
22	Denmark	2 (1.20)	2 (2)	325	162,50	186
23	Japan	3 (1.81)	1 (1)	675	225,00	153
24	Israel	1 (0.60)	-	232	232,00	147
25	Taiwan	1 (0.60)	-	573	573,00	136
26	Brazil	1 (0.60)	1 (1)	157	157,00	105

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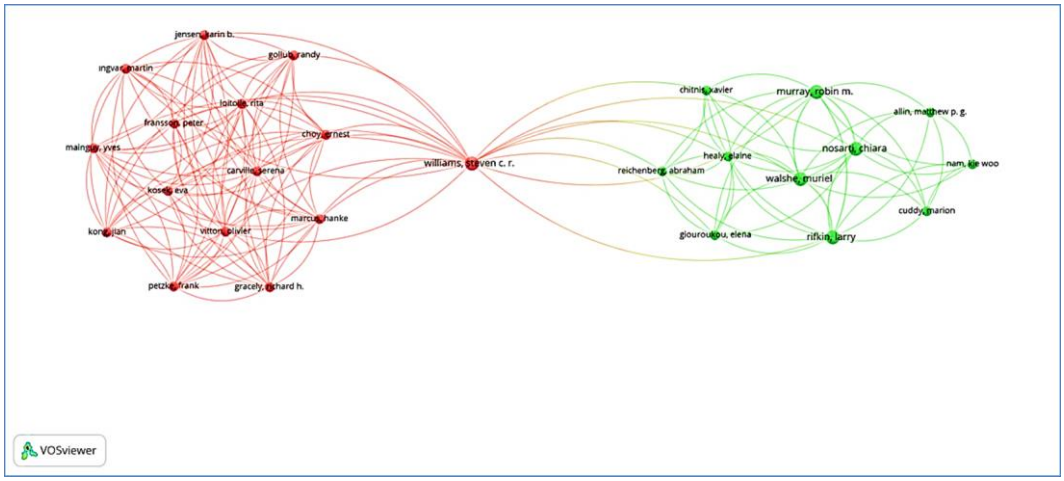

Figure 3. Co-authorship Network Analysis

Tablo 2. List of most cited authors

Ranking	Author	Documents	Citiations	The average number of citations per article	Total link strength
1	Snyder, Abraham Z.	4	1428	357,00	19
2	Shimony, Joshua S.	3	1123	374,33	15
3	Alexander, Daniel C.	2	915	457,50	17
4	Parker, Geoff J. M.	2	915	457,50	17
5	Hammers, Alexander	2	809	404,50	10
6	Rueckert, Daniel	2	809	404,50	10
7	Feng, Jianfeng	2	786	393,00	14
8	Li, Chiang-Shan R.	3	703	234,33	4
9	Lu, Guangming	2	639	319,50	20
10	Fox, Michael D.	2	619	309,50	9
11	Raichle, Marcus E.	2	619	309,50	9
12	Zhang, Dongyang	2	619	309,50	9
13	Schuepbach, Michael	2	573	286,50	18
14	Zhang, Sheng	2	570	285,00	3
15	Brody, Al	2	558	279,00	15
16	Smith, Ec	2	558	279,00	15
17	Calhoun, Vince D.	2	550	275,00	13
18	Pearlson, Godfrey	2	550	275,00	13
19	Butson, Christopher R.	2	545	272,50	4
20	Mcıntyre, Cameron C.	2	545	272,50	4
21	Williams, Steven C. R.	2	496	248,00	22
22	Jeanmonod, Daniel	2	465	232,50	7
23	Murray, Robin M.	2	457	228,50	14
24	Nosarti, Chiara	2	457	228,50	14
25	Rifkin, Larry	2	457	228,50	14
26	Walshe, Muriel	2	457	228,50	14
27	Gustin, Sylvia M.	3	451	150,33	18
28	Henderson, Luke A.	3	451	150,33	18
29	Barker, Gareth J.	2	447	223,50	17
30	Duncan, John S.	2	447	223,50	17
31	Koepp, Matthias J.	2	447	223,50	17
32	Symms, Mark R.	2	447	223,50	17
33	Paulson, Olaf B.	2	325	162,50	11
34	Murray, Greg M.	2	308	154,00	14
35	Peck, Chris C.	2	308	154,00	14
36	Wilcox, Sophie L.	2	308	154,00	14
37	Ide, Jaime S.	2	292	146,00	3
38	Deuschl, Guenther	2	283	141,50	14
39	Herzog, Jan	2	283	141,50	14
40	Volkmann, Jens	2	283	141,50	14

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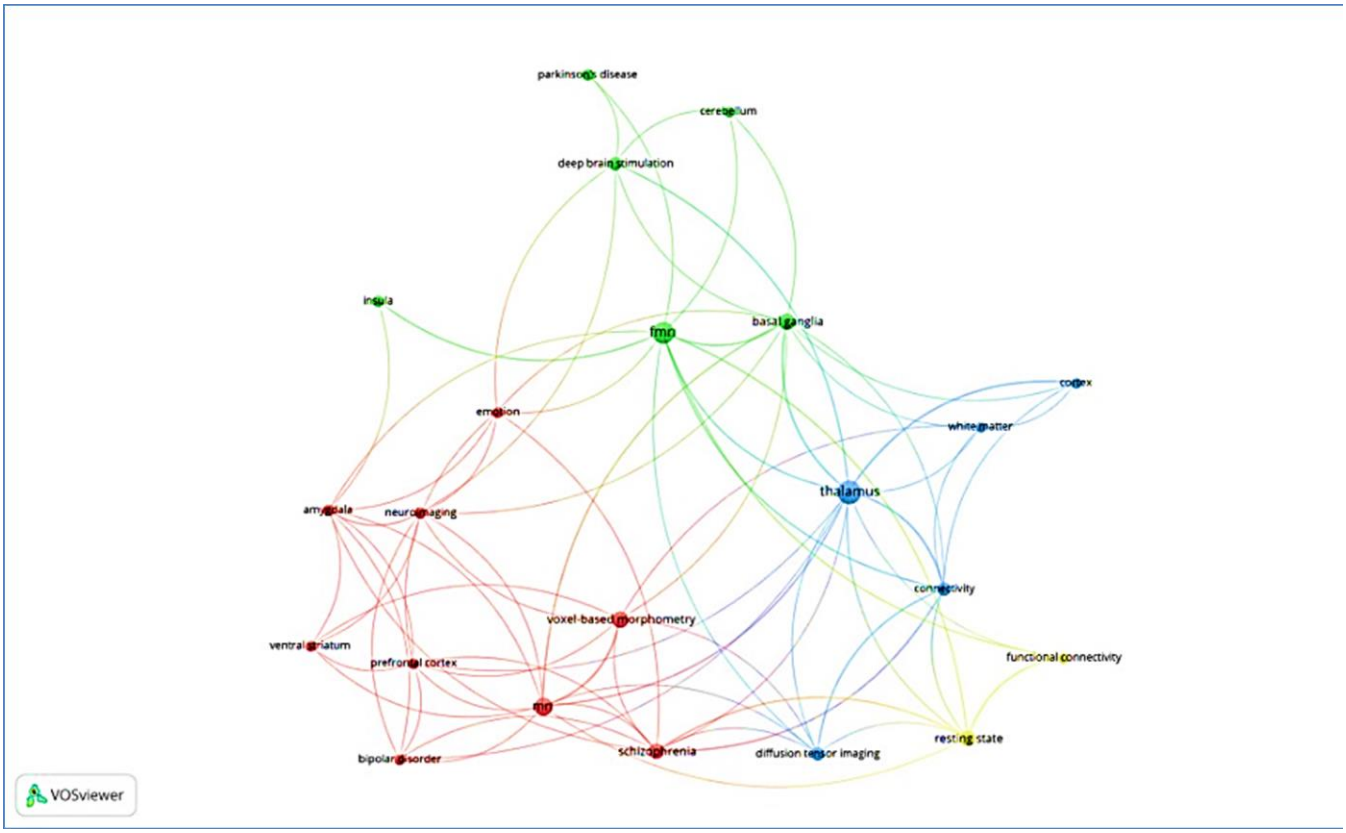
Keyword Mapping Co-Occurrence Analysis

Keywords give an idea about the topic, purpose and method of scientific research (19). This makes it easier for researchers to quickly scan for literature in the related field without having to read the full text of the article. The co-occurrence refers to the number of occurrences of two keywords in the same article. During the analysis, words with the same meaning were combined into a single word (e.g. "magnetic resonance imaging" and "mri"). Keywords with at least 3 co-occurrences were included in the analysis. The size of the circles correlates with the overall frequency of co-occurrence of the keyword. Keywords indicated with larger circles are topics more closely related to the anatomy of the thalamus. The lines between the keywords indicate the strength of the association between the keywords. When analyzing the co-occurrence of keywords, 4 different clusters were obtained. In the green cluster, FMRI (TLS=17), basal ganglia (TLS=15) and deep brain stimulation (TLS=7) are the top 3 keywords by TLS size. The top 3 keywords in the red cluster are MRI (TLS=15), schizophrenia (TLS=12) and amygdala (TLS=10). In the blue cluster, the top 3 keywords with the highest TLS scores are thalamus (TLS=23), connectivity (TLS=14) and cortex (TLS=6). The yellow cluster consists only of the keywords resting state (TLS=13) and functional connectivity (TLS=6) (Figure 4). The green cluster shows the relationship between the thalamus and related anatomical structures; the red cluster shows the connection between the thalamus and psychiatric disorders and mood disorders; the blue cluster shows the relationship between the thalamus and the cerebral cortex; and finally, the yellow cluster shows the function of the thalamus.

Co-citation Network Analysis of the Documents

A co-citation occurs when two different documents are cited by another document. In other words, it is an analysis of the frequency with which a group of publications is cited together in other publications. It is based on the co- occurrence of references in a bibliography. With this analysis, it is possible to identify articles that provide guidance and different perspectives in a field of study (29). This analysis is useful to explore the knowledge base of a particular research group (30). In this study, we used this analysis to identify the most frequent references to documents related to the anatomy of the thalamus in the last 20 years and presented them in a table and visualization. Documents that were cited at least 5 times were included in the analysis (Figure 5). The size of the circles in the figure shows the frequency of co- cited documents, while the connections between the circles represent the relationship between the references. The top 3 studies with the highest total link strength are Fox MD's "The human brain is intrinsically organized into dynamic, anticorrelated functional networks" (31); Behrens TEJ's "Non-invasive mapping of connections between human thalamus and cortex using diffusion imaging" (32) and Biswal B's "Functional connectivity in the motor cortex of resting human brain using echo-planar MRI" (33). The top three most frequently cited studies are "Non-invasive mapping of connections between human thalamus and cortex using diffusion imaging"(32), "Co-planar stereotaxic atlas of the human brain" (34) and "Parallel organization of functionally segregated circuits linking basal ganglia and cortex" (35) studies (Appendix 2).



Şekil 4. Keyword co-occurence of thalamus anatomy documents

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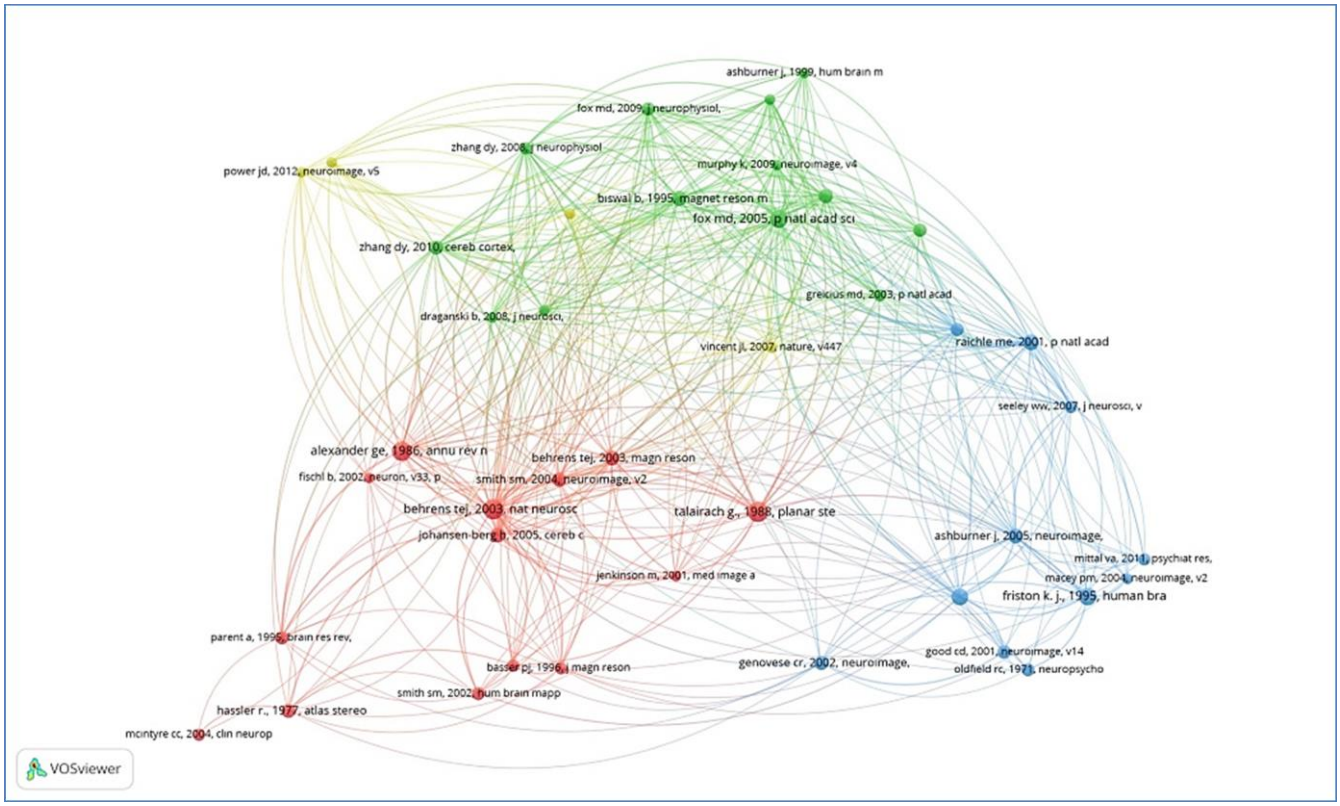

Şekil 5. Highly Cited Documents and Co-citation

DISCUSSION

This study performed a bibliometric analysis of the top 100 most cited articles published between 2004 and 2023 on the thalamus's anatomy. As a result of the study, it was found that the average number of citations of 100 articles was 229.14 and the most cited study was by Heckemann. Most of the most cited articles on the anatomy of the thalamus were published in NeuroImage. The USA is the country with the strongest bibliographic matching. The author with the most collaboration is Williams Steven C. R. In addition, Snyder Abraham Z. is the most productive author in the related field. When analyzing the co-occurrence of keywords, 4 different clusters were obtained. Among the first 100 articles listed, the study with the highest TLS score in terms of co-citations is "The human brain is intrinsically organized into dynamic, anti-correlated functional networks", while the study with the highest number of citations is "Non-invasive mapping of connections between human thalamus and cortex using diffusion imaging".

The most cited paper on the anatomy of the thalamus between 2004 and 2023 presents a procedure for automating anatomical segmentation based on MRI images of the human brain. The study experimentally determines how label propagation and decision fusion can be combined to automate the segmentation of the human brain. Although the procedure described in the study has been described previously, Heckemann's study is the first comprehensive study using data from 30 subjects. The authors also found that the propagation-fusion approach provides consistent segmentations for most macroscopic brain structures, such as the hippocampus, thalamus and orbitofrontal cortex, which will shed light on functional research and the study of disease progression in related brain structures (36).

It is believed that such anatomical modelling studies of the thalamus have attracted more attention from researchers and the number of citations of such studies has increased because they can be used to better understand the structure and functions of the thalamus, to examine different regions of the thalamus and their connections to the cortex, and to investigate diseases in which the thalamus plays a role and develop treatments. Also, the fact that 3 of the top 5 most cited studies (36-38) in this bibliometric study were related to anatomical modelling of the thalamus supports this theory that studies on anatomical modelling of the thalamus are attracting more attention from researchers.

NeuroImage ranks first among the journals in which the most frequently cited studies on the anatomy of the thalamus are published. Brain and Cerebral Cortex follow in 2nd and 3rd place on the list. All three journals have in common that they have the Open Access publishing option and are fundamentally concerned with research on the brain, even if their aims are different (39-41). NeuroImage focuses on neuroimaging studies investigating the relationships between structure- function and brain- behaviour (39). Brain is a journal in the fields of clinical neurology and translational neuroscience with a broad spectrum of topics ranging from studies to elucidate disease mechanisms to new clinical trials for brain disorders (40). Cerebral Cortex publishes articles on the development, organization, plasticity and function of the cerebral cortex, including the hippocampus, as well as on thalamocortical relationships and cortical-subcortical interactions (41).

Brain has the highest impact factor of the three journals. However, most of the most cited articles on the anatomy have been published in NeuroImage because anatomical imaging studies are among the most frequently cited studies.

Looking at the countries in which the top 100 most cited articles in the field of thalamus anatomy were conducted, the countries with the strongest relationship in terms of bibliographic similarity are the USA, England and France respectively. At the same time, these countries have the highest number of citations and publish the most articles in the related field. As the number of studies conducted in the countries has increased, so has the strength of the cross-country research network and the number of citations. Indeed, this result is not surprising given the socio-economic level of the countries concerned and the amount of funding allocated to scientific research in the countries (42).

As a result of the analysis conducted in this study found that Williams Steven C. R., Lu Guangming, and Snyder Abraham Z. were the top 3 authors who collaborated most frequently with other authors. In addition, Snyder Abraham Z. was found to be the most productive and most cited author in the field. Although Williams, Steven C. R. contributed 2 studies on the anatomy of the thalamus, we assume that his collaboration with other authors is high based on his studies with multiple authors. The author has contributed to studies on neurodevelopmental brain differences in adolescents with a history of prematurity and on pain-related functional connectivity in individuals with fibromyalgia (43, 44). Lu, Guangming also has 2 studies on epilepsy (45) and brain network changes in mental illness (46). He also participated in studies with several authors, so the degree of collaboration with other authors is high. Snyder Abraham Z. has 4 documents in the related field. The studies in which the author is involved are on the development of neuronal networks in preterm infants (47), functional relationships between cerebral cortex and thalamus (48), differences in gray matter between smokers and non-smokers (49) and affected brain structures in Parkinson's disease (50). Since he was involved in articles with fewer authors than the other authors, we think that his influence on the collaboration as co-author is less than that of the others. In addition, the highest citation rate may be associated with a larger number of documents.

Co-occurrence analysis of keywords resulted in four different themes: the thalamus and its relationship with related anatomical structures, the connection between the thalamus and psychiatric and mood disorders, the relationship of the thalamus with the cerebral cortex, and the function of the thalamus. All of these topics summarize the topics covered in the most frequently cited publications on the anatomy of the thalamus. The anatomical connections of the thalamus with other brain regions, its association with diseases, and the active multifunctional role of the thalamus in the regulation of all senses except olfaction, motor function, cognitive function, mood and motivation (51) are likely to increase the diversity of topics in the literature on the anatomy of the thalamus. The fact that the functional connectivity of the thalamus is so extensive will contribute to multidisciplinary studies by researchers from different fields.

When reviewing the co-references of the top 100 most cited papers in the field of thalamus anatomy, the study with the highest TLS score is the one that uses fMRI to examine how the human brain is organized at rest. The study shows that the activation and deactivation dichotomy routinely observed in attentional tasks is also present in the resting human brain without the presence of a task or behavior. Furthermore, the study changes the perspective on the functional role of the brain by suggesting that the brain behaves like a sensory autonomic system that responds to changing conditions (31). The fact that such studies investigating the brain's functional networks provide important data for researchers in the fields of anatomy, psychology and psychiatry has strengthened their relationship with other cited studies. In addition, based on the bibliometric data of this study, it was found that the most co-cited document was "Non-invasive mapping of connections between human thalamus and cortex using diffusion imaging". This study by Behrens et al. focuses on the specific connections between the human thalamus and cortex. The study provides the first quantitative evidence of mapping anatomical connections between the grey matter structures of the human thalamus and cortex using diffusion imaging. In addition, this study provides a comprehensive description of the connections between the subregions of the human thalamus and cortex (32). Therefore, it is believed to be of interest for studies on the anatomy of the thalamus.

CONCLUSION

In conclusion, it is recommended that neuroimaging studies may be more popular and multidisciplinary studies in different fields related to the anatomy of the thalamus can be carried out.

Acknowledgements: None

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author Contributions: EH: Designed and directed the study, Literature search, Data collection, Statistics **EH:** Article writing, Final revisions. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval: The present study was conducted in strict accordance with the principles outlined in the Declaration of Helsinki.

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Medical Science and Discovery
ISSN: 2148-6832

Pigmentation of the Tongue, Nails, and Gingiva Following Adriamycin Therapy: A Literature Review and Clinical Insights

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ABSTRACT

Objective: Hyperpigmentation in the mucosa of the tongue and mouth may also occur with the administration of combination chemotherapy containing doxorubicin (Adriamycin). Chemotherapeutic agents may occasionally necessitate discontinuation, either temporarily or permanently, despite the fact that most of these side effects are purely cosmetic and resolve following treatment. The return of nail growth and coloration a few weeks or months after therapy cessation suggests the involvement of chemotherapeutic drugs. Following Adriamycin administration, pigmentation observed in the tongue, nails, and oral mucosa typically diminishes upon discontinuation of the medication without the need for additional treatment. However, careful monitoring is essential to ensure that no alternative explanations are overlooked.

Conclusion: To enhance awareness and facilitate the exchange of experiences regarding the management of this rare side effect, we present four cases of patients who developed nail, oral mucosa, and gingiva pigmentation following combination therapy with Adriamycin and cyclophosphamide in our clinic.

Keywords: adriamycin, hyperpigmentation, chemotherapy, side effects, nail, tongue, oral mucosa

INTRODUCTION

One relatively uncommon cutaneous side effect of chemotherapy drugs is nail toxicity. Despite the fact that most of these side effects are merely cosmetic and resolve following treatment, chemotherapeutic medications may occasionally necessitate discontinuation, either temporarily or permanently (1).

Hyperpigmentation in the mucosa of the tongue and oral mucosa may also occur with the administration of combination chemotherapy containing doxorubicin (Adriamycin).

Chemotherapeutic drugs' antimitotic action readily targets the constantly dividing nail matrix cells and oral mucosal cells (2). The mechanism of pigmentation remains unknown. The return of nail growth and coloration a few weeks or months after therapy cessation suggests the involvement of chemotherapeutic drugs (3).

To enhance awareness and facilitate the exchange of experiences regarding the management of this uncommon side effect, we present four cases of patients who developed nail, oral mucosa, and gingiva pigmentation following combination therapy with Adriamycin and cyclophosphamide in our clinic.

Review Article

Received 27-02-2023

Accepted 17-03-2024

E-Pub: 28-03-2024

Issue Publication: 30-03-2024

http://dx.doi.org/10.36472/msd.v11i3.1137

CASE SERIES

Case 1:

A 37-year-old female patient presented to the hospital with a palpable mass in her left breast. Mammography revealed a 24*19 mm spiculated contoured Birads 5 mass at the periphery of the left breast. Trucut biopsy pathology confirmed invasive ductal carcinoma. In January 2023, she underwent a sentinel lymph node biopsy (SLNB) and partial mastectomy. Pathology results showed a 3 cm tumor, CerbB-2 negative, 80% (2+) estrogen receptor (ER), 35% Ki67, and 60% (2+) progesterone receptor (PR). Sentinel lymph nodes were reactive. Surgical margins were clear of tumor cells. Adjuvant chemotherapy was initiated due to the elevated Ki67 to reduce the risk of recurrence. The patient received four cycles of dose-dense Adriamycin+cyclophosphamide (ddAC) followed by four cycles of dose-dense paclitaxel. During her visit for the third course of ddAC, the patient presented with small, dark-colored macules on the edges of her tongue, dark pigmentation in her upper and lower gums, and bilateral nail bed pigmentation on her fingers and toes (Figure 1). Blood pressure and vital signs were normal, and biochemical measurements did not show any abnormalities. Evaluation for adrenal insufficiency revealed normal cortisol and ACTH levels, and thyroid function tests were within normal limits. Apart from chemotherapy, no other medications were implicated in the onset of these symptoms. Treatment continued under the assumption that the lesions were related to Adriamycin administration.

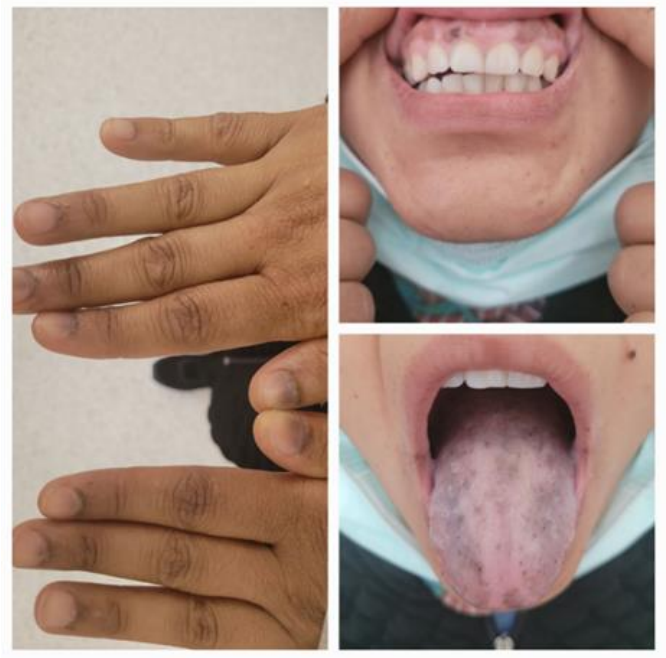

Figure 1. Figure 1. Pigmentation of tongue, upper and lower gingiva and upper extremity nails (case 1)

Case 2:

A 43-year-old woman presented to the hospital with complaints of hardness in her right breast. Mammography revealed hypermetabolic metastatic lymph nodes in the right axillary area and a soft tissue lesion in the upper inner-upper outer quadrant of the right breast, extending throughout the breast's parenchyma without a clear nodular pattern. Breast trucut biopsy results showed 20% Ki67, 80% PR (3+), 90% ER (3+), and CerbB-2 negative invasive ductal carcinoma. Malignant cytology was confirmed from a fine needle aspiration biopsy (FNAB) performed on the right axillary lymph node. The patient was scheduled to receive neoadjuvant chemotherapy consisting of 4 cycles of ddAC and 12 weeks of paclitaxel. Upon arrival for the fourth cycle of ddAC, black discoloration localized in the nail beds of the patient's bilateral fingernails was observed (Figure 2). No visible pigmentation was noted in any other area of her body. Biochemical tests were within normal limits, and vital signs were normal. Doxorubicin was considered the primary cause of the pigmentation. Her medical management continued accordingly.

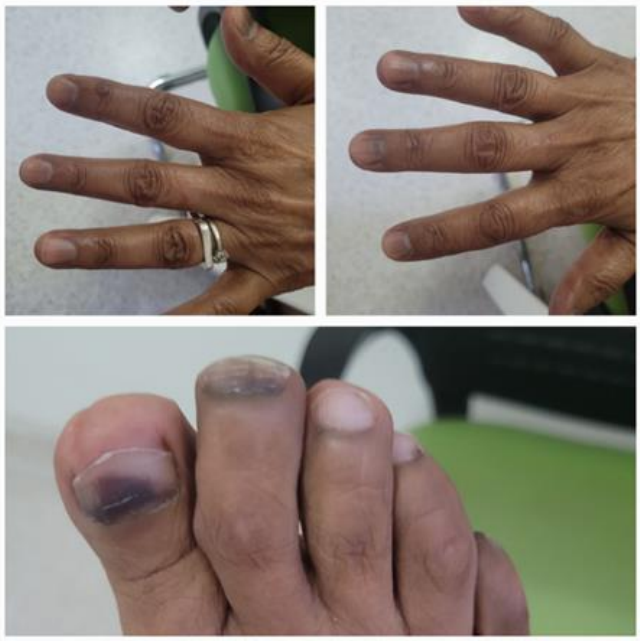

Figure 2. Black pigmentation on bilateral lower and upper extremity nails (case 2)

Case 3:

A 72-year-old female patient presented with a palpable lump in her left breast. Mammography revealed a 16 mm mass lesion with irregular outlines in the upper outer quadrant of the left breast. No lymphadenopathy was observed in the axillary region on breast ultrasonography. Trucut biopsy confirmed invasive ductal carcinoma along with ductal carcinoma in situ; PR was 100% (3+), Ki67 was 15%, ER was 100% (3+), and CerbB-2 was negative. In May 2023, the patient underwent partial mastectomy and SLNB. Histological analysis revealed hormone-positive, HER2-negative invasive ductal carcinoma and one metastasis to an axillary nonsentinel lymph node. Adjuvant chemotherapy was initiated, consisting of 4 cycles of ddAC followed by 12 weeks of paclitaxel. Brown-black pigmentation was observed in the bilateral fingernail beds during the fourth cycle of ddAC (Figure 3). No pigmentation was noted in other parts of her body. Vital signs and blood biochemistry parameters were normal. Paclitaxel therapy was continued. The pigmentation resolved upon completion of treatment, as confirmed during follow-up appointments.

http://dx.doi.org/10.36472/msd.v11i3.1137

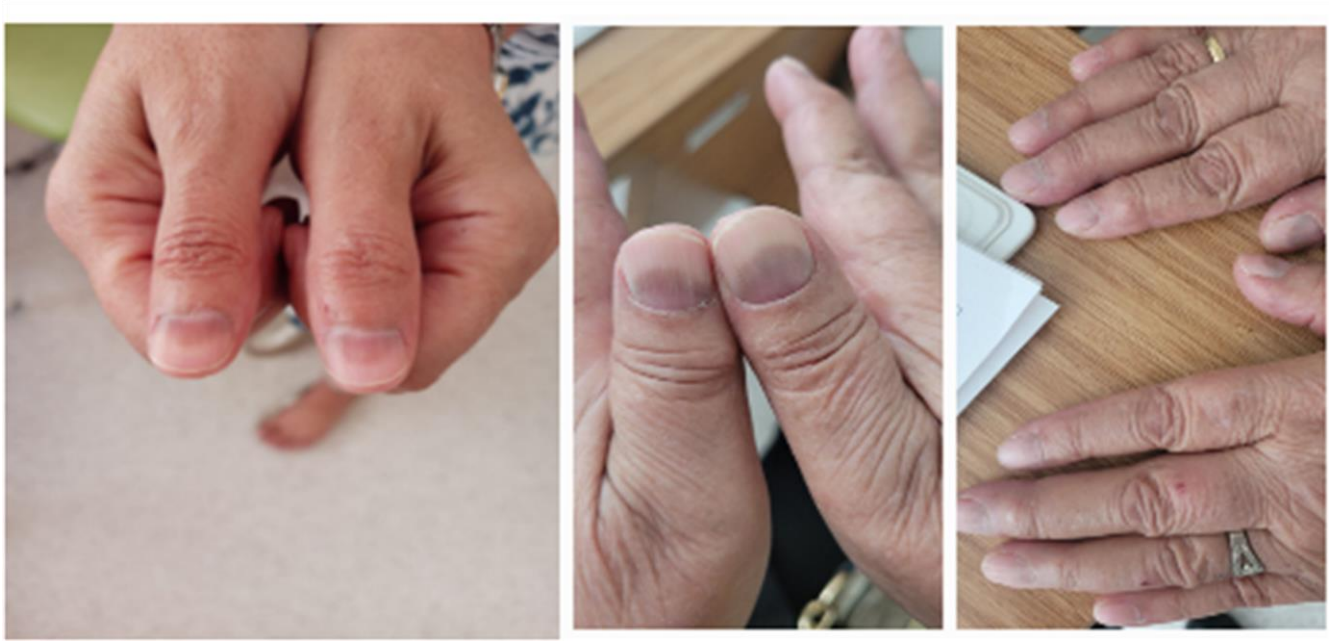

Figure 3. Brown-black discoloration of bilateral upper extremity nail beds (case 3); Black pigmentation of bilateral upper extremity nail beds (case 4)

Case 4:

A 73-year-old woman presented after discovering a lump in her right breast. Mammography revealed numerous pathological lymphadenopathies in the right axilla and an irregularly bounded nodular opacity, measuring 18 mm, in the upper middle quadrant of the right breast. Breast tru-cut biopsy confirmed glycogen-rich invasive apocrine carcinoma; CerbB-2, ER, and PR were negative, while Ki67 was 20%. Fine needle aspiration biopsy of the right axillary lymph node showed benign cytology. PET/CT showed no evidence of distant metastases. In July 2023, the patient underwent right partial mastectomy and SLNB. Pathology revealed grade 2 invasive ductal carcinoma plus ductal carcinoma in situ (high grade), measuring 15 mm, with extranodal dissemination (right sentinel lymph node sample) and carcinoma metastases in 2 out of 5 lymph nodes; ER, PR, and CerbB2 were 10% (2+), and Ki67 was 25%. HER2 FISH tests yielded negative results. Adjuvant chemotherapy consisting of 4 cycles of ddAC followed by paclitaxel for 12 weeks was scheduled for the patient with T1N1M0. By the end of the fourth treatment visit, intense, nearly black pigmentation was observed in all nail beds, particularly in the bilateral thumb nails (Figure 4). No pigmentation was noted in any other area of the patient's body. Vital signs and biochemical markers remained stable. Therapy was continued accordingly.

DISCUSSION

Breast cancer stands as the most prevalent illness affecting women globally, a multifactorial condition influenced by both genetic and environmental factors, and remains the leading cause of cancer-related mortality (1). Adriamycin and cyclophosphamide combination are commonly employed chemotherapeutic agents in neoadjuvant, adjuvant, and metastatic stages of breast cancer treatment (4, 5). Nail abnormalities induced by medications can manifest a wide array of clinical symptoms, many of which are dose-dependent and resolve upon discontinuation of the drug (6).

Nail changes and hyperpigmentation have been documented following cytotoxic chemotherapy (such as doxorubicin, cyclophosphamide, fluorouracil, docetaxel) and tyrosine kinase inhibitors like gefitinib. Additionally, hyperpigmentation can manifest in the mucosa and tongue (7).

While some nail modifications may cause discomfort and affect appearance, others can be painful and impede daily activities or mobility (8). Certain side effects, apart from purely cosmetic ones, may necessitate alterations in chemotherapy regimens.

In a study involving 205 patients, diffuse nail hyperpigmentation emerged as the most common side effect with the combination of cyclophosphamide and adriamycin (1). A prospective cohort study evaluating the toxicity profile of 146 breast cancer patients receiving paclitaxel following ddAC revealed skin hyperpigmentation in 96.6% of the patients (9). The FAC and AC-P regimens are widely utilized in breast carcinoma treatment. A comparative study showed hyperpigmentation development in 49 patients receiving the FAC regimen compared to only one patient on the AC-P regimen (10). Taxanes and anthracyclines therapy, such as doxorubicin, may result in painful onycholysis and subungual abscesses in the skin (11). Although the exact pathophysiology remains unclear, several chemotherapy drugs, including doxorubicin, may elevate melanocyte-stimulating hormone (MSH) levels, potentially explaining why this side effect is more prevalent in individuals with darker skin tones (12). Pigmentation observed in the tongue, nails, and oral mucosa typically regresses following cessation of Adriamycin without requiring additional treatment (6). Numerous case reports and case series have documented this type of hyperpigmentation, which tends to resolve weeks to months after discontinuation of doxorubicin (13). However, vigilant observation is essential to ensure that alternative explanations are not overlooked.

CONCLUSION

Recognized side effects of anticancer drugs may necessitate dose adjustments or discontinuation, potentially compromising their efficacy. Hence, ongoing research endeavors should explore novel treatments or combinations that can deliver high response rates while mitigating the frequency and severity of side effects.

Acknowledgements: None

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author Contributions: AG, OO, FPA, BK, EG: Designed and directed the study, Literature search, Data collection, Statistics **AG:** Article writing, Final revisions. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval: The present study was conducted in strict accordance with the principles outlined in the Declaration of Helsinki. Ethical approval for the study was obtained from the appropriate ethics committee. Informed consent was obtained from all participants of this study.

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Medical Science & Discovery

International Journal of
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Open Access Scientific Journal
ISSN: 2148-6832
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