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Address: Gaziantep University, School of Medicine, Journal Office, 27310 Şehitkamil, Gaziantep, Turkey
Phone: +90 342 3606060/77751
Fax: +90 342 3601617
E-mail: info@eurjther.com

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Address: Büyükdere Cad., 105/9 34394 Mecidiyeköy, Şişli, İstanbul, Turkey
Phone: +90 212 217 17 00
Fax: +90 212 217 22 92
E-mail: info@avesyayincilik.com
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# Instructions to Authors

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Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.
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Address: Gaziantep University, School of Medicine, Journal Office, 27310 Şehitkamil, Gaziantep, Turkey
Phone: +90 342 3606060 / 77751
Fax: +90 342 3601617
E-mail: info@eurjther.com

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# Contents

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Review

# The Assessment of microRNA Role in Kidney Transplantation

Aslı Özkızılcık Koçyiğit[1,2], Mustafa Soyöz[1,2], Tülay Kılıçaslan Ayna[1,2]

[1]Department of Medical Biology and Genetics, İzmir Katip Çelebi University School of Health Sciences, İzmir, Turkey

[2]Tissue Typing Laboratory, İzmir Tepecik Training and Research Hospital, İzmir, Turkey

**ABSTRACT**

miRNAs are short, single stranded, noncoding RNA molecules with approximately 22 nucleotides. They play crucial roles in post-transcriptional regulation. To date, more than 21,000 mature miRNAs have been discovered from 193 species. miRNAs are important in renal physiology. Their expressions may be specific to different organs and tissues, or the same miRNAs may be expressed in different organs. With this discovery, it has been reported that tissue-specific miRNAs can be used as biomarkers for allograft rejection and ischemia reperfusion injury following renal transplantations. Although allograft biopsy is accepted as a gold standard, scientists have been developing novel approaches to prevent the associated risks, such as bleeding and infection, following biopsy. Recently, several attempts have been made in terms of the use of urine as a noninvasive material instead of biopsy samples. However, no procedure is available for routine testing. Therefore, further investigations are warranted.

**Keywords:** Kidney, rejection, graft, microRNAs

## INTRODUCTION

MicroRNA (miRNA) is a short (~22 nucleotides), noncoding, and single-stranded RNA molecule that plays a significant role during posttranscriptional regulation in various physiological processes ranging from development to oncogenesis. It was first discovered in 1993 from *Caenorhabditis elegans* as "miRNA lin-4." More than 21,000 mature miRNAs from a total of 193 species have been identified till date. miRNAs play critical roles in cell proliferation, apoptosis, lipid metabolism, neuronal process, hematopoietic differentiation, and immunity (1, 2). However, its role in the regulation of plant and animal gene expression was unknown until a decade ago. Previous studies found that this molecule acts as a repressor for a number of genes (2, 3).

The first miRNA database included only 218 miRNA loci. However, the number increased to >21,264 in plants and animals owing to the developments in miRNA expression profiling techniques (3). According to recent study, 2588 mature miRNAs belonging to Homo sapiens are found in the miRBase database (http://www.mirbase.org/cgi-bin/browse.pl?org=hsa, 26.12.2016).

miRNAs are synthesized from primary miRNAs (pri-miRNAs) in animals. This synthesis occurs in two steps, and two RNase III-type proteins, namely "Drosha" and "Dicer," play valuable roles in the nucleus and cytoplasm during this process, respectively. Pri-miRNA is transcribed from miRNA genes through RNA polymerase. This pri-miRNA is spliced to precursor miRNA (pre-miRNA) by Drosha in the nucleus. Thereafter, pre-miRNA is transported to the cytoplasm with the help of transport proteins. In the cytoplasm, it is spliced by Dicer protein, which is loaded on Argonaut (Ago) proteins (generating effector RNA-induced silencing complex (RISC)), and mature miRNA is generated (2).

In miRNA expression studies, there are some miRNAs that have been reported to be expressed, particularly, in adult kidneys (miR-215, miR-146a, and miR-886). Additionally, it has been shown that some of the other miRNAs, such as miR-192, miR-194, and miR-21 have been expressed in the kidneys and other organs. miRNAs play a number of crucial roles in the physiology of the kidneys. Unusual miRNA splicing in the ureteric bud leads to enormous cell proliferation and apoptosis. Nevertheless, ciliogenesis is destroyed on the ureteric bud epithelium, and, as a result, renal cysts are produced. In addition, miRNAs have critical roles in podocyte homeostasis. They have unique roles in a number of processes, including blood pressure control, body fluid, and electrolyte homeostasis (4).

In this review, the effects of miRNAs on immune response to allograft after kidney transplantation were evaluated.

**ORCID IDs of the authors:** A.Ö.K. 0000-0002-0015-7070; T.K.A. 0000-0001-7993-978X; M.S. 0000-0001-5159-6463.

**Corresponding Author:** Aslı Özkızılcık Koçyiğit **E-mail:** asli.ozkizilcik@gmail.com

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## CLINICAL AND RESEARCH CONSEQUENCES

**miRNA Synthesis in Humans**

All animal miRNAs are first processed in the nucleus followed by their transcription by RNA polymerase II. The transcript is called pri-miRNA. After the hairpin structure of pri-miRNA is cleaved, the released hairpin structure (60-70 nucleotides) is called pre-miRNA. A protein "Drosha" and its cofactor protein DGCR8 (DiGeorge syndrome critical region on the eighth gene) are involved in the process during this step. Pri-miRNAs contain 33 base-paired (bp) stem, loop, and single-stranded segments. DGCR8 protein interacts with these single-stranded segments and leads to Drosha to cut pri-miRNA (Figure 1) (2, 5).

Pre-miRNAs are transported to the cytoplasm for further processing. Transport occurs through nuclear pore complexes (large channels) with the help of RanGTP-dependent nuclear transport receptor exportin-5 (EXP5) protein. It was reported that transport begins when EXP5 recognizes a double-stranded RNA loop structure (14 bp) followed by binding of Ran protein (GTP-bound cofactor). Then, pre-miRNA-EXP5 complex is transported to the cytoplasm, and pre-miRNA is released by GTP hydrolysis (Figure 1) (2, 6).

In the cytoplasm, pre-miRNA is processed by Dicer protein (endonuclease cytoplasmic RNase III enzyme), and mature miRNA is produced. Dicer protein cuts miRNA (approximately 22 nucleotides), and this miRNA molecule combines with Ago protein complex. Generally, one of the strands of miRNA is destroyed, whereas the other strand with Ago protein complex becomes a mature miRNA, which is called RISC (Figure 1) (2, 3, 6).

After mature miRNAs are produced, they are ready to be transported to the regions where they function. For instance, if a miRNA plays a role in the nucleus (i.e., miR-29b), then it is transported back into the nucleus (6). On the other hand, if its specific function is in the cell of another distant tissue, it is carried through plasma-derived vesicles, such as exosome and microparticles in the bloodstream. Previous studies on miRNA transport showed that they are carried by exosomes. However, subsequent studies reported that microparticles also play roles in cell communication, which is based on miRNAs (7). In addition, various studies have revealed that high-density lipoprotein (HDL) and low-density lipoprotein (LDL) help miRNA transport in plasma (7, 8). It was indicated that some miRNAs are carried by exosome, HDL, and LDL, whereas others are transported by only one of them (7). miRNAs may be found in peripheral blood cells (miR-142-5p, miR-155, and miR-223), plasma (miR16, miR-210, miR21, miR-155, and vb), and/or urine (miR-10a, miR-10b, and miR-210) (9).

**Detection of miRNA Expression Profiles**

Detection of miRNA expression profiles may help to identify miRNAs. miRNAs are well preserved in plasma, serum, urine, and formalin-fixed tissue blocks and can be measured more sensitively than proteins. Thus, they may be used as a biomarker in various molecular diagnosis applications, such as cancer, cardiovascular, and autoimmune diseases (10). They can be isolated from cells, tissues, and body fluids (plasma, serum, and urine), and their expressions can be analyzed by RNA sequencing, quantitative real-time polymerase chain reaction, or microarray applications (Figure 2). miRNA profiling studies may provide benefits in the discovery of novel miRNAs, analysis of miRNA-mRNA, and miRNA-protein associations.

**Role of miRNA in Solid Organ Transplantations**

The alterations in miRNA expressions in allograft or serum of the recipient during acute or chronic rejection episodes have been studied after the findings of the role of miRNA in immune cell development and activities. In addition, its diagnostic and prognostic biomarker features have been investigated to estimate the status of allograft and to develop individual-specific immunosuppressive drug strategy (11).

Figure 1. miRNA synthesis steps

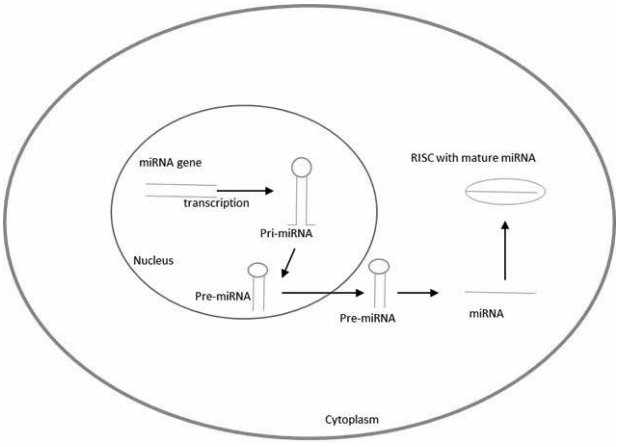
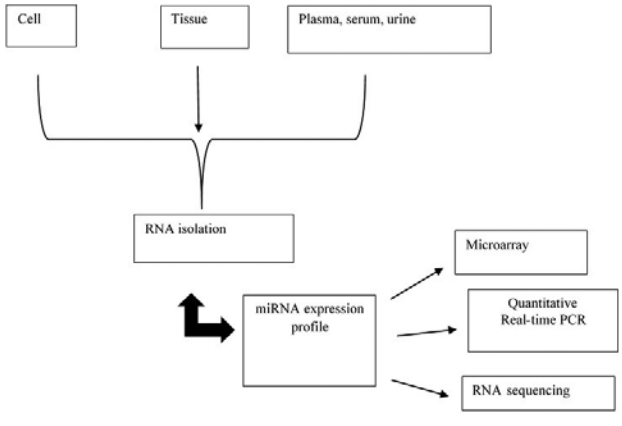



Figure 2. miRNA profile study pathway. RNA, ribonucleic acid; PCR, polymerase chain reaction



Dendritic cells, which have significant roles in transplant immunity, present alloantigens to T lymphocytes and trigger immune rejection. In addition, they function as regulators of immune reactions. miRNAs regulating dendritic cell activation were discovered in a previous study. For example, it was observed that miR-155 expression increases during the maturation of these cells. Moreover, it was shown that dendritic cells fail in antigen presenting and cannot activate T cells effectively owing to costimulatory function in miR-155 deficiency (11, 12).

The most frequently observed activity in allograft rejection is T cell-mediated reaction. It was reported that seven miRNAs are most commonly expressed in CD8+ T cell subgroup, and it was assumed that these miRNAs may regulate the genes that direct cytotoxic T cell functions. miRNA expression profile is also altered during CD4+ T cell activation. It was presented that miR-181a regulates T cell activation by altering T cell receptor signal strength and cut-off value. miR-155 also functions in T cell response by playing a crucial role in the regulation of T helper 1/2 cell differentiation. T-regulator cells can control a number of immune responses and may function in the prevention of rejection. It was observed that there are miRNAs (i.e., miR-155) playing crucial roles in T-regulator cell activation (11).

B cells are also important defenders in graft rejection. It was reported that graft rejection is associated with, particularly, CD20+ B cells; however, the mechanism has not been clearly elucidated yet (13). miR-155 is the well-studied miRNA in B cell response, and it was assumed that germinal center development, immunoglobulin class switching, and antibody production may be affected by miR-155 (11).

Major histocompatibility complex class I-related gene A (MICA) and gene B (MICB) are ligands for the receptors on natural killer and CD8+ T cell surfaces. MICA and MICB are expressed by the cells under stress because of infection or inflammation. It was reported that miRNAs that downregulate MICA and MICB expressions are found, and these miRNAs play roles in MIC-related rejections (11).

Researchers have been studying miRNAs as rejection-related biomarkers as well as therapeutic targets in various solid organ transplantations (e.g., heart, small intestine, and kidneys). Allograft quality is important for graft survival in organ transplants. There have been a number of studies associated with graft quality, ischemia-reperfusion injury, and miRNA expressions. It was reported that alterations in miRNA expression might be used for ischemia-reperfusion injury. The results revealed that there are lymphocyte-independent alterations in miRNA expression profiles of ischemia-reperfusion injury (14, 15).

Scian et al. (16) assessed miRNA expressions related to chronic allograft dysfunction in urine samples in parallel to allograft tissues. Genes showed different expression levels of miR-142-3p, miR-204, miR-107, miR-211, and miR-32 in tissue and urine. These findings suggest that miRNAs can be used as a noninvasive marker for chronic allograft dysfunction.

**Role of miRNA in Kidney Transplantation**

Kidney transplantation is the only treatment for end-stage renal failure. Human leukocyte antigen compatibility is critical for transplantation, and the recipients with more mismatches may produce de novo antibodies against donor graft because of alloimmunizations (pregnancy, blood transfusion, and previous transplants) and result in graft rejection (17). There are various methods to detect the antibodies during/after rejection, such as complement-dependent cytotoxicity crossmatch and solid-phase assays (e.g., flow cytometric crossmatch, Luminex crossmatch, and panel reactive antibody screening) (18, 19). However, early prediction of rejections is very important to prevent graft failure.

Ischemia-reperfusion injury is an inevitable result of kidney transplantation. Ischemia results in ATP deficiency, ion gradient failure, cell swelling, and increase in toxic products because of nutrition and oxygen failure. Ischemia-reperfusion injury is also associated with acute and chronic rejections (20). Godwin et al. (21) reported that miR-21, miR-20a, miR-146a, miR-199a-3p, miR-214, miR-192, miR-187, miR-805, and miR-194 are expressed differently in ischemia-reperfusion injury; therefore, they could be used as biomarkers of this injury. Currently, miRNAs, which can be used in ischemia-reperfusion injury, are not used in routine methods. Research is ongoing to address this issue.

miRNAs play important roles in immune processes, including T and B cell differentiation, cytokine production, T cell and Toll-like receptor signalization, and antigen processing and presenting. In studies on acute rejection, it was reported that there are differently expressed miRNAs (let-7c, miR-10a, miR-10b, miR-125a, miR-200a, miR-30a-3p, miR-30b, miR30c, miR30e-3p, and miR-32) in biopsy samples. Some of these miRNAs were upregulated, whereas others were downregulated (22).

It was also highlighted that miRNAs that are expressed differently in renal biopsy samples in chronic allograft dysfunction (chronic rejection) are miR-142-3p, miR-32, miR-204, miR-107, and miR-211 (16).

Two parameters have been analyzed in serum to determine renal graft status after kidney transplantation. One of these parameters is serum creatinine, which determines glomerular filtration rate, whereas the other one is proteinuria, which identifies if there is an injury in glomerular filtration barrier. However, to identify these parameters, the injury should progress gradually. Under this condition, biopsy, which is the golden standard method, is used; however, this method has approximately 3% risk for patients. Thus, recent studies have been performed to analyze miRNAs as biomarkers in a noninvasive sample "urine" (23). Maluf et al. (24) assessed miRNA expressions in 191 samples.

They found that 22 of 1733 mature miRNAs, which were tested by microarray analyses, were expressed differently between the groups. They reported that some of these miRNAs might be used as biomarkers because they could be detected at an early stage after kidney transplantation before the histological progression of allograft injury.

Discovery of miRNAs was a milestone in various areas, such as system biology, immunology, and cellular biology. miRNAs play crucial roles as regulators in various cellular functions ranging from cell development to apoptosis.

miRNAs have also been analyzed in solid organ transplantations. It has been shown that miRNAs may be associated with allograft rejection, ischemia-reperfusion injury, and fibrosis after kidney and other solid organ transplantations. Some miRNAs may be expressed in tissues of several organs, such as the kidneys, liver, and lung. However, the important issue in miRNA studies is its tissue and/or body fluid specificity. In kidney transplantations, the specificity of miRNAs to kidney tissue is crucial, and with the discovery of the specific miRNAs, the idea of using miRNA as a biomarker has emerged owing to their stability and resistant structure. Currently, biopsy samples are collected to analyze tissue-specific miRNAs; however, this method has some risks, such as bleeding and infection. Therefore, miRNAs have been studied as noninvasive biomarkers. Further studies have suggested that urine may be used as a noninvasive material to assess graft rejections at an early stage before kidney injury. There are studies indicating thatmiRNA expressions are compatible in urine. The studies are usually performed by evaluating all patient groups. However, results may not be representative of all patients when we consider that their lifestyles, diseases, and habits are different from each other. Thus, the evaluation of miRNA profiles individually may be useful particularly in studies dealing with transplantation.

## CONCLUSION

The role of miRNAs in kidney transplantations is discussed in the current study. Although there have been several studies conducted regarding this subject, the effective mechanisms of miRNA and their role in cell communication have not been completely understood yet. Thus, the use of miRNAs as biomarkers is not yet included in the routine diagnosis. Further investigations with broad range should be performed.

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Systematic Review

# Evaluation of the Effectiveness of Web-based Intervention for Patients with Breast Cancer

Birgül Özkan[1], Reyhan Eskiyurt[1], Dilek Öztaş[2]

[1]Department of Nursing, Ankara Yıldırım Beyazıt University School of Health Science, Ankara, Turkey

[2]Department of Public Health, Ankara Yıldırım Beyazıt University School of Medicine, Ankara, Turkey

**ABSTRACT**

**T**he study was conducted to define web-based patient education programs and to evaluate the effectiveness of a web-based intervention for patients with breast cancer. In this systematic review, we searched the Web of Science, Wiley Online Library, PubMed, and MEDLINE electronic databases for abstracts using the keywords "breast cancer," "web-based intervention," "online intervention," and "web-based intervention." There was no date limitation in the study, and the reviews were completed in December 2016. We identified 15 articles within the scope of this review. Web-based intervention included social support, exercise and dietary practices, self-help guides, self-management, and symptom management, which were detected to have a positive effect on quality of life, fatigue, pain, self-sufficiency, depression, and stress management. In conclusion, this systematic review showed that web-based interventions are effective in psychosocial treatment and care process and that they are eligible for use in this area.

**Keywords:** Breast cancer, web-based intervention, patient, psychoeducation

## INTRODUCTION

Breast cancer, the most frequently diagnosed cancer in women, has been identified as the second leading cause of deaths from cancer in women. More than a million women are annually diagnosed with breast cancer (1, 2).The survival rate is increasing; however, patients may have to deal with not only the disease itself but also the adverse effects of treatments (3). In this context, the internet can be a way to overcome the problems experienced. Individuals with the disease can refer to the internet for health information (4). In the 2011 Pew Report, 80% of online users stated that the internet is an important source of information for them, after physicians (5).

Web-based support groups have several advantages compared with those of face-to-face support groups. First, web-based methods are very flexible. This is because the transfer of information can be synchronous or asynchronous. Second, web-based systems have several convenience features. In comparison with face-to-face interventions, they facilitate access to care services for individuals that cannot join face-to-face groups due to distance, social anxiety, or health problems, and they make scheduling easier. The cost is low because relatively fewer resources are required for the management of a group. On the other hand, the disadvantages are that individuals should know how to use the computer and internet and have good reading and writing skills in the language spoken by the group. It was also suggested that the participant can become more dependent on web-based relationships, resulting in increased social isolation (6). Web-based programs are being developed for the management of adverse effects of the disease to provide informative and emotional support, to help in the planning of cancer treatment care, and to develop psychological and emotional coping abilities. It was observed from previous studies that all web-based programs improve the quality of life, increase social support, decrease anxiety and depression, and encourage patients to participate in health management (7-9).

This systematic collection was compiled to define the web-based patient education programs available for individuals with breast cancer and to assess the efficacy of the intervention.

## METHODS

### Investigation Strategies

Several studies on the evaluation of the effectiveness of physical, psychological, social, and spiritual interventions conducted on individuals with breast cancer were found in the literature. This systematic collection was compiled to define the web-based patient education programs available for patients with breast cancer and to assess the efficacy of the intervention. The electronic databases, including Web of Science, Wiley Online Library, and PubMed, were reviewed for the articles dating up until December 2016 using the keywords "meme kanseri," "web tabanlı," "müdahale," "breast cancer," "web-based intervention," "online intervention," and "Internet-based intervention." The review was divided into five categories: 1. study design (e.g., intervention and

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**ORCID IDs of the authors:** R.E. 0000-0003-0421-4914; D.Ö. 0000-0002-8687-7238.

**Corresponding Author:** Reyhan Eskiyurt **E-mail:** reyhan.ryhn.reyhan@gmail.com

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psychoeducation), 2.thefocus of the intervention (e.g., to provide information or social support or to improve the quality of life), 3.thetype of cancer (e.g., breast cancer), 4.patients or survivors, and5. Web-based or online access management.

Inclusion criteria were;
1. Written in English or Turkish,
2. Published as a full-text,
3. Samplecomprising individuals with breast cancer, and
4. The application of web-based intervention.

Exclusion criteria comprised periodicals and unreported intervention results. In total, 15 studies were reviewed for the purpose of this systematic collection.

## RESULTS

**Properties of Web-based Interventions**

The web-based "cancer management" programs are designed to improve the health-related quality of life. In this context, it was seen that web-based interventions are used to improve the management of the adverse effects of the disease;toprovide emotional, informational, and social support; and to develop coping abilities (7, 8).

**Web-based Self-control Intervention Programs**

Web-based self-control intervention programs are designed to change daily diet and exercise behaviors. These programs can provide an alternative method effective in terms of weekly exercises, improving the daily intake of vegetables and fruits, diet quality, physical functionality, and avoiding the loss of appetite and fatigue (10). A web-based program (WSEDI, web-based exercise and diet intervention, self-control program) comprises evaluation, education, action plan, and automatic feedback sections (10). The educational content in diet and exercise intervention programs comprises the information aimed at improving the diet and exercise behaviors of patients, highlighting the importance of maintaining a normal weight, addressing obstacles in exercise and diet behaviors, providing exercise and diet guidelines for patients with cancer, and describing the effects of regular exercise and balanced diet on the quality of life (10). The Telerehabilitation program developed by Galiano-Castillo et al. (11) comprises general and special access interfaces (e-CUIDATE). The general section is the main page of the website and includes the latest information about breast cancer, whereas the special access section can be accessed with a username and password. The exercise program comprises three sessions, each of which lasts for a maximum of 90 min/day, and includes 1) warm-up, 2) resistance/aerobic exercises, training, and 3) cooling down. The web-based system allows participants to send a message and log in to video chat sessions (11).

Lately, most women are living with breast cancer as a chronic disease and are exposed to drug treatments. After breast cancer treatment, women with the disease can switch from being patients to being survivors. During this switch-over process, these women may face various issues regarding physical and emotional improvement, including their return to work and the fear of disease relapse (12). In this scope, BREATH (Breast cancer e-health), a web-based self-control intervention, was developed to enable women to psychologically adapt to the primary treatment by reducing stress and increasing their strength. Communication with an advisor is not available in the Self-help Program, which is the continuation of BREATH Intervention comprising cognitive-behavioral therapy modules. New materials will be sent weekly by a standard e-mail reminder so that psychological support previously obtained can be sustained. The elements of the intervention are based on cognitive-behavioral therapy and comprise homework, evaluation, and video sections (13). WebChoice, created to develop self-control in individuals with cancer, is a disease management support system based on patient-oriented principles and includes evaluation, recommendations, information, and contact sections. Patients can observe their symptoms and problems and can share their experiences through the system. Internet-based patient-provider communication is a program wherein patients can ask questions to oncology nurses, obtain a recommendation, and share their experiences (14). Psychological and cognitive properties of the patients have been determined through interactive empowerment intervention to improve the interaction between patients and physicians by providing recommendations specific to the coping abilities of each patient (3).

**Web-based Psychoeducation Intervention Programs**

During the care period of patients with breast cancer, their expectations as to how much information they need may vary. While in the early phase, patients may want to learn the effects the treatment will have on their body and eventually become interested in self-care methods (15). Furthermore, they desire to learn about the effectiveness of a treatment, the phases of the disease, treatment options, and the prognosis of the disease (16).

The Breast Cancer Patient Pathway program, patient training program, was developed by Ryhänen et al. (17) to inform patients about their own treatment and care following the diagnosis of cancer. The program provides all the information about cancer and includes biophysiological, functional, experience, ethical, social, and financial sections. The biophysiological section contains physiological indicators and symptoms and the information on how these are managed; the functional section includes the information on how a patient can secure functional control of the condition and that on the control methods for health problems; the experience section comprises the information on how to use previous experiences to help control health problems; the ethical section discusses how every patient's experience is unique and how it should be respected; the social section emphasizes on how one must feel part of a social support group rather than focusing on health problems; and the financial section contains the information on how to manage finances while facing a health problem (17). Johnson-Turbes et al. (18) provided psychoeducation on the genetic tests related to cancer and its treatment via online intervention, the information about how to cope with the emotional burden of the disease and treatment, and sexual and reproductive health problems that might eventually occur in the course of the disease. The-Optimal-Lymph-Flow health system, a web-based intervention, has a patient-oriented

design and focuses on developing self-care abilities that are research-based, innovative, reliable, applicable, and easily adaptable to daily life to decrease the burden on the lymphoedema symptoms (18). Self-care methods for managing lymphoedema symptomsinclude symptom assessment, daily lymphatic exercises, the strategies for optimal body mass index, and situational self-care strategies (19).

**Web-based Psychosocial Support Intervention Programs**

When women are diagnosedwithbreast cancer, they usually experience psychological stress, and the symptoms of stress can persist for a long time. Most women join breast cancer support groups to cope with the disease, which ranks third among the online support groups related to breast cancer (20).

Participants can learn novel methods of coping with the disease, how to receive support, how to offer support, and how to honestly and openly express their thoughts via web-based intervention (6). It was stated that adjuvant psychological therapy, developed by Owen et al. (9), was commonly used among patients with cancer; it reduces emotional stress and improves the quality of life. The CHESS online program was developed to provide social support and information services and to help patients make decisions while coping with a health crisis (9). Intervention was provided to the online support group using a cognitive adaptation and emotional exposure-habit models, and it was suggested that this can be beneficial for providing an insight into stress and negative emotions (21). Web Choice was designed to provide self-control in patients with cancer and to improve patient-oriented care. The website allows patients to monitor their health problems and symptoms and provides various services, including the information on how to manage disease-related problems and symptoms, an e-mail function that allows them to ask personal questions, a diary for writing personal notes, and participatory group sessions with other patients with cancer (22). The training on self-coping abilities comprises a series of websites and symptom management training and contains educational topics entitled active and passive coping methods, communication with families and friends, the identification of the relationship among stress, emotions, and behaviors, stress management programs, assertiveness training, and structured problem-solving training (23).

**Web-based Cognitive-Behavioral Intervention Programs**

Thecognitive-behavioral stress management intervention approach by Carpenter et al. (24) comprises an introductory section and 10 other sections covering subjects, including cognitive-behavioral coping strategies and supportive interactive exercises, relaxation training, meditation techniques, expressive writing exercise, and weekly assignments aimed for developing new coping methods in daily life. BREATH Intervention, a self-control program based on cognitive-behavioral therapy elements, comprises psychoeducation, cognitive reconstruction, target planning, and process evaluation elements. Intervention is structured according to four different post-breast cancer recovery stages and comprises forgetting the past, emotional process, empowerment, and looking forward to the future sections (25).

**Results of the Web-based Interventions**

**Quality of life**

In terms of the quality of life, it was found that there wasan improvement in the areas of physical functionality and the loss of appetite as a result of the improvement in the frequency of exercise and the quality of nutrition encouraged through web-based intervention (10). In the study conducted by Galiano-Castillo et al. (11), it was seen that web-based intervention is a beneficial tool for changing health-related behaviors and cognitive development and that it improves the quality of life. Owen et al. (9) observed that online intervention affects the quality of life in the areas of dysfunctional thoughts related to cancer, symptom prevalence, the assessment of health status, and the concerns about breast cancer.

**Fatigue**

It is suggested that exercise training and a high protein diet can be effective in reducing fatigue in patients with cancer (26). Lee et al. (10) found that the severity of fatigue decreases if patients exercise and improve their diet quality. Galiano-Castillo et al. (11) noted that the intervention group participants' perception of fatigue declines as a result of the physical activity program provided in 10 sessions through their telerehabilitation program and that this decline was maintained for sixmonths. It was determined that the severity of fatigue among the patients participating in the online self-help program is reduced (13).

**Physiological symptoms**

In the study by Galiano-Castillo et al. (11) wherein a telerehabilitation program was applied, it was observed that web-based programs had an effect on the severity of pain. It was identified that at the end of the study, the participants in the intervention group had less severe pain than those in the control group(11). In the study conducted by Fu et al. (19), online self-care intervention was applied to patients with breast cancer to enable them to manage their symptoms followingsurgery. It was found that online self-care had a positive effect on reducing pain, aches, sensitivity, and lymphoedema symptoms in patients.

**Self-sufficiency**

It was found that self-sufficiency improves as a result of the web-based intervention (10, 24). Carpenter el al. (24) concluded that cognitive-behavioral stress management intervention is effective in enabling patients to cope with cancer, manage negative emotional states, and improve their independence. It was determined that online intervention improves motivational readiness and perceived independencein terms of exercise and nutrition (10). Additionally, it was stated that applying self-regulation strategies, maintaining a diary, emphasizing targets, and receiving feedback regarding progression can improve motivation and perceived independence (27).In online groups, revealing insights became more effective at improving health benefits than revealing emotions (21). The revelation of insights is related to the improvement of emotional wellness and the reduction of negative emotions and concerns related to cancer (28). Shim et al. (21) achieved significant results related to independence in terms of health, emotional wellness, functional wellness, and de-

creased concern about breast cancer as a result of the revelation of insights through online group intervention.

**Expectation of information**

It was stated that online intervention provides helpful information (18). The areas wherein patients largely expect information before web-based intervention have been identified as biophysiology and function, whereas the information on social aspects the least expected. It was found that there is an improvement in the areas of social and experience. A conclusion was reached that the intervention group is better informed about breast cancer (17). The stress caused by lymphoedema symptoms and concerns about the development of lymphoedema is defined as daily concerns for women with breast cancer (29). Online healthcare intervention performed by Fu et al. (19) was found to be beneficial by patients with breast cancer and was detected to be effective and convenient in teaching patients self-care strategies about lymphoedema and its symptoms.

**Psychosocial problems**

It was suggested that web-based support programs are effective in reducing depression, anxiety, and symptom stress in patients with cancer (30, 31). In a study on web-based programs, these programs were found to be effective in reducing depression, perceived stress, and cancer-related trauma in patients (6). It was suggested that web-based intervention provides an online group environment, thus being effective in reducing the social isolation of patients. In a support group, participants can easily express their life problems and provide specific coping recommendations. In the online intervention applied by Ruland et al. (22) for patients with breast cancer, it was concluded that stress about symptoms is reduced. It was suggested that reduced stress is a key finding as patients first experience their disease through symptoms. Patients who participate in online patient management programs for breast cancer are less stressed about their symptoms (14).

## DISCUSSION

In this systematic collection, the web-based interventions applied for breast cancer were reviewed, and the effect of web-based intervention programs on the care of patients with breast cancer was assessed. Internet and communication technologies are becoming increasingly important in healthcare. The internet can be an important tool in educating patients with breast cancer because, in places where breast cancer prevalence is high, women's skills regarding the use of the internet are also high (32, 33). We should take advantage of this option and find the best way to educate patients with breast cancer via the internet.

There are some limitations in the articles reviewed within the scope of this systematic collection. They include the characteristics of the individuals included in the study, the technical features of the website, and the features of the web-based interventions. It was stated in s studies that individuals with advanced age, low education level, and low income cannot independence use websites effectively. The fact that the time between the diagnosis of individuals who agreed to participate in the study and the beginning of their treatment process is different effects the psychological functions of individuals, and different drug adverse effects are observed in different phases of treatment. Therefore, the resulting findings cannot be generalized to the whole group. The technical problems experienced such as the disruption of the internet connection during education and the short duration of the programs such as exercise and diet programs provided through web-based intervention are the other limitations of the studies.

It is plausible to suggest that there is a positive relationship among the level of knowledge, the satisfaction of patients with cancer, and the patient education provided through web-based interventions. The results show that web-based interventions are associated with a competent knowledge of the subject of health and that online chat groups increase social supportamong patients. It is thought that interventions can be made more effective and beneficial through the planning of all elements such as the characteristics of an individual and disease, treatment process, and website design in such programs.

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Original Research

# Echocardiographic and Laboratory Findings of Turkish Children during the First Attack of Acute Rheumatic Fever

Erman Çilsal[1], Ayhan Pektaş[2], Bilgehan M. Pektaş[3], Buğra H. Koca[4]

[1]Clinic of Pediatric Cardiology, Adana City Training and Research Hospital, Adana, Turkey

[2]Department of Pediatric Cardiology, Afyon Health Sciences University School of Medicine Hospital, Afyon, Turkey

[3]Department of Pharmacology, Afyon Health Science University School of Medicine, Afyon, Turkey

[4]Department of Medical Biochemistry, Afyon Health Science University School of Medicine, Afyon, Turkey

**ABSTRACT**

**Objective:** In this study, we aim to determine demographic features and laboratory data of Turkish children who are diagnosed with acute rheumatic carditis and to evaluate echocardiographic indices of the left ventricle (LV) and right ventricle (RV) systolic-diastolic functions at the time of diagnosis.

**Methods:** This is a retrospective review of 100 children who were hospitalized because of the diagnosis of acute rheumatic fever (ARF)-related acute-stage carditis and 100 healthy children who were matched with respect to age and body mass index. All subjects initially underwent detailed two-dimensional (2D), pulsed Doppler, and tissue Doppler for assessment of ventricular functions.

**Results:** Internal diameter of LV, measured during end-systole, was significantly increased and the mitral annular plane systolic excursion was decreased in patients compared with those in controls (p=0.005). Although E′ velocity derived from septal mitral annulus were significantly lower, isovolumetric contraction time and myocardial performance index were increased in the carditis group compared with that in the control group. Peak early diastolic (E′), late diastolic (A′), and systolic (S′) myocardial velocities; isovolumetric contraction time; and myocardial performance index measured from the tricuspid annulus were similar in the carditis and control groups, although isovolumetric relaxation time was significantly prolonged. The carditis group had significantly lower mean corpuscular and platelet volume than the control group, although erythrocyte sedimentation rate and serum C-reactive protein levels were higher.

**Conclusion:** We detected a subclinical reduction in the systolic and diastolic function of both ventricles in children with the first attack of ARF. "Pulsed" and tissue Doppler techniques in pediatric patients with ARF have an important role in assessing cardiac functions.

**Keywords:** Acute rheumatic fever, carditis, children, Doppler echocardiography

## INTRODUCTION

Acute rheumatic fever (ARF) is an inflammatory disease caused by a delayed autoimmune response to a preceding Group A streptococcal infection of the throat. It is still an endemic disease, particularly observed among school-aged children in developing countries, and the incidence of an ARF episode following streptococcal pharyngitis is 0.5%-3% (1-3).

Being a multisystem disease, ARF can affect different tissues and cause a wide spectrum of clinical features, including carditis, arthritis, chorea, subcutaneous nodules, and erythema marginatum. The most clinical presentation of ARF is migratory arthritis with severe pain, observed in approximately 80% of patients. Major joints, like the knees, ankles, elbows, or shoulders, are often affected. Sydenham chorea, a neurologic expression of ARF, is the most common cause of acute chorea in pediatric population despite declining incidence of ARF (4-6).

Carditis is the most important manifestation of rheumatic fever, affecting 30%-50% of ARF patients. In fact, ARF is the leading cause of acquired heart diseases in children and adolescents worldwide. Because rheumatic fever can involve the pericardium, myocardium, and free borders of cardiac valve cups, it may develop into chronic and progressive valvular lesions because of immune-mediated damage and thus, result in significant morbidity and mortality. The mitral valve is the most affected valve, which is followed by the aortic and tricuspid valves, respectively. Moreover, carditis with progressive congestive heart failure, a new murmur, or pericarditis may indicate unrecognized previous ARF episodes (4, 7). This disease is more frequent in developing countries where low living standards and poor public health conditions are observed (8, 9).

The present study was designed to evaluate demographic features and laboratory data of patients with acute rheumatic car-

**Corresponding Author:** Erman Çilsal **E-mail:** ermancilsal@gmail.com

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ditis. We also aimed to compare echocardiographic indices of LV and RV systolic and diastolic function in newly diagnosed patients with those of controls.

## METHODS

This study was approved by the institutional review board and ethical committee of Afyon Kocatepe University Hospital where it was conducted at the department of pediatric cardiology between January 2015 and December 2015. Written informed consent was obtained from each participant and their parents.

### Study Population

We retrospectively studied 100 patients presenting with their first attack of rheumatic carditis to our clinic over 12 months. All subjects had evidence of ongoing rheumatic activity, including elevated acute phase reactants. We also recruited 100 healthy subjects who were referred for evaluation of an innocent murmur over the same period.

Diagnosis of ARF was based on the revised Jones criteria, whereas acute rheumatic carditis was defined as the presence of a new murmur, tachycardia, gallop rhythm, cardiomegaly, or congestive heart failure and is diagnosed by echocardiography. The World Health Organization Expert Committee specified four criteria of mitral and aortic regurgitant jets to distinguish between normal and pathological regurgitation on echocardiography and between normal and pathological mitral and aortic regurgitation. Accordingly, the regurgitant jet should be at least 1 cm in length, seen in at least two planes, have a peak velocity of 2.5 m/s, and should persist throughout the systole or diastole (10, 11).

The patients who had previously been diagnosed with congenital or rheumatic heart disease were excluded from this study. In addition, patients who had abnormal hepatic or renal functions, those with myeloproliferative disorders and malignancies, and those who used any medications (such as aspirin) that might have caused platelet or coagulation abnormalities during the last 2 months before blood sampling were excluded from the study.

### Echocardiography Examination

Echocardiography examination was performed using equipment with 3- and 5-MHz transducers (Vivid S6, GE Healthcare, UK). All subjects underwent echocardiographic examination within 24-48 h after the diagnosis of ARF and before starting anti-inflammatory treatment.

### Standard Echocardiographic Assessment

Parasternal long-axis views provided two-dimensional M-mode images. All children's interventricular septal wall thickness left ventricular internal diameters and left ventricular posterior wall thickness measurements were determined. Using the shortening fraction, we evaluated systolic functions of the left ventricle (LV). The Teichholz method was utilized for calculating the ejection fraction (12).

Mitral and tricuspid annular plane systolic excursion (MAPSE and TAPSE, respectively) were measured using the standard M-mode technique. TAPSE and MAPSE were measured in an M-mode examination in the apical four-chamber view during systole, at the junction of the right ventricle (RV) and LV with the tricuspid and mitral valve, and expressed in mm.

"Pulsed" Doppler measurements were performed with the transducer from the apical 4-chamber view. The LV and RV-inflow pattern at the tips of the mitral valve and tricuspid provided peak early filling velocity (E) and peak late filling velocity (A) and E/A ratio.

Measurements of "pulsed"tissue Doppler were attained with the transducer from the apical 4-chamber view by aligning the Doppler beam perpendicular to the plane of the lateral tricuspid and lateral and septal mitral annulus. The off-line analysis used the average measurements of the peak systolic (S'), early diastolic (E'), and late diastolic (A') myocardial velocities in 2-3 cardiac cycles.

The isovolumic contraction time (IVCT: interval between the end of the A' wave and the beginning of the S' wave) and the isovolumic relaxation time (IVRT: interval between the end of the S' wave and the beginning of the E' wave) were measured for both sides of the mitral annulus and lateral side of the tricuspid annulus on the tissue Doppler. The following formula was used for calculating the myocardial performance index [MPI: isovolumic relaxation time + isovolumic contraction time/LV ejection time (defined as the duration of the S' wave)] (13).

### Laboratory Studies

Complete blood count, including hemoglobin level, mean corpuscular volume (MCV), red cell distribution width (RDW), leukocyte count, neutrophil/lymphocyte ratio, platelet count, and mean platelet volume (MPV), was calculated using an automated counter (Coulter analyzer, Max Instruments Laboratory, Milan, Italy). Erythrocyte sedimentation rate (ESR) was determined by the Westergren method. Serum C-reactive protein (CRP) and mannan-binding lectin (MBL) levels were measured by enzyme-linked immunosorbent assay, whereas serum 25-hydroxy-vitamin D levels were recorded by radioimmunoassay (DSL Diagnostic Systems Laboratories, USA).

### Statistical Analysis

Data are presented as mean±SD. Comparisons between the groups were calculated using nonparametric tests (Mann-Whitney U test) for non-normally distributed data and parametric tests (Student's t-test) for normally distributed data. Kolmogorov-Smirnov test was used for checking the distribution of the variables. A $p<0.05$ was considered to be significant. All statistical analyses were performed using SPSS (Statistical Package for Social Sciences) version 18.0 (IBM Corp.; Armonk, NY, USA).

## RESULTS

### Clinical Features

A hundred children with rheumatic carditis (41 male; 59 female) and 100 healthy controls (33 male; 67 female) were recruited. The mean age of the patients and controls were 13.2±3.0 and 13.9±2.3 years, respectively. Table 1 summarizes the demographic characteristics of the children enrolled in the study. Both the

**Table 1.** Demographic Characteristics of the Study Population

| | Carditis Group (n=100) | Control Group (n=100) | p |
|---|---|---|---|
| Age (years) | 13.2±3.0 | 13.9±2.3 | 0.133 |
| Male/Female (%) | (41/59) | (33/67) | 0.115 |
| Body weight (kg) | 48.9±12.7 | 48.6±12.2 | 0.196 |
| Body height (cm) | 157.7±15.0 | 157.2±11.2 | 0.214 |
| Body mass index (kg/m$^2$) | 19.3±3.3 | 19.4±3.2 | 0.157 |

Data are presented as mean±SD; *p<0.05 was accepted to be statistically significant.

**Table 2.** Echocardiography Findings of the Study Population

| | Carditis Group (n=100) | Control Group (n=100) | p |
|---|---|---|---|
| Ejection fraction (%) | 74.1±10.6 | 72.2±10.6 | 0.127 |
| Fractional shortening (%) | 43.2±10.1 | 42.2±9.0 | 0.177 |
| Systolic volume | 55.0±19.0 | 50.7±16.8 | 0.146 |
| End-diastolic volume | 73.0±22.1 | 70.2±21.9 | 0.230 |
| Left ventricle internal diameter end-systole (cm) | 2.3±0.5 | 2.1±0.5 | 0.005* |
| Left ventricle internal diameter end-diastole (cm) | 3.6±1.2 | 3.9±0.6 | 0.248 |
| Left ventricle posterior wall diameter-systole (cm) | 1.5±0.3 | 1.5±0.3 | 0.214 |
| Left ventricle posterior wall diameter-diastole (cm) | 1.1±0.5 | 1.1±0.2 | 0.219 |
| Interventricular septum systole (cm) | 1.8±1.1 | 1.4±0.3 | 0.484 |
| Interventricular septum diastole (cm) | 1.0±0.2 | 1.1±0.8 | 0.092 |

Data are presented as mean±SD; *: p<0.05 was accepted to be statistically significant.

carditis and control groups were statistically similar with respect to age, body weight, body height, body mass index, and gender distribution (p>0.05 for all). Of the 100 patients, 68 had mild mitral, 24 had moderate mitral, and 8 had mild mitral and aortic regurgitation.

**Conventional and Doppler Echocardiographic Parameters (Standard Echocardiographic Evaluation)**

Table 2 shows echocardiographic findings of the study population. The carditis and control groups were statistically similar with respect to echocardiographic parameters, including ejection fraction, fractional shortening, systolic volume, end-diastolic volume, LV internal diameter at end-diastole, LV posterior wall diameter at systole and diastole, and interventricular septum diameter at systole and diastole. The carditis group had significantly greater LV internal diameter at end-systole than the control group. Values of MAPSE were significantly lower in the carditis

**Table 3.** Pulsed and Tissue Doppler Echocardiography Parameters of the Study Population

| | Carditis Group (n=100) | Control Group (n=100) | p |
|---|---|---|---|
| Mitral E (m/s) | 1.3±0.3 | 1.2±0.2 | 0.052 |
| Mitral A (m/s) | 0.8±0.3 | 0.7±0.1 | 0.001* |
| Mitral E/A | 1.6±0.4 | 1.8±0.4 | 0.001* |
| Mitral annular plane systolic excursion (cm) | 3.0±0.5 | 3.2±0.6 | 0.049* |
| Tricuspid E (m/s) | 1.0±0.2 | 0.9±0.2 | 0.019* |
| Tricuspid A (m/s) | 0.6±0.2 | 0.6±0.3 | 0.906 |
| Tricuspid E/A | 1.6±0.4 | 1.6±0.5 | 0.085 |
| Tricuspid annular plane systolic excursion (cm) | 3.7±0.6 | 3.9±0.7 | 0.165 |
| Lateral mitral annulus | | | |
| E' (m/s) | 0.15±0.09 | 0.15±0.03 | 0.621 |
| A' (m/s) | 0.12±0.05 | 0.11±0.03 | 0.314 |
| S' (m/s) | 0.13±0.03 | 0.14±0.02 | 0.387 |
| Isovolumetric relaxation time (ms) | 61.8±14.1 | 65.0±15.6 | 0.225 |
| Isovolumetric contraction time (ms) | 58.8±15.4 | 61.5±15.3 | 0.154 |
| Myocardial performance index | 0.49±0.13 | 0.47±0.11 | 0.168 |
| Interventricular septum | | | |
| E' (m/s) | 0.18±0.03 | 0.19±0.04 | 0.036* |
| A' (m/s) | 0.08±0.00 | 0.07±0.02 | 0.186 |
| S' (m/s) | 0.11±0.02 | 0.11±0.03 | 0.272 |
| Isovolumetric relaxation time (ms) | 57.3±12.7 | 60.1±14.0 | 0.107 |
| Isovolumetric contraction time (ms) | 65.9±12.6 | 62.1±12.7 | 0.033* |
| Myocardial performance index | 0.52±0.11 | 0.47±0.13 | 0.021* |
| Lateral tricuspid annulus | | | |
| E' (m/s) | 0.13±0.03 | 0.13±0.04 | 0.831 |
| A' (m/s) | 0.07±0.02 | 0.07±0.02 | 0.061 |
| S' (m/s) | 0.08±0.02 | 0.08±0.02 | 0.082 |
| Isovolumetric relaxation time (ms) | 62.6±13.0 | 58.3±13.3 | 0.011* |
| Isovolumetric contraction time (ms) | 64.6±11.7 | 65.8±14.0 | 0.661 |
| Myocardial performance index | 0.53±0.15 | 0.54±0.19 | 0.756 |

Data are presented as mean±SD; *: p<0.05 was accepted to be statistically significant

**Table 4.** Laboratory Findings of the Study Population

| | Carditis Group (n=100) | Control Group (n=100) | p |
|---|---|---|---|
| Hemoglobin (g/dL) | 13.1±1.1 | 13.4±1.3 | 0.161 |
| Mean corpuscular volume (fl) | 82.9±8.6 | 84.3±8.8 | 0.046* |
| Red cell distribution width | 14.1±1.7 | 14.1±2.2 | 0.193 |
| Leukocyte count (/mm$^3$) | 7158.0±1735.6 | 7083.3±1513.0 | 0.966 |
| Neutrophil count (/mm$^3$) | 5567.6±1192.9 | 5563.9±1026.0 | 0.918 |
| Lymphocyte count (/mm$^3$) | 3306.9±1019.2 | 3434.8±1178.4 | 0.645 |
| Neutrophil/Lymphocyte ratio | 1.97±1.15 | 1.82±0.81 | 0.873 |
| Platelet count (/mm$^3$) | 301240±81377 | 278447±63433 | 0.058 |
| Mean platelet volume | 7.54±1.07 | 8.10±0.98 | 0.001* |
| Erythrocyte sedimentation rate (mm/h) | 27.0±6.5 | 9.2±2.3 | 0.001* |
| C-reactive protein (mg/L) | 3.84±1.99 | 0.60±0.34 | 0.001* |
| Mannan-binding lectin (ng/mL) | 12.7±7.7 | 11.8±6.5 | 0.506 |
| 25-hydroxy-vitamin D (ng/mL) | 17.6±4.7 | 16.6±3.5 | 0.148 |

Data are presented as mean±SD; *: $p<0.05$ was accepted to be statistically significant.

group, whereas those of TAPSE were similar between the groups. The E velocity of the mitral annulus was significantly higher in the carditis group, and the E/A ratio of the mitral annulus was significantly higher in the control group, whereas mitral E velocity was similar for both groups. The E/A ratio of the tricuspid annulus was significantly higher in the control group, whereas E and A velocity were similar between groups (Table 3).

**Tissue Doppler Echocardiography (TDI)**

Table 3 demonstrates the Doppler echocardiography findings of the study population. Comparison of TDI parameters measured from the lateral mitral annulus demonstrated similar E', A', and S' velocities for the groups. Although E' velocity and IVCT time derived from the septal mitral annulus were significantly lower, MPI was prolonged in the carditis group compared with that in the control group. E', A', and S' velocities; IVCT; and MPI measured from the tricuspid annulus were found to be similar in the carditis and control groups, although IVRT was significantly lower.

**Laboratory Findings**

Laboratory findings of the study population are enlisted in Table 4. The carditis group had significantly lower MCV and MPV values and higher ESR and CRP levels than the control group (p=0.001). Both groups had statistically similar measurements for hemoglobin, RDW, neutrophil/lymphocyte ratio, platelet count, MBL, and 25-hydroxy-vitamin D values.

## DISCUSSION

Pathogenesis of ARF is very complicated because of underlying environmental and genetic factors in the etiology. After a specific bacterial infection, the antigen initiates an adaptive immune response in a susceptible host and obviously, this clinical entity is the result of activating innate immunity (14, 15).

The present study aims to assess demographic, clinical, and biochemical features of Turkish children who were diagnosed with acute rheumatic carditis and evaluate cardiac functions using echocardiography at the time of diagnosis. The predominant manifestation of rheumatic carditis is valvular involvement, particularly mitral and aortic regurgitation. Because of mitral and aortic regurgitation, resultant volume overload occurs during the acute phase of the disease. This overload was evidenced by significantly increased end-diastolic and - the systolic volume of LV (16). Children with acute carditis had statistically similar echocardiography findings on M-mode measurements, except the LV internal diameter at the end-systole that was significantly shorter in the carditis group. In our study, the carditis group had significantly greater LV internal diameter at the end-systole, but there were no significant changes in LV internal diameter at the end-diastole. Lack of significant changes in the diameter of LV at end-diastole may be due to the small number of patients with active carditis, and these findings also suggest that reviewed patients having mild disease and cardiac functions were not severely impaired. Such discrepancy may be also attributed to the fact that most indices used in practice, such as the ejection fraction and fractional shortening as a marker of ventricular function, do not clearly reflect the contractile power of the ventricle. As reported before, the ejection fraction and internal dimensions of LV do not reliably predict systolic functions of LV after surgical correction of mitral regurgitation (17).

In our study, we also investigated changes in the function of the ventricle presented after the first attack of ARF. As for 'pulsed' Doppler echocardiography findings, the carditis group in this study had significantly higher mitral A and tricuspid E velocities and lower mitral annulus E/A ratio and MAPSE. These patterns show that there is a subclinical diastolic dysfunction of LV (18). Various pediatric and adult studies have described subclinical

impairment of systolic functions with changes in MPI, isovolumetric contraction, and relaxation time (19). In our study, increase in MPI and decrease in isovolumetric contraction and relaxation time also indicate a subclinical systolic dysfunction of both ventricles. These findings indicate that there is a subclinical systolic dysfunction of both ventricles, and Doppler echocardiography is useful for specification of mild alterations in cardiac functions of pediatric patients with ARF. In a previous Turkish study, 30 healthy children and 82 children with ARF-related carditis were compared with respect to tissue Doppler findings, and subclinical systolic dysfunction of LV was demonstrated in children with a primary episode of rheumatic carditis (20). Thus, it has been concluded that tissue Doppler imaging is a quantifiable indicator that can be used for assessing the cardiac function during clinical follow-up of the disease. In addition, assessment of systolic indices of the mitral annulus with tissue Doppler imaging has previously been shown to provide a global estimate of the left ventricular systolic function (21).

Mean platelet volume is a good sign of the platelet size and rate of platelet production in the bone marrow. Thus, MPV may be used as an indicator of platelet activation and multiplicity of inflammation. Recently, increased MPV values have been used as a simple marker for the severity of inflammatory disorders, such as familial Mediterranean fever, rheumatoid arthritis, asthma, hypertension, diabetes mellitus, myocardial infarction, and secondary pulmonary hypertension (22). It has been hypothesized that decreased MPV values may indicate the intensity of the inflammatory process in conditions with elevated inflammatory markers. Excessive production of cytokines, such as IL-6 and acute phase reactants, may affect the platelet production and suppress the size of the platelets released from the bone marrow. Moreover, IL-6 release and/or intensive consumption of larger platelets in the areas of inflammation may contribute to the low MPV during acute ARF attacks (23). The results of this study can further support this suggestion that inflammatory markers ESR and CRP were significantly elevated in children with ARF-related carditis. In contrast, Özdemir et al. (24) were unable to detect a significant alteration in MPV values in children with acute rheumatic carditis. Likewise, Sert et al. (25) found lower MPV values in patients with ARF. This study also pointed out significantly lower MPV values in patients with ARF-related carditis.

It has been well established that ARF attacks may lead to an increase in acute phase reactants, including leukocyte count, ESR, CRP, and IL-6, and neutrophil-lymphocyte ratio. These reactants can be normalized because of suppression of cytokines by anti-rheumatic treatment (26). Only ESR and CRP values were found to be significantly increased in patients with rheumatic carditis in our study. This finding may be a result of the relatively small cohort size and presumably high prevalence of mild disease.

Mannan-binding lectin is an acute phase inflammatory protein that is involved in primary defense against microorganisms. Circulating MBL binds to the surface of numerous pathogens, including Group A streptococci. MBL deficiency is associated with an increased risk of infectious and autoimmune diseases (27,28).

The power of the present study is limited by its retrospective design in the absence of longitudinal data and lack of subgroup analysis with respect to the severity of the disease. Large-scale longitudinal studies should be conducted for clarifying the demographic, clinical, and biochemical characteristics of Turkish children with ARF-related carditis.

## CONCLUSION

This study showed that assessment of both ventricular functions using tissue Doppler imaging demonstrated significant differences in cardiac parameters measured by echocardiography in patients with ARF-related carditis. The ultimate future goal will be to accurately identify these patients with speckle-tracking echocardiography using a more sensitive imaging method. Adequately powered, well-designed clinical trials are necessary for clearly defining echocardiographic indices of the valvular involvement of the disease.

**Ethics Committee Approval:** Ethics committee approval was received for this study from institutional review board and ethical committee of Afyon Kocatepe University (Approval Date: 17.04.2014; Approval No: 2014/06-118)

**Informed Consent:** Written informed consent was obtained from all patients who participated in this study.

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Original Research

# Lateral Sagittal Infraclavicular Block for Orthopedic Surgery: One Year Experience

Onur Palabıyık[1], Tuğba Çağlar[2], Fikret Bayar[2], Havva Sayhan[1], Ayça Taş Tuna[1], Yaşar Toptaş[2], Yakup Tomak[1], Ümit Karadeniz[1]

[1]Department of Anesthesiology and Reanimation, Sakarya University School of Medicine, Sakarya, Turkey

[2]Department of Anesthesiology and Reanimation, Sakarya University Training and Research Hospital, Sakarya, Turkey

**ABSTRACT**

**Objective:** Lateral sagittal infraclavicular block (LSIB) is commonly used as a regional anesthetic technique for below the mid-humerus region in upper-limb surgery. The primary aim of the present study was to analyze the success rate of LSIB for orthopedic surgery.

**Methods:** We retrospectively analyzed orthopedic surgical procedures and identified patients who were applied LSIB between January 2015 and December 2015. Patient age, gender, American Society of Anesthesiologists (ASA) classification, diagnosis, surgery time, premedication regimens, serious complications, and guidance type [ultrasound (US) or nerve stimulator (NS)] were recorded. Need for additional sedatives and analgesics, laryngeal mask airway anesthesia, and general anesthesia was documented. The successful block was defined as the block sufficient to perform the surgery without any additional anesthetic and analgesic methods.

**Results:** We identified 233 patients who underwent 244 orthopedic procedures. US-guided and NS-guided LSIB were applied in 170 (69.7%) and 74 (30.3%) procedures, respectively. Mean age, gender, ASA classification, surgery time, and premedication regimens were similar in both the groups. The success rates of US-guided and NS-guided LSIB were 95.3% and 83.8%, respectively, and this difference was significant as statistically.

**Conclusion:** US-guided LSIB had been gradually increased in our daily practice. Moreover, US-guided LSIB had a higher success rate than NS-guided LSIB.

**Keywords:** Infraclavicular block, ultrasound, nerve stimulator, success rate, orthopedic surgery

## INTRODUCTION

Lateral sagittal infraclavicular block (LSIB), a technique for regional anesthesia developed by Klaastad et al. (1), is frequently used below the mid-humerus region in upper-limb surgery. Nerve stimulator (NS)-guided LSIB has been used for many years. Recently, ultrasound (US)-guided LSIB has been used to view the nerves and advance the needle during injections, because peripheral nerve blocks (PNBs) can be applied more easily and involves lesser risk using US than using NS (2). Thus, the aim of this retrospective study was to analyze the use of LSIB for orthopedic surgeries of the elbow, forearm, wrist, and hand for comparing the success rate of LS-guided and US-guided LSIB.

## METHODS

We retrospectively analyzed orthopedic surgical procedures and identified patients who were applied LSIB between January 2015 and December 2015 at a single institution after Local Ethics Committee approval had been obtained (31.03.2016-E.4563).

We recorded patient age; gender; American Society of Anesthesiologists (ASA) Physical Status classification; diagnosis; surgery time; premedication regimens; guidance type; additional drug requirements; and serious complications, such as convulsion, local anesthetic drug toxicity, and pneumothorax. All values of patients were obtained from computers with hospital information management system and anesthetic charts.

The inclusion criterion was that the patients who underwent only unilateral LSIB had to be aged 18 years and older. The exclusion criteria were as follows: patients aged less than 18 years; those who received multiple PNBs; those who received multiple anesthetic techniques, such as LSIB and general anesthesia; those with central neuraxial blocks; those with perineural catheter placement; and those with inadequate data.

### Block Techniques and Applications

All blocks were performed by anesthesiologists experienced in LSIB and residents who were trained for at least 3 years under observing of the same anesthesiologists.

### Nerve stimulation guidance technique

The needle was connected to the active lead of the nerve stimulator, and 1.5-mA current impulses of 0.1-ms in duration at 1-Hz

The study was presented in Anesthesiology and Reanimation Specialists' Society Congress Balkan States Anesthesia Days III: Orthopedic Anesthesia and Intensive Care (May 18–21, 2016, Skopje, Macedonia).

**ORCID ID of the author:** O.P. 0000-0003-3876-4279

**Corresponding Author:** Onur Palabıyık **E-mail:** mdpalabiyikonur@yahoo.com

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frequency were delivered. The needle was inserted caudally in the sagittal plane 45° from the skin on a horizontal plane at the intersection between the clavicle and the coracoid process. When the second and third finger flexion of the median nerve response was observed, 20 mL of a bupivacaine (0.25%) and lidocaine (1%) mixture was injected.

**Ultrasound guidance technique**

The needle was inserted in a plane with the US probe at the intersection between the clavicle and the coracoid process. The axillary artery and chords of the nerves were identified. The needle was directed posterolateral of the axillary artery, and 20 mL of a bupivacaine (0.25%) and lidocaine (1%) mixture was injected until the local anesthetic mixture surrounded the artery in a U-shaped pattern.

**Statistical Analysis**

All analyses were performed using the SPSS (Statistical Package for Social Sciences) for Windows version 22.0 (IBM Corp.; Armonk, NY, USA). Data were expressed as mean±standard deviation (SD), percentage, or n, as appropriate. Shapiro–Wilk tests were used for normality assumption of data. Student's t-test was used to compare numeric parameters that showed a normal distribution, and the Mann–Whitney U-test was used to compare numeric parameters that did not show a normal distribution. Categorical parameters were compared using Pearson's chi-square and Fisher's exact tests. $p<0.05$ was considered statistically significant.

## RESULTS

We identified 233 patients who underwent 244 orthopedic procedures. Of them, nine patients were operated two times and one patient was operated three times. US- and NS-guided LSIB were applied in 170 (69.7%) and 74 (30.3%) procedures, respectively. LSIB was applied for distal humerus or olecranon surgeries, ulna or radius surgeries, hand or wrist surgeries, and implant removals and revisions in 27 (11.1%), 69 (28.3%), 137 (56.1%), and 11 (4.5%) patients, respectively.

Demographic and clinical data of the patients are shown in Table 1.Mean ages, genders, ASA classifications, surgery times, and premedication regimens were similar in both the groups ($p>0.05$). In total, 45 patients had no premedication (18.4%); 130 (53.3%) patients used midazolam, 40 (16.4%) patients used midazolam and fentanyl, and 29 (11.9%) patients used midazolam and ketamine.

The success rate of US-guided LSIB (95.3%) was higher than that NS-guided LSIB (83.8%). General anesthesia was applied in 3 (1.8%) and 4 (5.4%) patients, laryngeal mask airway (LMA) anesthesia was applied in 1 (0.6%) and 5 (6.8%) patients and additional sedatives and analgesics were applied in 4 (2.3%) and 3 (4.0%) patients under US-guided and NS-guided LSIB, respectively. No convulsion, local anesthetic drug toxicity, or pneumothorax was associated with inadvertent intravascular injection in either group.

**Table 1.** Demographic and clinical data to guidance type

| | US-guided LSIB (n=170) | NS-guided LSIB (n=74) | p |
|---|---|---|---|
| Age | 38.22±19.1 | 42.84±17.9 | 0.079 |
| Surgery time | 76.48±44.7 | 80.19±45.3 | 0.554 |
| Gender (male/female) | 120/50 | 50/24 | 0.637 |
| ASA class (I/II/III/IV) | 82/68/18/2 | 26/39/9/0 | 0.181 |
| Patients with premedication | 141 | 58 | 0.398 |
| Success rate (%) | 162 (95.3) | 62 (83.8) | 0.020* |

Data are presented as mean±SD, n, or percentage. ASA: American Society of Anesthesiologists; SD: standard deviation; n: number of cases; %: percentage; p: statistically significant

## DISCUSSION

Lateral sagittal infraclavicular block is a safe and effective technique for forearm surgery that can be easily applied with low risk of complications (3). LSIB can be NS guided or US-guided. The success rate of NS-guided LSIB is variable (73%–92.5%) (4, 5). Using a multiple-injection technique allows for increased success rates (6, 7). It is well known that US-guided LSIB provides increased success rates and decreased complications. The success rate of US-guided LSIB is variable (83%–100%) (5, 8).

In a prospective, randomized, single-blinded study, Dhir et al. (8) reported the success rates of US-guided and NS-guided infraclavicular catheter placement to be 83.2% and 81.4%, respectively. Another study comparing US-guided and NS-guided LSIB reported higher success rates at 95% and 92.5%, respectively (4). Sauter et al. (9) reported that the success rates of US-guided and NS-guided LSIB to be 95% and 85%, respectively. Further, Brull et al. (10), consistent with the former studies, reported the success rate of US-guided and NS-guided LSIB to be 92% and 80%, respectively. As indicated by these studies, the differences in the success rates are not significant (4, 8-10). Contrary to the results of previous studies, in our study, US-guided LSIB had a significantly higher success rate than NS-guided LSIB. However, this may be because our study retrospective in nature.

Vascular punctures and pneumothorax are serious complications that can result from LSIB (11-13). Vascular punctures were reported in 2%–33% LSIB procedures (9, 11), and this number increases in NS-guided LSIB (4, 9). In the present study, we could not measure the incidence of vascular punctures that accidentally occurred because they were not recorded; nevertheless, no convulsion or local anesthetic drug toxicity related to accidental intravascular injection was reported. Further, pneumothorax is rarely observed (14-16); in our study, pneumothorax was not observed in either group.

The major limitation of this study is that the data were collected retrospectively. Another limitation is that we did not use a standardized premedication protocol because PNBs were applied by different anesthesiologists.

## CONCLUSION

US-guided LSIB was gradually applied in our practice without serious complication. In addition, the success rate of US-guided LSIB was significantly higher than that of NS-guided LSIB. However, to add value to these findings, prospective and randomized large-scale studies are required.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the Sakarya University School Of Medicine Local Ethics Committee (Approval Date: 31.03.2016; Approval Number: E.4563).

**Informed Consent:** Since this was a retrospective study, informed consent could not be taken from the patients.

**Peer-review:** Externally peer-reviewed.

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Original Research

# Evaluation of Pulmonary Vein Variations Using Multidetector Computed Tomography

Mehmet Demir, Feyza Gelebek Yılmaz
Department of Radiology, Gaziantep University School of Medicine, Gaziantep, Turkey

**ABSTRACT**
**Objective:** In this study, we aimed to identify variations in pulmonary veins (PVs) that are important for preparation before a radiofrequency ablation (RFA) using 64 multidetector computed tomography (MDCT) and to classify their incidence and drainage types.
**Methods:** In total, 503 patients, including 312 males and 191 females who were examined by abdominal computed tomography angiography for various reasons between January 2011 and December 2016 were included in this study. A 64-section CT device was used for scanning. Two-dimensional multiplanar reformats were created from axial images, and 3-dimensional images were created using maximum intensity projection and volume rendering methods. PV anatomic variations were identified.
**Results:** A pulmonary venous drainage pattern with the classical pattern, i.e., four ostia with two ostia on the right and two on the left, was observed in 44.8% of the patients. The remaining patients had varying anatomies on the right or on the left. In addition, 3.4% had right top PV, 72.4% had the classical type with two ostia on the right, whereas 27.6% had the varying type with one ostium or more than two ostia on the right. In addition, 61.5% had the classical pattern with two atrial ostia on the left, whereas 38.5% had the varying drainage patterns with one atrial ostium or three atrial ostia on the left. Our study is important in terms of being the largest series to date with 503 patients. The type that involves three separate atrial ostia on the left classified as L3 (Left) is not included in Marom's classification.
**Conclusion:** MDCT accurately identifies pulmonary venous anatomy in detail, which is important in RFA preparation.
**Keywords:** Pulmonary vein, variation, Multidetector Computed Tomography

## INTRODUCTION

Like all the veins in our body, the pulmonary vein (PV) shows a different pattern in each individual, called normal variation. Moreover, the PV is an important source of ectopic atrial electrical activity and frequently initiates paroxysmal atrial fibrillation (AF) (1, 2).

Increasingly, selective radiofrequency ablation (RFA) of these arrhythmogenic foci is performed to treat patients with refractory AF. The effectiveness of the RFA procedure relies on mapping the location and complete disconnection of the arrhythmogenic foci on the atrial tissue. Therefore, detailed knowledge of pulmonary venous anatomy and relationships between the PVs and the left atrium is highly necessary for mapping and ablation treatment (3).

Increasing the success of RFA and treating ectopic foci is possible by knowing the common variations in the pulmonary anatomy (4, 5). Therefore, cross-sectional imaging modalities are necessary for mapping pulmonary anatomy and recognizing variant veins before RFA.

Multidetector computed tomography (MDCT) can help visualize the anatomy of the left atrium as well as the number, localization, and diameters of PVs (6-8). Moreover, examinations during planning are used as reference images for detecting complications after RFA treatment. In diagnosing and identifying pulmonary venous anomaly coexisting with isolated and cardiac pathologies, pulmonary vein CT angiography (PVCTA) was reported to have results similar to cardiac catheterization (9, 10).

In our study, we aimed to identify the variations in PVs that are important in RFA preparation and to classify their incidence and drainage patterns using 64-MDCT.

## METHODS

In this study, 503 patients who underwent cardiac and thoracic angiography between 2011 and 2016 were retrospectively evaluated. The approval of the ethics committee was obtained from Gaziantep university ethics committee (27.03.2017/43).

Thoracic MDCT examinations were performed to evaluate suspected pulmonary embolism, coronary artery disease, or bypass grafts. Patients who underwent a lung operation and those not suitable for anatomical evaluation due to atelectasis, radiation fibrosis, or hilar mass were excluded from the study. Because of its being a retrospective study we didn't take written patient informed consents

**ORCID IDs of the authors:** M.D. 0000-0002-5031-6324; F.G.Y. 0000-0002-9900-5981.
**Corresponding Author:** Feyza Gelebek Yılmaz **E-mail:** feyzagelebekyilmaz@hotmail.com
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**Table 1.** Distribution according to the number of orifices and types on the right

| | 1 | 2 | | | 3 | | | 4 | | 5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **O.S.** | **R1** | **R2A** | **R2B** | **R2C** | **R3A** | **R3B** | **R3C** | **R4A** | **R4B** | **R5A** | **R5B** | **Total** |
| N | 7 | 159 | 181 | 24 | 86 | 14 | 1 | 8 | 16 | 5 | 2 | 503 |
| % | 1.4 | 31.6 | 36 | 4.8 | 17.1 | 2.8 | 0.2 | 1.6 | 3.2 | 1 | 0.4 | 100 |
| N | 7 | | 364 | | | 101 | | 24 | | 7 | | |
| % | 1.4 | | 72.3 | | | 20.1 | | 4.8 | | 1.4 | | |

O.S.: number of orifices; T: type; N: number of cases

Images were obtained using a 64-section (General Electric, Milwaukee ABD) CT device. Bolus administration of 120 mL nonionic contrast agent at 4 mL/s was followed by a bolus administration of 40 mL physiological serum at 4 mL/s through the right or left antecubital vein via an automatic injector (Covidien, ABD), wherein the contrast agent contained 300 mgI/mL iodine. In scanning, 40-mm (64×0,625) collimation, 0.35-s rotation time (s), 1-pitch value, 100-120 kilovolt (kV), 150-600 miliamper (mA), 0.625-mm detector width, and 0.625-mm reconstruction interval were used. Three-dimensional (3D) images were created at the workstation (Vitrea) from all axial images, which were analyzed for anatomic variations by two radiologists and subsequently reported after reaching a mutual decision.

**Statistical Analysis**

Data were loaded on the SPSS (Statistical Package for Social Sciences) software and evaluated using chi-square method in this software. Some types were grouped to enable statistical evaluation. A $p<0.05$ was considered statistically significant.

## RESULTS

Patients were aged 1-91 years (mean, 50.7±19.6 years). The study included 191 women (38%; mean age, 50.3±19.4 years) and 312 men (62%; mean age, 50.9±19.8 years). Left atrium and PVs were imaged clearly using 64-section MDCT in all patients. There was no statistically significant difference among them in terms of gender.

In identifying and classifying pulmonary venous anatomy, we used the classification of Marom et al. (1), which relies on both the number of venous ostia and drainage patterns.

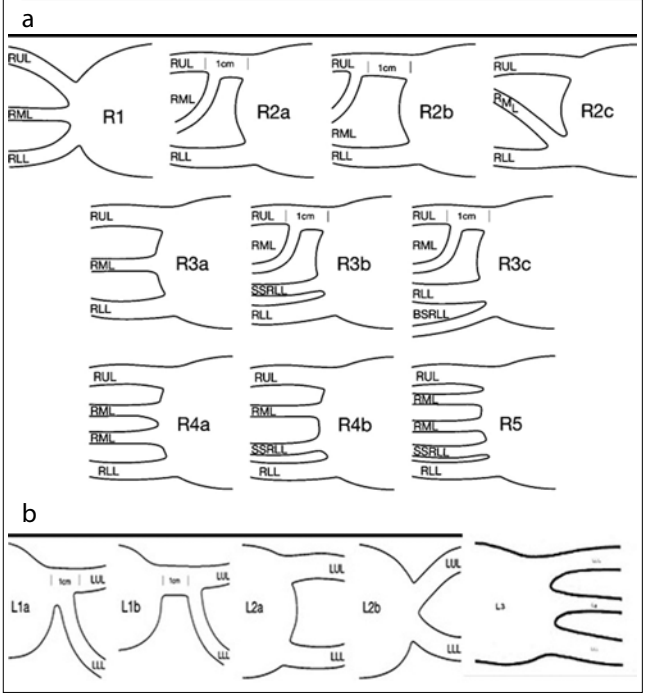
In Marom et al. (1) classification, the first letter (L: Left, R: Right) indicates the side of the drainage, the following number (1-6) indicates the number of ostia, and the last letter (A, B, and C) defines the variation.

We identified two different types of PV variations that are not included in Marom et al. (1) classification. Therefore, we called these L3 for five patients on the left and R5B for two patients on the right and created two new classes that include these types (Figure 1 a, b).

**Right Pulmonary Venous Drainage Patterns**

Among 503 patients, 364 (72.3%) had two atrial ostia on the right (Table 1), 181 patients (36%) had R2B, and 159 patients (31.6%) had R2A with high incidence rates. Patients with two orifices on the right also had two orifices on the left at a high incidence rate (44.8%, Table 2, Figure 1). Only seven patients (1.4%) had one ostium (Table 1, Figure 2). The incidence rate of three or more ostia was 26.3% (Figures 3, 4). There was no significant difference in the evaluation of the number of ostia in terms of gender distribution (Table 3).

Figure 1. a, b. Right Pulmonary Vein Types [Marom et al. (1)] (RUL: right upper lobe; RLL: right lower lobe; RML: right middle lobe; SSRLL: right lower lobe superior segment, BSRLL: right lower lobe basal segment) (a); Left Pulmonary Vein Types [(Marom et al. (1)] (LUL: left upper lobe; LLL: left lower lobe, Lg: lingual) (b)




In the present study, 17 patients (3.4%) with right PV (RTPV) drainage patterns that branch from the top of the right PV as an accessory was imaged (Figure 5). RTPV coexisted with six (35.2%) R2B, five (29.4%) R3A, three (17.7%) R2A, one (5.9%) R2C, one (5.9%) R3B, and one (5.9%) R4B types. The incidence of drainage patterns on the left in patients with RTPV was as follows: L1B in eight patients (47%), L2B in five patients (29.4%), L2A in three patients (17.7%), and L1A in one patient (5.9%).

**Table 2.** Number of left and right orifices together

| | | Left | | | |
|---|---|---|---|---|---|
| O.S. | | 1 | 2 | 3 | Total |
| Right | 1 | 4(0.8%) | 3(0.6%) | 0(0%) | 7(1.4%) |
| | 2 | 136(27%) | 225(44.8%) | 3(0.6%) | 364(72.4%) |
| | 3 | 36(7.1%) | 64(12.7%) | 1(0.2%) | 101(20%) |
| | 4 | 10(2%) | 13(2.6%) | 1(0.2%) | 24(4.8%) |
| | 5 | 3(0.6%) | 4(0.8%) | 0(0%) | 7(1.4%) |
| | Total | 189(37.5%) | 309(61.5%) | 5(1%) | 503(100%) |

O.S.: number of orifices

**Table 3.** Gender distribution according to the number of orifices on the right

| O.S. | N | Female | (%) | Male | (%) |
|---|---|---|---|---|---|
| 1 | 7 | 3 | (42.8%) | 4 | (57.2%) |
| 2 | 364 | 132 | (36.2%) | 232 | (63.8%) |
| 3 | 101 | 42 | (41.6%) | 59 | (58.4%) |
| 4 | 24 | 10 | (42.5%) | 14 | (57.8%) |
| 5 | 7 | 4 | (57.2%) | 3 | (42.8%) |
| Total | 503 | 191 | (37.9) | 312 | (62.1%) |

O.S.: number of orifices

**Table 4.** Distribution according to the number of orifices and types on the left

| O.S. | 1 | | 2 | | 3 | |
|---|---|---|---|---|---|---|
| T | L1A | L1B | L2A | L2B | L3 | Total |
| N | 60 | 129 | 166 | 143 | 5 | 503 |
| % | 12 | 25.6 | 33 | 28.4 | 1 | 100 |
| N | 189 | | 309 | | 5 | 503 |
| % | 37.6 | | 61.4 | | 1 | 100 |

O.S.: number of orifices; T: type; N: number of cases

**Table 5.** Gender distribution according to the number of orifices on the left

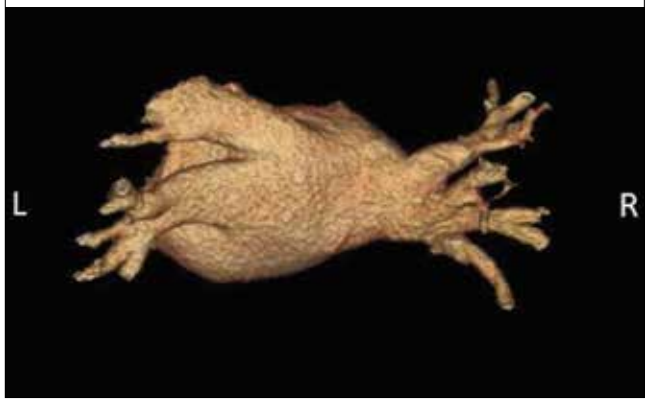
| O.S. | N | Female | (%) | Male | (%) |
|---|---|---|---|---|---|
| 1 | 189 | 76 | (40.2%) | 113 | (59.8%) |
| 2 | 309 | 113 | (36.5%) | 196 | (63.5%) |
| 3 | 5 | 2 | (40%) | 3 | (60%) |
| Total | 503 | 191 | (34.1%) | 312 | (64.9%) |

O.S.: number of orifices

In our study, the addition to Marom et al. (1) classification, i.e., R5B was seen in two patients (0.4%). Five different ostia on the right drained the right upper lobe, middle lobe, right lower lobe, lower lobe superior segment, and lower lobe basal segment. Unlike the variation referred to as R5 in Marom et al. (1) classification, it contains one middle lobe vein instead of two middle lobe veins, and the other vein drains the lower lobe basal segment (Figures 6, 7). Two R5Bs that we added to the classification were accompanied by L2A in one and L2B in the other on the left (Table 3, Figure 8).

Figure 2. R2A/L2A. The first letter indicates the side of the drainage, the following number (1-6) indicates the number of ostia, and the last letter (A, B, and C) defines the variation (L: Left, R: Right)


Figure 3. R1/L2B. The first letter indicates the side of the drainage, the following number (1-6) indicates the number of ostia, and the last letter (A, B, and C) defines the variation (L: Left, R: Right)




**Left Pulmonary Venous Drainage Patterns**

Among the 503 patients, classically, 309 (61.4%) had two atrial ostia that drained the upper and lower lobe veins (Table 4). There was no statistically significant difference in gender distribution. Similarly, there was no statistically significant difference in the distribution among genders when grouped according to the number of orifices and type ($p>0.05$; Table 5). Patients with two orifices on the right generally (44.8%) had two orifices on the left (Figure 9). A high number of ostia was less prevalent on the left compared to the right (Table 2).

Figure 4. R3B/L2B. The first letter indicates the side of the drainage, the following number (1-6) indicates the number of ostia, and the last letter (A, B, and C) defines the variation (L: Left, R: Right)

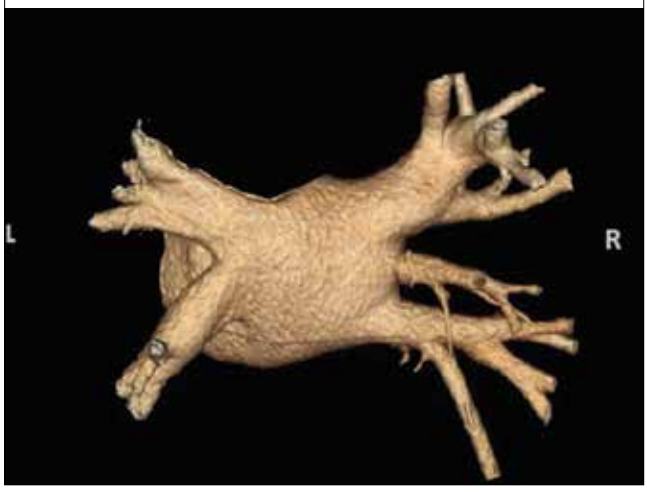



Figure 5. R4B/L1B (OBLIQUE). The first letter indicates the side of the drainage, the following number (1-6) indicates the number of ostia, and the last letter (A, B, and C) defines the variation (L: Left, R: Right).

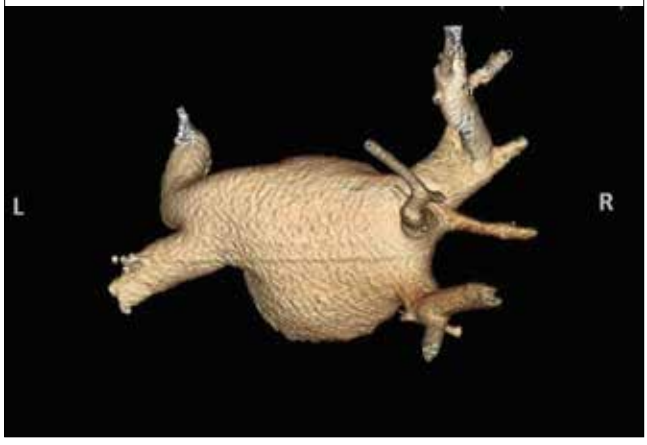



Figure 6. *RTPV
*top of the right pulmonary vein


Figure 7. R5A/L2B. The first letter indicates the side of the drainage, the following number (1-6) indicates the number of ostia, and the last letter (A, B, and C) defines the variation (L: Left, R: Right)


Figure 8. R5B/L2A. The first letter indicates the side of the drainage, the following number (1-6) indicates the number of ostia, and the last letter (A, B, and C) defines the variation (L: Left, R: Right)


Figure 9. R2B/L1B. The first letter indicates the side of the drainage, the following number (1-6) indicates the number of ostia, and the last letter (A, B, and C) defines the variation (L: Left, R: Right)

Figure 10. R4A/L1B. The first letter (L: Left, R: Right) indicates the side of the drainage, the following number (1-6) indicates the number of ostia, and the last letter (A, B, and C) defines the variation

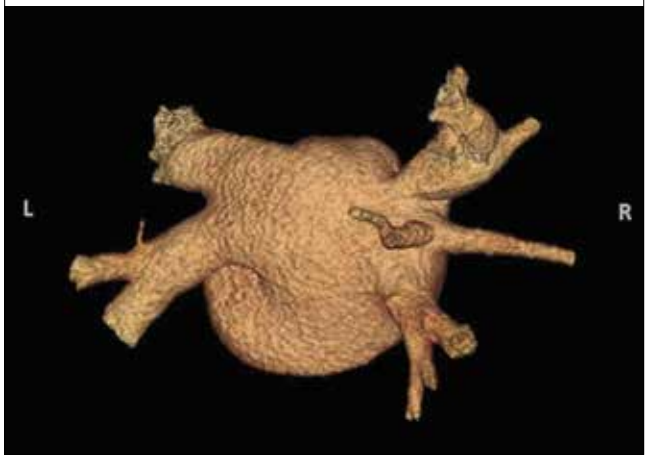



Figure 11. R2B/L3. The first letter indicates the side of the drainage, the following number (1-6) indicates the number of ostia, and the last letter (A, B, and C) defines the variation (L: Left, R: Right)

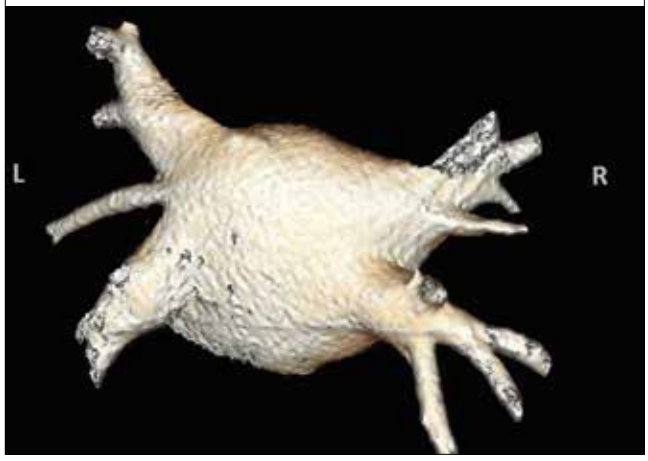



**Table 6.** Coexistence of right and left pulmonary vein types

| | L1A | L1B | L2A | L2B | L3 | Total |
|---|---|---|---|---|---|---|
| R1 | 0 (%) | 4 (0.8%) | 0 (%) | 3 (0.6%) | 0 (%) | 7 (1.4%) |
| R2A | 21 (4.2%) | 39 (7.8%) | 51 (10.1%) | 48 (9.5%) | 0 (0%) | 159 (31.6%) |
| R2B | 15 (3%) | 47 (9.3%) | 64 (12.7%) | 52 (10.3%) | 3 (0.6%) | 181 (35.9%) |
| R2C | 4 (0.8%) | 10 (2%) | 6 (1.2%) | 4 (0.8%) | 0 (0%) | 24 (4.8%) |
| R3A | 15 (3%) | 15 (3%) | 30 (6%) | 25 (5%) | 1 (0.2%) | 86 (17.1%) |
| R3B | 2 (0.4%) | 4 (0.8%) | 3 (0.6%) | 5 (1%) | 0 (0%) | 14 (2.8%) |
| R3C | 0 (0%) | 0 (0%) | 1 (0.2%) | 0 (0%) | 0 (0%) | 1 (0.2%) |
| R4A | 0 (0.2%) | 5 (1%) | 1 (0.2%) | 2 (0.4%) | 0 (0%) | 8 (1.6%) |
| R4B | 0 (0%) | 5 (1%) | 7 (1.4%) | 3 (0.6%) | 1 (0.2%) | 16 (3.2%) |
| R5A | 3 (0.6%) | 0 (0%) | 2 (0.4%) | 0 (0%) | 0 (0%) | 5 (1%) |
| R5B | 0 (0%) | 0 (0%) | 1 (0.2%) | 1 (0.2%) | 0 (0%) | 2 (0.4%) |
| Total | 60 (12%) | 129 (26%) | 166 (33%) | 143 (28%) | 5 (1%) | 503 (100%) |

The L1B type with a single orifice constituted the majority of the cases. In L1B, a lingular vein drained into the proximal inferior PV, and these two combined to form a wide common truncus to the superior PV and opened into the left atrium (Figure 10). On the right, R2B was most frequently concurrent with L1B.

In the L3 type on the left seen in five (1%) patients, three ostia were formed by upper, lower, and lingual veins and these three ostia were separated by the left atrial wall (Figure 11). Five patients with L3 type were males. However, a statistical comparison in terms of gender distribution could not be performed because the number of cases was low. The L3 type was accompanied by R2B on the right in three patients, R3A in one patient, and R4B in one patient (Table 6).

## DISCUSSION

The incidence of AF in the general population is 1%-2%, wherein the patients are characterized by an increased risk of stroke, thromboembolic complications, cardiac insufficiency, and mortality. (11)Aging is an important risk factor for AF. The cause of AF is the independently formed ectopic electrical foci in the atrium, and these are generally seen around the orifices of the PVs. Other foci localizations comprise the superior vena cava, crista terminalis, sinus coronaries, and interatrial septum. In literature, studies have reported that 94% of these foci are around the PVs (12). However, no connection was identified between variations and diameters of these PVs and AF (1, 13). In RF treatment, the aim was to electrically insulate target PVs by creating a linear scar tissue. In AF recurrences, after RFA treatment, it is considered that PVs became reconnected, and complete recovery after re-insulation was reported in up to 90% of the selected patients (2).

It has been shown that arrhythmogenic focus forms in abnormal veins and the ablation of these veins could be used to successfully treat atrial arrhythmia (14). Therefore, mapping of PVs and identifying abnormal veins before the procedure are thought to be beneficial. Although before radiofrequency catheter ablation became an important treatment for atrial arrhythmia, pathology and surgical literature were not defined the variant pulmonary venous anatomy; thoracic surgeons were aware of the anatomical variations, such as the drainage of the right middle lobe into the lower PV, and that it might lead to destructive results during

lower right lobectomy (15). Therefore, variations in pulmonary venous drainage were not well-defined until radiofrequency catheter ablation became an important treatment for atrial arrhythmia. Results of our study confirm that there are significant variations in pulmonary venous anatomy, particularly on the right side. In our series, the variation was on the right venous anatomy in 32% of the patients, and 25% of these patients had a separate orifice for the right middle lobe vein. In our series, the incidence of variant pulmonary venous anatomy is within the range of the studies that employed magnetic resonance imaging (MRI), ultrasonography, and CT scan (31%-38%) (2, 14, 16, 17). In previous studies on RFA, the focus was on the identification and mapping of four primary PVs (4, 18). However, these variations cannot optimally assess complex pulmonary venous anatomy most of the time (19, 20). Because of these reasons, cross-sectional images from CT or MRI can be requested before the ablation procedure.

Although angiography is the gold standard in imaging the pulmonary venous system, it has disadvantages, such as being invasive, causing radiation exposure to the patient and physician, and having a high cost. While transthoracic and transesophageal echocardiography enables imaging the left atrium, they are insufficient in showing the PVs. Therefore, the two noninvasive methods CT and MRI stand out in imaging the PVs and the left atrium (21, 22). In MRI, although the patient is not exposed to radiation, there are drawbacks, such as the long duration of the scan, motion artifacts, and claustrophobia. Moreover, MRI constitutes a contraindication for patients with metallic prostheses and implants. The PVCTA has advantages, such as a shorter duration, good patient compliance, and high-resolution images. The limitations of PVCTA include allergic reactions to the contrast agent, renal failure, and radiation.

Transverse, coronal, and coronal oblique images should be evaluate carefully in pulmonary anatomy investigation. This is because, in some complex situations, for instance, to show whether a vein opens into an orifice or into two closely located ostia, axial images are insufficient. A complex variant pulmonary anatomy can be seen in an easier manner in two- or three-dimensional reconstruction multiplanar images (1). Two- or three-dimensional reconstruction multiplanar images also reduce the radiation exposure of the patient and duration of the scan (1, 16, 23).

Pulmonary vein variations are significantly more prevalent in comparison to pulmonary artery variations. The number of ostia opening into the atrium, the location of the right lung middle lobe vein, and the fact that venous returns in both lung segments are not always into their own lobar veins are the reasons PV variations are more prevalent (24, 25).

Pulmonary vein variations were first defined systematically by Marom et al. (1) in the study published in 2004, which investigated the relationship between PVs and PVCTA with the left atrium and AF. This classification is alphanumeric and relies on the number of PVs opening into the left atrium and the position of the right lung middle lobe vein. According to Marom et al. (1) classification, the highest incidence belongs to L2A and R2A. Many limitations were reported in Marom et al. (1). First, because the CT scan performed on patients aimed to eliminate pulmonary embolism, it was reported that the results did not reflect the entire population. In addition, pulmonary venous anatomy is not expected to differ substantially between patients with suspected pulmonary embolism and the general population. In our study, thoracic and coronary CTAs were performed with various indications. Second, a very small patient group with atrial arrhythmia has been reported. In our study, patients could not be evaluated in terms of atrial arrhythmia. This study was prepared as an anatomical study.

Marom et al. (1) was interested in determining whether there was a relationship between any venous drainage pattern and atrial arrhythmia. Tsao et al. (14) reported a high incidence of variation in right middle lobe venous drainage in patients with refractory AF. In Marom et al. (1) study, sinus rhythm was observed in 70% of the patients in R2A and R2B right venous anatomy. One-half of the patients with atrial arrhythmia (50%) had typical venous anatomy. Values in Marom et al. (1) study were not statistically significant. Patients with R3A, R4A, R4B, and R5 types on the right (separate ostium for middle lobe PVs) were more prone to arrhythmia than other patients (18). A similar result was not found for left pulmonary anatomy, wherein there was no statistically significant finding between left variant anatomy and arrhythmia (1). There was no difference between gender distribution and drainage pattern in terms of arrhythmia ($p>0.155$). Similarly, in our study, there was no statistically significant relationship between female-male drainage patterns.

Marom et al. (1) stated that there were drawbacks because their classification was open to improvement. In addition, Marom et al. (1) provided a detailed explanation and drawing for each case that was investigated. We included the new patterns R5B that has five orifices on the right and L3 that has three orifices on the left in the classification as additions to Marom et al. (1) classification. Because R5B type was identified in two of 503 patients, it was not suitable for statistical evaluation. The variant structure defined as L3 on the left could not be compared with other groups in terms of the significance of gender distribution and its coexistence with other types on the right because it was identified in five (1%) patients. A larger series is required to achieve statistically significant results for the newly defined R5B and L3 types.

Yazar et al. (26) conducted a study on 30 cadavers in 2002 to investigate the drainage patterns of the right middle lobe vein and defined five drainage patterns of middle lobe vein. In type 1 (53.3%) and type 2 (16.6%), the middle lobe vein drains into the upper lobe vein; in type 3 and 4 (26.6%), the right middle lobe vein drains directly into the left atrium; and in type 5 (3.3%),the right middle lobe vein drains into the lower lobe vein (21). In our study, type 1-2 (R2A, R2B, R3B, and R3C) was seen in 70.5% of the patients, type 3 and 4 (R3A, R4A, R4B, R5A, and R5B) in 32.8% of the patients, and type 5 (R2C) in 4.8% of the patients. The type (R1) in which upper, middle and lower lobe veins open into the left atrium with a single ostium by forming a truncus was seen in 1.4% of the patients, and it was not reported in the previous study (26).

Right PV was seen in 16 patients (4%) in the study by Kaseno et al. (27) conducted in 2008 involving 428 patients. Lickfett et al. (28) reported the same to be 3%, whereas, in our study, RTPV was found in 17 (3.4%) of 503 patients.

In our study, apart from the classic anatomy involving L2A and L2B on the left, 35.2% of the patients had the anatomical types considered variants, whereas variations are less prevalent on the left side according to literature (1). Because of the similarity of left and right variation rates in our study, the left side should also be carefully evaluated in terms of variations.

In our study, L2B variation had the highest incidence on the left with a rate of 28.4%, wherein this variation was most frequently accompanied by R2B on the right. L1B had the second highest incidence with a rate of 25.6%, and in this variation, a lingular vein drained into the proximal inferior PV, and these two veins drained into the superior PV and opening into the left atrium. However, in the study of Marom et al. (1) including 201 patients, L1B was identified in only one patient.

Before the study by Marom et al. (1), variations in the number and structure of the PVs were only reported as case presentations (29, 30). Our study was based on the classification of Marom et al. (1). However, it reflects the general population because the scans were performed with different indications, and it is the largest study due to being conducted with 503 patients. Another importance of our study is that it reflects the variant pulmonary venous structures in the Turkish population.

## CONCLUSION

Knowing the pulmonary venous anatomy before both RFA and surgical intervention is of utmost importance. MDCT is very beneficial in evaluating the variations in pulmonary venous drainage because it is noninvasive and easy to tolerate.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the ethics committee of Gaziantep University (Approval Date: 27.03.2017; Approval No: 43).

**Informed Consent:** N/A

**Peer-review:** Externally peer-reviewed.

**Author contributions:** Concept - F.G.Y.; Design F.G.Y.; Supervision - F.G.Y.; Resource - F.G.Y.; Materials M.D.; Data Collection and/or Processing M.D.; Analysis and/or Interpretation - M.D. F.G.Y.; Literature Search - M.D.; Writing - M.D. F.G.Y..; Critical Reviews - F.G.Y.

**Conflict of Interest:** The authors have no conflicts of interest to declare.

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Original Research

# Comparison of Analgesic Effects Induced by Different Strengths of Extremely Low-Frequency Electromagnetic Fields

Ayşe Demirkazık[1], Ercan Özdemir[2], Yavuz Türkay[3], Aykut Pelit[4], Olca Kılınç[1], Ahmet Şevki Taşkıran[2], Gökhan Arslan[2]

[1]Department of Biophysics, Cumhuriyet University School of Medicine, Sivas, Turkey

[2]Department of Physiology, Cumhuriyet University School of Medicine, Sivas, Turkey

[3]Department of Electrical and Electronics Engineering, Cumhuriyet University School of Engineering, Sivas, Turkey

[4]Department of Biophysics, Çukurova University School of Medicine, Adana, Turkey

**ABSTRACT**

**Objective:** Our aim was to compare the results of the most commonly used analgesic measurement techniques and to determine the time and intensity at which the analgesic effects of the magnetic field (MF) are most effective.

**Methods:** This study compared the analgesic effect of MF strengths (1, 5, and 10 mT) in 30 adults, male Wistar albino rats weighing 200-250 g. The analgesic effects were measured using tail-flick (TF) and hot-plate (HP) tests. To determine the optimum MF strength, rats were assigned into four groups: sham group and exposed to 1, 5, and 10 mT MF groups. Rats were placed in a solenoid, and MF of 50 Hz for 165 min was administered daily for 15 days. All four groups were kept in the solenoid for 165 min/15 days and exposed to MF. However, the analgesic effect was measured only on day 0, 4, 7, 11, and 15 using TF and HP tests. The latencies of analgesia were converted to a percentage of maximal antinociceptive effects (% MPE).

**Results:** When the maximum analgesic effect of the 5 mT MF was determined on the seventh day, the% MPEs were 5.37±0.51, 13.66±1.27, 25.89±3.00, and 25.37±2.41 in the sham, 1 mT, 5 mT, and 10 mT groups, respectively. The optimum effect was observed with 5 mT MF on the seventh day and with 90 min in the solenoid.

**Conclusion:** We didn't find any differences between the analgesic responses to the TF and HP tests.

**Keywords:** Analgesic effects, extremely low-frequency magnetic fields, tail-flick test, hot-plate test, Wistar albino rats

## INTRODUCTION

It is known that extremely low-frequency magnetic fields (ELF-MFs) can modify human and animal behaviors, such as orientation, learning, nociception, and anxiety-related behaviors (1-3). The International Association for the Study of Pain defines pain in humans as "an unpleasant, sensory, and emotional experience associated with actual or potential tissue damage or described in terms of such damage" (4, 5). Recently, a majority of studies have particularly focused on the mechanism of magnetic antinociception. A previous study in this laboratory showed that ELF-MFs affected the acute and chronic effects of pain in experimental animals (6). We measured thermonociceptive sensitivity in diabetic (treated with insulin) rats under repeated exposure of ELF-MFs for 30 days. Other studies have shown that land snail and mice inducted analgesia through repeated, daily exposure to oscillating fields or to specific pulsed MFs (7, 8). Various tests have been suggested to assess MF antinociception in animals. A majority of tests measure a specific group of nociceptors or particular sites of the central nervous system for nociceptive processes. Therefore, a difference in the response may be observed due to different testing methods. In our opinion, the two most popular nociceptive tests, namely rodent tail-flick (TF) and hot plate (HP) have not been sufficiently compared with literature, and the intensity of MF, which affects the most effective analgesia, has not been studied in either of the MF intensities. Hence, our aim was to compare the results of the most commonly used analgesic test measurement techniques and to determine the time and intensity at which the analgesic effects of MF are most effective.

## METHODS

### Animals and Electromagnetic Field Exposure Conditions

The experimental procedures applied in this study were confirmed by the Institutional Review and Animal Use Committee of the Cumhuriyet University School of Medicine, and the study was organized and designed by following the guidelines for

**ORCID IDs of the authors:** A.D. 0000-0001-7224-2832; E.Ö. 0000-0001-8231-1053; Y.T. 0000-0002-4263-8286; A.P. 0000-0003-3916-0207; A.S.T. 0000-0002-5810-8415; O.K. 0000-0001-9621-7543; G.A. 0000-0003-4186-2478.

**Corresponding Author:** Ayşe Demirkazık **E-mail:** dmrkzk@yahoo.com

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the care and use of laboratory animals for research. Thirty adult male Wistar albino rats weighing 200-250 g were tested. Rats were maintained at 25°C±1°C and under a 12-h light/dark cycle. All experiments were performed during the light cycle (10:00-14:00).

The electromagnetic field (EMF) exposure system consists of a power supply, solenoid with plexiglass cage, and timer (Figure 1). Before the EMF exposure, all rats were accustomed to their environment for 1 week. The MF groups were left in a solenoid with an MF of 50 Hz, and strengths of 1, 5, and 10 mT were applied daily for 165 min. They were exposed to two nonstop pulses of 30 min with 15 min intervals. The animals were subjected to repeated exposures of alternating 50 Hz EMF for 15 days, which was performed in three different MFs. The EMF that was generated in a specially designed solenoid (500 mm in length and 210 mm in diameter, 1400 turns of insulated 1.4 mm copper wire). An electrical current (50 Hz, respectively 25, 120, and 220 V) was passed through the device (with a time relay) at strengths 1, 5, and 10 mT. The animals were exposed to EMF with an alternating current for four, 30-min implementations deactivated by 15-min intervals; thus, the entire EMF sessions were carried out during the same time period (9.00-11.00 a.m.) and lasted 165 min daily. The EMF intensity in the solenoid was measured using a digital tesla meter with an axial probe (PHYWE 8010 Model Digital Teslameter; Figure 2 a-c). The solenoid was always retained in the north-south direction, and its temperature was maintained at 22.0°C±2°C. The plexiglass rat cage (dimensions, 40×17×13 cm) was placed in the solenoid. Four rats were simultaneously placed in the cage to be exposed to EMF. The control group rats were also placed in the animal cage, but they were not exposed to EMF. The plexiglass cage was designed to supply food and water for rats.

Figure 1. Schematic representation of placement of the rats in the solenoid with the pulsed MF. At the bottom, the schematic of MF that the rats are exposed to for a total of 165 min with 30 min of exposure and 15 min of silencing is indicated. a) MF experimental setup at 1 mT; b) MF experimental setup at 5 mT; c) MF experimental setup at 10 mT

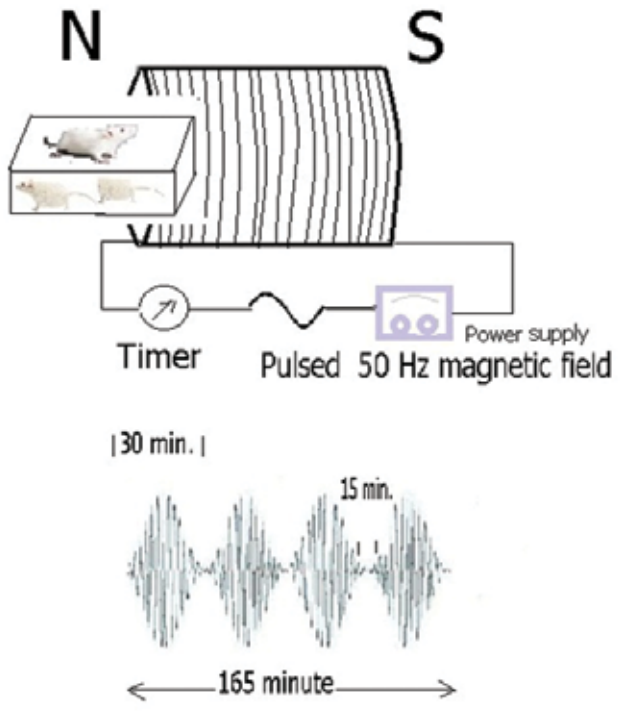
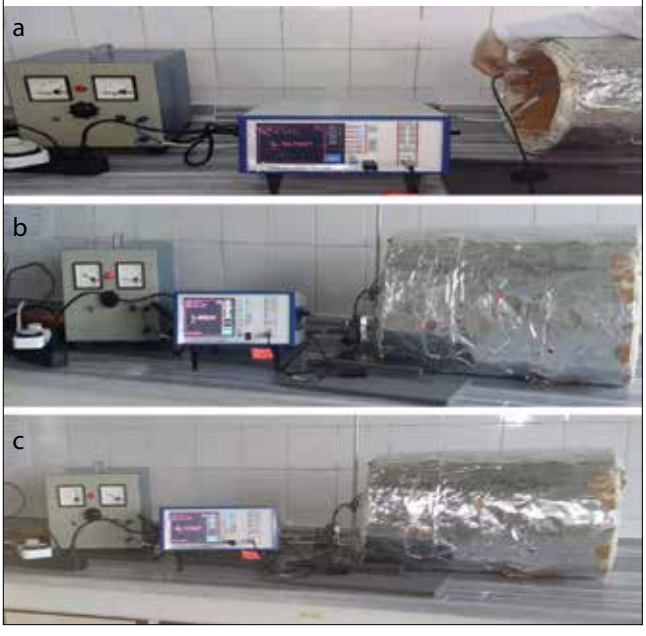



Figure 2. a-c. Experimental set up (a, b, c) for MF strengths (1, 5, and 10 mT). The solenoid was powered by a power supply




**Experimental Protocols**

First, the following procedure was established to determine MF strength and the day on which the most effective analgesia was induced. Rats were randomly delivered to one of four groups: sham (the control group, placed in the solenoid but not exposed to MF) and exposed to a 1, 5, and 10 mT MF. The antinociceptive effects of three different EMF strengths (1, 5, and 10 mT) were evaluated at 30-min intervals (0, 30, 60, 90, and 120 min) using TF and HP test in rats (n=6). Initially, the maximum analgesic effect of EMF was detected in 15 days.

**Antinociception Tests**

**TF test**

Nociceptive responses in all groups were measured using the radiant-heat TF test. This test is generally used to specify sensory functions under different strengths of MF since it is comparable with certain quantitative sensory tests used in clinics. The TF test mostly projects the activity of simple spinal reflex arcs. It ensures information on peripheral nerves and spinal functions in relative isolation from higher nociceptive processing and cognitive systems (9). The nociception response was measured using a tail-flick apparatus (May TF 0703 Tail-Flick Unit, Commat, Turkey). Animals were individually placed on a plate with the temperature adjusted to 51°C±1°C. The cut-off time was 15 s to prevent damage to their tails (10). The analgesia response measured on the HPis considered originating from a combination of central and peripheral nervous system mechanisms (11). Animals were

Figure 3. Analgesic effects of three different EMF strengths (1, 5, and 10 mT) on rats measured using the TF (a) and HP tests (b). The maximal analgesic effect of MF was observed on day 7 in all groups of rats: ELF-EMF (1,5, and 10 mT). The analgesic activity of MF of 1, 5, and 10 mT were significantly higher than control group rats (p<0.01). Each point represents the mean±SEM of % MPE for six rats. HP, hot plate; TF, tail-flick; SEM, standard error means; ELF-EMF, extremely low-frequency electromagnetic fields; % MPE, the percentage of maximal antinociceptive effects. *p<0.01 compared to the control, **p<0.05 compared to 1 mT group

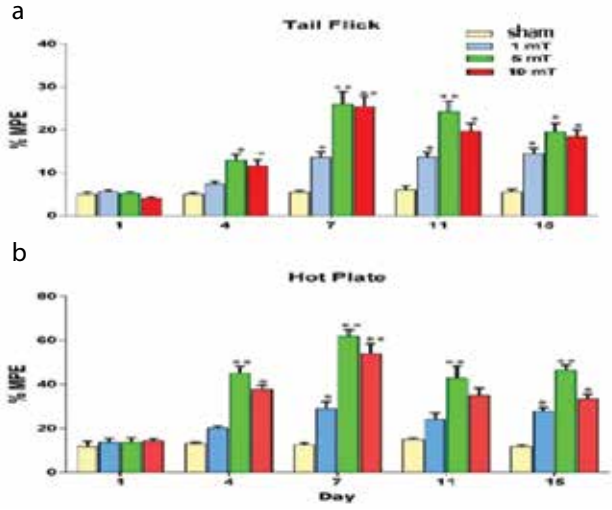
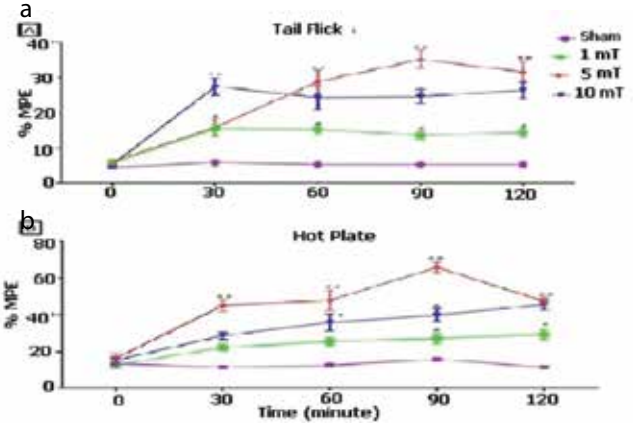



Figure 4. Time-dependent change of EMF analgesic effects. The effect of EMF in the TF (a) and HP tests (b). The maximal analgesic effect was determined in the 5 mT group and at 90 min measurement for three different MF strengths on the seventh day. Each point represents the mean±SEM of % MPE for six rats. HP, hot plate; TF, tail-flick; SEM, standard error mean; ELF-EMF, extremely low-frequency electromagnetic fields. *p<0.01 compared to the control, **p<0.05 compared to 1 mT group




individually placed on anHP (May AHP 0603 Analgesic Hot-plate Commat, Turkey) with the temperature adjusted to 54°C±3°C. The latency to the first sign of paw licking or jumping to avoid the heat is treated as an index of the pain threshold; the cut-off time to avoid damage to the paw was 30 s (12).

**Data Analysis**

To calculate the percent of maximal antinociceptive effects (% MPE), the TF and HP latencies (in seconds) were converted to percent antinociceptive effect using the following equation:

% MPE=[(postdrug latency−baseline latency)/(cut-off value-baseline latency)]×100.

**Statistical Analysis**

Group sizes were based on the following equation: N=2+C(S/d)2, where N is the group size, C is the constant obtained according to α and β, S is standard deviation, and d is effect size. The statistical power was assumed to be 1, and α was 0.05. All results are expressed as a mean ± standard error of the mean (SEM). The effect of antinociception was measured and the mean of % MPEs in all groups was computed. Data were analyzed using the analysis of variance followed by Tukey test. All statistical tests were performed using the SPSS (Statistical Package for the Social Sciences) software version 22.0 (IBM Corp.; Armonk, NY, USA). A significant difference was defined as p<0.05 in comparison to the sham group.

## RESULTS

**Analgesic Effects of Different Strengths of MF**

Based on the TF and HP tests, we determined the strength and duration of MF that produced the most effective analgesia. The analgesic activity of MF 1 mT (TF: 13.66±127 and HP: 28.95±3.10), 5 mT (TF: 25.89±3.00 and HP: 61.73±2.95), and 10 mT (TF: 25.37±2.41 and HP: 53.85± 4.62) groups were significantly higher than control group rats ($F_{3,20}$=23.13, p<0.05 for TF and $F_{3,20}$=50.46, p<0.01 for HP; Figure 3). The maximal analgesic effect was determined at 5 mT group and 90-minute measurements (TF: 35.13 ± 2.63 and HP: 65.73 ± 2.92) for three different MF strengths ($F_{3,20}$= 555.51 for TF and $F_{3,20}$=766.03 for HP, p<0.001; Figure 4).

For the TF test, % MPE values were significantly high in all groups on day 7 ($F_{3,20}$=23.43, p<0.05) in comparison to the sham group. Tail -flick latencies decreased significantly on days 11 and 15 (p<0.05; Figure 3 a).

For the HP test, % MPE values increased significantly in all groups on day 7. Day in HP test ($F_{3,20}$=50.26, p<0.05). Figure 3 b represents that HP latencies decreased on days 11 and 15.

## DISCUSSION

Numerous studies have shown that EMF reduces pain. The differences among these investigations are MF intensities and application periods. The inhibitory effects of EMFs on pain have been demonstrated in a variety of studies (13, 14). Consistent with these findings, 15-30 min acute exposures to EMFs block the elevated pain responses in snails (15). Our results suggest that four times 30-min acute exposures to EMFs enhance the analgesic activity measured using theTF and HP tests in rats.

This study investigated pulsed MFs of three strengths to identify a potential dose-response relationship based on theTF and HP tests. There were significant changes in pain processing activity when exposed to a 5mT MF.

The effect peaked on day 7 and then decreased to control levels. These results indicated that the magnetic antinociception reached a maximum on days 6-7. From day 11 onward, the TF and HP tests began to show a progressive decline until they were distinct from the responses of the sham group. According to Tiffany and Maude-Griffin, this may be because of opioid tolerance, which is a typical effect of repeated administrations of opiates (16). There is no data on agonist-antagonist opiate receptors. Similar findings were observed in selected brain regions in rats after chronic MF exposure (-100 mT, 50 Hz, 8 h per a day, for 8 months) (17). This raises the possibility that MFs have a direct effect on opioid receptor number, binding activity, or functional activity. In agreement with this, showed that four a 4-day magnetic exposure increased the levels of beta-endorphin and substance P in the hypothalamus of rats (18). It seems possible to increase the antinociceptive effect on opiates by increasing the effect of the MF on the seventh day.

No difference was found between the results of the TF and HP tests. Hence, the MF uses pathways between the sensory, central, and peripheral nervous system similar to that used for creating analgesia. Langerman et al. (19) in 1995 created a design to compare the TF and HP tests for (a) evaluating the tolerance of morphine and (b) assessing the influence of repeated testing on morphine antinociception. The TF and HP responses were dissimilar for morphine infusion. This may be attributed to the differential effects of morphine on spinal and supraspinal sites (19).

We have noted a widespread expectation that a greater MF strength will increase the analgesic effect. However, the analgesic effect in our study peaked at 5 mT and decreased at 10 mT (Figure 3). Hence, we propose the concept of an effective MF dose. Robertson et al. (20) showed significant correlations between different MF strengths and a change in the BOLD activity in the anterior cingulate and ipsilateral insula, indicating that there is either a dose-response or a threshold effect of EMFs. They used EMF strengths of 100, 200, and 1000 μT and found significant increases in the analgesic effect at 100 and 200 μT but a decrease at 1000 μT. Similarly, our study showed an increasing analgesic effect with MFs of 1 and 5 mT and a decrease at 10 mT, which may be due to habituation. However, further research is needed to evaluate the cause of decreasing analgesic effect at higher MF strengths.

**Ethics Committee Approval:** Ethics committee approval was received for this study from Institutional Review and Animal Use Committee of the Cumhuriyet University School of Medicine (Approval Date: 19.06.2014; Approval No: 65202830/125).

**Informed Consent:** N/A

**Peer-review:** Externally peer-reviewed.

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**Conflict of Interest:** The authors have no conflicts of interest to declare.

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Original Research

# Risk Factors for Mother to Child Transmission of HIV in Southwest Ethiopia

Abiot Girma Sime, Birtikuan Tsehayneh Adamu, Fessahaye Alemseged Tesfamichael, Lamessa Dube Amentie, TsegayeTewelde Gebrehiwot

Department of Epidemiology, Jimma University Institute of Health Sciences, Jimma, Ethiopia

**ABSTRACT**

**Objective:** One in four Ethiopian children born to human immunodeficiency virus (HIV) positive mothers were found to have acquired the virus, although the country has been implementing the World Health Organization's(WHO) four-pronged prevention approaches. This study was therefore aimed at identifying the factors responsible for mother to child transmission of HIV among children who received HIV exposure care.

**Methods:** An unmatched case-control study was conducted on randomly selected 64 cases and 256 controls from December 2011 to May 2012. The cases were HIV positive children less than 18 months of age, and the controls were HIV negative children less than 18 months of age born to HIV positive mothers. Data on the parents'sociodemographic characteristics and parents' clinical profiles before the final child's HIV status determination were collected. A logistic regression was used to identify predictors.

**Results:** The records of 60 casesand 235 controls were included for analysis. Mixed breastfeeding(adjusted odd ratio [AOR]: 22.03; 95% confidence interval [CI]:5.31–91.49), maternal CD4 count <200 cells/mm$^3$ before delivery (AOR: 17.14, 95% CI: 4.73-62.06), no maternal WHO clinical staging after delivery (AOR: 3.38; 95% CI: 5-35.76), children born to mothers from rural areas (AOR:7.64; 95%CI: 2–29.22),and no paternalantiretroviral therapy(ART)enrollment or an unknown enrollment status (AOR: 11.11; 95% CI: 2.94-50) were factors independently associated with the child's HIV infection.

**Conclusions:** Mixed breastfeeding, maternal CD4 count, no maternal WHO clinical staging, children to mother from rural areas, and no paternal ART enrollment or an unknown status were independent predictors. Behavioral change communication should be intensified, and emphasis should be given for mothers with lower CD4 count and those from rural settings.

**Keywords:** Risk Factors,infectious disease transmission: vertical, HIV, Ethiopia

## INTRODUCTION

In resource-limited settings, a marked decrease of mother-to-child transmission (MTCT) of HIV was documented, from over 570,000 in 2003 to an estimated 220,000 children in 2014. More than 90% of this infection among infants was from sub-Saharan African countries (1-3).

Several studies confirmed that almost all new child HIV cases resulted from perinatal transmission of HIV during pregnancy, childbirth, and breastfeeding. In the absence of preventive interventions, about one-half of HIV-exposed children will acquire the virus from their mothers. About one-fourth of exposed children will acquire the virus during childbirth, while one-fifth of the exposed children will acquire it during pregnancy and breastfeeding depending on presence/absence of other parental and child-related risk factors (4-6).

The rupture of the membrane for more than4 hours, micro-transfusions across the placenta during labor contractions, genital tract infections, placental infection, and high maternal viral load/low CD4 cell count/advanced clinical stage, and mixed/prolonged breastfeeding were known to increase MTCT of the virus. However, the most important risk factors for MTCT of the virus are maternal plasma and breast milk viral load followed by maternal immunologic status and clinical stage as suggested by several studies among both breastfed and non-breastfed children. Even among mothers on antiretroviral (ARV) agents and who do not breastfeed their infants, the maternal viral load is directly correlated to the MTCT of HIV. In addition, anemia, maternal mastitis, gastrointestinal tract lesion of infants, paternal enrollment to antiretroviral therapy (ART) care, and acute maternal sero-conversion during pregnancy or during breastfeeding were associated with MTCT of HIV (7-12).

To eliminate child HIV infection globally, The World Health Organization (WHO) recommended four-pronged MTCT of HIV preventive strategies to all its member states. The strategies include primary prevention of HIV infection among women of child-bearing age, prevention of unintended pregnancy among HIVpositive women, prevention of MTCT of HIV from HIVpositive mothers, and continued care/support for infected mothers/infants/partner. It was shown that appropriate ARV drugs prophylaxis or Highly Active Antiretroviral Therapy (HAART) to the mothers/infant, avoidance of breast milk, and elective cesarean section can reduce the risk of MTCT of the virus to less than 5% among breast fed, HIV exposed children, while to less than 2% among non-breastfed, HIV exposed children (8-12).

**Corresponding Author:** Tsegaye Tewelde Gebrehiwot **E-mail:** tsegaye.tewelde@yahoo.com

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Although all WHO member states, including Ethiopia, have been implementing the approach, pediatric HIV continued to be an important cause of child morbidity and mortality in the sub-Saharan African countries (12). In Ethiopia, the trends of HIV infection among pregnant women is declining, but their enrollment to ART care for MTCT of HIV remained very low compared to other sub-Saharan African countries (13).

For example, in 2012/13, there were 2.9 million expected pregnancies and 80% of them utilized antenatal care(ANC) in Ethiopia; 17,742 were found to be HIV positive, but <70% of these mothers and their infants received an ARV drug for the prevention of MTCT of HIV. It is evident that the prevalence of HIV among ANC attendants aged 15-24 years decreased from 3.5% in 2007 to 2.1% in 2012, and the rate of MTCT was reduced from 35% in 2007 to 25% in 2013 after the breastfeeding period. However, it is still far beyond the global expected prevalence in breastfed children and very slow decline rate for elimination of vertical transmission 2020(12, 13).

As per the WHO recommendation of infant feeding in the context of HIV, Ethiopia adopted and promotes exclusive breastfeeding for the first 6 months of birth and complementary feeding plus breastfeeding until the child is two years of age (13). However, a study conducted in Zimbabwe indicated that breastfeeding for more than 6months was responsible for over two-third of HIV infection among infants who were tested using DNA polymerase chain reaction (DNA PCR) negative at 6 weeks of birth, with a higher risk of HIV infection rate among mixed breastfed infants compared with exclusively breastfed infants (14). In another comparative cross-sectional study conducted in Addis Ababa, only 15% of HIV-exposed children were mixed breastfeeding. The study further revealed that mixed breastfeeding and maternal breast problem were associated with infant HIV infection (15, 16).

In conclusion, mixed breastfeeding practice among HIV positive mothers was low (15%), approaching WHO recommended level (5%).Similarly, enrollment of mothers/their infants to ART care of pregnant mother and their infants are encouraging. However, little is known about why the rate of child's HIV infection in Ethiopia is not declining as theoretically expected. We hypothesized that the parental clinical profile before childbirth, during breastfeeding periods, and breastfeeding by itself with incomplete maternal/child's HAART coverage throughout the breastfeeding period play a crucial role for MTCT of the virus.

The identification of those local factors is important to devise policy recommendations of HAART utilization to prevent MTCT of HIV in the context of the universal breastfeeding community. In addition, the identification of those factors will help healthcare providers to grade the risk of HIV infection for a given child and accordingly plan the management in more efficient ways. This study was therefore aimed at identifying parental and child-related risk factors for MTCT of HIV in the area.

## METHODS

**Study Area and Study Period**

The study was conducted in two hospitals located 345-660km away from Addis Ababa, Ethiopia. Both hospitals serve as teaching and referral hospitals for an estimated 15 million population in the area. The hospitals in collaboration with International Centers for AIDS treatment and care Program, Ethiopia, provide HIV preventive and ART services since 2004. Between January 2004 and February 2012, a total of 683 children were born to HIVpositive mothers at both hospitals, and 86 of them were confirmed to be infected with HIV. The study was conducted between December 30, 2011, and May 05, 2012, on the original data generated for HIV exposed infants/children and their corresponding parents' care between January 2004 and February 2012.

**Study Design, Study Population, and Sampling Design**

A hospital-based unmatched case-control study was carried out on children aged 6 weeks-18 months. The cases were HIV positive children less than 18 months of age born to HIVpositive mothers, and the controls were HIV negative children less than 18 months of age born to HIV positive mothers. Children's and their parents' medical records and the HIV follow-up database was used as a data source. The clear HIV infection status of children and their mothers on either medical records or the databases were required for data extraction. However, the records or databasethat lacked information on maternal ARV prophylaxis status, child ARV prophylaxis, breastfeeding pattern, or at least one maternal CD4 count/WHO clinical staging before child HIV status determination were excluded. In cases where repeated measurements of predictor variables such as CD4 count, WHO clinical staging, were encountered, values measured nearest to the time at which infant was tested for HIV, was considered. Epi-info version 3.5.1was used to determine the appropriate sample size using the following parameters: proportion of maternal breast problem among controls, 6.4%; proportion of maternal breast problem among cases, 21.1%(17); 5% significance level; power of 80%; control-to-case ratio of 4:1; and incomplete records/database of 15%, leading to 64 cases and 256 controls. A simple random sampling technique was applied to both cases and controls using a computergenerated a random number from sampling the frame created by child's treatment card number and the corresponding mother's records were obtained.

**Data Collection Procedure and Data Analysis**

Four trained nurses were recruited and trained to collect the data using a pretested questionnaire. The questionnaire was adapted from the Ethiopian HIV-exposed infant follow-up guideline, which consists of sociodemographic characteristics of the mother and child (age of the mother, sex of the child, address, and birth year), clinical profiles of the mother (HIV status known time, CD4, survival status, prevention of mother to child transmission (PMTCT) prophylaxis, WHO clinical stage, and breast lesion), clinical profile of the child (age of the child at enrollment, age of the child at status determination, birth place, birth weight, infant ARV prophylaxis, breastfeeding pattern, and breastfeeding duration), and paternal clinical profile (HIV status and ART enrollment status).

To maintain the quality of data, one health officer was recruited to supervise the data collection activities with the principal investigator. In addition, the questionnaire was pretested to estimate the time and simplicity of data transfer from data sources.

**Table 1.** Child's clinical profiles before HIV infection status determination associated with child HIV infection, in Southwest Ethiopia, January 2004–February 2012

| Variables | Cases, N=60(%) | Controls, N=235(%) | COR(95%CI) |
|---|---|---|---|
| Child's HIV status confirmed the age | | | |
| 6weeks–6 months | 19(31.70) | 146(62.10) | 1 |
| 7–12 months | 17(28.30) | 50(21.30) | 2.61(1.26,5.42)* |
| 13–18 months | 24(40) | 39(16.50) | 4.73(2.35,9.50)* |
| Birthplace | | | |
| Health facility | 33(55) | 193(82.10) | 1 |
| Home | 27(45) | 42(17.90) | 3.76(2.05,6.91)* |
| Child's sex | | | |
| Male | 15(25) | 68(28.90) | 0.82(0.43,1.57) |
| Female | 45(75) | 167(71.10) | 1 |
| Birth weight | | | |
| ≤2500g | 28(46.70) | 105(44.70) | 1 |
| <2500 g | 9(15) | 37(15.70) | 0.91(0.39,2.11) |
| Unknown | 23(38.30) | 93(39.60) | 0.93(0.50,1.72) |
| Infant ARV | | | |
| Yes | 40(66.70) | 178(75.70) | 1 |
| No | 20(33.30) | 57(24.30) | 1.56(0.85,2.89) |
| Breast Feeding pattern | | | |
| Exclusive | 19(31.70) | 157(66.80) | 1 |
| Mixed | 23(38.30) | 12(5.10) | 15.84(6.80,36.87)* |
| Exclusive replacement fed | 18(30) | 66(28.10) | 2.25(1.11,4.57)* |
| Breast Feeding duration when child's HIV status Confirmed | | | |
| ≤6 months | 43(71.70) | 188(80) | 1 |
| >6 months | 17(28.30) | 47(20) | 1.58(0.83,3.02) |

*: p<0.25; COR: crude odds ratio; CI: confidence interval; ARV: antiretroviral; HIV: human immunodeficiency virus

A 1-day training was given for data collectors and supervisors on the data collection procedure before data collection. Collected data were analyzed using the Statistical Package for Social Sciences for window version 16. Simple frequencies were used to see the overall distribution of the study subjects with regard to the variables under study. All variables with a P value less than 0.25 in a bi-variable analysis were entered into multiple logistic regression models using the backward likelihood ratio variable selection method. In addition, predictor variables that had shown a significant association with child HIV infection in the final model were evaluated for multi-colinearity using a variance inflation factor and standard error of the parameter estimate. In addition, each predictor variables in the final model were also evaluated for interaction.

An approval from the ethical committee was obtained from the institutional review board of Jimma University on October 6, 2011, through an approval letter number CPHMS/078/2011, and a letter of permission was also secured from the concerned hospital officials to access the patients' records. Confidentiality was assured through securing private rooms for data collectors while extracting the data from the records, filled in the questionnaire were obtained on daily bases and kept in secure places. We did not obtain verbal or informed consent from study participant as we used secondary data (records) sources and patient records/information was anonymized and re-identified prior to analysis.

## RESULTS

### Socio-Demographic Characteristics and Children Clinical Profiles

After a stratified random selection of 64 cases and 256 controls, 4 case records and 21 control records were excluded from the analysis due to missing values. The male to female ratio was 1.2:1 in the reviewed records of children. HIV-infected children were enrolled on an average at 6.15 (±3.40) months, while HIV-uninfected children were enrolled in ART care on an average at 3.51 (±2.08) months of age. In total, 82% of HIV-uninfected children were born in a health facility compared to only 55% of HIV-infected children. Seventeen percent of mothers of HIV-infected children died compared to 10% of the uninfected cases. Forty

**Table 2.** Parents' clinical profiles before child HIV infection status determination, in Southwest Ethiopia, January 2004–February 2012

| Variables | Cases, N=60(%) | Controls, N=235(%) | COR(95%CI) |
|---|---|---|---|
| Maternal address | | | |
| Urban | 36(60) | 146(62.10) | 1 |
| Rural | 24(40) | 89(37.90) | 1.09(0.61,1.95) |
| Maternal HIV status confirmation time | | | |
| Before delivery | 32(53.30) | 146(62.10) | 1 |
| After delivery | 28(46.70) | 89(37.90) | 1.44(0.81,2.54)^ |
| Maternal CD4 before delivery | | | |
| <200 cells/mm$^3$ | 32(53.30) | 22(9.40) | 8.04(3.93,16.42)^ |
| 200–350 cells/mm$^3$ | 7(11.70) | 97(41.30) | 0.40(0.16,0.98)^ |
| >350 cells/mm$^3$ or unknown | 21(35) | 116(49.40) | 1 |
| Maternal CD4 after delivery | | | |
| <200 cells/mm$^3$ | 10(16.70) | 54(23) | 0.65(0.29,1.44) |
| 200–350 cell/mm$^3$ | 22(36.70) | 83(35.30) | 0.93(0.49,1.74) |
| >350 cells/mm$^3$ or unknown | 28(46.60) | 98(41.70) | 1 |
| Maternal survival status | | | |
| Died | 10(16.70) | 24(10.20) | 1.76(0.79,3.91)^ |
| Alive | 50(83.30) | 211(89.80) | 1 |
| Maternal PMTCT prophylaxis | | | |
| None or sdNVP | 34(56.70) | 138(58.70) | 1 |
| HAART or sdNVP+AZT+3TC | 26(43.30) | 97(41.30) | 1.09(0.61,1.93) |
| Maternal WHO clinical stage before delivery | | | |
| I–IV | 38(63.30) | 149(63.40) | 1 |
| Not staged | 22(36.70) | 86(36.60) | 1.00(0.56,1.81) |
| Maternal WHO clinical stage after delivery | | | |
| I–IV | 15(25) | 189(80.40) | 1 |
| Not staged | 45(75) | 46(19.60) | 12.33(6.32,24.02)^ |
| Maternal breast lesion | | | |
| Yes | 17(28.30) | 9(3.80) | 9.93(4.15,23.73)^ |
| No | 43(71.70) | 226(96.20) | 1 |
| Paternal HIV status | | | |
| Positive | 15(25) | 54(23) | 1.15(0.57,2.31) |
| Negative | 15(25) | 57(24.20) | 1.09(0.54,2.18) |
| Unknown | 30(50) | 124(52.80) | 1 |
| Paternal ART enrollment status | | | |
| Enrolled | 14(23.30) | 129(54.90) | 0.25(0.13,0.48)^ |
| Not enrolled or Unknown | 46(76.70) | 106(41.1) | 1 |

^: p<0.25; COR: crude odds ratio; CI: confidence interval, HIV: human immunodeficiency virus; ART: antiretroviral therapy; HAART: highly active antiretroviral therapy; PMTCT: prevention of mother to child transmission; sdNVP: single dose nevirapine; AZT: Zidovudine; 3TC: lamivudine

percent of mothers of the HIV-infected children were from rural settings compared to only 38% for uninfected children (Table 1).

**Parental Factors Associated with MTCT of HIV**

In a bi-variable analysis, cases of maternal CD4 count <200 cells/mm$^3$ before delivery, no maternal clinical staging after delivery, and unknown paternal ART enrollment status were more likely to transmit the virus to their children (Table 2).

**Factors Independently Associated with children HIV Infection**

After controlling for the effects of other variables entered in to the multiple logistic regression, mixed breastfeeding, maternal

**Table 3.** Factors independently associated with child HIV infection, in Southwest Ethiopia, January 2004–February 2012

| Variables | Cases, N=60(%) | Controls, N=235(%) | COR (95%CI) | AOR (95%CI) |
|---|---|---|---|---|
| Breastfeeding pattern | | | | |
| Exclusive breastfed | 19(31.7) | 157(66.8) | | 1 |
| Mixed breast fed | 23(38.3) | 12(5.1) | 15.8(6.8,36.8)** | 22.0(5.3,91.5)*** |
| Exclusive replacement fed | 18(30) | 66(28.1) | 2.3(1.1,4.6)* | 0.5(0.1,3.0) |
| Maternal WHO clinical stage after delivery | | | | |
| I–IV | 15(25) | 189(80.4) | | 1 |
| Not staged at all | 45(75) | 46(19.6) | 12.3(6.3,24.02)** | 13.4(5,35.8)*** |
| Maternal CD4 before delivery | | | | |
| <200 cells/mm$^3$ | 32(53.3) | 22(9.4) | 0.7(0.3,1.4)^ | 17.1(4.7,62.1)*** |
| 200–350 cells/mm$^3$ | 7(11.7) | 97(41.3) | 0.9(0.5,1.7)^ | 1.0(0.3,3.7) |
| >350 cells/mm$^3$ or unknown | 21(35) | 116(49.4) | 1 | 1 |
| Maternal address | | | | 1 |
| Rural | 24(40) | 89(37.9) | 1.1(0.6,1.95)^ | 7.6(2, 29.2)** |
| Urban | 36(60) | 146(62.1) | 1 | |
| Infant ARV | | | | |
| Yes | 40(66.7) | 178(75.7) | 1 | 1 |
| No | 20(33.3) | 57(24.3) | 1.6(0.9,2.9)^ | 4.7(0.9, 24.2) |
| Pate Paternal ART enrollment status | | | | |
| Enrolled | 14(23.3) | 129(54.9) | 1 | 1 |
| Not enrolled or unknown | 46(76.7) | 106(41.1) | 4(2.1,7.7)*** | 11.1(2.9,50)*** |

***: p<0.001; **: p<0.01 and *: p<0.05; ^: p<0.25, COR: crude odds ratio; CI: confidence interval; AOR: adjusted odds ratio; ART: antiretroviral therapy; ARV: antiretroviral

CD4 count <200cells/mm$^3$ before delivery, no maternal WHO clinical staging after delivery, children born to mothers from urban settings, and no paternal ART enrollment were identified as independent predictors of child HIV infection (Table 3).

## DISCUSSION

Child HIV infection continued to be a major contributor to a preventable cause of morbidity and mortalities among Ethiopian children. The identification of child- and parent-related risk factors for MTCT of HIV is a workable approach to decrease the incidence of child HIV infection and thereby attain the global new child infection targets. Mixed breastfeeding, mothers with CD4 count <200cells/mm$^3$ before delivery, mothers who were not staged after delivery, mothers from a rural setting, fathers who were not enrolled or with unknown enrollment status to ART care were found to be independently associated with child HIV infection.

The measurements of parent- and child-related factors were not subjected to recall bias, as the data was collected from records; misclassification of cases and controls is very unlikely since the DNA PCR was used on all of the study subjects, which have high sensitivity and specificity (18). However, all children were enrolled in follow-up care at various ages; this could lead to misclassifications of indicator factors as causal predicators. Therefore, the results should be interpreted with all these limitations in consideration.

HIV-exposed children who had been mixed breastfed were 22 times more likely (adjusted odds ratio [AOR]: 22.03, 95% confidence interval [CI]:5.31-91.49) to be infected with HIV compared to exclusively breastfed children. The finding implies that mixed breastfeeding is the predominant postnatal risk factor for MTCT of HIV in the studied population. This is consistent with the comparative cross-sectional study finding among HIV exposed children in Addis Ababa (AOR: 6.10, 95%CI: 1.40-25.70) and Zimbabwe (AOR: 3.79; 95%CI: 1.40-10.29) (14, 16). The difference in the magnitude of association might be attributable to social determinants, such as fear of status discovery by relatives or other community members by nonbreastfeeding mothers, (16, 17, 19, 20) which might have contributed to the difference. The other possible reason for the difference could also be due to differences in the study design, in which the comparability of cases and controls cannot be ascertained in a comparative study as that of a case-control study design. However, it was comparable with the study finding from Ivory Cost where mixed breastfed children in the first 6 months of life were 6 times more likely to be infected with HIV (AOR=6.3, 95% CI: 1.1-36.4) compared to exclusively breastfed children in the first six months of life (21). It was also comparable with other several studies in the sub-Saharan African countries, which had shown a reduction of HIV transmission to less than one-fourth among exclusively breastfed children compared to mixed breastfed (22).

Mothers with a CD4 count of <200cell/mm$^3$before delivery were 17 times more likely (AOR: 17.14, 95% CI: 4.73-62.06) to transmit

HIV to their children compared to mothers with a CD4 count >350cells/mm$^3$ or with an unknown CD4 count. This is because the maternal CD4 count is inversely related to the viral load in the maternal body fluids, including breast milk, which in turn affects the child's viral exposure during pregnancy, delivery, and breastfeeding, as reported elsewhere (20, 23-25). A lower maternal CD4 count before delivery implies prolonged child exposure to higher maternal viral load during pregnancy and child birth. It implies that HIV positive pregnant mothers are coming to health institutions at an advanced stage of the disease probably because of lower PMTCT service uptake of the mothers (13).

A nested case-control study conducted in France also indicated a comparable finding, in which mothers with a higher viral load or lower CD4 count before delivery were 23 times more likely (AOR: 23.2; 95% CI: 3.5-553) to transmit the virus to their children compared to mothers with a lower viral load or higher CD4 count(20). It was also consistent with other study findings elsewhere in Africa and America (20, 25).

Children from mothers with no WHO clinical stage after delivery were 13 times more likely (AOR: 13.38; 95% CI: 5-35.76) to be infected with HIV compared to those whose mothers were staged. This might be related to low postnatal service uptake in the general population of Ethiopia, which could also hold true for HIV-positive mothers and thus allow mothers miss the follow-up schedule for WHO clinical staging and other services (26). This is roughly comparable to mothers who received no preventive measures at all among mothers in developing countries (12). In addition, more than three-fourth of the mothers who were staged after delivery had been on HAART, while only a one-fourth of the mothers who were not staged had been on HAART; a retrospective cohort study conducted in Mozambique indicated that mothers who had been on HAART before delivery were more likely (AOR: 3.15; 95% CI: 1.02-9.73) (27) to bring their children for an early diagnosis and thereby have the opportunity for staging for their own health as well. Hence, mothers who were staged are in a better position to be evaluated for child- and parent-related risk factors and receive the corrective measures which will, in turn, decreases HIV infection risks in children.

Children born to mothers from rural settings were 7 times more likely (AOR: 7.64; 95%CI: 2-29.22) to be infected with HIV compared to children to mothers from urban settings. This might be due to poor PMTCT service utilization among rural mothers and poor quality of maternity care in primary healthcare facilities where most rural mothers get the services.

Children whose fathers were not enrolled or had an unknown enrollment status to ART care were 11 times (AOR: 11.11; 95% CI: 2.94-50) more likely to be infected with HIV compared to children whose fathers were enrolled. Although the direction of a relationship was similar and the CIs overlap, there is a stronger association when compared with the finding from a prospective cohort conducted in Nairobi (Adjusted Hazard Ratio (AHR) =1.79; 95%CI: 1.02-3.03) (15). In Ethiopia, fathers enrolled in HIV chronic care when their sexual partners disclose their HIV positive status. Studies indicated that a female partner who disclosed her status and helped her partner enroll in ART care is more likely to participate in PMTCT of HIV services and more likely to adhere to ART for her own health (16, 17, 20). This could, in turn, decrease the risk of MTCT. But the non-disclosure issue of the mother to her male partner is commonly reported in the studied area (16).

## CONCLUSION

Mixed breastfeeding, maternal CD4 before delivery, no maternal WHO clinical staging after delivery, children to mothers from rural settings, and no paternal ART enrollment or an unknown enrollment status were independent predictors of child HIV infection. Behavioral change communication should be intensified to address mixed breastfeeding, paternal ART enrollment, and postpartum retention in care for clinical staging. In addition, service providers in the studied hospitals need to devise strategies to enhance postpartum retention in care for clinical staging and give special attention for mothers with a lower CD4 count before delivery and for mothers from rural settings.

**Ethics Committee Approval:** Ethics committee approval was received from the institutional review board of Jimma University (Approval Date: 06.10.2011; Approval No: CPHMS/078/2011).

**Informed Consent:** N/A

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Original Research

# Polyclonal Antibody Production Against Hapten-Structured KDN Molecule by Using Different Adjuvants Alternative to Freund's Adjuvant

Sultan Gülçe İz, Pelin Sağlam Metiner, Ilgın Kımız, Çağlar Kayalı, Saime İsmet Deliloğlu Gürhan

Department of Bioengineering, Ege University School of Engineering, İzmir, Turkey

**ABSTRACT**

**Objective:** KDN (2-keto-3-deoxy-D-glycero-D-galacto-nononic acid), a member of the sialic acid family, is a hapten-structured low-molecular-weight monosaccharide on the cell membrane, which cannot induce immune responses without a carrier protein. Since it is over-expressed on the cancerous cells' membrane, it is thought to be a great target molecule for anti-cancer treatments. The aim of this study is to obtain high titersof the anti-KDN polyclonal antibody response without using any carrier protein against the hapten-structured KDN molecule alternative to Freund's adjuvants.

**Methods:** Montanide™ ISA 61 VG, a water-in-oil adjuvant; ISA 201 VG, a water-in-oil-in-water emulsion adjuvant; and IMS 1313 VG NPR, an aqueous-dispersion-based nanoparticle (50-200 nm) microemulsion adjuvant; and Freund's adjuvant were used as anti-KDN antibody response stimulators. FourBALB/c mice were used for each adjuvant group, and immunization was performed at eight different time points. Anti-KDN antibody levels induced after each immunization with different adjuvants were detected with indirect enzyme-linked immunosorbent assay.

**Results:** The adjuvant efficiency of Montanide™ ISA 61 VG water in oil adjuvant was 1.4 times higher than in Freund's adjuvant ($p<0.0001$), with a maximum anti-KDN level on Day 83.

**Conclusion:** It's shown that without any carrier protein conjugation molecules such as hapten-structured KDN, higher amount anti-KDN antibody titres could be obtained by using a more safe and effective Montanide™ ISA 61 VG water-in-oil adjuvant as an alternative to Freund's adjuvants. In this regard, it may be possible to produce high-antibody titers without using any carrier molecule, especially when commercial large scale monoclonal antibodies are desired to be produced against haptens as therapeutic approaches.

**Keywords:** KDN (2-keto-3-deoxy-D-glycero-D-galacto-nonionic acid), polyclonal antibody, ELISA

## INTRODUCTION

2-keto-3-deoxy-D-glycero-D-galacto-nononic acid, an abbreviation for 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid, was discovered in 1986 and is found in almost all glycoconjugates containing glycolipids, glycoproteins, and capsular polysaccharides. It has been reported that KDN has higher expression levels in fetal red blood cells than in adult red blood cells, as well as in ovarian tumor tissue cells, which have a higher KDN expression in comparison with normal ovarian tissue (1).

2-keto-3-deoxy-D-glycero-D-galacto-nononic acid, a member of the sialic acid family has distinctive properties such as insensitivity to various sialidases and the association of N-acetyl group with the hydroxyl group of NeuAc (N acetylneuraminic acid) has been shown to be related to human cancers (2-3). The synthesis of KDN by organic routes makes it possible to become an important target for anti-cancer therapies (4). Ketocidic bonds in the diamine sialic acid type KDN cause resistance to bacterial and animal sialidases and protect against bacterial and viral attacks (1). In addition, KDN exhibits metastasis and anti-Alzheimer activity as it stops the glycolipid synthesis as an immunoregulator (5). Due to these features, studies on the use of KDN as an early warning signal of disease or in case of disease recurrence have been reinforced (6). In addition, the KDN expression level is elevated in ovarian adenocarcinomas and is associated with metastasis (7).

2-keto-3-deoxy-D-glycero-D-galacto-nononic acid is a relatively low-molecular-weight molecule of hapten character (4). Haptens are small molecules capable of reacting with antibodies that are specific immune-response products, having antigenicity, but not immunogenicity alone, capable of producing an immunological response only after binding with a carrier macromolecule (e.g., protein) (2, 8, 9). In general, it is thought that only large molecules, infectious agents, and insoluble foreign substances cause immunological reactions in the body (10). For the application of vaccine

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**ORCID IDs of the authors:** S.G.İ. 0000-0002-9275-6272; P.S.M. 0000-0002-5726-1928; I.K. 0000-0002-9639-6550; Ç.K. 0000-0002-3266-1757; S.İ.D.G. 0000-0003-3841-6282.

**Corresponding Author:** Sultan Gülce İz **E-mail:** sultangulce@gmail.com

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production, the use of protein carriers is preferred in the conjugation of haptens (11). However, in the polyclonal or monoclonal antibody production, the antigen is expected to be pure enough to induce a specific immune response. In addition, the purification steps become difficult because of the carrier protein. Although in the laboratory scale production, the purification problem can be ignored, in large-scale production, the cost of purification steps will increase the overall cost of the antibody production process (11). In this context, the ultimate goal of the study is to find an alternative adjuvant to the protein conjugates for hapten-structured KDN.

Freund's adjuvants are emulsion adjuvants that are the most commonly used the oil-in-water emulsion for immunization studies (12, 13). The main disadvantages of them are toxicity, the formation of granulomas, inflammation, and lesions at the vaccination site. In this regard, the development of new adjuvants as an alternative to Freund's adjuvants is on the agenda(14, 15). Montanide™ adjuvants are non-toxic systems, optimized to increase the vaccine efficacy, formulation stability, and also to minimize side effects. There are commercialized vaccine registries using Montanide™ adjuvants, and they have been shown to be reliable in scientific studies(15-17; www.seppic.com/montanide-range). Montanide™ ISA 61 VG is a water-in-oil adjuvant; ISA 201 VG is a water-in-oil-in-water emulsion adjuvant, and IMS 1313 VG NPR is an aqueous dispersion based on nanoparticle(50-200 nm) microemulsion adjuvant with immune stimulators.

Although the Freund's adjuvants are the most used adjuvant systems in animal experiments, it is ethically controversial to use them due to their side effects (local acute or chronic inflammation, abscess at the injection site, a permanent nodule, wound, or lymphadenopathy)given the 3R rule (replacement, reduction, and refinement) in the use of experimental animals. Thus, using the Montanide™ adjuvants also address the same ethical issue of using Freund's adjuvants.

The aim of this study is to obtain a high titer anti-KDN polyclonal antibody response using safer and more efficient adjuvant systems that are alternative to Freund's adjuvants without using any carrier protein against the hapten-structured KDN molecule.

## METHODS

**Chemicals and Experimental Animals**

Montanide™ ISA 61 VG (Seppic, France), Montanide™ ISA 201 VG (Seppic, France), and Montanide™ IMS 1313 VG NPR (Seppic, France) adjuvants were obtained from (Seppic Turkey, YILBAK Tic. A.Ş.). KDN (Cat no: 60714), Freund's complete adjuvant (FCA; Cat no: F5881) and Freund's incomplete adjuvant (FICA; Cat no: F5506), NaCl, KCl, bovine serum albumin (BSA),o-phenylenediaminedihydrochloride (OPD), and citric acid were purchased from Sigma, USA; horseradish peroxidase (HRP)-labeled anti-mouse IgG was from Thermo Scientific, USA and $Na_2HPO_4.2H_2O$, Tween-20 and $H_2O_2$ from Merck, Germany. Six- to eight-week-old BALB/c mice were purchased from Uludağ University Research Center for Breeding Test Animals (DEHYUAM). This study has been approved by Ege University's Animal Experiments Local Ethics Committee on 25/11/2015 with an approval number of 2015-084.

**Immunization with KDN**

Immunization studies were carried out at the Ege University, Drug Development and Pharmacokinetics Research and Application Center (ARGEFAR) pre-clinical development unit. Five experimental groups including four mice in each group-Montanide™ ISA 61 VG, Montanide™ ISA 201 VG, and Montanide™ IMS 1313 VG NPR adjuvants, Freund's adjuvants, and the control group that were not immunized with KDN-were used. In the Freund's adjuvant group, FCA was used in the first immunization, and FICA was used in the following immunizations. Before the first immunization, blood was drawn from the tail of all mice as 0.2-0.5 mL/mouse to obtain the blood serum. In the first immunization, 100 µg KDN antigen in 100 µL saline was injected subcutaneously with 100 µL adjuvant in a total volume of 200 µL for each mouse. In the second immunization on Day 14, mice were injected intraperitoneally with 50 µg KDN/100 µL saline and 100 µL adjuvant in a total volume of 200 µL. The third immunization was performed on Day 28, the fourth immunization on Day 55, the fifth immunization on Day 72, the sixth immunization on Day 84, the seventh on Day 98, and the eighth day on Day 112, subcutaneously with 50 µg KDN/100 µL saline and 100 µL adjuvant in a total volume of 200 µL. For the indirect enzyme-linked immunosorbent assay (ELISA), blood was drawn from the tail after the third immunization at the 41$^{st}$, 69$^{th}$, and 83$^{rd}$ days, respectively, and from the heart on the 125$^{th}$ day.

**Blood Sample Collection and Serum Separation**

Primarily, mice tails were treated with warm water to make the vein more visible. When the vein became clearly visible, the tail was wiped with alcohol, and the intracath was introduced into the vein to collect 0.2- 0.5 mL of blood. For bloodletting from the heart, ketamine (60-90 mg/kg) and xylazine (4-7 mg/mouse) were administered to mice, and they were euthanized by cervical dislocation. All blood was collected as much as possible by injecting into the heart ventricle. Blood samples were incubated overnight at +4$^0$C. And were centrifuged at 3000 rpm for 10 minutes to separation. After centrifugation, the supernatant (serum) was removed and stored at -20°C for indirect ELISA.

**Indirect ELISA to determine anti-KDN antibody titer**

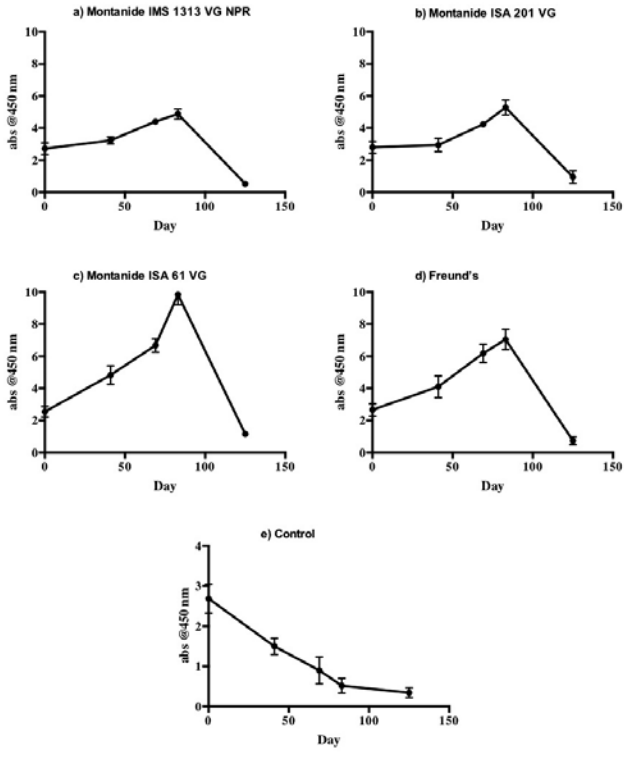
In indirect ELISA, the ≥99.0% thin-layer-chromatography-grade KDN molecule was used to coat the plates. Briefly, the KDN protein was diluted to a final concentration of 175 ng/well in phosphate buffered saline (PBS), added 100 µL into the ELISA plates, and incubated overnight at 37°C. The wells were spilled, and 100 µL blocking solution consisting of 0.5% (w/v) BSA in 0.1 M PBS was added to the wells and incubated for 1 hour at room temperature. The plates were washed three times with 150 µL TPBS (0.1 M PBS with 0.1% (v/v) Tween-20). The 100 µL blood serum samples and controls were added as the primary antibody, and plates were incubated for 1 hour at room temperature. The plates were washed three times with 150 µL tween phosphate buffered saline (TPBS). A 100 µL diluted conjugated secondary antibody (anti-mouse IgG conjugated with HRP was diluted in TPBS) was added, and plates were incubated 1 hour at room temperature. The plates were washed three times with 150 µL TPBS. 200 µLof substrate solution (30 mg of OPD was dissolved

**Table 1.** ELISA results of antibody titer against KDN obtained after immunization with different adjuvants

| | Absorbance @450 nm | | | | |
|---|---|---|---|---|---|
| Day | Montanide™ IMS 1313 VG NPR Adjuvant | Montanide™ ISA 201 VG Adjuvant | Montanide™ ISA 61 VG Adjuvant | Freund's Adjuvant | Control (without immunization with KDN) |
| 0 | 2.725±0.372 | 2.804±0.370 | 2.548±0.326 | 2.660±0.380 | 2.684±0.362 |
| 41 | 3.240±0.219 | 2.948±0.409 | 4.833±0.590 | 4.106±0.679 | 1.495±0.202 |
| 69 | 4.416±0.183 | 4.236±0.174 | 6.678±0.420 | 6.175±0.577 | 0.900±0.339 |
| 83 | 4.881±0.318 | 5.283±0.466 | 9.848±0.630 | 7.044±0.630 | 0.515±0.183 |
| 125 | 0.536±0.169 | 0.946±0.399 | 1.156±0.095 | 0.737±0.238 | 0.340±0.124 |

Montanide™ ISA 61 VG: water-in-oil adjuvant; Montanide™ ISA 201 VG: water-in-oil-in-water emulsion adjuvant; Montanide™ IMS 1313 VG NPR: aqueous dispersion based on nanoparticle (50-200 nm) micro emulsion adjuvant

Figure 1. Changes in anti-KDN pAb absorbance values of blood serum obtained after immunization using different adjuvants with respect to days: immunization with Montanide™ IMS 1313 VG NPR, which is an aqueous dispersion based on nanoparticle (50-200 nm) micro emulsion adjuvant (a); immunization with the Montanide™ ISA 201 VG water-in-oil-in-water emulsion adjuvant (b); immunization with the Montanide™ ISA 61 VGa water-in-oil adjuvant (c); immunization with Freund's adjuvant (d); control group without immunization with KDN (e)




in 75 mL 0.05 M of the phosphate-citrate buffer, and 30 µL fresh 30% [v/v] H2O2 was added immediately prior to use) was added, and the plates were incubated for 30 minutes in dark at room temperature. After 30 minutes, the absorbance was recorded at 450 nm wavelength with a UV-visible spectrophotometer (SpectraMax 190, VersaMax, USA).

**Statistical Analysis**

The obtained data were evaluated in the two-way variance analysis (two-way ANOVA) with ±95% confidence interval using GraphPad Prism 6.0e, taking into account the days of immunization and the different adjuvant types.

## RESULTS

The anti-KDN polyclonal antibody level according to the ELISA results obtained from Montanide™ ISA 61 VG, Montanide™ ISA 201 VG, Montanide™ IMS 1313 VG NPR, Freund's adjuvants, and the control group on Days 0, 41, 69, 83, and 125 is shown in the Table1.

According to Figure 1, all groups reached the highest level of anti-KDN polyclonal antibody on Day 83. With Freund's adjuvant, the highest absorbance was 7,044±0,630; with the Montanide™ adjuvants including IMS 1313 VG NPR, ISA 201 VG and ISA 61 VG; 4.881±0.318, 5.283±0.466, and 9.848±0.630 absorbances were obtained respectively after the last immunization. In the control group, which was not immunized with KDN, the absorbance decreased on a daily basis (Figure 1).

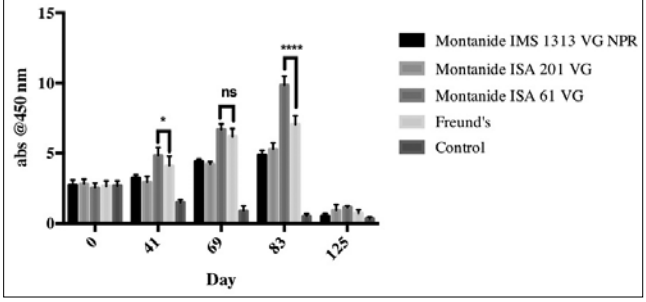
When evaluating the resulting anti-KDN titers, the Freund's adjuvant was taken as the basis and compared with different Montanide™ adjuvants whose efficacy in this study was to be compared on the same graph in Figure 2.

According to Figure 2, there was no statistically significant difference ($p>0.05$) between the blood samples taken from all groups before immunization (Day 0). Based on the results of the 41$^{st}$, 69$^{th}$, and 83$^{rd}$ days ELISA, it was determined that the polyclonal antibody titer obtained after using the Montanide™ IMS 1313 VG NPR and Montanide™ ISA 201 VG adjuvants was lower than the polyclonal antibody titer obtained with Freund's adjuvant. When the Montanide™ ISA 61 VG and Freund's adjuvants were compared, the level of the anti-KDN polyclonal antibody titer obtained using the Montanide™ ISA 61 VG adjuvant was higher at last three immunization points, especially 1.4 times higher on the Day 83 ($p<0.0001$).

## DISCUSSION

2-keto-3-deoxy-D-glycero-D-galacto-nononic acid is a sialic acid family specific monosaccharide found on the cell membrane.

Figure 2. Comparative study of the Montanide™ adjuvant groups activities according to Freund's group: Montanide™ ISA 61 VG is a water-in-oil adjuvant; Montanide™ ISA 201 VG is a water-in-oil-in-water emulsion adjuvant; Montanide™ IMS 1313 VG NPR is an aqueous dispersion based on nanoparticle (50-200 nm) micro emulsion adjuvant. *: p<0.05; ****: p<0.0001; ns: non-significant p>0.05




It has been discovered for the first time in a patient with ovarian cancer that the level of free KDN molecules is high; thus, it is thought to be an antigen that promotes positive malignancy with cancer development, because it is generally exaggerated at low levels in mammalian cells (1, 4, 6, 18). After that, several studies showed that the KDN expression is high in cancerous cells, and KDN expression is associated with tumoral, nodal, and metastatic differentiation of tumors (5, 6, 18). It has been found that while the hypoxic environment enhances the invasiveness and metastatic ability of cancer cells (e.g., HeLa; adenocarcinoma, CaCo-2; colorectal adenocarcinoma and LS174T Dukes' type B; colorectal adenocarcinoma), it also induces the production of KDN in these cells by creating changes in the sialic acid metabolism (4, 6).

With haptens, like with KDN, the immunological response is usually achieved by chemical modification with the hapten-carrier complex (e.g., BSA-bovine serum albumin) and modifications to introduce active groups. On this account, artificial antigens can be obtained and used to stimulate B-cell proliferation and T-cell differentiation to produce antibodies against the hapten molecules (10, 11, 19). BSA is the most common carrier protein in the macromolecular, stable structure of the single polypeptide chain without carbohydrates, which is preferred for haptens in the conjugation reactions due to various functional groups carried by the structure in the antibody formation(10, 20, 21).

The name adjuvants, one of the conjugates used to increase the immunity of the haptens, is derived from the Latin word adjuvare, which means help or healing (12). To be effective, adjuvants must affect dendritic and macrophage cells, which are antigen-presenting cells in the natural immune system, to stimulate T and B lymphocytes in the living organism (11, 22). Although there are many types of adjuvants, oil-water-based emulsions and micro-nanoparticles are classified as adjuvants for antigen transport, and cytokines and saponins are classified as adjuvants for immune stimulators according to their mechanism of action (12, 22).

Freund's adjuvants are the most commonly used systems in immunization studies, called incomplete Freund's adjuvant (FICA) when prepared with non-metabolic oils and complete Freund's adjuvant (FCA) when prepared with dead Mycobacterium tuberculosis (12). Freund's adjuvants are designed to provide a sustained release of the antigen needed to stimulate a strong and lasting immune response (13). However, the use of these adjuvants for antibody production usually causes very severe lesions in experimental animals (14). For this reason, many regulatory and supervisory rules for licensing have been rearranged to limit the use of such adjuvants. In this regard, the development of new adjuvants has come to the agenda as an alternative to Freund's adjuvants (15).

The Montanide™ adjuvants are systems that have been scientifically proven to be reliable and available with commercialized vaccine products already on the market. They are optimized to increase vaccine efficacy and formulation stability and also to minimize side effects. These adjuvants usually include a surfactant system: in general, emulsions such as water-in-oil, water-in-oil-in-water, oil-in-water, and in nanoparticulate form (15-17, www.seppic.com/montanide-range).

The Montanide™ ISA 61 VG used in this study is an adjuvant that provides a powerful cellular immune response in the form of water-in-oil emulsion, fortified with light mineral oils to enhance the Th1 response and production of IgG2, with the addition of mannitol and vegetable oleic acid, without carrying an animal component (23, 24; www.seppic.com/montanide-isa-w-o). The Montanide™ ISA 201 VG adjuvant is a water-in-oil-in-water emulsion of similar features to the Montanide ISA 61 VG adjuvant. It stimulates both humoral and cellular long immune response (23, 24; https://www.seppic.com/montanide-isa-w-o-w). The Montanide™ IMS 1313 VG NPR adjuvant is a formulation in the form of an aqueous dispersion based on nanoparticles (50-200 nm). It contains immunostimulatorygenerally recognized-as-safe substances, and it rapidly stimulates both humoral and cellular immunological response (25; www.seppic.com/montanide-ims).

Antigen-antibody interactions are important in ELISA, and antigen-antibody interfaces are primarily controlled by hydrophobic and electrostatic interactions (9). In this study, immunization was performed at eight-time points, the last one on the 125th day. The blood samples taken at regular intervals were tested by indirect ELISA to determine the level of antibody response to KDN. Freund's adjuvants, which are the most commonly used adjuvant systems in scientific studies (12, 13), and the alternative Montanide™ (ISA 201 VG, ISA 61 VG, and IMS 1313 VG NPR) adjuvants were compared statistically in this study by two-way ANOVA test. Among the obtained anti-KDN polyclonal antibody levels, the highest anti-KDN polyclonal antibody titer is obtained with the Montanide™ ISA 61 VG adjuvant, which is 1.4 times more than the antibody levels obtained withFreund's adjuvant (P<0.0001). The Montanide™ ISA 61 VG adjuvant is specially developed for studying low-immunogenicity antigens in the form of water-in-oil (23, 24), which makes it a candidate for working with haptens (Figure 2).

As Figure 2 indicates, the absorbance values of anti-KDN polyclonal antibody were measured at a certain level in all adjuvant groups including the control group on the 0th day due to the

fact that KDN is generally excreted at low levels in normal life span in mammalian cells and these values are not statistically different from each other (p>0.05) (5, 6). Thereafter, it was observed that the anti-KDN antibody level increased by following immunizations, reached the maximum value on day 83rd, and immunity was decreased by age due to the immunological senescence (1, 5, 26). In the control group in which no KDN protein was given, it was determined that there was a decrease in the antibody level during lifetime from this cause (Figure 2). İbrahim et al. (24) performed immunization experiments in mice with the Montanide™ ISA 201, 206, 61 and 50 VG adjuvants for the foot-and-mouth disease vaccine development studies. Antibody titers were compared by ELISA in blood samples collected during the 20-week trial period. When the Montanide™ ISA 201 and 61 VG adjuvants were compared, it was also reported that the highest antibody titer was achieved with Montanide ISA 61 VG, and when the time-varying antibody titers were examined, the titers of antibodies were increased until the 10th week and started to decrease since then with aging due to the immunological senescence (24).

It has been stated that low-molecular-weight hapten-structured carbohydrates such as KDN can only bind to the receptors present on the surface of B lymphocytes, whereas they cannot activate the helper T cells and thus cannot achieve an antibody response against them (8). For this reason, it is stated that suitable carrier protein conjugates should be used (9). However, this study showed that the desired antibody response could be obtained using the Montanide™ ISA 61 VG water-in-oil adjuvant as an alternative to Freund's adjuvant without conjugating any carrier protein such as BSA to the KDN molecule.

## CONCLUSION

It may be possible to produce high-antibody titers against hapten-structured proteins without using any protein carrier molecule when using alternative adjuvant systems. This is a promising result for a large-scale antibody production against haptens, especially for commercial therapeutic monoclonal antibody production process as means of antibody specificity and ease of purification steps.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the animal experiments local ethics committee (EUHADYEK) of Ege University (Approval Date: 25.11.2015; Approval No: 2015/084).

**Informed Consent:** N/A

**Peer-review:** Externally peer-reviewed.

**Author contributions:** Concept – S.G.İ., S.İ.D.G.; Design – S.G.İ., S.İ.D.G.; Supervision – S.G.İ.; Resource – S.G.İ., C.K.; Materials – S.G.İ., S.İ.D.G., C.K.; Data Collection and/or Processing – P.S.M.,I.K., C.K., S.İ.D.G.; Analysis and/or Interpretation – S.G.İ., S.İ.D.G., P.S.M., I.K.; Literature Search – S.G.İ., P.S.M., I.K.; Writing – S.G.İ., S.İ.D.G., P.S.M., I.K.; Critical Reviews – S.G.İ., S.İ.D.G.

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**Conflict of Interest:** The authors have no conflicts of interest to declare.

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Original Research

# Splanchnic Venous Thrombosis, with Spotlight on Occult Malignancies, Anticoagulation, and Bleeding

Elif Gülsüm Ümit, Mehmet Baysal, Hakkı Onur Kırkızlar, Ahmet Muzaffer Demir

Department of Hematology, Trakya University School of Medicine, Edirne, Turkey

**ABSTRACT**

**Objective:** Splanchnic venous thrombosis (SVT) conceptually embraces thrombosis in the portal, hepatic, splenic, and mesenteric venous system thrombosis. The SVT risk factors may be classified as abdominal disorders, underlying myeloproliferative neoplasms (MPN), inherited thrombophilic syndromes, and autoimmune disorders. The aim of our study is to evaluate the risk factors for SVT and their relations with the localization of involvement and anticoagulation during the acute period and relation to major bleeding.

**Methods:** All patients with portal vein thrombosis or splenic venous thrombosis in their radiologic evaluation report were included over a 5-year period.

**Results:** Of the 96 patients, 87 had an identifiable risk factor for SVT (90.6%). The major risk factor was cirrhosis (60 patients, 62.5%). Other risk factors included thrombophilic conditions (12 patients, 12.6%), 6 patients had the myeloproliferative disorder (6.3%), and most interestingly, 24 had occult malignancy for which SVT was the presenting factor (25%). Within the whole group, 51 patients (53.1%) received anticoagulant treatment. Within the whole group, 30 patients developed major bleeding (31.3%), and 20 of these patients did not receive anticoagulation therapy. Twenty-five of the patients with cirrhosis suffered bleeding, and 18 of them did not receive anticoagulation therapy.

**Conclusion:** Almost all patients with SVT had an identifiable risk factor. The follow-up and further treatments should be based on this risk factor. SVT may be the presenting finding of occult malignancies and occult malignancy should be investigated in every patient with SVT. Anticoagulation during the initial acute period should not be withheld, even in patients with the chronic liver disease with a concern for major bleeding.

**Keywords:** Splanchnic venous thrombosis, anticoagulation, bleeding, malignancy

## INTRODUCTION

Splanchnic venous thrombosis (SVT) refers to thrombosis in the portal venous system (as portal vein thrombosis [PVT]), the hepatic venous system (as Budd-Chiari syndrome [BCS]), splenic venous system, and mesenteric venous system (as mesenteric venous thrombosis [MVT]). As an unusual site for venous thrombosis (VT), the incidence of SVT in general population is 0.7 per 100,000 person/years for PVT, and 0.8 per 100,000 person/years for BCS, although the incidence of deep VT (DVT) is 100 per 100.000 person/years (1-3).

As all sites for SVT are accepted as different pathologic entities, concomitant involvement as well as acute, subacute, or chronic presentations may telescope. Patients may be asymptomatic, and the presentation in patients depends upon the extent and speed of the thrombosis and coexisting conditions. Acute SVT generally present with a sudden onset of abdominal pain, fever, nausea, vomiting, and diarrhea. Liver functions are generally preserved due to the rapid development of collateral veins and the compensatory capacity of hepatic arterial flow. Acute MVT may also present with intestinal bleeding due to mesenteric ischemia. Pain may radiate to the back, and ileus may be observed if the proximal branches of the mesenteric artery are occluded. Hematochezia may be the sign of intestinal infarction (4).

In patients suffering from cirrhosis, PVT is usually asymptomatic, but in patients presenting with complications of cirrhosis, such as hepatic encephalopathy, gastrointestinal bleeding, or deterioration of ascites, PVT should be suspected. Doppler ultrasonography has a sensitivity of 90% in the diagnosis of PVT and BCS, but in MTV, the overlying bowel gas may complicate the vision obtained by ultrasonography, and computed tomography or magnetic resonance imaging should be performed (5).

Previously defined as primary/secondary or provoked/unprovoked, the distinction of an underlying risk factor is challenging. True unprovoked primary SVT is reported as 15% to 27% of all patients (3).

The SVT risk factors may be classified as abdominal disorders, underlying myeloproliferative neoplasms, inherited thrombophilic

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**ORCID IDs of the authors:** E.Ü. 0000-0001-5589-3000; M.B. 0000-0001-7681-4623; H.O.K. 0000-0001-7523-8599; A.M.D. 0000-0002-2073-5405

**Corresponding Author:** Elif Gülsüm Ümit **E-mail:** elifgumit@trakya.edu.tr

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syndromes and conditions, autoimmune disorders, viruses, and hormones (6).

The aim of our study is to evaluate the SVT risk factors and their relations with localization of involvement and anticoagulation during the acute period and relation with major bleeding.

## METHODS

All patients with PVT or splenic VT stated in their radiologic evaluation report were retrospectively included over a 5-year period. Concurrent chronic liver disease (CLD), inherited thrombophilic disorders, and occult malignancies for which splanchnic thrombosis was the presenting symptom were also evaluated and recorded. Major bleeding was defined as fatal, leading to surgery, in a critical organ (intracranial, retroperitoneal, intraocular), overt bleeding with a hemoglobin decrease of 2 g/dL or greater, requiring a red blood cell transfusion of 2 units or more, and requiring hospitalization as defined by International Society of Haemostasis and Thrombosis (ISTH) (7).

All analyses were performed by the IBM SPSS version 20. Descriptive analysis, chi-squared, and Fisher's exact test were performed and all significant parameters were assessed by the logistical regression method. The study was approved by local ethical committee (2014/62) as a retrospective data collection study. Informed consent was obtained from every patient who have been reached and living.

The study has been conducted according to the Helsinki Declaration. As it was conducted as a retrospective study, all data were collected from the files, and patients' personal information was protected.

## RESULTS

Out of 96 of patients with SVT, 48 were female (50%), while 48 were male (50%). The mean age was 53.8 years (19–94). Sixty had PVT (62.5%), 10 had splenic VT (10.4%), 3 had BCS (3.1%), and 22 had multiple thromboses in the splanchnic area (22.9%).

Fifty were incidental, while 46 had concurrent known CLD. The main presenting symptom was an abdominal pain (64 patients 66.6%), whereas 32 patients were asymptomatic (33.3%). Seventy-five patients had acute SVT (78.1%), whereas 21 had chronic SVT (21.9%). Most of the patients with asymptomatic SVT had occult solid tumors and myeloproliferative neoplasms (MPN) (33 patients, 66%). The mean age of patients with MPN was 44.6 and 56.2 in patients with CLD.

Regarding the SVT etiology, 87 had an identifiable risk factor for SVT (90.6%). The major risk factor was cirrhosis (60 patients, 62.5%). Other risk factors included thrombophilic conditions (12 patients, 12.6%), 6 patients had MPN (6.3%), and most interestingly, 24 had occult malignancy for which SVT was the presenting factor (25%). In 36 patients, SVT was the main reason for the deterioration of the clinical condition (60%). Fifty-seven of the patients with CLD had PVT (95%), while almost all patients with MPN (70%) had multiple thromboses in the splanchnic area, and the majority of patients with tumors had PVT (18 patients, 75%).

## DISCUSSION

The major issue and finding of our study are about treatment and bleeding. Within the whole group, 51 patients (53.1%) received anticoagulant treatment (including the initial acute post-thrombotic period), and a significant percent of patients did not receive anticoagulant treatment. Within the whole group, 30 patients developed major bleeding (31.3%), and 20 of these patients did not receive anticoagulation (p=0.008). Again, regarding anticoagulation and bleeding, 2 of the patients with MPN suffered bleeding and were not on anticoagulation, 7 patients with a solid tumor had to bleed, and 3 of them did not receive anticoagulation (not statistically significant). None of the patients with thrombophilia had to bleed, and all were on anticoagulation (p=0.003), and 25 of the patients with cirrhosis had to bleed, and 18 of them did not receive anticoagulation (p=0.000).

The first noteworthy observation of this study is the high incidence of occult malignancies. Patients presenting with acute abdominal pain without a clinical history should be evaluated for these unexpected diagnoses (8). In these patients, the most common pre-diagnosis for the radiology request was surgical acute abdomen such as acute appendicitis. Likewise, patients with CLD are most likely to be disregarded as any chronic disease, and awareness of both the caregivers and patients should be encouraged. Likewise, any difference in the clinical condition should raise the concern for the SVT development (5).

Majority of the patients with SVT, with CLD or not, had been undertreated. This reluctance to include anticoagulation therapy in acute or chronic, or asymptomatic SVT, may be due to the lack of suggestive guidelines or reviews. Prospective studies and expert opinions are needed, especially in patients with asymptomatic SVT and chronic SVT without an identifiable etiologic factor.

Only 53.1% of patients with acute SVT received anticoagulation, and within this group, 10 patients had major bleeding. Indeed, major bleeding was observed more frequently in patients who did not receive anticoagulation therapy. The decision not to include such therapy may be due to the concerns about bleeding, and in patients with CLD, due to the condition called "already anticoagulated due to liver failure" This persuasion has been cleared away with a definition of "rebalanced hemostasis" in patients with CLD, since all levels of the hemostatic process may be impaired, including primary hemostasis with thrombocytopenia and platelet dysfunction, coagulation with an impaired coagulation factor production, and increased fibrinolysis due to increased levels of tissue plasminogen activator, decreased levels of alpha 2 antiplasmin, factor XIII, and thrombin-activatable fibrinolysis inhibitor, and increased levels of fibrin degradation products that may influence normal hemostatic process (9-11). However, in our study, patients with CLD who were not on anticoagulation had more episodes of major bleeding than patients who were on anticoagulation.

## CONCLUSION

Almost all patients with SVT had an identifiable risk factor, and the follow-up and further treatments should be based on this risk factor. SVT may be the presenting finding of occult malig-

nancies and should be sought in every patient with SVT. Anticoagulation during the initial acute period should not be withheld even in patients with CLD with a concern for major bleeding.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the local ethics committee of TUTF GOKAEK2014/62.

**Informed Consent:** Informed consent was obtained from parents of the patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

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Case Report

# Vein of Galen Aneurysmal Malformation Presenting with Macrocephaly

Hüseyin Gümüş[1], Halil Kazanasmaz[1], Mustafa Çalık[2]
[1]Department of Pediatrics, Harran University School of Medicine, Şanlıurfa, Turkey
[2]Department of Pediatric Neurology, Harran University School of Medicine, Şanlıurfa, Turkey

**ABSTRACT**
The vein of Galen aneurysmal malformation (VGAM) is a rare congenital intracranial vascular malformation found in children. Dilatation of the vein of Galen is a common feature of VGAM. This malformation is actually a vascular lesion that may present as different clinical pictures in childhood and is difficult to extract using classical surgical methods because of its locality and hemodynamic structure. Major complications of VGAM include heart failure, hydrocephaly, intracranial bleeding, and vascular steal phenomenon. Here the case of a 4-year-old girl who presented with macrocephaly and was diagnosed with VGAM is discussed in the light of literature.
**Keywords:** Vein of Galen malformation, macrocephaly, congenital

## INTRODUCTION

The vein of Galen aneurysmal malformation (VGAM) is a rare congenital vascular malformation characterized by the shunting of the arterial flow into an enlarged cerebral vein of Galen. Although VGAM cases constitute only 1% of all cerebral vascular malformation cases, they comprise up to 30% of all pediatric vascular malformation cases. The arteries feeding VGAM are the posterior cerebral, choroidal, and posterior perforating arteries (1, 2). VGAM may present with different clinical pictures in childhood. Approximately 95% of newborns die of heart failure and the remaining 5% die of hydrocephaly, subarachnoidal bleeding, or intraventricular bleeding. Older children present with different complaints, which most commonly includes a headache, followed by hydrocephaly, subarachnoidal bleeding, and various neurological signs. An early surgical intervention is necessary to reduce the rate of these complications and prevent particularly severe cerebral injury (3-5).

In this case report, we present the case of a 4-year-old girl patient who presented with macrocephaly and was diagnosed with VGAM.

## CASE PRESENTATION

A 4-year-old girl presented to our clinic with a headache and an enlarged head circumference. Her medical history revealed macrocephaly followed up since the age of 1. Her complaints had recently aggravated. A headache was localized in the frontotemporal region and was continuous. Upon a physical examination, her weight was evaluated to be 14 kg (<10$^{th}$ centile), height 93 cm (<3$^{rd}$ centile), and head circumference 52 cm (>97$^{th}$ centile). Her head appeared macrocephalic, and the frontal bossing was present. Systolodiastolic murmur was auscultated all around the head, particularly in the left temporal region. She also had a systolic murmur of II/VI severity, best auscultated in the mesocardiac area. An echocardiography revealed a secundum type of atrial septal defect, 4 mm in size. A brain magnetic resonance imaging (MRI) demonstrated that the nidus and the vein of Galen were enlarged (Figure 1). On MR angiography, the internal cerebral vein and the vein of Galen were observed to be dilated and an arteriovenous malformation (AVM) was noted originating from the interpeduncular cistern extending into the quadrigeminal cistern and lateral ventricle, nourished by the posterior cerebral artery, draining into the internal cerebral vein and vein of Galen. On cerebral angiography, a grade III AVM nourished from the bilateral posterior choroidal and thalamo-perforating arteries was observed in the middle axis, and a surgical operation was planned. The Galenic vein system was markedly dilated (Figure 2). A color Doppler ultrasonography of the vertebral and carotid arteries was normal. Other laboratory examinations of the patient were also within the normal ranges. A written informed consent was obtained from the patient's parents.

## DISCUSSION

The vein of Galen aneurysmal malformation is a congenital vascular malformation comprising 30% of all pediatric vascular anomalies and 1% of all pediatric congenital anomalies. It is believed to result from an insult to the cerebral vasculature at between 6 and 11 weeks of gestation after the development of the circle of Willis. Other venous anomalies commonly co-occur with VGAM, including anomalous dural sinuses, sinus stenoses, and an absence of the straight sinus (1, 2, 5). These anomalies com-

**ORCID IDs of the authors:** H.G. 0000-0002-9326-2194; H.K. 0000-0003-4671-4028; M.Ç. 0000-0002-6383-3355.
**Corresponding Author:** Hüseyin Gümüş **E-mail:** huseyingumus2163@hotmail.com
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Figure 1. Axial T2 weighted magnetic resonance imaging (MRI) demonstrating the arteriovenous malformation (AVM) of the nidus and an enlarged vein of Galen

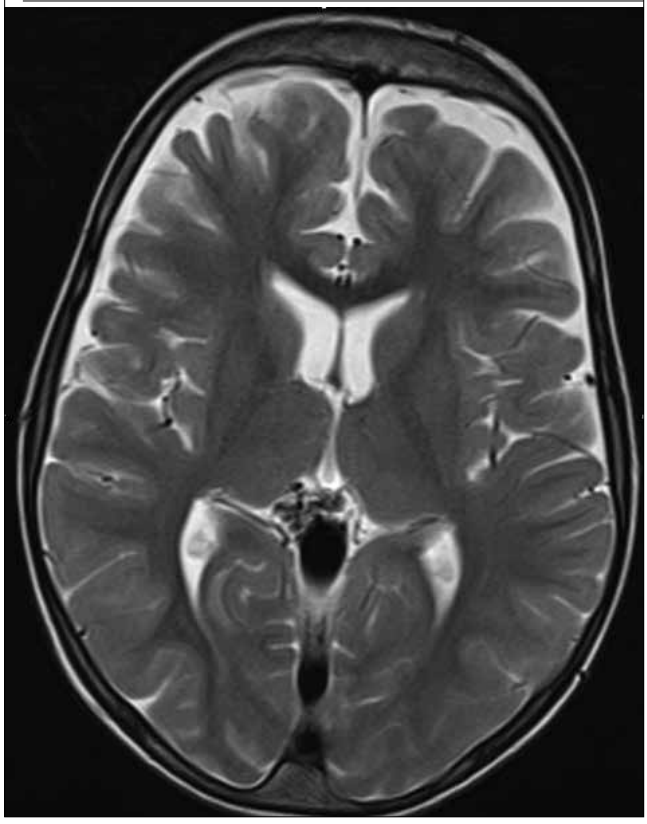
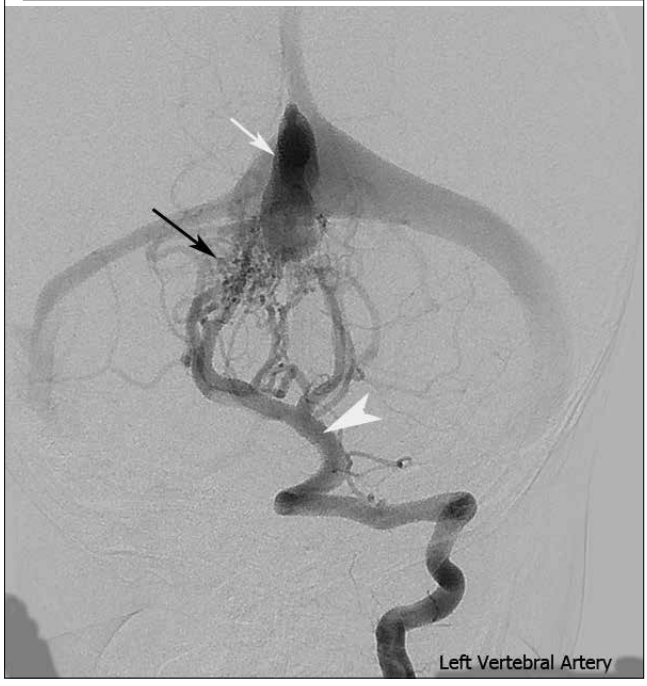


Figure 2. Left vertebral artery angiogram demonstrating AVM feeding artery (bacillary artery: arrowhead), nidus (black arrow), and draining veins (vein of Galen: white arrow)




monly present during the neonatal period, although they may also appear during early childhood. The major effects of VGAM include heart failure, hydrocephaly, intracranial bleeding, and vascular steal phenomenon (2, 6, 7).

In our patient, the main complaint upon presentation was a headache and an increased head circumference. Additionally, the systole-diastolic murmur was auscultated all around the head, particularly in the left temporal region. However, the physical examination and echocardiography did not demonstrate any finding of heart failure.

In their series of 59 cases, Lasjaunias et al. (8) classified VGAM into five different types, including parenchymatous AVM in 44%, choroidal AVM in 30%, mural AVM in 20%, vein of Galen varices in 7%, and dural AVM in 3% of cases, and reported that the pediatric population was particularly most sensitive to this shunt, regardless of the type (9). It has also been reported that the classification of this malformation is quite difficult in most cases.

In our patient, the cerebral angiography revealed a grade III AVM in the middle axis draining into the vein of Galen (approximately 2 cm in the longest diameter) (Figure 2). The case was considered to be complex, and surgery was planned.

Intracranial and vascular lesions might be treated using multimodalities, including surgery, chemotherapy, and conventional radiotherapy. However, complete excision might not be possible in all cases because of the anatomical localization of the lesion. Conventional radiotherapy and chemotherapy, as well as extended excision, may have adverse effects on the growth of child (10, 11).

Although the treatment of the aneurysm using endovascular methods is advantageous than that using surgery in several ways, it has certain drawbacks, including the use of a contrast material during the procedure and washing solution to prevent thrombosis, both of which adversely affect heart failure and require a close follow-up during anesthesia (12-14). VGAM can cause severe morbidity and mortality, particularly in neonates, but also in infants and older children. The mortality rate of VGAM is 9% and 50% in children and newborns, respectively, despite the administration of endovascular treatment (1, 2).

Endovascular surgery treatment was selected as the primary treatment in our case because of the localization of AVM. No complications developed after the surgery in our patient. After the discharge, the patient was followed up at the outpatient clinic.

Thus, VGAM must be considered in all children presenting with macrocephaly. Moreover, imaging studies must be performed in patients presenting with concomitant macrocephaly and headache. Endovascular surgery and various advanced surgical methods can be performed for the treatment of these cases.

**Informed consent:** Informed consent was obtained from parents of the patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author contributions:** Concept H.G; Design H.K; Supervision - M.Ç; Resource - H.G. Materials - M.Ç.; Data Collection and/or Processing - H.K.; Analysis and/or Interpretation - H.K.; Literature Search - H.G.; Writing - H.G.; Critical Reviews - H.K.

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Case Report

# Use of Mirror Therapy to Treat Psychogenic Tremors

Tomris Duymaz

Department of Physiotherapy and Rehabilitation, İstanbul Bilgi University School of Health Sciences, İstanbul, Turkey

**ABSTRACT**

Psychogenic tremors present with various clinical demonstrations in neurological practice. In mirror therapy, the patient concentrates on the image of the normal limb and learns to manage pain and movement. This study aimed to use mirror therapy for the treatment of psychogenic tremors. A 19-year-old female presented in the hospital with complaints of incompetence in manual skills, sluggish movements, reduction in muscle strength, weakness of fine motor activities, and presence of involuntary movements, such as tremors in the right upper extremity, which appeared approximately 2 years ago but worsened in the last 1 year. Both before and after treatment, we used a digital goniometer to assess the range of motion of the forearm and wrist, digital hand-held dynamometer to evaluate flexor and extensor muscles of the hand and wrist, hand grip dynamometer and pinchmeter to evaluate muscle strength, Disabilities of the Arm, Shoulder and Hand (DASH)questionnaire to assess the functional capacity of the upper extremity, Jebsen hand function test to evaluate the functionality of the hand, Grooved Pegboard test to evaluate manipulative skills, and functional independence measure (WeeFIM) to evaluate the quality of life. There was an improvement in all the parameters when compared before and after treatment. The results of this study showed that mirror therapy was a very useful approach in the treatment of psychogenic tremors, which significantly disrupt exercise compliance and are characterized by severe psychogenic tremors that reduce the quality of life.

**Keywords:** Mirror therapy, psychogenic, tremor

## INTRODUCTION

Psychogenic tremors present with various clinical demonstrations in neurological practice. It is sometimes difficult to differentiate psychogenic tremors from organic neurogenic movement disorders. Psychogenic tremors are phenomenologically encountered either as hyperkinetic movement disorders, such as tremor, dystonia, myoclonus, and tics or as hypokinetic movement disorders, such as parkinsonism, which is less frequent (1, 2).

In mirror therapy, the patient concentrates on the image of the normal limb and learns to manage pain and movement. In this study, we aimed to use mirror therapy for the treatment of psychogenic tremors.

## CASE PRESENTATION

A 19-year-old female (body mass index, 18.75 kg/m$^2$) presented in the hospital with complaints of incompetence in manual skills, sluggish movements, reduction in muscle strength, weakness of fine motor activities, and presence of involuntary movements, such as tremors in the right upper extremity, which appeared nearly 2 years ago but worsened in the past 1 year. According to the patient's personal medical history, she had been diagnosed with psychogenic tremors, which were not accompanied by any neurological symptom. No genetic disorder was detected in the family history. The patient was informed of the projected examination techniques and treatment method and was asked to sign the informed consent form before the initiation of the therapy. After implementation of necessary examination techniques, the patient underwent a rehabilitation program with mirror therapy. No medical treatment was administered, except exercise, mirror therapy, and occupational therapy. The patient's sociodemographic data were recorded. Both before and after the treatment, we used a digital goniometer to assess the range of motion of the forearm supination/pronation and wrist flexion/extension/ulnar deviation/radial deviation, digital hand-held dinamometer to evaluate flexor and extensor muscles of the hand and wrist, hand grip dynamometer and pinchmeter to evaluate muscle strength, DASH questionnaire to assess the functional capacity of the upper extremity, Jebsen hand function test to evaluate the functionality of the hand, Grooved Pegboard test to evaluate manipulative skills, and functional independence measure (WeeFIM) to evaluate the quality of life (3-6).

The patient received a total of 15 sessions of mirror therapy (5 sessions per week for 3 weeks). Moreover, she was requested to exercise in front of the mirror at home. Each session lasted for 45

**ORCID IDs of the author:** T.D. 0000-0003-0917-2098.

**Corresponding Author:** Tomris Duymaz **E-mail:** fzt_tomrisduymaz@yahoo.com

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min. She was also recommended to exercise for at least 45 min/day at home. A 45×30 cm mirror was used in the therapy.

This research was not approved by the ethics c mmittee, but the patient's and head doctor's in-hospital approvals were obtained as ethical considerations.

While implementing the mirror therapy for our patient with psychogenic tremors, the affected upper limb was hidden behind the mirror. The intact hand was used to perform exercises in front of the mirror, such as performing supination and pronation; flexing and extending the wrist and fingers; squeezing a ball; touching different floors; strengthening the wrist through flexion-extension using resistant springs and dumbbells; holding a pen; filling glasses with water; using spoons and forks; zipping, unzipping and fastening buttons; drawing different shapes; and exercising motor and hand skills with the Purdue Pegboard set.

Comparing the pre- and post-treatment evaluation results, there was an improvement in the patient's joint range of motion (wrist flexion increased from 75.1° to 88.4°, wrist extension from 59.2° to 67.8°, ulnar deviation from 31.1° to 35°, radial deviation from 15.7° to 18.2°, and supination from 78.1° to 88.6°), muscle strength (flexion strength increased from 6.6 to 8.8 kg, extension strength from 7.1 to 9.3 kg, handgrip measurement from 8.3 to 11.7 kg, and pinchmeter measurement from 2.6 to 3.6 kg), manipulative dexterity (Grooved Pegboard fitting activity increased from 34.19 to 57.68 s, removal activity from 12.12 to 24.91 s, JHFT writing score from 9.51 to 11.82, card turning activity score from 9.83 to 11.25, picking up small common objects score from 6.67 to 8.67, stimulated feeding from 11.15 to 12.25, stacking checkers from 7.29 to 8.06, moving empty cans from 5.56 to 6.86, and moving filled cans from 6.01 to 8.12 s), functional activities (DASH score increased from 19.75 to 25.5), and daily activities (WeeFIM social cognition subitem score increased from 20 to 21) along with a reduction in involuntary movements and pain experienced during the activities.

## DISCUSSION

This study presented the effectiveness of mirror therapy in the treatment of a patient who experienced psychogenic tremors. Increasing the ability to perform daily living activities and functions were the primary goals in the treatment of this subject.

The term psychogenic tremor is conventionally used to define the disorders arising from an emotional or psychiatric problem in the absence of any anatomical or neurochemical disease (7). Although there are no accurate data on the prevalence of psychogenic illnesses in Turkey, a study performed at a clinic on movement disorders in the USA reported that 28 (3.3%) of 842 successive patients who presented at the clinic were diagnosed with psychogenic tremors (8). The age of onset varies from study to study, but it is interesting to note that there is a clear preponderance of female patients (6).

The clinical signs of psychogenic tremors include onset with a sudden and triggering event, rapid start with maximum symptoms and static progress, features that are noncompliant with an organic disease, disappearance or change of a movement when attention is dissipated, presence of various abnormal movements, increase of the movement when the affected body is considered, intentional slowdown of movements, and reduction of movements when another region of the body is activated. Some of these features can be also seen in neurological diseases (9). Furthermore, a neurological disease that accompanies psychogenic tremors has been reported in 10%-15% of patients. However, the neurological disease was not detected in our patient. Brain magnetic resonance imaging (MRI) findings and dopamine levels were normal.

Mirror therapy is a neurorehabilitation technique that enables the neural network in the brain to reconfigure itself by creating a visual illusion. In other words, it is considered as the substitution of reduced/missing proprioceptive input with the help of a mirror. Mirror therapy involves the superposition of the movements of the unaffected side on the affected side while watching the reflection in the mirror. "Mirror neuron" is a neuron that fires both when a person acts and when the person observes the same action performed by another person. The activation of mirror neurons plays an important role in the enhancement of motor skills. Functional MRI studies have indicated that this method ensures plasticity in the brain (10).

In this study, consistent with the literature, we observed that the use of mirror therapy increased the range of motion and strength of the wrist and fingers, enhanced dexterity, and improved the patient's quality of life (11).

There are only a few studies suggesting the efficiency of mirror therapy on the upper limb in the context of psychogenic tremors. Based on the findings of the present study, we concluded that mirror therapy is an effective method for rehabilitation of the hand and upper limbs in patients with psychogenic tremors (12-14).

In this report, we presented a patient diagnosed with psychogenic tremors whose complaints were completely resolved with mirror therapy.

## CONCLUSION

There is no study in the literature on the use of mirror therapy for treating psychogenic tremors. Furthermore, we designed a patient-centered active physical and occupational therapy program with the primary goals of increasing physical functioning, decreasing movement disturbance, independently performing daily living activities, and improving the quality of life while considering the needs and requirements of the caregiver and subject. The exercise program helped in improving upper extremity functions in the subject. Larger trials will be needed to determine the success of this treatment.

This study showed that mirror therapy was a very useful approach in the treatment of psychogenic tremors, which significantly disrupt exercise compliance and characterized by severe psychogenic tremors that reduce the quality of life.

**Informed Consent:** Written informed consent was obtained from the patient who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Conflict of Interest:** The author has no conflicts of interest to declare.

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Case Report

# Odontogenic Keratocyst Masquerading as a Dentigerous Cyst in the Maxilla: A Case Report of an Unusual Presentation

Arvind Karikal[1], Tripthi Shetty[1], Arathi Kudthadka[2]

[1]Department of Oral and Maxillofacial Surgery, A.B. Shetty Memorial Institute of Dental Sciences, Karnataka, India

[2]Department of Oral Pathology, A.J. Institute of Dental Sciences, Karnataka, India

**ABSTRACT**

We encountered a case of an odontogenic keratocyst (OKC) that radiographically mimicked a dentigenous cyst and was associated with an impacted maxillary third molar. The common OKC location is in the mandible, with a lateral radiolucency. The attachment of the radiolucent mass to the coronal surface of the tooth in a maxillary molar is clinically highly unusual. The finding was pathologically confirmed after an excisional biopsy. The clinical and radiographic presentation is different than the typical description of OKC.

**Keywords:** Dentigenous cyst, odontogenic keratocyst, unusual, rare

## INTRODUCTION

Odontogenic keratocyst (OKC) is officially known as a true benign tumor, and it is aggressive in nature. First described by Philipsen in 1956 as "odontogenic keratocyst," it was renamed by the World Health Organization (WHO) in 2005 as the keratocystic odontogenic tumor (1, 2). Theentity was reverted back to be classified as a cyst in the 2017 WHO classification for the lack of evidence of being a tumor. It constitutes approximately 4%-16.5% of all cysts of the jaws (3). This aggressive cystic lesion has a high predilection for recurrence, higher than other odontogenic cysts (4). Many of the studies have shown that OKC occurs more in the mandible than in maxilla, with the posterior mandible being the commonest location according to published literature. The usual presentation is in the mandible and with a lateral radiolucency, and the entity spreads mesiodistally and has minimal buccolateral swelling (5, 6). This paper highlights a case of OKC occurring in the posterior maxilla in association with an impacted maxillary third molar, with a significant swelling in the maxillary vestibule. The radiological investigation showed coronal attachment of the radiolucent mass, typical of a dentigenous cyst.

## CASE PRESENTATION

A 24-year-old male patient reported to our department with a chief complaint of pus discharge from the upper-right back region of the jaw lasting for 1 year. He reported a history of pain and pus discharge in the upper-right back region of the jaw lasting for 1 year. The patient visited a local dental surgeon, where he underwent extraction with respect to the upper-right second molar. Unfortunately, the associated symptoms persisted as mild dull aching pain, which was intermittent nature. The patient had no significant medical or surgical history.

During the visit to our institute, the patient was subjected to radiological examination, by conducting a cone beam computed tomogram (Figure 1-4). The investigation showed an impacted maxillarymolar on the right side, with a significant envelope of a hazy mass attached to the coronal section of the impacted tooth. The radiologist gave a provisional diagnosis as dentigerous cyst since the mass was attached to the coronal head of the impacted tooth.

Written informed consent was obtained from the patient, and he was operated under general anesthesia. The lesion and the tooth were removed in completely. The hazy mass seen in the radiograph was a thick curdled pus fluid. The entire specimen was sent for a histopathologicalexamination.

The histopathological report in our case described the lesion as follows: The H and E sections of the submitted specimen showed a cystic odontogenic epithelial lining and an underlying connective tissue capsule. The odontogenic epithelial lining was the stratified squamous parakeratinized type, showing the areas of minimal corrugation (Figure 5, 6). The epithelium is six- to seven-cell-layers thick with basal cells showing the palisaded arrangement of the nuclei and nuclear hyperchromatism. In a few areas, the lining epithelium can be seen separating from the connective tissue. The underlying connective tissue capsule shows bundles of collagen fibers, fibroblasts along with chronic inflammatory cell infiltrate consisting of lymphocyte, and plasma cells,

**ORCID IDs of the authors:** A.K. 0000-0002-3444-9063; T.S. 0000-0002-3614-5709; A.K. 0000-0002-2978-6137

**Corresponding Author:** Arvind Karikal **E-mail:** arvikarikal@gmail.com

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Figure 1. Panoramic image from the CBCT imaging

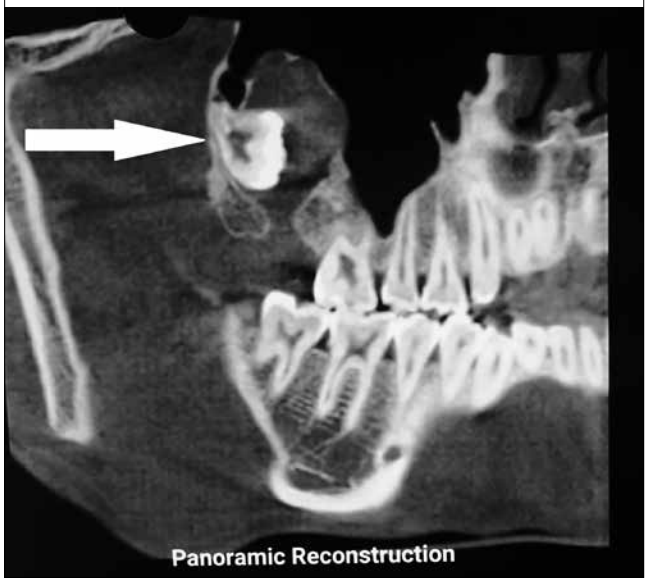



Figure 2. Axial section of CBCT showing the impacted maxillary molar with radiolucency attached to the coronal section of the tooth

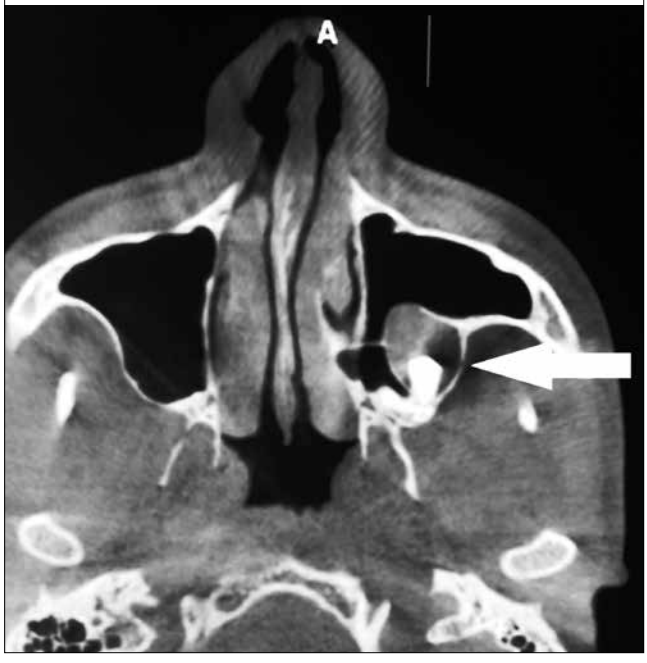



Figure 3. Coronal section of CBCT showing the impacted maxillary molar with unilocular radiolucency

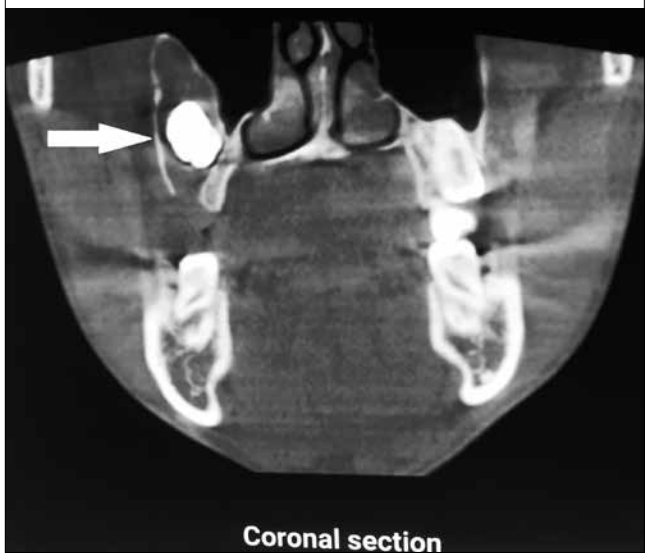
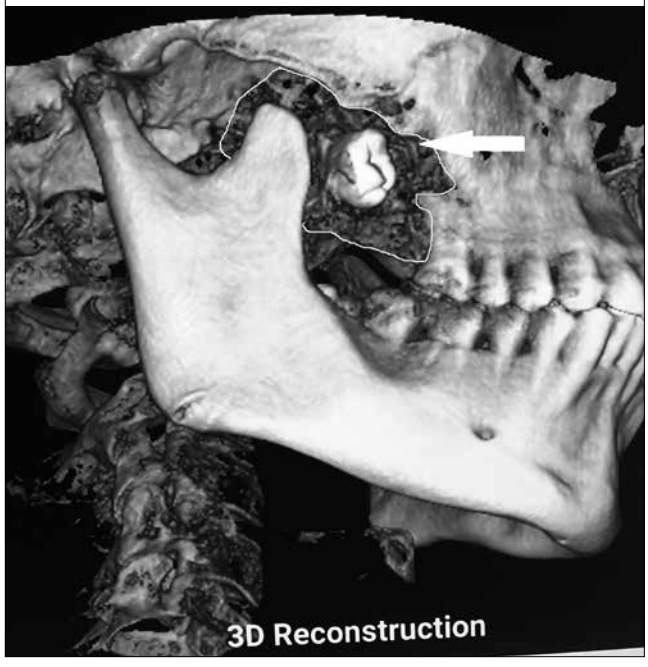



Figure 4. 3D reconstruction of CBCT showing the impacted maxillary molar, with surrounding radiolucency highlighted




blood capillaries, and extravasated RBC's. Daughter-cyst-like areas can also be seen in the connective tissue capsule, thus giving an impression of the lesion to be suggestive of the keratocystic odontogenic tumor (Figure 7-9).

Healing was uneventful with no untoward complications noted in the past 1 year of regular follow-ups.

## DISCUSSION

Dentigerous cysts are odontogenic cysts that are associated with the crowns of unerupted teeth.These cysts are termed dentigerous, which means "containing tooth," and this is the character description of the cyst. A characteristic feature of a dentigerous cyst is its appearance: The cyst typically surrounds the crown of an unerupted tooth, expands thefollicle, and is attached to the cemento-enamel junction of the unerupted tooth (7).

Dentigerous cysts most frequently involve unerupted upper and lower third molars and are generally seen in patients aged between 10 and 30 years. Males are more affected by an incidence rate of 1.6:1 (8). The cysts are usually solitary (9). The exact

Figure 5. Stained slide in 10× magnification showing parakeratinization

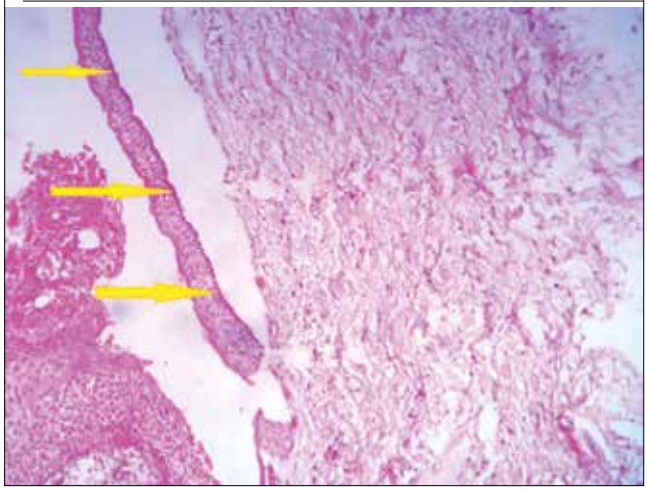


Figure 6. Stained slide in 40× magnification showing parakeratinization

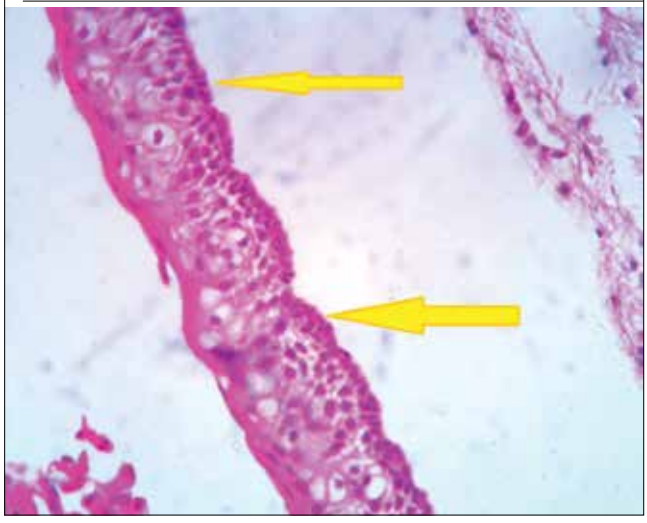


Figure 7. Daughter-cyst-like areas under 10×

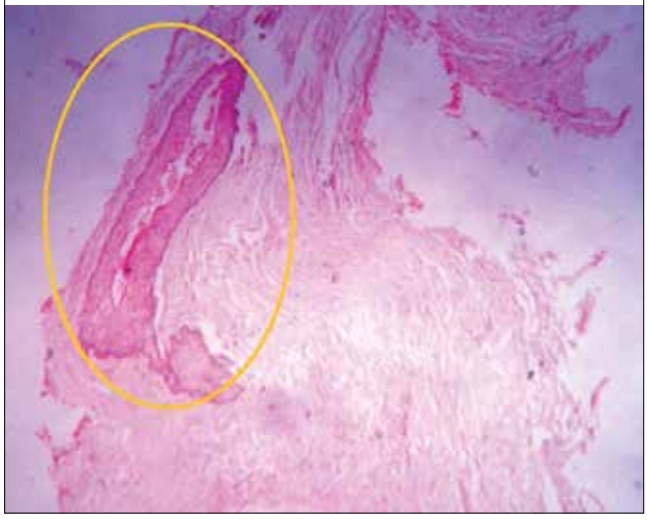


Figure 8. Daughter-cyst-like areas under 40×

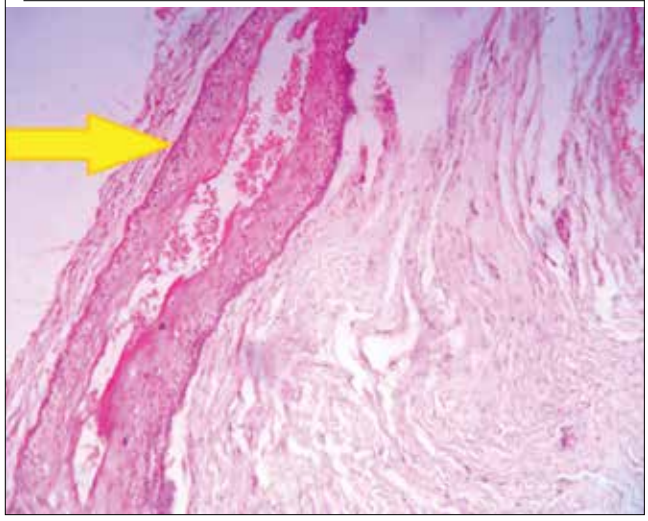
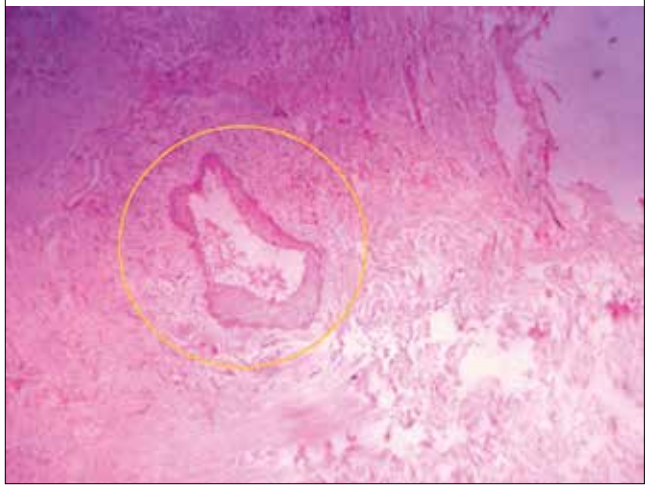


Figure 9. Daughter-cyst-like areas under 10× highlighted in the circle



pathogenesis of these cysts is unknown;however, it is assumed that they develop due to the accumulation of fluid between the reduced enamel epithelium and tooth crown (10-11). These cysts are generally without symptoms and exist for many years without being discovered.

Dentigerous cyst occurs radiographicallyas a well-defined unilocular radiolucency, often with a sclerotic border. As the epithelial lining is derived from the reduced enamel epithelium, in radiographs, the radiolucent shadow characteristically surrounds the crown of the tooth. A large dentigerous cyst may sometimes resemble a multilocular process (12).

Radiographically, dentigerous cyst can be described as;
1. The central variety, in which the tooth crown is enclosed by the radiolucency, and the crown protrudes into the cystic lumen;
2. The lateral variety in which the cyst occurs laterally along the tooth root, thus partially surrounding the crown; and

3. The circumferential variety exists when the cyst notonly surrounds the crown but also extends down along the root surface, thus giving the impression of the tooth within the cyst (13).

The above-mentioned features clinically and radiological are well known to represent a dentigerous cyst. A histological feature seen in the dentigerous cyst lining is usually a fibrous wall with two- to four-layer thickness of non-keratinized stratified squamous epithelial lining, and itconsists of the myxoid tissue, odontogenic remnants, and occasionally, the connective tissue wall resembles dental papillae and shows mild chronic inflammatory cell infiltrate with mild vascularity and areas of hemorrhage (13).

The typical radiographic features of OKC are unilocular, multi-locular, or multiple well-circumscribed radiolucent lesions surrounded by a thin radiopaque border with a smooth or loculated periphery.The lumen is frequently densely filled with keratin, causing the image to show a hazy appearance (14). An unerupted tooth is involved in the lesion in 25%-40% of OKC cases; in such instances, the radiographic features suggest the diagnosis of a dentigerous cyst. In these cases, the cyst haspresumablyarisen from a dental laminal cyst in the vicinity of an unerupted tooth and has grown to envelop the unerupted tooth. Altini and Cohen (15) introduced the term follicular primordial cyst (follicular keratocyst) for this group of lesions, this lesion was typically keratocyst on histology, but on macroscopic examination, it is seen surrounding the crown of the tooth and is firmly attached to the neck.

Histological examination accounts for the definitive diagnosis. The epithelial lining in thekeratocyst is highly characteristic with its features being unchanged even in different specimens. Five- to eight-cell-layer thick, regular keratinized stratified squamous epithelium without rete ridges lines the cyst. The form of keratinizationis usually parakeratotic (80%-90%), but it is sometimes orthokeratotic. The keratin formation amounts to no more than a thin eosinophilic layer of parakeratin in the parakeratotic variant.

The squamous epithelium has a clearly defined, palisaded layer of tall basal cells. The cells superficial to the basal layer are polyhedral and often exhibit intracellular edema.

Although the treatment of both the dentigerous cyst and OKC are excision, protocol calls foraggressive resection followed by chemical cauterization in cases of OKC owing to its high incidence rate. Extensive resection and use of the Carnoy's solution are considered the normal modality for the OKC treatment.

## CONCLUSION

Differential diagnoses of radiolucencies that occur in the maxilla and mandible cover a broad spectrum of cysts and tumors of odontogenic and non-odontogenic origin. An accurate diagnosis is based on the distinctive clinical, radiographic, and histopathologic aspects, but variations from these typical features also exist, and the clinician should be aware of the possibility of these variations in diagnosis and treatment of the radiolucent lesion occurring in the mandible and maxilla (15). Although the patient was treated as a case of dentigerous cyst with no routine protocol for an OKC treatment not being followed, the patient was placed on regular follow-ups after thehistopathological diagnosis as a precaution for further recurrence and repercussions. Regular radiographic monitoring was also done to establish the same.

This paper is an attempt to highlight the many possible clinical outcomes of a non-suspecting lesion and the need for clinicians to be aware of these masquerading lesions and to anticipate futurecomplications if any.

**Informed Consent:** Written informed consent was obtained from the patient who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author contributions:** Concept - A.K., T.S., A.Kudthadka; Design - A.K., T.S.; Supervision - A.K.; Resource - A.K., T.S., A.Kudthadka; Materials - A.K., T.S., A.Kudthadka; Data Collection and/or Processing - T.S., A.Kudthadka; Analysis and/or Interpretation - A.K., T.S., A.Kudthadka; Literature Search - T.S., A.Kudthadka .; Writing - A.K., T.S., A.Kudthadka; Critical Reviews - A.K., T.S., A.Kudthadka.

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Erratum

## Erratum

In the Turkish version of the article by Avcı and Altındağ, entitled "Evaluation the relationship between body mass index and kinesiology band application to acute grip strength on healthy men" (Eur J Ther 2018; 24(2): 5-11. DOI: 10.5152/EurJTher.2017.156) that was published in the March 2018 issue of European Journal of Therapeutics, some contextual errors has been detected. The specified corrections have since been implement on the online version of this article.

- The title of Table 5 has been corrected.
- The missing data in the Results section has been added.

You may access the updated version of this article at http://eurjther.com/sayilar/57/buyuk/5-112.pdf.