IgG4 Subclass of Immunoglobulins; Immunobiology and Roles in Relation to Human Diseases

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ABSTRACT

IgG4, a subclass of antibodies known as immunoglobulins have unique structural features, in particular, their Fc regions, that prevents their interactions with other receptors on effector cells and thus disabling them of activating complements system. IgG4 antibodies can undergo a process called Fab-arm exchange, wherein they exchange half-molecules with other IgG4 antibodies, thus forming bispecific monovalent antibodies. Isotypic switch in mature B cells in germinal centres of secondary lymphoid organs is controlled by Tfh subset of T cells. Functionally IgG4 antibodies exert immunomodulatory and blocking activities, modulating protective inflammation evolved by parasitic invasion and allergic inflammation. From the pathophysiological point of view, IgG4 autoantibodies are prominently observed in autoimmune diseases under the umbrella of IgG4-autoimmune diseases (IgG4-AID). Furthermore, IgG4-related diseases (IgG4-RD) are affecting various organs characterized by lymphoplasmacytic infiltrates and storiform fibrosis in tissues, together with elevated IgG4 levels in the blood. A better understanding of IgG4 immunobiology helps us diagnose and treat patients suffering from these rare forms of diseases.

KEYWORDS

IgG4 subclass; immunobiology; characteristics; IgG4 autoimmune diseases; IgG4 related diseases

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Received: 30 January 2025
Accepted: 28 February 2025
Published online: 4 April 2025

Acta Medica (Hradec Králové) 2024; 67(4): 101–106
https://doi.org/10.14712/18059694.2025.6

INTRODUCTION

The humoral arm of specific immunity, characterized by the antigen-driven terminal differentiation of mature B cells into plasmablasts and predominantly plasma cells to produce immunoglobulins, is indispensable for protective inflammation. Both inherited and acquired defects in antibody production are a cause of enhanced susceptibility to pathogens invasion, with severe impacts on human health. When homeostatic regulations of B cells differentiation are disrupted, it can result in production of autoantibodies causing damage to various self structures leading to a harmful inflammatory response. There are numerous mutual functional interactions between B cells and T cells. Majority of the antigen-driven B cell activities are regulated by the Th2 subset of polarized T cells mediated by direct cell-to-cell interactions and cytokines. This is true for two processes in B cells, regulating isotypic switching and somatic hypermutation. The isotypic switching is responsible for the production of other classes and subclasses of immunoglobulins instead of IgM class, which is mostly produced in the primary specific humoral response. The somatic hypermutation mediates a gradual increase in antibody affinity during antigen-induced B cell proliferation. The antigen switching is essential to produce IgG4 subclass of IgG antibodies. The immunobiological role of IgG4, the least produced subclass of IgG, is unique in many aspects, and still enigmatic. In addition, enhanced production of IgG4 is associated with the presence of fibroinflammatory lesions in various organs and the presence of IgG4 subclass autoantibodies with IgG4 autoimmune diseases. The aim of this review article is to summarise the unique immunobiological molecular characteristics of the IgG4 subclass, its production, and its roles in health and diseases.

MOLECULAR CHARACTERISTICS OF IgG4 SUBCLASS

It is important to note that approximately threequarters of daily immunoglobulin production is dedicated to IgA2 subclass of IgA immunoglobulins, which forms secretory IgA responsible for protecting mucosal surfaces. However, the IgG class of immunoglobulins is the most abundant in plasma with levels ranging from 5,0–16,0 g/l. The high level of IgG immunoglobulin in plasma and its long 21 days half-life are apparently maintained by production and cellular recycling using FcRn of neonatal IgG1 receptors (1). Based on differences in heavy chain structures, IgG class is further subdivided into four subclasses (2). Each subclass has distinct structural and functional properties leading to different effector functions comprising opsonization, classical pathway complement activation, antibody-dependent cytotoxicity, pathogen neutralization, and immunocomplexes handling (3). IgG4 is the least abundant subclass of IgG in blood and has unique functional features compared to other IgG subclasses. IgG4 has reduced affinity to many effector molecules expressed on immune cells, such as Fc and complement receptors. Furthermore, IgG4 has a significantly limited ability to activate the complement system. This is caused by the presence of several amino acids in the Fc part of molecules together with absent of N-glycans when compared to IgG1 which is highly active in this regard. However, the interactions of IgG4 with the FcγRII inhibitory receptor are not abrogated, contributing to its participation on dampening of inflammatory responses (4). In sharp contrast to other immunoglobulin classes and subclasses, IgG4 does not cross-link antigens. Although initially produced like other subclasses as bivalent, monospecific antibodies by plasma cells, IgG4 immunoglobulins undergo a unique process called "Fab-arm exchange". In this process, half-molecules of IgG4

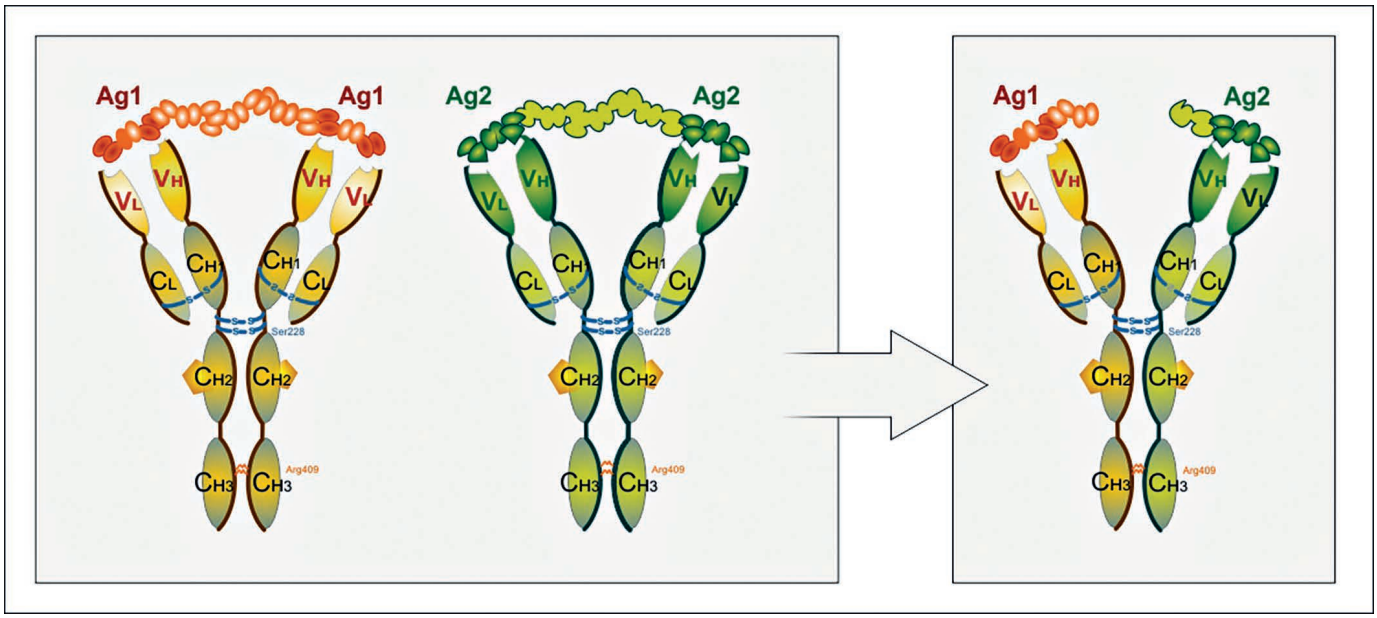

Fig. 1 Fab-arm exange in IgG4 subclass. Legend: IgG4 molecules are characterized by their unique molecular structure. Monomers of heavy (H) and light (L) chains are bound together by two disulfide bounds in hinge region with serin at position 228 and non covalent bond in CH3 domain with arginin at possition 409. Both features are required for Fab arm exchange. A functional consequence is the effective monovalency of IgG4. This eliminates the potential of the antibody to cross-link antigen, further minimizing the immune-activating potential of IgG4.

(made of one heavy and one light chain) are randomly exchanged with half-molecules from other IgG4 antibodies, forming bispecific molecules that target two different antigens (Figure 1). This process is facilitated by two unique amino acids, first one in hinge region and second one in terminal CH3 domain of IgG4. As this reaction does not require any additional proteins, it results in a highly dynamic process during which IgG4 molecule antibodies constantly exchange arms which generate ever-changing repertoire of hetero-bivalent IgG4 antibodies. Owing to the heterogeneity of the IgG4 pool, up to 99% of circulating IgG4 is considered bispecific (5). In summary, IgG4 subclass antibodies are considered immunomodulatory and referred to as "blocking antibodies" that might function to remove antigens to stop their active potential (6).

IGG4 IMMUNOGLOBULINS FORMATION

After antigen-independent B cells differentiation in primary lymphoid organs, mature naive B cells accumulate in B-cell-rich areas of secondary lymphoid organs, such as lymph nodes, spleen, and mucosa associated lymphoid tissues. These are sites of encounters for antigenic stimuli. When an antigen is specifically recognized via BcR receptors and a sufficient level of costimulatory signals provided by membrane-to-membrane contacts and soluble mediators is reached, the B cell undergoes clonal expansion and terminally differentiate to the antibody-producing plasma cells. The response to antigenic stimulation of B cells is inseparably linked to affinity maturation and isotypic switching (7). The initial response to antigen is predominantly driven by the extrafollicular pathway. It involves a subset of T follicular helper cells (Tfh) driving the differentiation of antigen-stimulated B cells into short-lived plasmablasts, which will maintain a primary antibody production for up to a week after the trigger (8). To create an immune response based on IgG4, B cells must undergo class switch recombination and somatic hypermutation. These processes are hallmarks of follicular response, which take part in germinal centres of lymph nodes and spleen. In the follicular pathway, B cells develop high affinity and specificity and become memory B cells or long-lived antibody-producing plasma cells predominantly residing in the bone marrow. The germinal centres are divided into dark and light zones, with functional differences. The dark zone promotes the proliferation of B cells, as well as somatic hypermutation and isotypic switch. (9) Given its mechanisms, switching into Ig class follows a segmental pattern from Cμ/Cδ constant genes adjacent to already rearranged VDJ genes segment, marking specificity to γ3, γ1, and α1, thus forming IgG3, IgG1 and IgA1 subclasses instead of IgM (7). A secondary switch gives rise to more distal classes of IgG2, IgG4, IgE and IgA2, respectively. The isotypic switch is under the control of a functionally polarized subset of Th2 T cells which provide stimuli to antigen-driven differentiated B cells in both humoral and membrane associated molecules, such as IL-4, IL-13, CD40 – CD40L, and ICOS-ICOSL (10). After this cooperation in between Th2 subsets and B cell clones, high affinity and specificity B-cell clones migrate to the light zone of lymph nodes where they undergo a selection according to interactions with follicular dendritic cells and highly specialized set of follicular T helper cells (Tfh). Once the highly specific clones of B cells are selected, they leave germinal centres and become long-lived plasma cells or memory B cells (3).

A specialized subset of helper T cells, Tfh cells, deserve special attention as they have a profound influence over specific humoral immune responses by guiding antigen-driven B cell differentiation, maturation, isotypic switching, and their terminal differentiation into IgG4 subclass producing plasma cells. Tfh cells are localized in germinal centres of secondary lymphoid organs but also dynamically recirculate across injured tissues, lymph nodes, and peripheral blood. The Subset of Tfh cells has emerged as pivotal orchestrators in the balance between immune tolerance and inflammation. They play a role in driving the production of IgG4 and IgE immunoglobulins which are crucial in both physiological and pathophysiological processes (11).

Tfh cells migrate aptly to CXCL13-rich B cells follicles using CXCR5 chemokine receptors to enhance formerly described fundamental B cells functions. Moreover, Tfh cells enhance overall B cell survival and differentiation by expressing CD40L (CD154). An indispensable role in programming early Tfh cell development is given to membrane expression of ICOS (CD278) costimulatory molecules. This process is checked by checkpoint inhibitory molecules such as PD-1 (CD279) interacting with PD-1L (CD274), expressed on B cells to modulate excessive activation within germinal centres (12). The master regulator of Tfh seems to be the Bcl-6 molecule with lower expression in peripheral blood circulating Thf. The crucial expression of Bcl-6 is regulated by the action of Aiolos transcription factor (13). The population of Thf could be subdivided into several functionally polarized subsets, such as Thf1, Thf2, Tfh13, Tfh17, and Tfhreg, respectively, thus partially reflecting functional polarization of the subpopulation of T helper T cells as a whole. Isotyping switching to IgG4 secretion and terminal differentiation into long-lived plasma cells require IL-4 produced by Tfh2 and IL-10 produced by Tfhreg T cells (14).

IGG4 ROLES IN PHYSIOLOGICAL IMMUNE REACTIVITY

Owning to Fab-arm exchange, and the effective monovalency of majority of the IgG4 molecules *in vivo* preventing the cross-linking of antigens together, with the overall reduced ability of IgG4 to activate Fcγ receptors and complement system, the IgG4 is often regarded as a natural type of "blocking" antibody dampening the inflammatory responses (4).

The best examples of this IgG4 response are parasitic infections. The host develops a strong innate and specific T and B cell response during the acute phase of infection. Specific response is dominated by Th1 T cells activity and the recruitment of eosinophils. If the pathogen fails to evade the host response this leads to parasite clearance. If the host effort fails, the chronic phase of infection is an-

ticipated. This phenomenon is associated with the switch from a potentially damaging Th1-driven response to a Th2 response which is sparing tissue integrity. In addition, Treg T cell activities are upregulated by parasites to escape from the Th1 immune response (15). The net result is that up to 90% of parasite-specific antibodies are IgG4 subclass in asymptomatic patients. Interestingly, it has been suggested in so-called "hygiene hypothesis" that IgG4-mediated attenuation of host immunity by the parasites may be a form of protection of the host from allergies and autoimmune diseases (9).

Allergic diseases comprise heterogeneous set of illnesses affecting almost any organ. They can develop in predisposed atopic individuals after exposure to specific allergens. The clinical presentation reflects the complexity of allergic inflammation. However, the role of allergen-specific IgE antibodies is very relevant in the immediate phase of allergic response. This is caused by the rapid release of numerous biologically active compounds stored in granules of mast cells and basophils induced by allergen crosslink of IgE molecules tightly bound to high-affinity receptors FcεRI. Tolerance to allergens can be achieved by long-term natural chronic exposure to allergens or by specific allergen immunotherapy. For almost all individuals with a history of allergic symptoms, the IgG4 can constitute more than 75% of allergen-specific IgG after continuous exposure to allergens (16). The protection against the symptoms of allergy mediated by IgG4 is thought to be the result of at least several actions. These are blocking activities of allergen-specific membrane-bound IgE by competing for allergen binding sites thus blocking mast cell degranulation, preventing the immune complex formation, and inhibiting antigen presentation to T cells by B cells and dendritic cells. There is a raise in IL-10 production by Treg which is to provide tolerogenic stimuli to T cells and also enhance isotypic switch in B cells to produce allergen-specific IgG4 antibodies (17).

THE ROLES OF IGG4 IN PATHOLOGICAL PROCESSES

A selective lack or extremely reduced plasma concentrations of IgG4 are rare and are often associated with recurrent respiratory and/or gastrointestinal tract infections, impaired immunity against fungal invasion, and increased risk of IgE-mediated pathologies (18).

There are clinically more challenging diseases in which autoantibodies against defined targets are formed in the IgG4 subclasses. This emerging subgroup of autoimmune diseases is called the IgG4 autoimmune diseases (IgG4--AID) (19).

On the other hand, immunopathological disorders characterized by elevated plasma level of IgG4 are in the majority patients concomitantly present with lymphoplasmacytic infiltration of the affected organs and storiform fibrosis of tissues (20).

Autoantibodies produced by autoreactive B cells under the regulation of functionally polarized Th2 T cells are key pathogenic players in many immunopathological disorders, with rheumatoid arthritis and connective tissue diseases being the most prevalent. They cause the immunopathological processes by a range of different effector mechanisms such as antibody-enhanced endocytosis and activation of complement cytotoxicity that are dependent on the engagement of the Fc part of antibodies. These effector functions are not available to the IgG4 subclass. The pathogenicity of IgG4 subclass autoantibodies in IgG4-AIDs is largely mediated by blocking spatially protein-protein cell-cell interactions thus competing with the physiological roles of targeted proteins (21).

IgG4-AIDs is heterogeneous group of immunopathological disorders that can affect many organs, depending on the major site of action of targeted autoantigens, including central and peripheral nervous systems, kidney, skin, and hematopoietic system. A recent comprehensive review on the IgG4-AID and autoantigens involved in this disorder is available (22). IgG4-AIDs is evidenced in animal models by passive transfer of autoantibodies comprising subtype of myasthenia gravis characterized by the presence of IgG4 autoantibodies targeting muscle-specific tyrosine kinase (MuSK) critical for the proper clustering and functionality of acetylcholine receptor at the muscle endplate of the neuromuscular junction (23). The presence of IgG4 autoantibodies specifically identifying epitopes on desmoglein 1 and desmoglein 3 is characteristic of pemphigus foliaceous and pemphigus vulgaris, respectively. Desmogleins are Ca2+-dependent transmembrane proteins localized in keratinocyte desmosomes and play a critical role in maintaining the integrity and cohesion of keratinocytes within the epidermis. Desmoglein 1 is primarily located in superficial layers, while desmoglein 3 is found in basal layers of the skin. The interaction with IgG4 autoantibodies results in the obstruction of keratinocyte-keratinocyte adhesion leading to formation of skin blisters (3). The last example of IgG4 AID is thrombotic thrombocytopenic purpura. In this immunopathology, ADAMTS13 metalloproteinase activity is blocked by IgG4 autoantibodies. ADAMTS13 is a proteinase found in blood circulation. It is responsible for the proteolytic cleavage of multimeric form of von Willebrand factor (vWF) and ensuring normal haemostasis. ADAMTS13 proteolytic activity is disturbed by the binding of IgG4 autoantibodies. This result is the accumulation of vWF multimers which cause platelet aggregation and the formation of microthrombi leading to the characteristic phenotype of microangiopathic haemolytic anaemia. It has to be emphasized that the autoimmune nature of IgG4-AID is based on significant HLA association, particularly HLA-II class molecules (7).

IgG4-related disease (IgG4-RD) is a systemic immunopathological reaction characterized by fibroinflammatory lesions in various organs. These lesions can mimic inflammatory disorders, infections, or malignancies. IgG4-RD is often accompanied by elevated IgG4 plasma levels, although not always (24). IgG4-RD is characterized by three major histopathological findings; dense lymphoplasmacytic infiltrates, fibrosis predominantly in storiform pattern, and obliterative phlebitis with an increased number of IgG4+ plasma cells in affected tissues. The comprehensive criteria to diagnose the IgG4-RD were established (25).

Five clinical phenotypes of IgG4-RD were identified based on patterns of affected organs. Group 1 with pancreatic-biliary lesions, is common in older patients and

Tab. 1 Clinical phenotypes of IgG4 – related disease.

	Group 1	Group 2	Group 3	Group 4	Group 5
Pattern	Pancreato-hepato biliary disease	Retroperitoneum and aorta	Head-and neck-limited	Mikulicz and systemic disease	Hematologic diseases
Presentation	Autoimmune pancreatitis sclerosing cholangitis	Retroperitoneal fibrosis aortitis large vessel disease	Salivary and/ or lacrimal gland enlargement adnexal orbital involvement	Symetric salivary gland enlargement with involvement in chest and/or abdomen	Lymphadenopathy Eosinophilia
Phenotype	Proliferative	Fibrotic	Proliferative	Proliferative	Proliferative/Fibrotic
Serum IgG4 level	Elevated	Normal or mildly elevated	Elevated	Very highly elevated	Mildly Elevated

may develop pancreatic and bile duct cancer due to chronic inflammation caused by autoimmune pancreatitis and IgG4-related sclerosing cholangitis. Group 2 with retroperitoneal fibrosis is predominantly found in older males associated with elevated plasma CRP levels and lower IgG4 levels compared to other groups of IgG4-RD. Group 3 with head and neck lesions predominantly in salivary and/or lacrimal glands and, group 4 with Mikulicz disease characterized also by lesions in salivary and lacrimal glands associated with systemic lesions, more prevalent in middle-aged females often accompanied by allergies with higher plasma levels of IgG4 compared to other groups (20, 26, 28).

Group 5 of IgG4-RDs are manifested in hematologic diseases, i.e. lymphadenopathy, eosinophilia, and polyclonal hypergammaglobulinemia. The disease can be easily mistaken, as patients may present with clinical problems that mimic disorders such as multicentric Castleman disease, lymphoma, plasma cell neoplasms, and hypereosinophilic syndromes. When IgG4-RD is suspected, a firm histological diagnosis is essential to confirm the diagnosis and to rule out mimickers. The central histopathological features are a dense, polyclonal, lymphoplasmacytic infiltrate enriched with IgG4-positive plasma cells (with an IgG4/IgG ratio >40 %), storiform fibrosis, and obliterative phlebitis. It is of high importance to haematologists, that the latter two features are seen in all tissues except bone marrow and lymph nodes, making these two sites suboptimal for histological confirmation. Many patients follow an indolent course and respond well to treatment, but a significant proportion may have highly morbid or fatal complications such as periarteritis, severe retroperitoneal fibrosis, or pachymeningitis. Corticosteroids are effective, and the initial response rates to rituximab are high but durable remissions are rare (29).

ACR (American College of Rheumatology) / EULAR (European League Against Rheumatism) classification of clinical criteria for IgG4-RDs was developed and validated in 2019. These criteria should contribute substantially to future clinical, epidemiologic, and basic science investigations. A 3-step classification process was developed. Firstly, it must be demonstrated that a potential case with is involved in one of 11 possible organs in a manner consistent with IgG4-RD classification. Secondly, exclusion criteria which consists of clinical, serologic, radiologic, and pathologic items must be applied, and the presence of any of these criteria eliminates the patient from IgG4-RD classification. Thirdly, 8 weighted inclusion criteria domains, addressing clinical findings, serologic results, radiology assessments, and pathology interpretations, are applied. These criteria were shown to have robust test characteristics over a wide range of thresholds (25).

Current studies using the most advanced molecular biological methods yield evidence that support the pathogenesis of IgG4-RD is an antigen-driven process mediated by B and T cell interactions. Many studies suggest the role of autoantigens, such as carbonic anhydrase II, lactoferrin, annexin A11, galectin 3 (7).

One of the key histopathological features commonly observed in affected organs and tissues in IgG4-RD is the formation of tertiary lymphoid tissues, a process believed to induce the mass formation and swelling of these organs parallel with an increased number of IgG4 plasma cells. Tertiary lymphoid organs are characterized by the accumulation of B cells interacting with Tfh cells expressing CXCR5 chemokine receptor, PD1, and TIGIT regulatory membrane molecules which are stimulated by follicular dendritic cells. Somatic hypermutations and isotypic switching to IgG4 production drives the cellular and cytokine microenvironment (27). Extrafollicularly, there is the presence of macrophages polarized to the M2 subset producing TGFβ to stimulate fibroblasts to produce extracellular matrix molecules, leading to storiform fibrosis. There is also an accumulation of cytotoxic CD8+ T cells together with a unique subset of cytotoxic T helper T cells. Cytotoxic CD4+ T cells are mainly found within effector, effector/memory, antigen-experienced highly differentiated T cells that downregulate costimulatory receptors such as CD27 and CD28. Using specific transcription factors, they store cytotoxic compounds such as various granzymes and perforins in cytotoxic vesicles in parallel with surface molecules expressed on innate lymphoid cells (30).

CONCLUSION

Both IgG4-autoimmune diseases and IgG4-related diseases have low prevalence fulfilling the criteria of orphan diseases. However, the individual burden of these diseases could be very high. Being entirely different in their clinical presentation, IgG4-autoimmune diseases have a lot in common. They share the substantial contribution of the

IgG4 subclass of IgG immunoglobulins with its unique immunobiological characteristics in immunopathogenesis. The correct diagnosis of such diseases is an important basis for their effective treatment. Regardless of the role of IgG4 in immunopathology, numerous positive roles of IgG4 antibodies in normal physiological immune responses have not to be omitted.

ACKNOWLEDGMENT

This work was supported by the Cooperatio Program, research area IMMU.

ABBREVIATIONS

CD	Cluster of Differentiation
Fab	Antigen binding fragment of immunoglobulins
Fc	Constant fragment of immunoglobulins
FcRn	Neonatal receptor for IgG1
HLA	Human Leucocyte Antigen
IgG4	IgG4 subclass of IgG immunoglobulins
IgG4-AID	IgG4-AutoImmune Diseases
IgG4-RD	IgG4-Related Diseases
Tfh	T follicular helper T cell

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Deleterious Effect of Fructose on the Heart Function of Hypertriglyceridemic Rats

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ABSTRACT

A high-fructose intake (HFI) in food, sweetened beverages, and soft drinks appears to be one of the risk factors that worsens human metabolic and cardiovascular health, although the more accurate mechanism remains unclear. Hypertriglyceridemic (HTG) rats represent a suitable animal model of metabolic syndrome where the consumption of an HFI could have an additional aggravating impact. We aimed to study the effect of fructose on the heart functions. Male HTG rats had HFI or a standard diet for five weeks. Heart function was tested *ex vivo* on the perfused heart using the Langendorff technique. Isolated hearts underwent 25 min ischemia (I) and 30 min reperfusion (R). Left ventricular developed pressure (LVDP), ventricular premature beats, and dysrhythmias were monitored during R. At the end of the R, ventricular fibrillation (VF) was evoked electrically. Systolic blood pressure, glucose level, serum total cholesterol (TC), triglycerides (TAG), and thiobarbituric acid reactive substances (TBARS) in the kidney were determined. The LVDP showed a reduced return to the input values, the duration of VF in R increased, and the threshold for VF induction decreased. Serum TC, TAG, and kidney TBARS were increased. The effect of HFI on heart ventricular impairment was associated with the reduced threshold for induction of VF and aggravated dyslipidemia. The results point to the adverse impact of dietary high-fructose intake in rats with hypertriglyceridemia.

KEYWORDS

high-fructose intake; heart function; aortic endothelium-dependent relaxation; lipid profile; hypertriglyceridemic rats

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Received: 25 November 2024
Accepted: 18 January 2025
Published online: 4 April 2025

Acta Medica (Hradec Králové) 2024; 67(4): 107–112
https://doi.org/10.14712/18059694.2025.7

INTRODUCTION

Metabolic syndrome (MetS) is characterized by a cluster of several simultaneously present risk factors as abdominal obesity, hypertension, hyperlipidemia, hyperglycemia, and insulin resistance. These disorders consequently lead to the development of cardiovascular diseases, diabetes mellitus type 2, non-alcoholic fatty liver disease, etc. (10, 39). The increased prevalence of MetS is related to an unhealthy lifestyle, mainly sedentary work, low physical activity, and consumption of unhealthy food with high amounts of saturated fats, added sugar, and low-fiber food. The introduction of high-fructose corn syrup in the 1970s accelerated fructose consumption. Fructose is sweeter than sucrose, and its production is less expensive (1). Over the past few decades, epidemiological studies have demonstrated that a high-fructose intake is an etiological factor of MetS. Fructose is metabolized in the liver to lipids, and finally, it increases triacylglycerol concentration here thus a high-fructose intake is partly analogous to a high-fat diet and endangers health (25). A high-fructose intake evokes the expression of all main features of MetS (11). A changed serum lipid profile characterized by dyslipidemia was documented, especially the increased levels of low-density lipoprotein cholesterol linked to cardiovascular diseases (17, 38). Some evidence suggests that a high-fructose intake increases cardiovascular risk, although the mechanism remains unclear. It was reported that high-fructose feeding elicits insulin resistance, hyperinsulinism, and hypertension in normal mongrel dogs (22), in experimental mice and rats resulted in high triglyceride levels, glucose intolerance, insulin resistance, and obesity (18). Some differences in fructose overload effects were observed depending on the rat strain. Fructose in drinking water (10%) administered to Wistar and Sprague-Dawley (SD) rats for eight weeks resulted in hypertension and hypertriglyceridemia in SD rats. However, no metabolic changes were seen in the Wistar rats. These differences could be attributed to the active behavior of Wistar rats causing a higher metabolic rate (7). As hereditary hypertriglyceridemic (HTG) rats are considered a suitable animal model for the study of MetS-like conditions (14), we focused on the impact of a high-fructose intake as an additional stress on the organism, which is already burdened by the presence of several MetS risk factors in HTG rats (41). Our study aimed to find the effect of high-fructose intake on the heart function of HTG rats. In addition, some biometric, biochemical, and physiological parameters were determined.

MATERIAL AND METHODS

ANIMALS AND DESIGN OF EXPERIMENT

All experimental procedures involving animals conformed to Directive 2010/63/EU on the protection of animals used for scientific purposes and were approved by the Ethical Committee of the Centre of Experimental Medicine of the Slovak Academy of Sciences, and the Animal Health and Animal Welfare Division of the State Veterinary and Food Diet Administration of the Slovak Republic (the number of the permit 385/18-221/3). Adult male HTG rats, 15 weeks old at the onset of the experiment, weighed 293 ± 4 g, n = 28 were used in a chronic 5-week lasting experiment. Rats were divided into two groups: a control group fed a standard diet (Control; n =14), and a group fed a high-fructose diet with 60% of fructose in the pellets (Fructose; n = 14). A standard rodent diet (1g/13.26 kJ) was produced by the certified pellet producer (Centrum of Experimental Medicine, Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Dobrá Voda, Slovakia). A modified high-fructose diet (1g/14.57 kJ) was prepared by the same pellet producer with a 60% fructose addition and omitting an adequate amount of wheat and barley. Rats were kept on a 12h/12h light/dark cycle, placed in cages of five animals and they had food and water *ad libitum*. The amount of pellets and water was recorded *per* cage and recalculated to the consumption of one rat/day.

HEART FUNCTION

The heart function was tested *ex vivo* on the perfused heart under constant pressure (15) using the Langendorff technique. The hearts of 7 rats from each group were retrogradely perfused via cannulated aorta in Langendorff mode with oxygenated Krebs-Henseleit solution (mmol/l: 120.0 NaCl; 4.2 KCl; 1.75 $CaCl_2$; 1.25 $MgSO_4 . 4H_2O$; 12.5 glucose; 25.0 $NaHCO_3$; pH 7.4; temperature 37.0 °C). To assign basal diastolic pressure to the left ventricle, a water-filled latex balloon was inserted into the left ventricular cavity, and adjusted to the value of 80 mmHg (1 mmHg = 133.32 Pa). After 10 min of the heart equilibration (a stabilization period), ischemic conditions (I) were induced by stop-flow (25 min), and reperfusion (R) was applied for a further 30 min. Left ventricular developed pressure (LVDP), number of ventricular premature beats (VPB), and heart dysrhythmias (ventricular tachycardia; VT and ventricular fibrillation; VF) were monitored. At the end of the R period, myocardial susceptibility to persistent VF was determined by electrical stimulation via electrodes located at the epicardium of the right ventricle. The heart was subjected to programmed electrical stimulation: current strength 10 mA, train duration 2s, stimulation rate 100 pps, and stimulus duration 0.2 ms was used (Electrostimulator ST-3, Medicor, Hungary). In case of sustained VF lasting > 2 min was not induced, the stimulus intensity was increased in 5 mA steps until a maximum of 50 mA to detect the fibrillation threshold. The system BioLab F ver.1 (Institute of Measurement Science, Slovak Academy of Sciences, Bratislava, Slovakia) was used for data collection and offline analysis.

ENDOTHELIUM-DEPENDENT RELAXATION OF THE AORTA

The thoracic aorta was prepared and placed in a modified Krebs solution (mmol/l: NaCl 122.2; KCl 5.9; $NaHCO_3$ 15.0; D-glucose 11.0; $MgCl_2$ 1.25; $CaCl_2$ 1.25). Two millimeter-long rings were clamped between two hooks of a triangular shape and immersed into Krebs solution gassed with 95% O_2 and 5% CO_2, pH 7.4 at 37 °C. The holder with an aorta ring was connected to a tensiometry sensor. Rings were stabilized at an optimal tension of 10 mN for 60 min

(A static-dynamic apparatus M 1101, Czech Republic). During the stabilization period, the rings were washed out several times with Krebs solution. Rings were contracted by Krebs solution with 100 mM KCl, washed out, and stabilized. Phenylephrine (10^{-6} mol/l) was added to induce contraction. At the plateau of contraction, acetylcholine was administered cumulatively in concentrations of 10^{-8} to 10^{-5} mol/l, and the endothelium-dependent relaxation response was monitored (Kutesz 185, Hungary).

SERUM LIPID PROFILE

The blood was collected from the retro-orbital plexus after 14 hours of starvation. Diagnostics kits for total cholesterol and triacylglycerols (Erba Lachema Ltd, Czech Republic) were used to determine the lipid profile from rat blood serum. The absorbance of resulting colored compounds was measured spectrophotometrically (LabSystems 352 Multiscan MS Microplate Reader, ThermoFisher Scientific, USA).

THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS) ASSAY

The thiobarbituric acid (TBA) test was used as an index for lipid peroxidation based on the reactivity of the final lipid peroxidation product malondialdehyde with TBA to form a red adduct (9). The double heating method, according to Draper and Hadley (8) was used to determine TBARS. The trichloroacetic acid solution was added to the kidney tissue, homogenated into centrifuge tubes, and placed in a hot water bath. After 15 min, the mixture was cooled with water and centrifuged for 10 min at 3000 × *g* and 4 °C. The supernatant was added to the TBA solution and placed in a hot water bath of 95 °C for 15 min. Subsequently, the solution was cooled in water and its absorbance was measured on microplates spectrophotometrically (Labsystem Multiscan RC, Canada).

FASTING GLUCOSE

The blood was drawn from the retro-orbital plexus after 14 hours of starvation (the same collection as for serum lipid profile determination), and the level of glucose was measured in a drop of blood by glucose meter (Contour, Bayer, Germany).

BLOOD PRESSURE

Blood pressure was measured by non-invasive tail-cuff plethysmography (PowerLab 4/30, AD Instruments, USA). Rat-friendly modification of blood pressure measurement according to Liptak et al. (2017) was used to reduce the stress of animals. Systolic blood pressure was calculated as the average of five consecutive measurements.

STATISTICAL EVALUATION

Data were expressed as means ± SEM. An unpaired *t*-test was used to compare the means of two unrelated groups and determine a significant difference between them. The level of $p < 0.05$ was considered a statistically significant difference.

RESULTS

The rats consumed the same amount of food per g/rat/day regardless of the type of diet (Tab. 1). The addition of 60% fructose to the pellets meant a subsequent reduction of saccharides, fats, and protein content to 40% in the fructose-fed group. However, the fructose group had a higher energy intake (Table 1).

Yet, this increased energy intake lasting five weeks in the Fructose group of rats resulted in a not quite significant increase in the body weight gain compared to Control rats. HTG rats with a high-fructose intake drank on average significantly less water compared to Controls. Concerning the impact of a high-fructose intake on cardiovascular damage, a marked deterioration of LVDP was found during the reperfusion of the heart represented by a significantly slower return to the input values compared to Controls (Fig. 1A).

Moreover, the duration of VF was several times longer than in Control group of rats (Table 1). At the end of the 30-minute reperfusion period, VF was evoked by electri-

Tab. 1 Impact of a high-fructose intake on basic parameters and heart function in hypertriglyceridemic rats.

Parameter *(units)*	**CONTROL**	**FRUCTOSE**	**Significance**
Food consumption *(g/day/rat)*	28.68 ± 0.73	28.06 ± 0.81	n.s.
Energy intake *(kJ/day/rat)*	380.32 ± 4.33	408.86 ± 11.02	$p \leq 0.05$
Water Drinking *(ml/day/rat)*	26.23 ± 0.53	21.16 ± 0.78	$p \leq 0.001$
Body weight gain *(g/5 weeks/rat)*	55.64 ± 3.83	66.93 ± 5.12	n.q.s.
VPB in R *(number)*	474.36 ± 121.79	394.44 ± 115.38	n.s.
VT duration in R *(s)*	160.26 ± 83.33	243.59 ± 153.85	n.s.
VF duration in R *(s)*	115.38 ± 89.74	673.08 ± 230.77	$p \leq 0.05$
VF stimulation threshold *(mA)*	29.10 ± 0.31	18.81 ± 2.24	$p \leq 0.05$

Values are expressed as means ± S.E.M. VPB – ventricular premature beats; VT – ventricular tachycardia; VF – ventricular fibrillation; TBARS – thiobarbituric acid reactive substances; R – reperfusion; n.s. not significant; n.q.s. not quite significant. Basic values, $n = 14$ rats/group; values obtained by Langendorff technique, $n = 7$ rats/group. Student *t*-test.

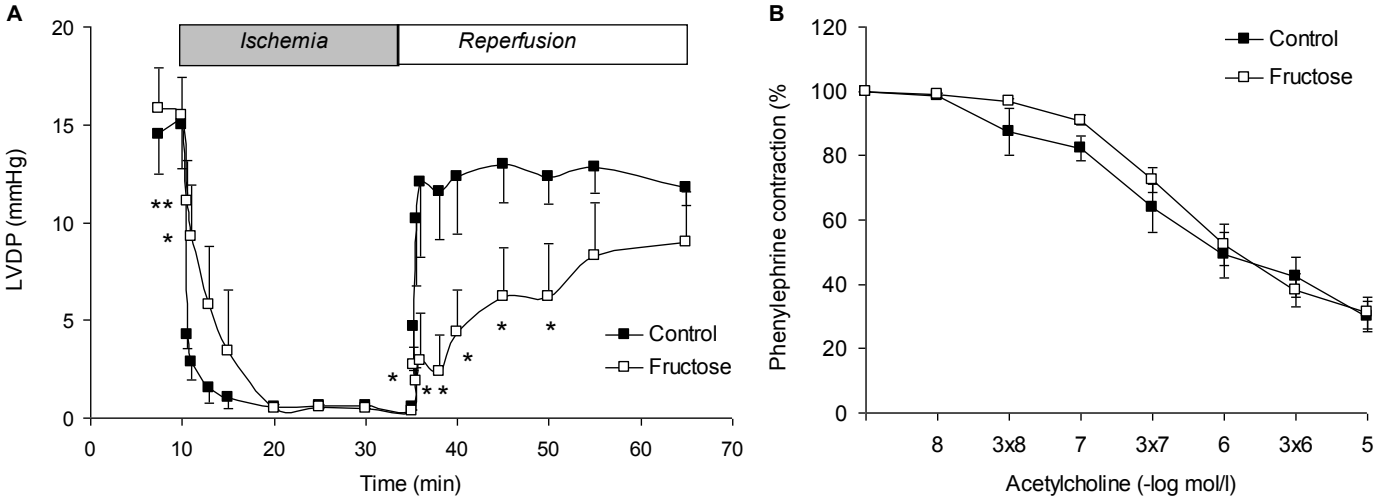

Fig. 1 Effect of a high-fructose intake on **(A)** the left ventricular developed pressure (LVDP). Isolated rat hearts according to the Langendorff technique underwent a stop-flow ischemia (25 min) followed by reperfusion (30 min). During the reperfusion period, a return of LVDP to the onset values was significantly slower in the Fructose group (n = 7) compared to the Control rats (n = 7; *p < 0.05). Effect of a high-fructose intake on **(B)** the thoracic aorta endothelial-dependent relaxation. Phenylephrine-precontracted vessels were relaxed by cumulatively adding acetylcholine (10^{-8} to 10^{-5} mol/l). No significant differences were observed between the responses of Control (n = 14) and Fructose group (n = 14). Values are means ± S.E.M. Student t-test.

cal stimulation. As a substantial finding of 5 weeks lasting high-fructose intake impact, we consider an increased sensitivity to the induction of life-threatening VF, as the electrical stimulation intensity needed to evoke VF was significantly lower comparing Control rats. A high-fructose intake did not deteriorate endothelium-dependent relaxation of the thoracic aorta (Fig. 1B). A significant increase was found in the total serum cholesterol (TC) level (1.52 ± 0.05 *vs*. 1.38 ± 0.04 mmol/l; $p \leq 0.05$) and the triacylglycerol (TAG) level (3.41 ± 0.19 *vs*. 2.44 ± 0.17 mmol/l; $p \leq 0.001$) due to a high-fructose intake compared controls at the end of the experiment. Similarly, significantly increased oxidative stress, measured as TBARS level, was observed in the kidneys of rats exposed to a high-fructose intake (6.30 ± 0.28 *vs*. 5.50 ± 0.20 mmol/mg protein; $p \leq 0.05$). The systolic blood pressure and the blood fasting glucose level were not affected by the 5-week lasting high-fructose intake comparing controls.

DISCUSSION

The simultaneous presence of several risk factors of MetS elevates rates and severity of cardiovascular diseases including coronary atherosclerosis and calcification, microvascular dysfunction, cardiac dysfunction, myocardial infarction, and heart failure (10, 17, 32, 39). Hereditary HTG rats are characterized by hypertriglyceridemia, mild hypertension, and insulin resistance and they have some disturbances in glucose metabolism (41). The occurrence of the aforementioned metabolic disturbances allows their use as a model of MetS and related cardiovascular diseases, even though they are non-obese. We assumed that the consumption of high-fructose intake could have an aggravating impact on rats with metabolic disturbances. This would mimic the situation when people with MetS consume a lot of fructose in soft drinks and industrially processed food and sweets. There is the suggestion that oxidative stress and inflammation may play a key role in high fructose-induced cardiac dysfunction. Cardiac inflammation due to high-fructose intake was induced via macrophage recruitment in cardiomyocyte (37). High-fructose intake increased the expression of TNF-α, IL-6, IL-1β, and NF-κB in rat hearts, resulting in cardiac inflammation in fructose-fed diabetic rats (16). Further, fructose exposure induced oxidative stress, mitogen-activated protein kinases (MAPK) signaling, mitochondrial signaling pathway, and inflammatory signaling, increased NLRP3 inflammasome activation due to the induction of CD36 expression and heart tissue oxidative stress (13, 37), stimulated the expression of p38, ERK1/2 and JNK (40).

We focused on heart and vessel dysfunctions. In the present work, marked deterioration of LVDP represented by a slower return to the input values and many times increased durations of life-threatening VF during the reperfusion period was shown due to a high-fructose intake compared to the control group. Impairment of ventricular diastolic function in rats due to the fructose-rich diet was found by Xing and co-workers (2010). An increased susceptibility to myocardial I/R injury was shown by Maarman et al. (2017). Topçu and co-workers (2022) fed SD rats with high fructose for four weeks and their hearts underwent 30 min ischemia followed by 60 min reperfusion *ex vivo*. Similarly, they observed the left developed ventricular pressure was still depressed at the end of 60 min reperfusion compared to the control group. 60% fructose intake increased cardiac fibrosis, cardiomyocytes size, and relative wall thickness of the left ventricle in adult C57BJ/L6 mice (37).

Our original and most important finding is that the threshold for VF induction decreased, thus the sensitivity to the heart functional disorders induction increased. The lowering of the threshold for the VF induction was not accompanied by either an increase in the blood pressure or a change in glucose level, therefore it seems the only measurable indicator of approaching impairment of heart

function by fructose is presented by an increased level of TAG. Even though TAGs are highly atherogenic (24, 33), no detrimental effect on the endothelium-dependent relaxation of the thoracic aorta was observed here. The reason could be the short duration of the experimental diet to develop vessel impairment, even though the 60% fructose in pellets represents an intensive diet. It was suggested that an increase in plasma TAG levels is associated with an increase in cardiovascular risk, therefore plasma TAG levels may serve in the prognosis of the heart disease risk (12). The negative influence of increased TAG levels was reported in humans by Tikhonoff et al. (2024), who determined a prognostic cutoff value for increased cardiovascular risk events to 89 mg/dl of TAG. In the present work, a significant increase in the serum TAG levels was found with an additional TC level increase due to a high-fructose intake. The mechanism of the harmful effect of a high-fructose intake on heart function found here could be associated with observed dyslipidemia. Therefore therapies targeting dyslipidemia, and the TAG-lowering action may be useful in improving cardiovascular outcomes (26) even in high-fructose intake-induced heart dysfunctions.

The deleterious effect of high-fructose feeding may be related to renal damage (6), and can lead to oxidative stress in the kidneys (28). We also determined increased lipid peroxidation in the kidney tissue in the present work. Consistent with our observation, a negative effect of drinking 10% fructose, lasting 15 weeks, was documented in the kidneys in a recent study by Vrbjar et al. (34). These authors have found an increase in lipid peroxidation and a decrease in thiol-redox balance estimated by determining the GSH/GSSG ratio. In connection with this finding, in our previous work, we have demonstrated that the combination of antioxidants (Vitamin E, 100 mg/kg/day and the pyridoindole antioxidant coded SMe1EC2, 15 mg/kg/day, *p.o.*) suppressed the occurrence of serious dysrhythmias (VT+VF) within the group of HTG rats fed a high-fat-fructose diet (1% cholesterol, 7.5% pork lard, 10% fructose) in comparison to the control HTG animals fed standard diet (3). Thus an oxidative stress-reducing diet may have a positive effect on unhealthy high fat and sugar-rich intake sequale.

In addition to the main effect of the increased level of TAG in response to a high-fructose intake, it could lead to induction of insulin resistance by inhibiting the insulin signaling pathways (2), induction of non-alcoholic fatty liver diseases as increased fat is stored within the liver cells (27), and may exhibit proapoptotic effects *via* inhibition the PI3K/Akt axis, and thus contribute to the death of cardiomyocytes (4, 19). Modern concepts offer a new hypothesis regarding the direct myocardiotoxic effects of fructose (19, 23, 29). This might be the base for corresponding changes in molecular mechanisms that could lead to systemic consequences ending up in cardiovascular impairment. So far studies elucidating such backgrounds are infrequent (5, 29, 36).

CONCLUSIONS

Results from the present experimental study show the substantial impact of uncontrolled unhealthy fructose consumption which can trigger the progression of cardiovascular events and ventricular arrhythmogenesis associated with dyslipidemia. We proved that a high-fructose intake increased the duration of life-threatening VF and weakened LVDP during the rat heart reperfusion followed by ischemia. Most importantly, a decreased threshold for inducing VF was observed.

ACKNOWLEDGMENT

The work was supported by the VEGA grants 2/0018/23 and 2/0104/21. The authors thank Mrs. Katarína Vandáková, Júlia Poláková, and Monika Srnová for their technical assistance.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Updated Meta-Analysis of VDR FokI and TaqI Variants and Their Association with Melanoma Risk

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ABSTRACT

Background: Research suggests that melanoma patients with low vitamin D levels exhibit a higher risk of tumor ulceration and increased tumor mitotic rates. This has led to investigations into the vitamin D receptor (VDR) gene concerning its potential link to melanoma susceptibility. This meta-analysis aims to explore the association between VDR FokI and TaqI polymorphisms and melanoma risk, with an emphasis on the need for research in diverse populations to enhance our conclusions regarding interactions between skin phenotypes and VDR variations.

Methods: A comprehensive literature search was conducted in databases, including PubMed, Scopus, and Web of Science, for studies linking VDR polymorphisms to melanoma risk, up to February 1, 2024. Keywords used included "Melanoma", "VDR", and various genetic terms. Quantitative synthesis was performed with Comprehensive Meta-Analysis (Version 4.0) and a significance threshold set at $p < 0.05$.

Results: A total of twenty-one case-control studies involving 8,813 melanoma cases and 7,973 controls were included. Twelve studies on FokI had 4,642 cases and 4,534 controls, while nine TaqI studies included 4,171 cases and 3,439 controls. The results show a significant association between the VDR FokI polymorphism and increased melanoma risk across four genetic models (allele model: OR = 1.128, 95% CI 1.026–1.241; P = 0.013; homozygote model: OR = 1.166, 95% CI 1.020–1.332; P = 0.025; heterozygote model: OR = 1.255, 95% CI 1.046–1.507; P = 0.015; dominant model: OR = 1.243, 95% CI 1.052–1.470; P = 0.011). In contrast, the TaqI polymorphism showed no significant association with melanoma risk in the general population.

Conclusions: This meta-analysis suggests that the VDR FokI polymorphism is linked to an increased susceptibility to melanoma, while the TaqI variant does not show a significant association. Future research should explore the interactions between VDR polymorphisms, skin phenotypes, and melanoma risk in diverse populations, with larger and more varied studies needed to confirm these findings and enhance our understanding of genetic factors affecting melanoma susceptibility.

KEYWORDS

melanoma; vitamin D; VDR; polymorphism; genetic susceptibility; meta-analysis

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Acta Medica (Hradec Králové) 2024; 67(4): 113–124
https://doi.org/10.14712/18059694.2025.8

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Received: 6 March 2024
Accepted: 24 February 2025
Published online: 4 April 2025

INTRODUCTION

Melanoma, the most lethal form of skin cancer, arises from transformed melanocytes and affects people of all ages, genders, and ethnicities, with its global incidence rising steadily (1, 2). In 2020, around 325,000 new melanoma cases were reported worldwide, exhibiting significant geographic variation. Australia and New Zealand had the highest rates, with 42 cases per 100,000 person-years for males and 31 for females, while Western and Northern Europe, as well as North America, recorded lower but substantial rates ranging from 17 to 19 for males and 14 to 18 for females. In contrast, many African and Asian countries reported incidence rates below 1 per 100,000 person-years (3, 4). The overall prevalence tends to be higher in males, underscoring the need for continued awareness and prevention efforts. If current trends continue, new cases could surge to 510,000 by 2040, resulting in 96,000 deaths . The World Health Organization noted about 287,723 new cases globally in 2018, projecting a 14% increase in incidence by 2035 (3). Risk factors for melanoma include both environmental and genetic components, such as UV radiation exposure, fair skin, history of sunburns, numerous moles, family history, immunosuppression, specific genetic mutations, and certain occupational exposures to substances like coal tar or arsenic (5–7). Individuals with skin influenced by the melanocortin 1 receptor (MC1R), particularly those with lighter skin or red hair, are more vulnerable to UV damage. A higher number of acquired melanocytic nevi also correlates with increased risk (8, 9). Environmental influences, especially intermittent sun exposure during childhood or adolescence, further elevate melanoma risk, and the use of tanning beds significantly increases this risk among younger individuals (10, 11). Furthermore, a personal history of sunburn raises the likelihood of developing melanoma, and those with immunosuppression due to organ transplants exhibit significantly higher incidence rates compared to the general population. The presence of other skin cancers can also elevate risk, highlighting the intricate relationship between genetic predisposition, environmental factors, and overall health in the development of melanoma (5, 12).

The VDR gene plays a vital role in regulating cell cycle progression, differentiation, and apoptosis, which are all key factors in cancer development (13). Studies suggest that vitamin D deficiency is associated with a higher cancer risk, while elevated vitamin D levels correlate with reduced cancer incidence and mortality (14, 15). Many cancer patients are vitamin D deficient due to factors like impaired physical function, poor diet, chemotherapy, and limited sunlight exposure, prompting increased screenings for deficiencies over the past two decades. Vitamin D interacts with the Vitamin D receptor (VDR), essential for numerous physiological functions, while 25-hydroxyvitamin D [25(OH)D] is the primary marker of vitamin D status (16). As a nuclear macromolecule, the VDR mediates the effects of 1alpha, 25-dihydroxyvitamin D3 [1,25(OH)2D3] and regulates around 2,000 vitamin D-responsive genes involved in cell growth, differentiation, and apoptosis. Research indicates that the VDR gene significantly affects cancer growth and progression. The VDR gene, located on chromosome 12q13.1, has a complex structure with a large promoter region that generates various tissue-specific transcripts (17). It comprises 11 exons over approximately 75 kb: exons 1A, 1B, and 1C are in the 5' upstream non-coding region, while exons 2 to 9 encode the VDR protein, which contains six functional domains. The ligand-binding region interacts with 1,25(OH)2D3 and the retinoic acid X receptor (RXR) (18).

Research indicates that reduced expression of VDR in melanoma is associated with enhanced tumor growth, increased metastatic potential, and poorer survival rates. This suggests that dysregulation of VDR may play a role in the progression of melanoma and could serve as a potential therapeutic target (19, 20). VDR signaling pathways influence key aspects of melanoma biology, such as proliferation, apoptosis, angiogenesis, and invasiveness, highlighting their importance for disease management and treatment outcomes (21, 22). Over 900 genetic variations have been documented in the VDR gene, with polymorphisms like FokI, TaqI, BsmI, and ApaI being the most studied in relation to melanoma (23). However, the precise connection between VDR polymorphisms and melanoma risk is unclear, particularly for FokI (rs2228570) and TaqI (rs731236), which have yielded inconsistent results across studies. In 2020, Birke et al. performed a meta-analysis of 14 studies on VDR polymorphisms and melanoma risk, suggesting that the FokI, ApaI, and BsmI variants may affect susceptibility. Specifically, the BsmI polymorphism was associated with a 15% decrease in malignant melanoma risk, while FokI and ApaI were linked to an increase in risk by 22% and 20%, respectively (24). Other VDR polymorphisms had minimal impact on melanoma risk. Conversely, a meta-analysis by Lee et al. (2015) involving over 8,000 participants found no significant associations between VDR polymorphisms and melanoma risk (25), aligning with earlier findings by Mocellin et al. (2009) on TaqI but differing regarding FokI (26). Similarly, results

for the TaqI polymorphism are inconsistent; some studies suggest that Tt and tt genotypes may reduce melanoma risk by 30%, while others find no significant correlations, complicated by TaqI's strong linkage disequilibrium with other VDR variants (27). These discrepancies highlight the need for further research on ethnic variability in allele frequencies and their effects. To address these inconsistencies, our study undertook a meta-analysis employing systematic review and meta-analysis methodologies, involving comprehensive literature searches, quality assessments, and statistical analyses, to clarify the associations between VDR FokI and TaqI variants and melanoma risk, while also investigating potential ethnic differences.

MATERIALS AND METHODS

SEARCH STRATEGY

The meta-analysis followed PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines to guarantee a transparent and thorough review process. To identify relevant studies investigating the relationship between VDR polymorphisms and melanoma risk, we performed a comprehensive search across various online databases. The databases included PubMed, Web of Science, Elsevier, Europe PMC, ResearchGate, Cochrane Library, EMBASE, SciELO, Chinese Medical Current Contents (CMCC), Google Scholar, Wanfang Data Company, Chaoxing, VIP Information Consulting Company (VIP), Sinomed, Chinese Medical Citation Index (CMCI), Chinese Biomedical Database (CBD), Chinese National Knowledge Infrastructure (CNKI), Scientific Information Database (SID), and ClinicalTrials.gov. The search was restricted to studies published until February 1, 2024. To enhance the breadth of our search, we utilized a diverse array of specific keywords and terms, including "Skin Cancer", "Melanoma", "Cutaneous Melanoma", "Malignant Melanoma", "Cutaneous Malignant Melanoma", "Vitamin D Receptor", "VDR", "Polymorphisms", "FokI", "Taq1", "rs2228570", "rs731236", "Gene", "Genetics", "Single-Nucleotide Polymorphism", "SNPs", "Genotype", "Allelic Variation", "Mutation", "Mutant", "Allele", "Variant", "Risk Factors", "Susceptibility", "Epidemiology", "Molecular Genetics", "Environmental Factors", "Genetic Variation", "Cohort Studies", "Case-Control Studies", "Meta-Analysis", "Gene-Environment Interaction", and "Melanoma Epidemiology". In addition to the electronic search, we manually reviewed the reference lists of all eligible articles and reviews to uncover any pertinent studies that may have been overlooked in the initial search. Importantly, only articles published in English were included to maintain consistency in the meta-analysis framework. Informed consent was not applicable for this study as it did not involve individual participants.

INCLUDING AND EXCLUDING CRITERIA

All studies included in this analysis adhered to predetermined criteria to ensure the relevance and quality of the research. Only case-control or cohort design studies published in English that specifically explored the association between VDR polymorphisms and the risk of cutaneous melanoma in preterm neonates were considered. To qualify, studies needed to provide sufficient and accessible data for calculating odds ratios (ORs) and 95% confidence intervals (CIs). Exclusion criteria included case reports, case series, letters, editorials, comments, reviews, animal studies, in vitro experiments, conference papers, and meta-analyses. Studies that did not present genotype frequency data for VDR polymorphisms, had fewer than 30 participants, or had a follow-up period of less than one year were also excluded, as these factors could compromise the reliability of findings. Additionally, research that lacked appropriate statistical analysis or did not control for potential confounders such as age, sex, and environmental influences was removed. Finally, studies with overlapping data or duplicated analyses were excluded to ensure the uniqueness and validity of the results included in this analysis.

DATA EXTRACTION

Based on the specified inclusion and exclusion criteria, it was determined that two independent authors were responsible for extracting data from the eligible studies. This extraction process was meticulously carried out using a standardized Microsoft Excel spreadsheet. In cases of disagreements or discrepancies during the data extraction, the third author was consulted for resolution. The extracted information from each individual case-control study included the first author's name, year of publication, country of origin, ethnicity, genotyping methods used, source of controls, total number of cases and controls, genotype frequencies of cases and controls for each available VDR polymorphisms, Hardy-Weinberg equilibrium (HWE) test results, and minor allele frequencies (MAFs) observed in the controls. In instances where multiple studies were published by the same investigator(s) and featured duplicated or overlapped data, only the most recent published data or the one with the largest sample size was included in the analysis.

QUALITY SCORE ASSESSMENT

The Newcastle-Ottawa Score (NOS) evaluated the quality of studies in a meta-analysis by examining the methodological aspects of observational research, including case selection, group comparability, and exposure determination, each assessed through eight specific items. Studies with excellent selection and exposure received one star, while comparability could earn up to two stars. Quality was rated on a nine-star scale, with zero indicating poor quality and nine representing high quality. Studies scoring seven or more were considered high quality, while those with at least five points were suitable for meta-analysis. Disagreements were resolved through discussion and consensus.

STATISTICAL ANALYSIS

Quantitative data synthesis was performed using Comprehensive Meta-Analysis (Version 4.0) software developed by Biostat. A two-sided p-value below 0.05 was considered

statistically significant in this study. The study investigated the association between VDR FokI and TaqI polymorphisms and the risk of cutaneous melanoma by calculating ORs with 95% CIs. A Z-test was utilized to assess the statistical significance of pooled data by comparing population means with sample means. The meta-analysis incorporated five genetic models: allelic (B vs. A), homozygote (BB vs. AA), heterozygote (BA vs. AA), dominant (BB + BA vs. AA), and recessive (BB vs. BA + AA). A chi-square test evaluated heterogeneity, with significance set at $p < 0.05$. Following Cochrane's guidelines, heterogeneity among studies was measured on a scale from 0 to 100%. If the I^2 value exceeded 50%, random-effect models (DerSimonian-Laird method) were applied; otherwise, fixed-effect models (Mantel-Haenszel method) were used. To ensure the robustness of the findings, sensitivity analysis was conducted by systematically excluding one study at a time to observe its impact on the overall results. Publication bias was assessed using Begg's test, which plotted the standard error (SE) of each study against its corresponding OR, supplemented by Egger's test and visual inspection of the funnel plot for asymmetry. In cases where publication bias was identified, the Duval and Tweedie non-parametric "trim-and-fill" method was employed to adjust the results. The HWE for the control group in each study was evaluated using the chi-square test, assisted by the online software GenePop (http://genepop.curtin.edu.au), which provides functionality for HWE calculations within its genetic data analysis. A p-value below 0.05 was considered significant regarding HWE. Data analysis was conducted using Python, enabling the calculation of ORs, CIs, and metrics for heterogeneity.

RESULTS

CHARACTERISTICS OF SELECTED STUDIES

Figure 1 illustrates the process of selecting appropriate studies. A comprehensive search initially yielded 541 potentially relevant articles. After removing duplicate literature and carefully reviewing titles and abstracts, 318 articles underwent a full-text review. Among these, 190 articles were excluded due to lack of relevance and adequate data. Ultimately, a total of 21 case-control studies, which were derived from eleven publications (28–38), were deemed eligible based on the predefined inclusion criteria. These studies involved a large number of participants, with 8813 individuals identified as melanoma cases and 7973 individuals as controls. A comprehensive overview of the characteristics and genotype distribution of the eligible studies can be found in Table 1. Twelve studies concentrated on FokI, comprising 4642 cases

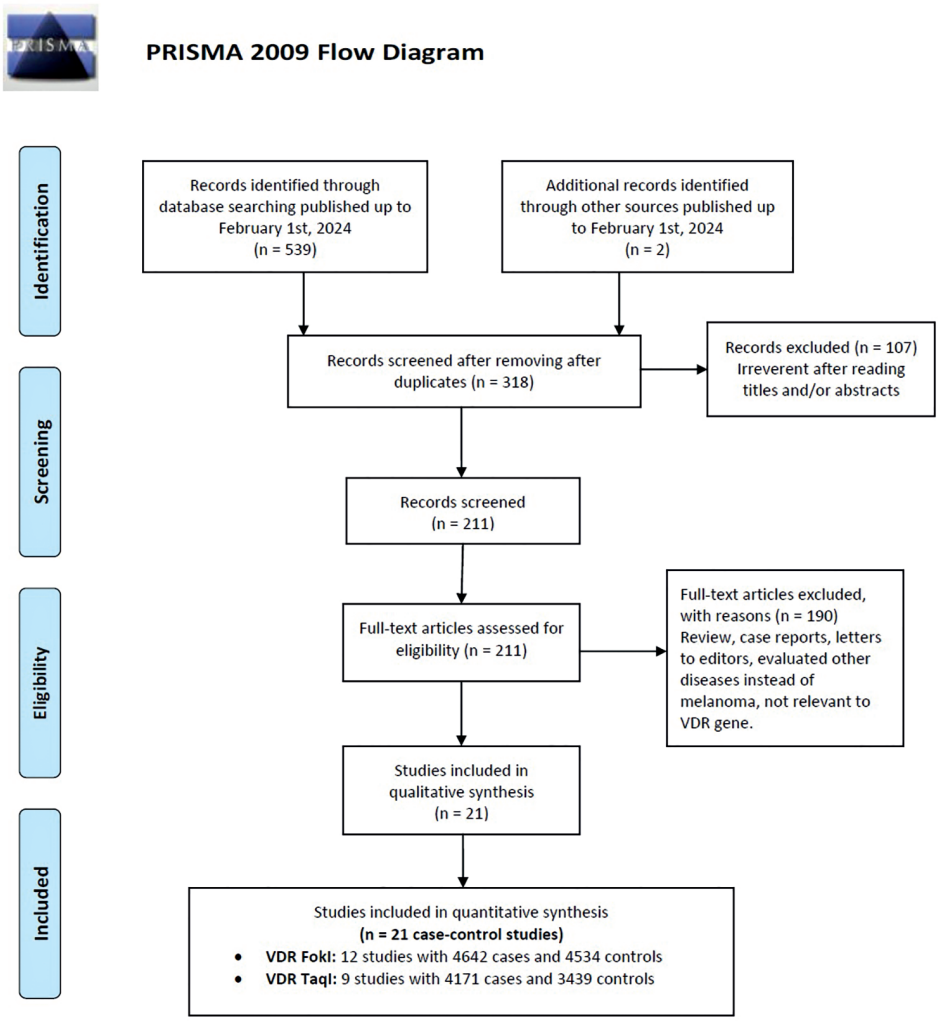

Fig. 1 Flow diagram of the study selection process.

Tab. 1 Characteristics of studies examined in the meta-analysis.

First Author	Country (Ethnicity)	SOC	Genotyping Technique	Case/ Control	Melanoma Cases					Healthy Subjects					MAFs	HWE	NOS
					Genotypes			Allele		Genotypes			Allele				
FokI					CC	TC	TT	C	T	CC	TC	TT	C	T			
Hutchinson 2000	UK (Caucasians)	HB	PCR-RFLP	293/108	105	142	46	352	234	52	44	12	148	68	0.314	0.563	7
Santonocito 2007	Italy (Caucasians)	PB	PCR-RFLP	101/101	47	41	13	135	67	41	46	14	128	74	0.366	0.869	7
Han 2007	USA (Caucasians)	PB	TaqMan	215/854	77	101	37	255	175	325	418	111	1068	640	0.374	0.193	6
Li 2008	USA (Caucasians)	HB	PCR-RFLP	805/841	287	427	91	1001	609	344	396	101	1084	598	0.355	0.424	8
Randerson-Moor 2009	UK (Caucasians)	PB	AS-PCR	1028/402	381	489	158	1251	805	161	176	65	498	306	0.380	0.151	8
Randerson-Moor 2009	UK (Caucasians)	PB	AS-PCR	299/560	96	139	64	331	267	225	255	80	705	415	0.370	0.058	8
Barroso 2008	Spain (Caucasians)	HB	TaqMan	283/245	135	121	27	391	175	110	108	27	328	162	0.330	0.949	7
Gapska 2009	Australia (Caucasians)	HB	TaqMan	763/752	240	377	144	857	665	252	357	143	861	643	0.427	0.408	7
Pena-Chilet 2013	Spain (Caucasians)	HB	AS-PCR	500/309	217	225	58	659	341	140	130	39	410	208	0.336	0.308	6
Zeljic 2014	Serbia (Caucasians)	NA	TaqMan	117/122	40	60	17	140	94	46	62	14	154	90	0.368	0.312	5
Cauci 2017	Italy (Caucasians)	NA	PCR-RFLP	120/120	47	60	13	154	86	54	50	16	158	82	0.341	0.418	6
Aristizábal-Pachón 2022	Colombia (Mixed)	HB	PCR-RFLP	120/120	23	86	11	132	108	65	40	15	170	70	0.292	0.034	6
TaqI					TT	CT	CC	T	C	TT	CT	CC	T	C			
Hutchinson 2000	UK (Caucasians)	HB	PCR-RFLP	261/93	94	127	40	315	207	39	41	13	119	67	0.360	0.674	7
Randerson-Moor 2009	UK (Caucasians)	PB	AS-PCR	1028/402	369	484	175	1222	834	144	194	64	482	322	0.400	0.920	8
Randerson-Moor 2009	UK (Caucasians)	PB	AS-PCR	299/560	107	150	42	364	234	187	273	100	647	473	0.422	0.983	8
Barroso 2008	Spain (Caucasians)	HB	TaqMan	283/245	98	137	48	333	233	91	117	37	299	191	0.389	0.951	7
Li 2008	USA (Caucasians)	HB	PCR-RFLP	805/841	330	355	120	1015	595	269	422	150	960	722	0.429	0.485	7
Gapska 2009	Australia (Caucasians)	HB	TaqMan	760/762	315	351	94	981	539	324	350	88	998	526	0.345	0.656	6
Pena-Chilet 2013	Spain (Caucasians)	HB	AS-PCR	498/294	186	248	64	620	376	109	141	44	359	229	0.389	0.884	5
Zeljic 2014	Serbia (Caucasians)	NA	TaqMan	117/122	33	62	22	128	106	59	48	15	166	78	0.319	0.291	6
Aristizábal-Pachón 2022	Colombia (Mixed)	HB	PCR-RFLP	120/120	106	14	0	226	14	81	39	0	201	39	0.163	0.033	6

Abbreviations: SOC – source of control; PB – population-based; HB – hospital-based; PCR – Polymerase chain reaction; AS – allele-specific; RFLP – restriction fragment length polymorphism; SSP – sequence-specific primers; MAF – minor allele frequency; HWE – Hardy-Weinberg equilibrium; NOS – Newcastle-Ottawa Scale.

and 4534 controls, while nine studies examined TaqI, with 4171 cases and 3439 controls. It is worth mentioning that 19 of the studies included individuals of Caucasian descent, while the rest featured mixed populations. The research, conducted and subsequently published, spanned a considerable timeframe from 2000 to 2022, covering numerous scientific endeavors and inquiries. The study's focus was limited to seven specific countries: the United Kingdom, Italy, the United States of America, Spain, Australia, Serbia, and Colombia. Various genotyping techniques, such as PCR-RFLP, AS-PCR, and TaqMan, were employed throughout these studies. Additionally, it is noteworthy that the genotype distributions among the groups of healthy individuals in a particular FokI study and another TaqI study exhibited deviations from the anticipated proportions according to the HWE principles, as illustrated in Table 1.

QUALITY OF INCLUDED STUDIES

The meta-analysis evaluated the quality of included studies by considering various factors such as sample size, control type, genotyping methods, and Newcastle-Ottawa Scale (NOS) scores. Most studies had substantial sample sizes, notably Li 2008 with 805 melanoma cases and 841 controls, and Randerson-Moor 2009 with 1028 melanoma cases and 402 controls, enhancing data reliability. The studies primarily employed hospital-based (HB) or population-based (PB) controls; while PB controls offer greater generalizability, HB controls may introduce bias if not representative of the general population. MAFs varied within plausible ranges, indicating sufficient power for detecting associations, and compliance with HWE standards was observed, with p-values above 0.05. NOS scores ranged from 5 to 8, with most studies scoring 7 or higher, reflecting sound study design principles. However, the predominance of Caucasian participants may limit generalizability to non-Caucasian populations. Common genotyping methods included PCR-RFLP and TaqMan, noted for their reliability, while allele-specific PCR was effectively used in several studies, showcasing methodological diversity. Variations in genotyping methodologies could lead to differences in sensitivity and specificity, impacting findings' reliability. Addressing these methodological limitations will enrich the discussion and suggest future research directions to enhance genetic assessments. Overall, the studies demonstrate good methodological quality, but biases associated with HB controls and limited ethnic diversity should be taken into account when interpreting results, underscoring their contributions to understanding the genetic basis of melanoma.

DATA SYNTHESIS

Table 2 presents a meta-analysis of the VDR FokI and TaqI polymorphisms and their relationship with melanoma risk. The FokI analysis includes data from 12 studies with 4,642 melanoma cases and 4,534 controls, while the TaqI analysis draws from nine studies with 4,171 cases and 3,439 controls. The findings indicate a significant association between the table and melanoma risk across four genetic models: allele model (T vs. C: OR = 1.128, 95% CI 1.026–1.241; P = 0.013, Figure 2A), homozygote model (TT vs. CC: OR = 1.166, 95% CI 1.020–1.332; P = 0.025, Figure 2B), heterozygote model (TC vs. CC: OR = 1.255, 95% CI 1.046–1.507; P = 0.015, Figure 2C), and dominant model (TT + TC vs. CC: OR = 1.243, 95% CI 1.052–1.470; P = 0.011). In contrast, the TaqI polymorphism shows no significant associations, indicating no increased melanoma risk. Ultimately, the FokI variant appears to have a greater impact on melanoma susceptibility than the TaqI variant, highlighting the importance of VDR polymorphisms in cancer genetics.

EMPLOYING PYTHON FOR AN IN-DEPTH ANALYSIS OF FOKI AND TAQI POLYMORPHISMS

The study employed Python for a comprehensive analysis of FokI and TaqI polymorphisms in relation to melanoma risk, examining twelve studies on FokI with a total of

Tab. 2 Summary of pooled data on the association between VDR polymorphism and melanoma risk.

Subgroup	Genetic Model	Type of Model	Heterogeneity		Odds Ratio				Publication Bias	
			I^2 (%)	P_H	OR	95% CI	Z_{test}	P_{OR}	P_{Beggs}	P_{Eggers}
FokI										
Overall	T vs. C	Random	49.32	0.027	1.128	1.026–1.241	2.480	0.013	0.837	0.380
	TT vs. CC	Fixed	19.48	0.252	1.166	1.020–1.332	2.248	0.025	1.000	0.553
	TC vs. CC	Random	69.26	≤0.001	1.255	1.046–1.507	2.440	0.015	0.945	0.356
	TT + TC vs. CC	Random	67.19	≤0.001	1.243	1.052–1.470	2.553	0.011	0.631	0.311
	TT vs. TC + CC	Fixed	12.38	0.323	1.054	0.933–1.192	0.850	0.395	0.731	0.937
TaqI										
Overall	C vs. T	Random	77.04	≤0.001	0.973	0.833–1.137	-0.340	0.734	0.602	0.839
	CC vs. TT	Random	59.11	0.017	0.995	0.781–1.267	-0.044	0.965	0.173	0.072
	CT vs. TT	Random	76.33	≤0.001	0.960	0.766–1.204	-0.352	0.725	0.348	0.700
	CC + CT vs. TT	Random	79.14	≤0.001	0.961	0.765–1.207	-0.343	0.732	0.465	0.686
	CC vs. CT + TT	Fixed	9.25	0.359	0.956	0.837–1.093	-0.659	0.510	0.265	0.210

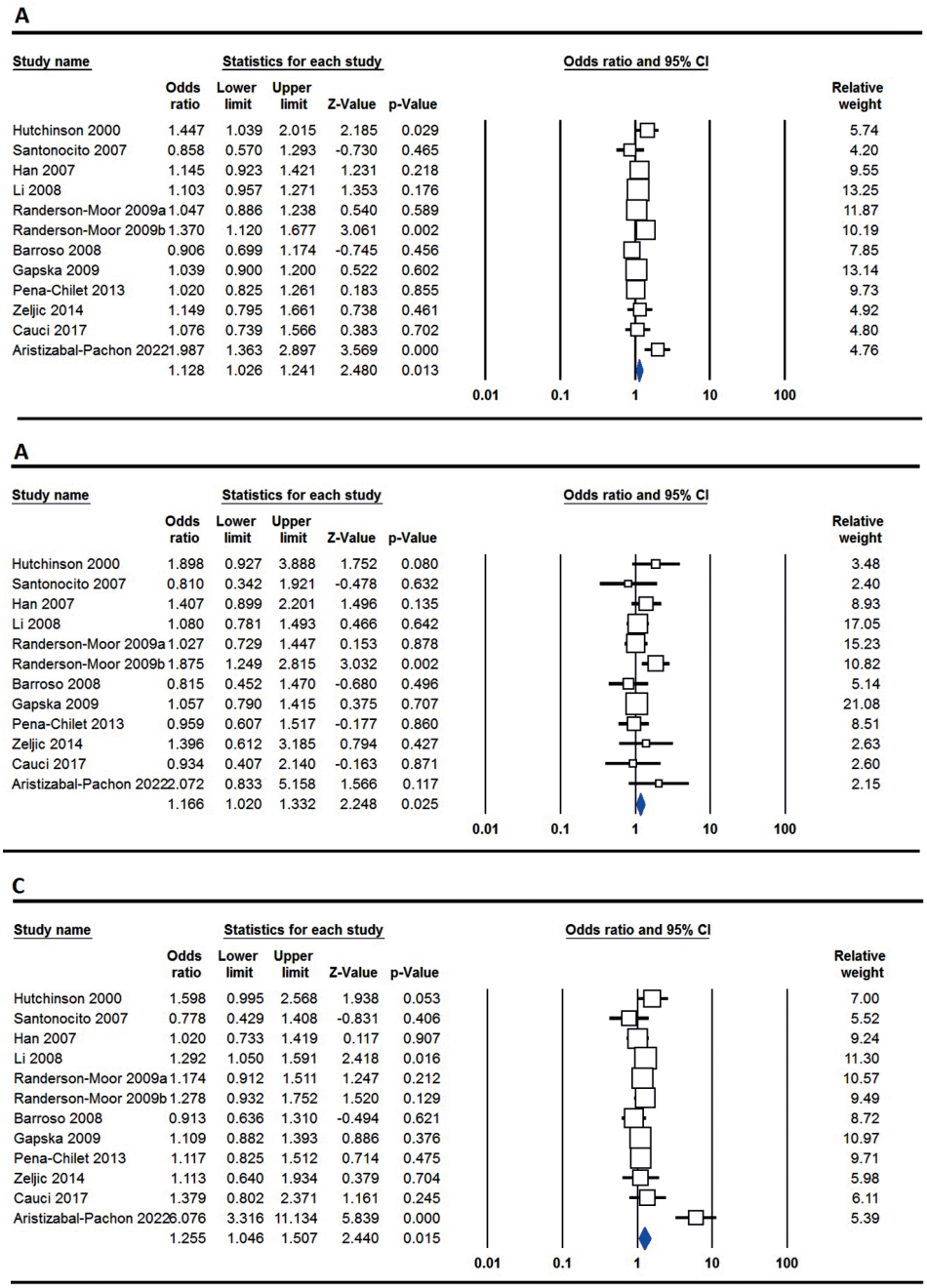

Fig. 2 Forest plots illustrating the correlation between VDR FokI polymorphism and melanoma risk: A: allele model (AA vs. AC + CC); B: homozygote model (AA vs. AC + CC); C: heterozygote model (AA vs. AC + CC).

4,642 cases and 4,534 controls, and nine studies on TaqI involving 4,171 cases and 3,439 controls, leading to a collective analysis of over 9,000 subjects. Significant findings were noted with FokI, particularly in ORs, revealing a 3.37% increase in odds when comparing T vs. C to TT vs. CC, and an 11.29% rise when assessing TC vs. CC relative to T vs. C. Conversely, a decrease of 7.07% was observed when comparing TC vs. CC to TT vs. CC. In contrast, the TaqI subgroup showed no significant association with melanoma risk, as indicated by overlapping CIs, such as for the comparisons of C vs. T (0.833–1.137) and CC vs. TT (0.781–1.267), highlighting a consistent absence of significant difference across various comparisons. The meta-analysis confirmed that nearly 65% of studies investigating TaqI supported similar non-significant results, with high heterogeneity noted for TaqI ($I^2 > 50\%$) indicating variability among studies, whereas moderate heterogeneity for FokI suggested significant overall associations remain. The average OR for FokI was approximately 1.173, suggesting its relevance in melanoma risk, while TaqI's average was around 0.973, indicating no increased risk. This thorough analysis highlights the importance of scrutinizing genetic factors influencing disease risk, as recent data estimates that genetic factors could account for up to 30% of melanoma risk, emphasizing the significance of this research in the broader context of skin cancer studies. Visual representations in Figure 3 illustrate the distinct differences between FokI and TaqI models, with FokI demonstrating strong, statistically significant positive associations across all comparisons, while TaqI exhibited more variability and

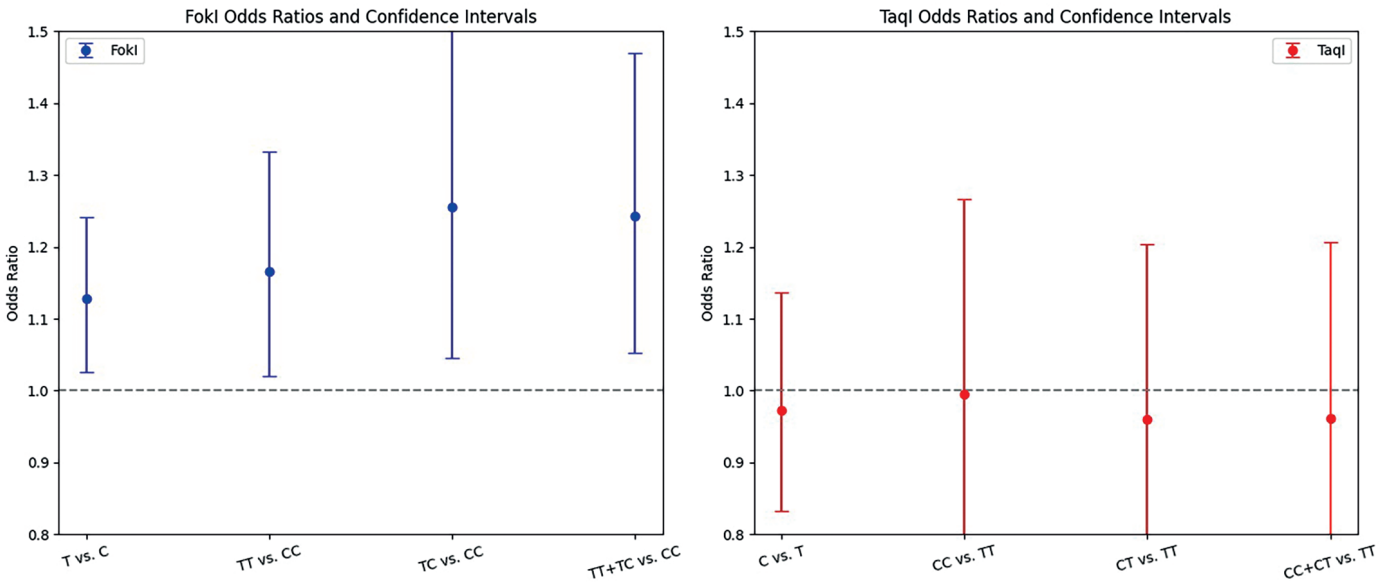

Fig. 3 Comparison of ORs and CIs for FokI and TaqI models. The left plot shows significant positive associations in the FokI model (blue circles), while the right plot indicates greater variability in the TaqI model with some near-significant comparisons (red circles). The dashed line at 1.0 represents no effect.

potential lack of significance in certain areas, suggesting the need for further investigation to better understand these dynamics. Overall, the results indicate that the FokI model provides clearer evidence of beneficial effects, whereas the TaqI model presents a more complex relationship meriting deeper analysis.

HETEROGENEITY

Table 2 highlights significant heterogeneity in research results concerning VDR FokI and TaqI polymorphisms across different genetic models. The I^2 statistic and p-values (P_H) provide valuable insights into these polymorphisms. For the FokI polymorphism, the T vs. C comparison has an I^2 of 49.32, indicating moderate heterogeneity, with a significant p-value of 0.027. In contrast, the TT vs. CC comparison shows low heterogeneity (I^2 = 19.48) and a non-significant p-value of 0.252. The TC vs. CC and TT + TC vs. CC comparisons demonstrate substantial heterogeneity, with I^2 values of 69.26 and 67.19, respectively, both statistically significant ($p \leq 0.001$). The TaqI polymorphism shows considerable heterogeneity in the C vs. T comparison (I^2 = 77.04, $p \leq 0.001$). The CC vs. TT and CT vs. TT comparisons also reveal substantial heterogeneity (I^2 values of 59.11 and 76.33) and are statistically significant (p-values of 0.017 and ≤0.001). The CC vs. CT + TT comparison, however, shows low heterogeneity (I^2 = 9.25, p = 0.359). Overall, the TaqI model exhibits significant heterogeneity across most comparisons, reflecting variability in the studies or populations, while the FokI model shows moderate to substantial heterogeneity in certain comparisons.

PUBLICATION BIAS

The analysis of publication bias regarding the association between VDR polymorphisms and melanoma risk yielded mixed results across different genetic models. For the FokI polymorphism, the Begg's test showed no significant publication bias in overall comparisons (PBeggs = 0.837) or specific contrasts like TT vs. CC (PBeggs = 1.000) and TC vs. CC (PBeggs = 0.945). Similarly, the Egger's test indicated no significant bias, with values ranging from 0.553 for TT vs. CC to 0.937 for TT vs. TC + CC. In the TaqI analysis, the overall genetic model showed no evidence of publication bias per the Begg's test (PBeggs = 0.602), though specific contrasts, such as CC vs. TT and CT vs. TT, exhibited more variable results (PBeggs = 0.173 and 0.348, respectively). The Egger's test for TaqI suggested potential bias in certain contrasts, particularly CC vs. TT (PEggers = 0.072). Figure 4 displays Begg's funnel plots for assessing publication bias in VDR FokI under the allele model (Figure 4A) and VDR TaqI under the recessive model (Figure 4B).

SENSITIVITY ANALYSIS

Sensitivity analyses were conducted by removing each eligible study to evaluate their impact on the overall results. The findings indicated that the statistical significance of the combined ORs for VDR FokI and TaqI polymorphisms remained consistent across all five genetic models, reinforcing the reliability of our results. Furthermore, excluding studies that deviated from HWE did not result in significant changes to the combined ORs. These findings underscore the robustness of our conclusions regarding the association between VDR FokI and TaqI polymorphisms and the risk of the condition investigated. The comprehensive sensitivity analyses enhance the credibility of our research.

HARDY-WEINBERG ANALYSIS

The evaluation of HWE for FokI and TaqI polymorphisms reveals significant variability across studies, countries, and methodologies. For the FokI polymorphism, out of

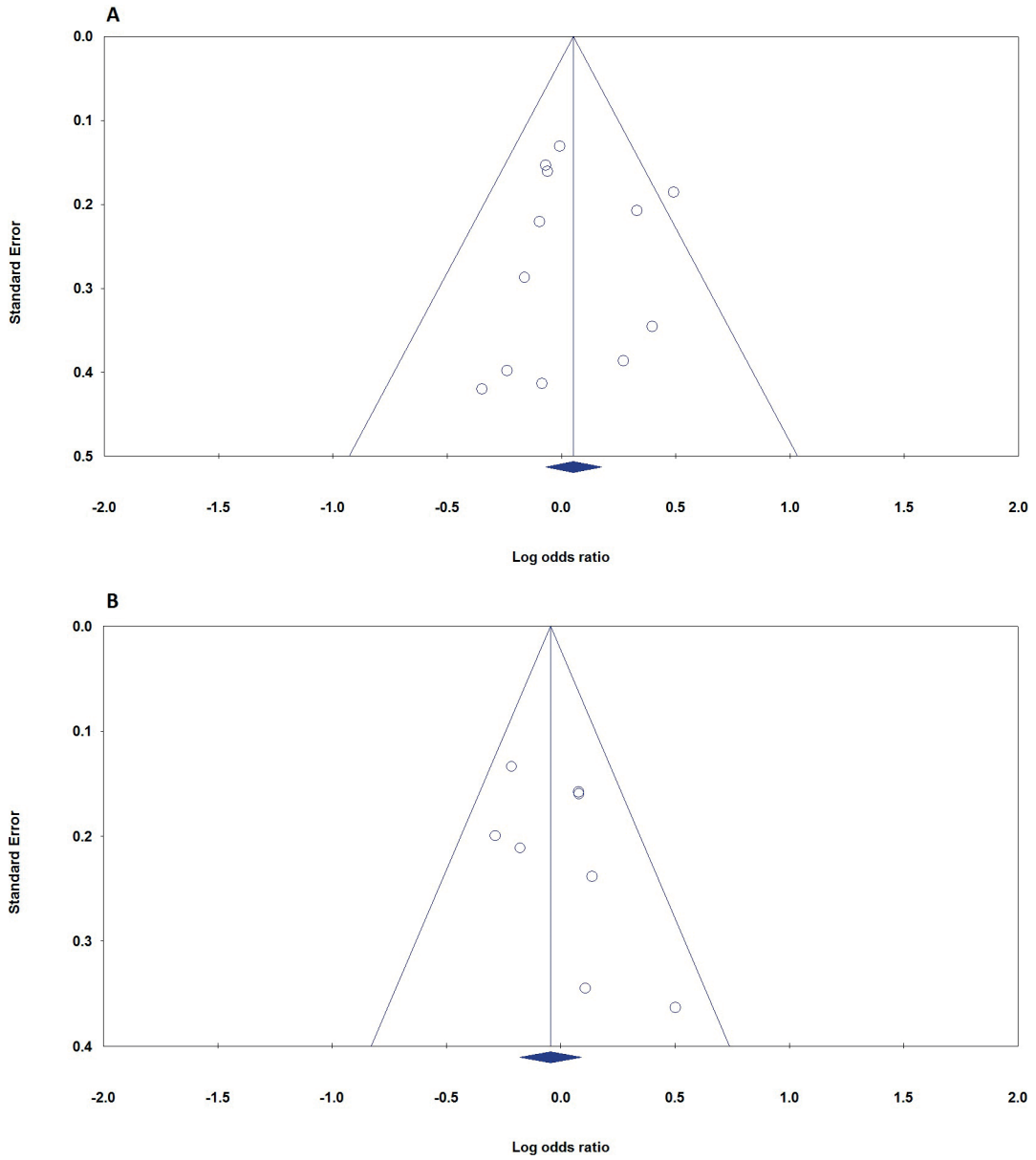

Fig. 4 Begg's funnel plots assessing publication bias in VDR polymorphisms associated with melanoma risk: A: VDR FokI under the allele model (AA vs. AC + CC); B: VDR TaqI under the recessive model (AA vs. AC + CC).

the assessed studies, four demonstrated equilibrium with p-values greater than 0.05 – namely Santonocito (0.869), Barroso (0.949), Gapska (0.408), and Zeljic (0.312) – while seven studies displayed deviations, including Hutchinson (0.563), Han (0.193), and Aristizábal-Pachón (0.034). Geographically, the UK studies generally suggested a lack of HWE, whereas Spain yielded mixed outcomes. Ethnic analysis indicated Caucasian studies showed several deviations from HWE, and genotyping methods like PCR-RFLP were correlated with more departures from equilibrium compared to TaqMan techniques. For the TaqI polymorphism, only two studies were in HWE (Randerson-Moor at 0.920 and Barroso at 0.951), with five studies reporting deviations, including Aristizábal-Pachón at 0.033. Overall, hospital-based controls exhibited greater deviations from HWE than population-based controls, and the data suggests that TaqMan genotyping might perform more favorably in achieving HWE status compared to PCR-RFLP across both polymorphisms.

MINOR ALLELE FREQUENCIES

The evaluation of MAFs for the polymorphisms FokI and TaqI reveals significant variation across different dimensions, including overall averages, by country and ethnicity, genotyping methods, and source of controls. For FokI, the overall average MAF is 0.358, while TaqI has a slightly higher average of 0.366. Country-specific evaluations for FokI show that the USA has the highest MAF at 0.386 among Caucasians, while Colombia, representing a mixed ethnicity, has a lower MAF of 0.292. In contrast, TaqI demonstrates a peak MAF of 0.429 in the USA, with the lowest observed in Colombia at 0.163. Analyzing by genotyping methods indicates that PCR-RFLP provides a consistent MAF for FokI and TaqI, while TaqMan generally yields higher averages. Furthermore, the source of controls impacts MAFs as well, with Population-Based studies often revealing higher frequencies compared to Hospital-Based studies. These evaluations underscore the complexity of genetic diversity linked to FokI and TaqI

polymorphisms, emphasizing the importance of considering multiple variables when interpreting genetic data.

DISCUSSION

Genetic variations, altered expression levels, and dysregulated signaling pathways all contribute to cutaneous melanoma susceptibility and outcomes. The link between VDR FokI and TaqI polymorphisms and melanoma predisposition has been extensively studied. Zeljic et al. conducted research indicating that FokI and TaqI polymorphisms in the VDR gene could serve as potential biomarkers for melanoma susceptibility (37). Similarly, Li et al. discovered a connection between VDR polymorphisms (TaqI and FokI) and cutaneous melanoma risk in a case-control study involving non-Hispanic white patients (39). In contrast, Beysel et al. proposed that the VDR FokI gene polymorphism raises the susceptibility to prostate cancer, while BsmI polymorphism does so for malignant melanoma, and TaqI increases the risk for renal cell carcinoma (40). This indicates that the influence of VDR polymorphisms may vary depending on the type of cancer. Additionally, the study by Marra et al. assessed VDR protein expression and VDR gene polymorphisms, including FokI, BsmI, ApaI, and TaqI, in cutaneous melanoma tissues, offering further proof of the significance of these polymorphisms in melanoma (41). Moreover, the meta-analysis by Rezaiian et al. suggested a potential positive association between VDR FokI and BsmI polymorphisms and non-melanoma skin cancer risks, underscoring the possible role of VDR polymorphisms in skin cancer predisposition (42). Evidence from these studies indicates that VDR FokI and TaqI polymorphisms may indeed influence melanoma predisposition. However, the specific impact of these polymorphisms may differ across various cancer types and populations, highlighting the intricate nature of genetic predisposition to melanoma and the necessity for further research in this domain. By elucidating the intricate relationship between the VDR gene and melanoma, this research paves the way for personalized and effective prevention and management strategies for this lethal disease.

Our analysis investigated the association between the VDR FokI polymorphism and melanoma, synthesizing data from 12 studies involving 4,642 melanoma cases and 4,534 controls. The findings revealed a significant link between the FokI polymorphism and increased melanoma risk, with the mutated allele (T) associated with higher susceptibility, shown by an OR of 1.128 (95% CI 1.026-1.241; P = 0.013). In contrast, a review of nine studies involving 4,171 cases and 3,439 controls found no significant association between the DR TaqI polymorphism and melanoma susceptibility in the general population. In 2020, Birke et al. conducted a meta-analysis of 14 studies assessing the relationship between seven VDR gene polymorphisms and melanoma risk, revealing that VDR variants FokI, ApaI, and BsmI may influence susceptibility. Specifically, the BsmI polymorphism was associated with a 15% reduction in the risk of malignant melanoma, while FokI and ApaI polymorphisms were linked to increased risks of 22% and 20%, respectively. However, no significant associations were found for other VDR gene polymorphisms, suggesting their minimal or non-existent influence on melanoma risk (24). Further investigation by Aristizabal-Pachon et al. focused on two SNPs of the VDR gene in a Colombian cohort of 120 patients and 120 matched healthy controls. Their findings indicated that the FokI polymorphism was associated with a significantly increased melanoma risk (OR: 5.10, 95% CI: 2.85–9.14), whereas the TaqI polymorphism appeared protective (OR: 0.27, 95% CI: 0.14–0.53) in the dominant model analysis. These results suggest that both polymorphisms can influence melanoma risk. Conversely, a meta-analysis by Lee et al. (2015) involving 4,413 patients and 4,072 controls of European descent found no significant associations between FokI, TaqI, ApaI, BsmI, and EcoRV polymorphisms and melanoma risk across 11 studies (25). The recent findings on VDR polymorphisms and melanoma risk reveal both similarities and differences compared to previous meta-analyses. While confirming a significant association between FokI and melanoma risk, the current study reiterates the lack of significant association with TaqI, echoing earlier work by Mocellin et al. (2009) (26) but diverging from Lee et al. (2015) regarding FokI (25). Overall, there is a consensus on the importance of certain VDR polymorphisms, highlighting the complexity of genetic influences on melanoma susceptibility, with varying degrees of association and specific risks attributed to each variant.

LIMITATIONS

The study presents several limitations that need to be acknowledged, starting with the limited number of studies available for inclusion in the meta-analysis, which may impact the overall robustness and reliability of the findings. Significant variations in study designs and methodologies, including participant selection and data collection methods, could introduce bias and affect the validity of the results. Additionally, considerable heterogeneity was revealed during the analysis, particularly in the FokI polymorphism subgroup, where high I^2 values indicated a significant level of inconsistency across studies, raising concerns about the reliability of the pooled results. Many of the included studies had small sample sizes, which led to unstable ORs and reduced statistical power to detect true associations between VDR polymorphisms and melanoma risk. Furthermore, evidence of publication bias was suggested by p-values from Begg's and Egger's tests, indicating that significant findings are more likely to be published, potentially skewing the results toward positive associations and affecting the overall interpretation. The analysis also did not sufficiently control for confounding variables such as environmental exposures, geographical differences, and other genetic variants, which could influence melanoma risk. Moreover, the analysis was primarily restricted to Caucasian populations, limiting the ability to generalize findings to other ethnic groups and raising uncertainties about the applicability of the results across diverse populations. The reliance on single-factor unadjusted ORs due to missing data on important factors such as age, gender, and lifestyle habits may also compromise

the accuracy of the findings. Additionally, the inability to assess the combined effects of VDR FokI and TaqI polymorphisms due to data inadequacy restricts the understanding of their interactions in melanoma pathogenesis. Variations in the time frames of the studies could further affect the detection and reporting of melanoma cases, leading to inconsistencies in outcomes. Lastly, while the study concentrated on VDR polymorphisms, it did not consider other relevant genetic variants that may contribute to melanoma risk, limiting the comprehensiveness of the investigation. The use of different genetic models, such as fixed vs. random effects, may not adequately capture the complexity of the genetic architecture related to melanoma risk, potentially oversimplifying the analysis. Addressing these limitations in future research is essential to enhance the understanding of VDR polymorphisms and their role in melanoma susceptibility, ultimately guiding more effective prevention and treatment strategies.

CLINICAL IMPLICATIONS

The significant association found for certain FokI models, particularly the TC versus CC genotypes, has important clinical implications for genetic screening and risk stratification in melanoma. With varying ORs indicating that individuals with specific FokI polymorphisms may have an elevated risk, this information is vital for genetic counseling and identifying at-risk populations. Additionally, the findings support the concept of personalized medicine, suggesting that high-risk individuals should undergo more proactive monitoring and preventive measures, such as increased dermatological screenings and education on sun protection. However, the inconsistent evidence surrounding the TaqI polymorphism highlights the need for further research to confirm these associations and understand the biological mechanisms involved, potentially leading to new therapeutic targets and preventive strategies. Moreover, these insights can inform the design of clinical trials that explore VDR-targeted therapies or vitamin D supplementation for populations with a higher prevalence of risk alleles, which may influence treatment protocols. Finally, understanding the genetic susceptibility linked to VDR polymorphisms can guide public health initiatives aimed at reducing melanoma incidence in genetically vulnerable groups.

CONCLUSIONS

In summary, this meta-analysis suggests that the VDR FokI polymorphism is linked to melanoma susceptibility, while the VDR TaqI polymorphism does not show a significant correlation with melanoma. Understanding the relationship between VDR gene polymorphisms and melanoma has important clinical implications, especially in personalized medicine. Furthermore, uncovering the mechanisms behind VDR-mediated melanoma risk could lead to novel therapeutic approaches. It is important to note that genetic factors interact with environmental factors to influence melanoma risk. Therefore, further studies with large sample sizes in diverse ethnic groups are needed to enhance our understanding of the role of VDR polymorphisms in melanoma development and to explore interactions among genetic, lifestyle, and environmental factors.

DECLARATIONS

Ethics approval: This article does not involve any studies with human participants or animals conducted by the authors.

Consent to participate: Not applicable for this manuscript.

Availability of data and material: The dataset utilized and/or examined in this study can be obtained from the corresponding author upon reasonable request.

Competing interests: The authors confirm no conflicts of interest.

Funding: No funding was received from any donor organization for this study.

Authors' Contributions: Study concept and design: Nazila Farnoush, Amirhossein Rahmani; Research: Mehdi Khosravi-Mashizi, Fatemeh Asadian; Data analysis and interpretation: Seyed Masoud HaghighiKian; Drafting of the manuscript: Maryam Aghasipour, Kazem Aghili; Critical review of the manuscript: Ahmad Shirinzadeh-Dastgiri, Mohammad Vakili-Ojarood, Amirhosein Naseri; Statistical analysis: Maedeh Barahman, Amirmasoud Shiri; Study supervision: Abolhasan Alijanpour, Hossein Neamatzadeh. All authors consented to submission to the current journal, provided final approval of the version to be published, and agreed to be accountable for all aspects of the work.

ACKNOWLEDGEMENTS

We thank all researchers whose studies contributed to this meta-analysis and appreciate the reviewers for their insightful comments that enhanced the quality of our work.

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P(A)SH Syndrome: Case Presentation and Short Update of Related Disorders

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ABSTRACT

Hidradenitis suppurativa (HS) is a chronic inflammatory disease that is frequently associated with syndromes, such as those within the PAPA spectrum. Syndromic HS presents unique management challenges, as it often shows resistance to conventional therapies. Pyoderma gangrenosum is a rare inflammatory neutrophilic dermatosis that is often seen in association within the spectrum of autoinflammatory diseases.

The PAPA spectrum disorders include a group of autoinflammatory diseases characterized by mutations in the PSTPIP1 gene or by clinical manifestations that closely resemble or overlap with those of PAPA syndrome. Each syndrome (PASH, PAPASH, PsAPASH, PASS, PAC, and PAMI syndrome) in this spectrum highlights specific inflammatory pathways and symptoms, providing insight into targeted therapeutic approaches.

Here, we present a rare case of incomplete PASH (pyoderma gangrenosum and hidradenitis suppurativa) syndrome successfully managed with a standard combination of antibiotics (ceftriaxone and metronidazole) and corticosteroids (methylprednisolone), followed by immunosuppressant (azathioprine) and corticosteroids (dexamethasone). We review both novel and established/standard treatment options, with an emphasis on treatment outcomes. Conventional therapies remain both effective and affordable, providing valuable alternatives for patients.

KEYWORDS

PAPA syndrome; PASH syndrome; pyoderma gangrenosum; hidradenitis suppurativa; antibiotics; azathioprine; corticosteroids

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Received: 3 November 2024
Accepted: 22 December 2024
Published online: 4 April 2025

Acta Medica (Hradec Králové) 2024; 67(4): 125–132
https://doi.org/10.14712/18059694.2025.9

INTRODUCTION

Hidradenitis suppurativa (HS) is a chronic inflammatory disease affecting the major skin folds, marked by recurrent suppurative lesions that lead to progressive tissue destruction and fibrosis (1). Its association with other complex clinical syndromes, though uncommon, adds a layer of therapeutic complexity, making effective management challenging (1). Patients with syndromic HS often exhibit signs of systemic inflammation, atypical cutaneous involvement, and resistance to conventional therapies (1).

Pyoderma gangrenosum is a rare inflammatory neutrophilic dermatosis that manifests as painful ulcers with violaceous, undermined edges, particularly on the lower extremities (2). Both pyoderma gangrenosum and its syndromic forms can be observed within the spectrum of autoinflammatory diseases (2).

"Autoinflammatory diseases" is a term used in the literature to describe a group of disorders caused by tissue damage resulting from overactivation of the innate immune system, occurring in absence of autoreactive T cells or antibodies (3).

Pyoderma gangrenosum (PG), pyogenic arthritis and acne (PAPA) syndrome is a rare autosomal dominant autoinflammatory disease caused by mutations in proline-serine-threonine phosphatase interacting protein 1 (PSTPIP1) gene (4). PAPA spectrum disorders represent a group of autoinflammatory diseases caused by mutations in the PSTPIP1 gene or marked by clinical findings that are similar to or overlap with those of PAPA syndrome (4). These disorders include PASH (PG, acne and hidradenitis suppurativa), PAPASH (involves all clinical findings of PASH plus pyogenic sterile arthritis), PsA-PASH (includes the features of PASH along with psoriatic arthritis), PASS (defined by PG, acne, ankylosing spondylitis, with or without hidradenitis suppurativa), PAC (involves PG, acne and ulcerative colitis), and PAMI syndrome (PSTPIP1-associated myeloid-related-proteinemia inflammatory syndrome) (4).

Incomplete PASH syndrome was discussed within the broader context of the PAPA spectrum syndromes, that was successfully managed with standard therapeutic combination of antibiotics (ceftriaxone, metronidazole), immunosuppressant (azathioprine) and corticosteroids (methylprednisolone, dexamethasone). With this case report we will explore PAPA, PASH, and PAPASH syndromes, focusing on their complex pathogenesis and reviewing the currently available treatment options. While acknowledging the effectiveness of conventional therapies, we aim to highlight the additional benefits that newer treatment strategies may offer to patients.

CASE REPORT

A 61-year old male presented in October 2024 to the dermatology department with primary complaints of painful, non-healing wounds on both lower legs. These wounds first appeared in 2018, and although local treatment with antiseptics and antibiotics initially led to improvement, the wounds reappeared.

The patient also reported a history of acne inversa (hidradenitis suppurativa), which began in the axillary region in 2016 as abscesses with purulent discharge. A year later, similar lesions developed in the genital area. Antibiotic therapy and local disinfection were used with satisfactory results.

In 2021, the patient underwent treatment with adalimumab 40 mg, starting with once-a-month injections administered for three months, followed by once a week injections for another three months. He observed slight improvement in his skin during this period. The patient also reported chronic back, knee, and ankle pain since 2018, along with endoscopic ligation for Mallory-Weiss syndrome in 2023, colon surgery for benign tumor in 2023, and surgery for postoperative ventral hernia in 2024.

At the time of the consultation the patient requested a physical examination and further therapeutic approach to be established.

Routine evaluations included blood and urine tests, biochemical analysis, and serum protein electrophoresis analysis (Table 1).

Tab. 1 Routine evaluations: blood and urine tests, biochemical analysis, and serum protein electrophoresis analysis.

Test	Result	Status
Routine Blood Tests		
WBC (white blood count)	18.2 × 10^9/l	Elevated
HGB (Hemoglobin)	118.0 g/l	Decreased
HCT (Hematocrit)	0.37 l/l	Decreased
MCV (Mean Corpuscular Volume)	75.0 fL	Decreased
MCH (Mean Corpuscular Hemoglobin)	23.9 pg	Decreased
RDW-CV (Red Cell Distribution Width)	15.8%	Elevated
PLT (Platelet Count)	513.0 × 10^9/l	Elevated
PDW (Platelet Distribution Width)	8.3%	Decreased
Granulocytes %	80.9%	Elevated
Lymphocytes %	15.2%	Decreased
ESR (Erythrocyte Sedimentation Rate)	72 mm/h	Elevated
Urine Tests		
Red Blood Cells (RBCs)	24.0 u/l	Elevated
WBC (White Blood Cells)	25.0 u/l	Elevated
Squamous Cells	49.0 u/l	Elevated
Hyaline Casts	4.0 u/l	Elevated
Granular Casts	24.0 u/l	Elevated
Biochemical Analysis		
HDL (High-Density Lipoprotein)	1.26 mmol/l	Elevated
Urea	1.8 mmol/l	Decreased
Uric Acid	470.0 micromol/l	Elevated
Total Protein	92.0 g/l	Elevated
C-Reactive Protein (CRP)	84.1 mg/l	Elevated
Iron Levels	1.4 micromol/l	Decreased

Albumin Levels	26.0 g/l	Decreased
Serum Protein Electrophoresis		
Albumin Fraction	36.20%	Decreased
Alpha 1 Fraction	6.30%	Elevated
Alpha 2 Fraction	12.30%	Elevated
Beta 1 Fraction	7.50%	Slightly elevated
Beta 2 Fraction	10.30%	Elevated
Gamma Fraction	27.40%	Elevated
Albumin Electrophoresis	27.04 g/l	Decreased
Alpha 1 Globulins	4.71 g/l	Elevated
Alpha 2 Globulins	9.19 g/l	Elevated
Beta 1 Globulins	5.60 g/l	Slightly elevated
Beta 2 Globulins	7.69 g/l	Elevated
Gamma Globulins	20.47 g/l	Elevated
C3 Complement	0.84 g/l	Decreased
ANA Screening	Borderline	

The patient was diagnosed with secondary iron deficiency anemia and hypoalbuminemia. Iron III-hydroxide polymaltose complex 100mg/ folic acid 0.35 mg two chewable tablets a day was prescribed.

From the consultation with a rheumatologist, HLA-B27 testing resulted negative. X-ray findings indicated unilateral sacroiliitis, possible lumbar syndesmophytes, and a compression fracture of T12, with suspicion of inflammatory changes in the thoracic vertebrae. The patient has shown a relatively poor response over the years to methylprednisolone and sulfasalazine. From the orthopedic consultation, en face and lateral X-rays of both knee joints were obtained, revealing early osteoarthritic changes bilaterally. Therapy with etoricoxib 90 mg once daily after meal and esomeprazole 20 mg one tablet before meal was suggested.

The dermatological examination revealed multiple undermined ulcerative lesions on both lower legs (regio cruris), some of which were confluent and irregularly shaped, covered with purulent-hemorrhagic crusts. A violet halo and local erythema were noted around the lesions (Fig.1a–c). Additionally, cicatricial changes and numerous deep and superficial abscesses exuding pus-like material were present in the genital and gluteal areas (Fig. 2a). Additional multiple dysplastic nevi were noted on the trunk (Fig. 2b) and back regions (Fig. 2c). In the axillae, only cicatricial and fibrous changes were observed, with no active inflammatory process. Onychomycosis was also noted. Enlarged lymph nodes were not palpable.

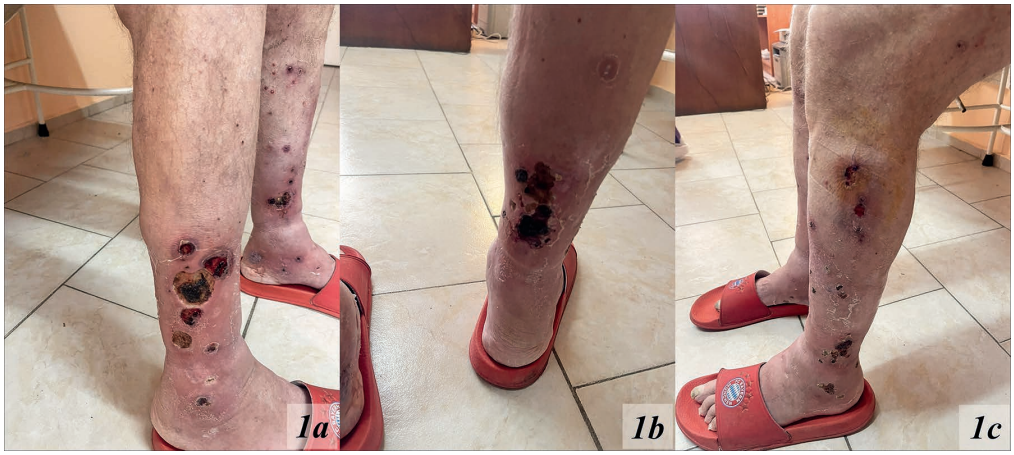

Fig. 1a–c Pyoderma gangrenosum: Multiple undermined ulcerative lesions on both lower legs (regio cruris), some of which were confluent and irregularly shaped, covered with purulent-hemorrhagic crusts. A violet halo and local erythema were also noted around the lesions. **1a** Lateral view of pyoderma gangrenosum lesions on the right lower leg. **1b** Posterior view of pyoderma gangrenosum lesions on the right lower leg. **1c** Lateral view of pyoderma gangrenosum lesions on the left lower leg.

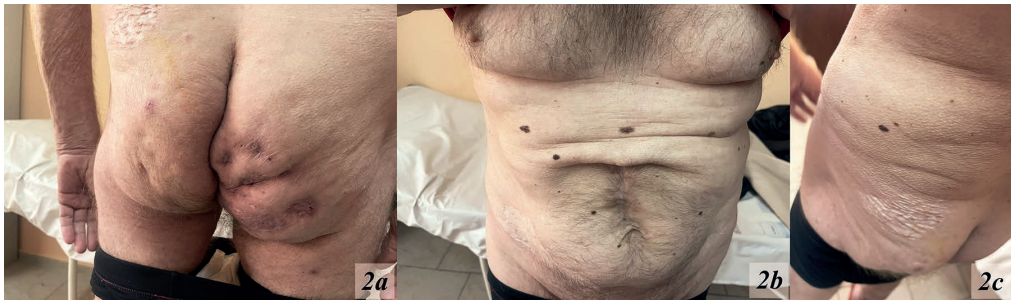

Fig. 2a–c Dermatological examination. **2a** Hidradenitis suppurativa: Cicatricial changes and numerous deep and superficial abscesses exuding pus-like material in the gluteal area. **2b** Multiple dysplastic nevi on the trunk region. **2c** Dysplastic nevus located in the lower left back region.

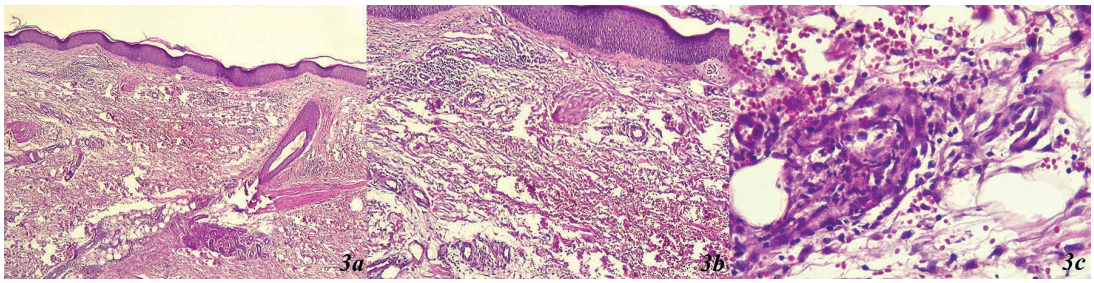

Fig. 3a–c Pyoderma gangrenosum: Orthohyperkeratosis, uneven acanthosis with smoothing of dermo-epidermal undulations, and a myxoid papillary dermis. There was also a moderate interstitial and perivascular round cell inflammatory infiltrate, with occasional eosinophils present in the upper and middle dermal layers. **3a** Pyoderma gangrenosum HEx40. **3b** Pyoderma gangrenosum HEx100. **3c** Pyoderma gangrenosum HEx200.

A biopsy was conducted from an erythematous-infiltrative ulcer with a raised edge and a fibrinous center located on the lower leg (regio cruris). The histological examination revealed orthohyperkeratosis, uneven acanthosis with smoothing of dermo-epidermal undulations, and a myxoid papillary dermis. There was also a moderate interstitial and perivascular round cell inflammatory infiltrate, with occasional eosinophils present in the upper and middle dermal layers. The histological findings were consistent with pyoderma gangrenosum (Fig. 3a–c).

Treatment was initiated with ceftriaxone 2 g i.v., metronidazole 500 mg i.v. twice daily, nadroparin calcium 0.4 ml subcutaneously, and metamizole sodium ampoules as needed. Local dressings with potassium permanganate were applied to the lower legs. Given the histologically confirmed diagnosis of pyoderma gangrenosum and the presence of hidradenitis suppurativa, a diagnosis of incomplete PASH syndrome was established, prompting the initiation of treatment with methylprednisolone 60 mg i.v. and famotidine 40 mg twice daily. Due to deviations in serum electrophoresis indicators, an immunofixation electrophoresis of serum proteins and urine was performed. Paraproteinemia was ruled out. Antibiotic and immunosuppressive therapies were administered, leading to a regression of the pyoderma gangrenosum lesions (Fig. 4a–d).

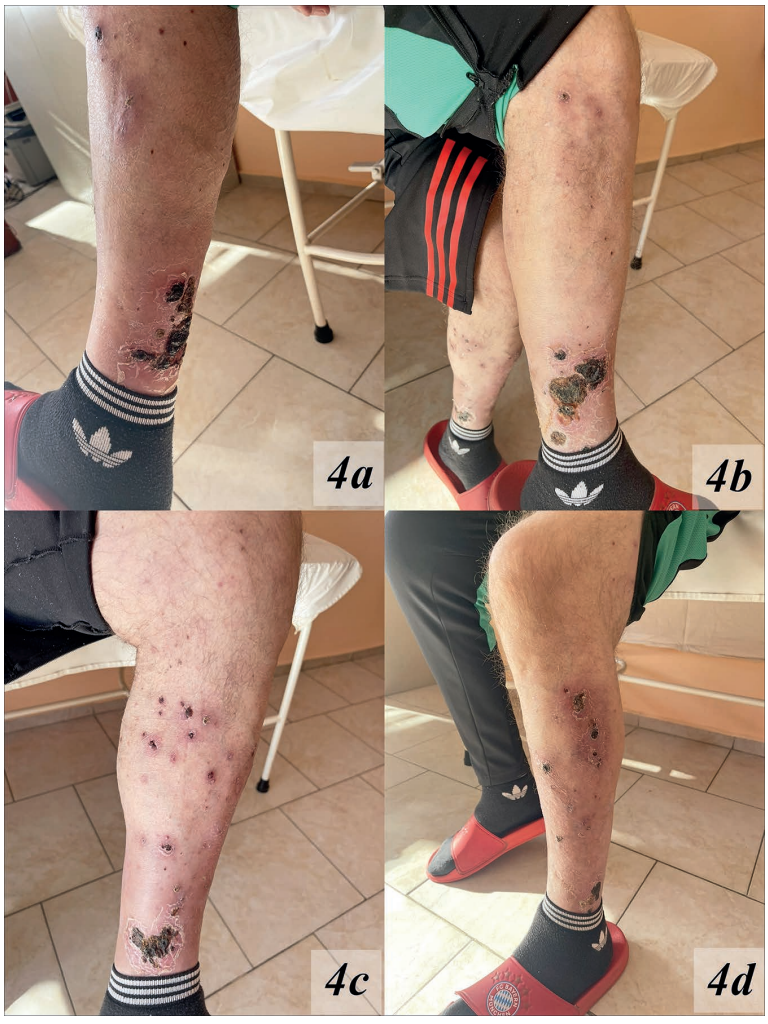

Fig. 4a–d Dermatological findings: Regression of pyoderma gangrenosum lesions following antibiotic and immunosuppressive therapies, with subsequent crust formation. **4a** Medial-posterior view of the lesions located on the right lower leg. **4b–c** Medial view of the lesions located on the left lower leg. **4d** Lateral view of the lesions located on the left lower leg.

Outpatient treatment included azathioprine 50 mg twice daily, to be taken after meals, and dexamethasone 4 mg after food, with a gradually reducing regimen: 1 tablet in the morning and half at lunch during the first week, followed by half a tablet in the morning and half at lunch during the second week. Esomeprazole 40 mg was prescribed twice before meals, along with bilastine 20 mg, to be taken at 5 p.m. For pain management, metamizole sodium was recommended, and local dressings with pale pink potassium permanganate solution were to be applied 2-3 times daily for 30 minutes. A hydrating intensive gel-cream was advised for the surrounding skin.

DISCUSSION

In 1975, a 14-year-old boy with "streaking leukocyte factor", arthritis, and pyoderma gangrenosum was documented as the first reported case in the literature, marking the initial description of what is now known as pyogenic arthritis, pyoderma gangrenosum, and acne (PAPA) syndrome (5).

PAPA syndrome is a rare, hereditary, autosomal dominant autoinflammatory disorder resulting from missense mutations in the proline-serine-threonine phosphatase interacting protein 1 (PSTPIP1)/CDBP1 gene located on chromosome 15q (6). These mutations lead to an overproduction of interleukin-1beta (IL-1beta) through hyperphosphorylated PSTPIP1 protein, which disrupts its role in the inflammasome activation, a key component in IL-1beta production (6). In addition to IL-1beta overproduction, PAPA syndrome has also been associated with elevated serum levels of tumor necrosis factor-alpha (TNF-alpha) (7). In the case of "streaking leukocyte factor", arthritis, and pyoderma gangrenosum (5), trauma or other stimuli may induce the accumulation of a serum factor that enhances the random leukocyte migration in vitro. This accumulation in certain tissues could lead, as suggested by the authors, to excessive leukocyte influx and increased local leukocyte activity in the inflammatory exudates (5). According to a study by Mistry et al. (8), neutrophils and low-density granulocytes exhibit increased formation of neutrophil extracellular traps (NET) compared with control neutrophils. The overproduction of NET formation and decreased degradation may contribute to prolonged or heightened inflammatory responses and overall immune dysregulation (8). Additionally, IL-17 and IL-6 have been suggested as potentially pathogenic cytokines in PAPA syndrome (8).

The clinical findings can vary, ranging from early onset, painful flares of recurrent sterile arthritis to skin ulcerations, pyoderma gangrenosum, and severe cystic acne (6). The initial sign is typically joint involvement, which presents as painful recurrent monoarticular arthritis that may be triggered by trauma or occur spontaneously (9). The cutaneous manifestations are generally more severe in adults compared to those in children (9). In children with recurrent or recurrent pyogenic arthritis/osteomyelitis, an underlying immunological disorder should always be considered (10). PAPA syndrome has been also linked with other disorders, including Crohn's disease and primary sclerosing cholangitis/autoimmune hepatitis overlap (11), as well as hypogammaglobulinemia (7).

While blood tests are not diagnostic for PAPA syndrome, they can reveal a non-specific elevation in acute phase reactants, such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), particularly during episodes of arthritis (4). Additionally, increased levels of IL-1beta and TNF-alpha can be observed in peripheral blood mononuclear cells (4).

Interestingly, cases of suspected PAPA syndrome lacking the PSTPIP1 mutation have been identified (6). As summarized by Smith et al. (6), PSTPIP1 mutations were confirmed in only 3 out of 8 cases studied. Among the mutation-negative cases, three exhibited the full clinical characteristics of PAPA syndrome, one was presented with alleged pyoderma gangrenosum and severe bleeding that responded to corticosteroids, and another showed severe pauciarticular corticosteroid responsive arthritis and recurrent, destructive pyoderma gangrenosum (6). A homolog of PSTPIP1, known as proline-serine-threonine-phosphatase-interacting protein 2 (PSTPIP2), was found in both humans and mice (12). Although data is limited on whether PSTPIP1 and PSTPIP2 proteins share a common or distinct pathways, the possibility of PSTPIP2 mutations may be considered in cases of PAPA syndrome that lack identifiable mutations (6). Based on these findings, it can be concluded that not all cases of PAPA syndrome are associated with identifiable causative mutation. This implies that 1) genetic testing may not always provide a definitive diagnosis, and 2) cases of PAPA syndrome can occur without presenting the full spectrum of clinical symptoms.

A personalized therapeutic approach is advisable for effective disease management. In some instances, the response of pyoderma gangrenosum to systemic corticosteroids may be poor (13, 14), posing a challenge particularly when 1) only a partial response is achieved, or 2) alternative treatments are cost-restrictive or unavailable in some countries. A one month regimen of high-dose corticosteroid treatment with prednisone at 60 mg daily, combined with regular wound care, resulted in rapid progression of the patient's pyoderma gangrenosum ulcers (13). The combination of etanercept and vacuum-assisted closure devices ultimately proved to be an effective therapy for managing the patient's condition (13). A rapid and lasting response to pyoderma gangrenosum in a patient with PAPA syndrome was achieved through target therapy with anakinra, a recombinant human interleukin-1 receptor antagonist (14).

A 14-year-old patient with PAPA syndrome, previously unresponsive to multiple therapies, experienced dramatic improvement in pyoderma gangrenosum after one infusion of infliximab, a chimeric anti-TNF alpha monoclonal antibody (15). A second infusion subsequently led to resolution of the condition (15).

A table by Lu et al. (16) summarized the effective drugs based on their gene mutation sites. The following agents have demonstrated good efficacy: corticosteroids, azathioprine, sulfasalazine, leflunomide, tumor necrosis factor-alpha inhibitors, and interleukin-1beta antagonist (16). In the same study, a 9-year-old boy with PAPA syndrome was treated with tocilizumab, a humanized anti-IL-6 receptor monoclonal antibody (16). The symptoms subsided within a week of treatment but reappeared after

six months (16). Following this, adalimumab, a TNF-inhibitor, was administered and controlled the symptoms for six months before a relapse occurred (16). Rapid symptom relief was achieved with intra-articular triamcinolone hexacetonide (a corticosteroid) during one of the patient's episodes, with no subsequent cutaneous symptoms observed (16). Unfortunately, due to the high cost of this treatment plan, more cost-effective therapies will need to be considered (16). The initial treatment for a patient with PAPA syndrome involved adalimumab 40 mg every two weeks, which led to partial clinical improvement in the pyoderma gangrenosum lesions and acne (17). Subsequently, the treatment was modified to include tacrolimus, administered at a dose of 3 mg twice daily, in combination with adalimumab (17). This combination resulted in more significant improvement in the lesions (17). Rare onset of new pyoderma gangrenosum lesions was managed with topical dapsone and/or topical steroids (17).

A patient with a PAPA-like syndrome, primarily exhibiting cutaneous manifestations such as pyoderma gangrenosum and acne fulminans, was treated with canakinumab, leading to the resolution of the dermatological symptoms (18).

Given the high cost of biological agents, cost-effectiveness and accessibility are important considerations for patient care. Sardana et al. (19) reported a case of PAPA syndrome with accompanying arthritis and worsening ulcerations that was successfully managed using minocycline, dapsone, deflazacort and methotrexate (19). This approach resulted in complete healing of the ulcers and sustained results over a two-year-follow-up period (19).

PASH syndrome is a rare autoinflammatory condition that is clinically characterized by the triad of pyoderma gangrenosum, acne, and hidradenitis suppurativa (acne inversa) (9). The first documented cases of the disease appeared in 2012, involving two patients who exhibited no signs of inflammatory joint involvement (20). These cases presented with the characteristic triad of severe Hurley III hidradenitis suppurativa, cystic acne, and pyoderma gangrenosum, along with systemic inflammation indicated by elevated serum amyloid A and CRP levels (20). The initial manifestation of the disease is typically acne, with the onset of PASH generally observed in the third or fourth decade of life (4, 21). Although no triggering factors or associated conditions were noted in the initial description by Braun-Falco (20), PASH syndrome has since been reported as a potential complication following bariatric surgery for morbid obesity in one case and as associated with Crohn's disease in another (21).

The histopathological findings of pyoderma gangrenosum are considered nonspecific but can be further categorized according to its clinical subtypes (22). In the early stages, a characteristic neutrophilic infiltrate is typically observed (22). However, in cases of vegetative pyoderma gangrenosum, the histopathological presentation may include neutrophilic and eosinophilic histiocytic mixed infiltrate (22). Although systemic pyoderma gangrenosum is not typically associated with tissue eosinophilia, eosinophilia may still be observed peripherally, particularly in conditions such as idiopathic hypereosinophilic syndrome (23).

Some authors suggest that vasculitis may also be observed in pyoderma gangrenosum, making its histological findings generally "problematic" (22). In addition to neutrophilic infiltration, observed in all cases in the case series by Moxda Suresh Patel et al. (22), the following histological findings were also noted: vaculitis in 11 patients (57.89%), lymphoplasmacytic infiltrate in 6 patients (31.57%), pseudoepitheliomatous hyperplasia in 5 patients (26.31%), mixed inflammatory infiltrate in 4 patients (21%), epidermal ulceration in 4 patients (21%), and mitotic activity in 3 patients (15.78%). Given the complex histological findings in pyoderma gangrenosum, a comprehensive diagnostic approach is essential. This includes both clinical and histological evaluations, with particular emphasis on integrating detailed patient anamnesis to support precise diagnosis and personalized therapeutic approach.

Based on the information above, we diagnosed our patient with incomplete PASH syndrome, despite the absence of severe acne in the clinical presentation. This conclusion is further supported by: 1) the presence of pyoderma gangrenosum and hidradenitis suppurativa, 2) chronic inflammatory markers (elevated CRP, ESR, WBC) and systemic inflammatory complications (iron deficiency anemia and hypoalbuminemia), 4) partial response to immunosuppressive treatment (adalimumab), and a 5) history of endoscopic ligation for Mallory-Weiss syndrome. Although this condition is not directly associated with PASH syndrome, the frequent vomiting characteristic of Mallory-Weiss syndrome (24) could suggest underlying gastrointestinal issues, which might arise from conditions that cause chronic inflammation.

Genetic testing for mutations in the autoinflammatory genes MEFV, NLRP3, TNFRSF1A, and PSTPIP1, resulted negative, except for an increased number of CCCTG microsatellite repeats in the promoter region of the PSTPIP1 gene (1, 20). Similar to PAPA syndrome, PASH is associated with the overactivation of the innate immune system, resulting in an increased production of interleukins and "sterile" neutrophil-rich cutaneous inflammation (9). Mutations in the PSTPIP1 gene have also been documented (25). Additionally, mutations in MEFV, NOD2, and NLRP3 have been identified in seven patients diagnosed with PASH syndrome (1, 26). Despite the mutations presented above, the genetic basis of PASH syndrome remains shrouded in mystery (27). However, PASH has been associated with testicular cancer (28) and ulcerative colitis (29), suggesting a link between this neutrophilic dermatosis and conditions such as inflammatory bowel disease and certain malignancies (27).

In a study by Marzano et al. (21), serum levels of IL-1beta, TNF-alpha, and IL-17 – key proinflammatory cytokines – were found to be within the normal ranges in the peripheral blood of patients with PASH syndrome. However, in lesional skin, particularly in the ulcerative lesions of pyoderma gangrenosum, elevated levels of proinflammatory cytokines, chemokines, and tissue-damaging effector molecules were observed (21). This finding suggests that the inflammatory response in PASH syndrome is predominantly localized to the skin, with no detectable proinflammatory activity in the bloodstream (21).

In the management of each patient with PASH syndrome, it is essential to consider the potential hyperactivity of the innate immune system and to employ a combination of multimodal anti-inflammatory therapies (30).

Systemic corticosteroid therapy or antibiotics with anti-inflammatory properties, such as dapsone and tetracyclines, are considered as first-line treatment options for pyoderma gangrenosum (31). Furthermore, immunosuppressive agents like azathioprine, cyclosporine, and mycophenolate mofetil may also be regarded as initial therapeutic strategies (31). Anti-tumor necrosis factor-alpha agents, such as adalimumab and infliximab, are frequently used in clinical practice, exhibiting favorable outcomes in the majority of patients with PASH syndrome, although not in every case (32). These agents typically promote rapid remission of pyoderma gangrenosum; however, hidradenitis suppurativa lesions tend to be more resistant to treatment (32).

In a study by Yan et al. (33), hidradenitis suppurativa lesions appeared several years before the onset of pyoderma gangrenosum and the initial diagnosis of PASH syndrome. This pattern aligns with our case report, adding further support to our diagnostic conclusion. If adalimumab proves ineffective or if the patient has more severe clinical findings, high-dose infliximab can be used to achieve disease control (33). This approach is often paired with intralesional corticosteroids, systemic corticosteroids, and vitamin supplementation (33). In the study, three out of the eight patients with PASH syndrome were found to have deficiencies in either zinc, vitamin A, or both (33). Some studies suggest that low vitamin D levels may contribute to the pathogenesis of PASH and PAPASH syndromes, indicating that vitamin D supplementation could serve as a potential additional treatment option (34).

A multimodal treatment regimen with infliximab, cyclosporine, and dapsone was administered to a 22-year-old woman with PASH syndrome, leading to prolonged improvement in her clinical symptoms (35). Previously, her symptoms had proven resistant to treatment with etanercept, adalimumab, fumaric acid and the IL-1 receptor antagonist anakinra (35).

The IL-23 inhibitor tildrakizumab, a monoclonal antibody used for treating moderate to severe psoriasis (36), has been proposed as a promising new therapeutic option for managing PASH syndrome (37). Gul et al. (38) reported remission of refractory PASH syndrome with a treatment regimen consisting of ixekizumab, an IL-17A inhibitor, and doxycycline (38). Guselkumab, an IL-23 inhibitor used for the treatment of moderate to severe plaque psoriasis (39), has also been reported in the literature as a therapeutic option for PASH syndrome (40).

Partial improvement was achieved in a patient with various treatment modalities, including isotretinoin, cyclosporine, azathioprine, and adalimumab (41).

In addition to biologic therapies, the management of PASH syndrome involves managing pyoderma gangrenosum and hidradenitis suppurativa (27). Some of the treatment options include oral antibiotics such as doxycycline, rifampin, moxifloxacin, amoxicillin, linezolid, and metronidazole, as well as immunosuppressants like cyclosporine, sulfasalazine, and corticosteroids (27). Immunomodulators, including thalidomide and dapsone, may also be employed, along with surgery (27).

Ead et al. (42) highlighted the significance of antibiotic use and wound care, suggesting that PASH syndrome may be a bacterial biofilm disease – a dysregulation of the host microbiota that leads to a chronic inflammatory state (27).

Systemic corticosteroids, in combination with azathioprine, cyclosporine, or mycophenolate, have been described in the literature as effective treatments in some cases (20, 43). Four patients with refractory PASH syndrome have achieved remission using target antibiotic therapy, which included different regimens including ceftriaxone, metronidazole, ertapenem, amoxicillin and other antibiotics (4, 44).

Given the high cost of newer therapies and/or the lack of disease control with adalimumab in our patient, we developed a therapeutic regimen that included antibiotics (ceftriaxone and metronidazole) and corticosteroids (methylprednisolone) during hospitalization, which resulted to an improvement in the pyoderma gangrenosum lesions. For outpatient management, the regimen was continued with an immunosuppressant (azathioprine) and corticosteroid (dexamethasone) resulting in an improvement.

PAPASH syndrome is characterized by the presence of pyoderma gangrenosum, acne, suppurative hidradenitis and pyogenic sterile arthritis, and is classified within the PAPA spectrum disorders (45). This syndrome is associated with missense mutations found in exons 10 and 11 of the PSTPIP1 gene (4, 45). A study reported successful treatment outcomes in two cases of PAPASH syndrome using infliximab and methotrexate (4, 46). In addition to the pyogenic arthritis, the other manifestations of the syndrome have been addressed in terms of their management.

CONCLUSION

We are currently in an era where innovative treatments, such as the monoclonal antibodies, for rare diseases and syndromes are emerging at a rapid pace. However, older treatment modalities should not be overlooked as they remain effective and are often more affordable than newer alternatives. In fact, conventional therapies and their treatment combinations are gaining renewed recognition, often proving to be the primary treatment option that patients may require.

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Contrast-Induced Sialadenitis: An Under-Recognized Adverse Reaction in Radiology and Clinical Practice

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ABSTRACT

Contrast-induced sialadenitis (CIS), a rare inflammatory reaction of the salivary glands, occurs after exposure to iodinated contrast media (ICM). This self-limiting condition typically manifests as glandular swelling and pain, with variable severity, from hours to days post-contrast administration. Its etiology includes inflammatory edema, ductal obstruction, and pseudoallergic or idiosyncratic reactions. Non-ionic, low-osmolar agents such as Iohexol and Iodixanol are frequently implicated. Risk factors include iodine allergy, renal dysfunction, and inadequate premedication. Diagnostic imaging via CT or ultrasound reveals characteristic findings such as glandular enlargement, periglandular fat stranding, and heterogeneous enhancement, aiding differentiation from other causes like infection or neoplasms. This case series presents three patients who developed sialadenitis following contrast-enhanced CT scans. Presentations ranged from mild, localized submandibular swelling to rapid-onset bilateral glandular inflammation involving the parotid and submandibular glands. All cases highlight the need for prompt recognition and adherence to preventive measures, including premedication with corticosteroids and hydration, especially in high-risk patients. Management is largely supportive, involving corticosteroids, antihistamines, and NSAIDs, with severe cases requiring closer monitoring. By raising awareness of this underreported condition, this article underscores the importance of early recognition and differentiation from other conditions by radiologists, emphasizing their role in timely diagnosis and management. It also calls for further research to optimize prevention and management strategies.

KEYWORDS

computed tomography; X-ray; contrast media; sialadenitis

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Received: 4 January 2025
Accepted: 18 February 2025
Published online: 4 April 2025

Acta Medica (Hradec Králové) 2024; 67(4): 133–136
https://doi.org/10.14712/18059694.2025.10

INTRODUCTION

Sialadenitis is an inflammation of the salivary glands characterized by their enlargement and, often, pain. Sialadenitis has multiple potential etiologies, which can be categorized as follows: obstructive, infectious, autoimmune, granulomatous, and post-treatment (1). The most common cause of sialadenitis is the formation of an obstructing calculus in the floor of the mouth, most commonly affecting the submandibular gland, followed by the parotid and sublingual glands (1). In contrast, bacterial or viral sialadenitis predominantly affects parotid glands. Post-treatment etiologies include radiation-, contrast-, and anesthesia-induced salivary gland enlargement (1). Sialadenitis is diagnosed clinically and confirmed with imaging. According to the American College of Radiology Appropriateness Criteria, non-pulsatile, non-parotid masses typically require initial imaging with contrast-enhanced CT or MRI of the neck, while parotid masses are usually evaluated with ultrasound. Differential diagnoses include sialolithiasis, sialocele, reactive lymph node, salivary abscess, osteomyelitis, and neoplasm (2).

Contrast-induced sialadenitis is a rare adverse reaction to iodinated or other contrast agents, primarily discussed in case reports (3). Such reactions may develop from 30 minutes to one week after contrast administration. The most commonly implicated agents include iodine-containing iopromide, iohexol, ioversol, iodixanol, iopamidol, and diatrizoate compounds (4). He proposed pathogenesis of iodinated contrast medium (ICM)-induced sialadenitis also known as “iodide mumps”, may include inflammatory edema leading to salivary duct obstruction (5).

Case reports indicate that ICM-induced sialadenitis can develop following coronary or cerebral angiography, coronary angioplasty, fistulograms, and contrast-enhanced CT scans of the brain, thorax, and abdomen (6). This case series describes three patients who developed neck edema suggestive of sialadenitis within hours to days after exposure to contrast agents during CT examinations.

CASE REPORTS

CASE 1

A 68-year-old woman presented with neck pain and swelling two days after a contrast-enhanced computed tomography (CT) scan of the abdomen and pelvis performed for epigastric pain. The patient had a documented iodine allergy and was prescribed a premedication protocol, which she only partially followed, taking a single dose of prednisone and diphenhydramine one hour before the scan. On physical examination, bilateral submandibular gland swelling was noted, with no associated dysphagia, dyspnea, or systemic illness.

A non-contrast CT scan of the neck confirmed the diagnosis of bilateral submandibular sialadenitis, showing diffuse edema and periglandular fat stranding, consistent with an inflammatory process (Figure 1). This case emphasizes the critical importance of strict adherence to premedication protocols in patients with known iodine allergies and underscores that sialadenitis can present as a delayed reaction to iodinated contrast exposure.

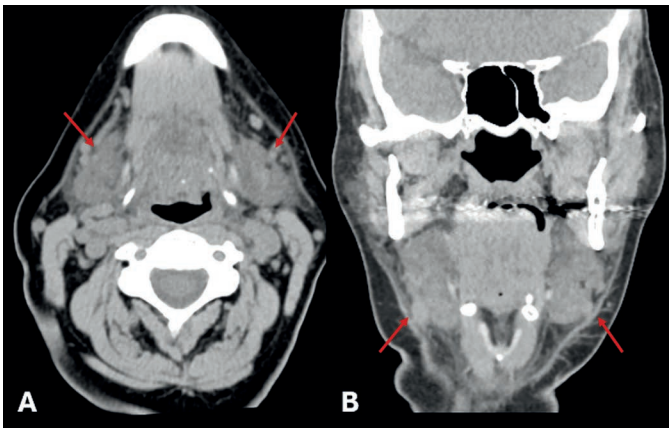

Fig. 1 Computed tomography (CT) of the neck non-contrast, axial (A) and coronal (B) images. Edema/swelling of the submandibular glands (red arrows), with associated periglandular fat stranding and reactive thickening of the platysma muscles.

CASE 2

A 61-year-old woman presented with mild submental edema and throat discomfort within 24 hours of a contrast-enhanced CT scan of the abdomen. She denied any known allergies to iodine or contrast agents and had not received premedication. Her symptoms were mild, localized, and without systemic manifestations. On physical examination, mild submandibular gland swelling was noted.

Subsequent CT imaging of the neck revealed early-stage sialadenitis, characterized by mild glandular swelling and adjacent fat stranding (Figure 2). This case illustrates the variable severity of of contrast-induced sialadenitis, highlighting the role of early recognition and imaging in differentiating it from other causes of glandular swelling, such as infectious or autoimmune conditions..

CASE 3

A 69-year-old woman presented with bilateral neck pain and swelling within six hours of undergoing a CT angiogram of the head and neck as for preoperative evaluation for embolization of a frontal arteriovenous malformation. Her symptoms, which were rapid in onset and more severe than in previous cases, included prominent bilateral swelling of the submandibular and parotid glands.

CT imaging revealed marked glandular enlargement with heterogeneous enhancement, significant vascular prominence, and fat stranding, findings consistent with acute sialadenitis (Figure 3). This case underscores the variable severity of contrast-induced sialadenitis and highlights the importance of considering glandular inflammation in the differential diagnosis of acute neck swelling following contrast imaging. The rapid onset of symptoms after iodinated contrast administration suggests a possible idiosyncratic or hypersensitivity reaction.

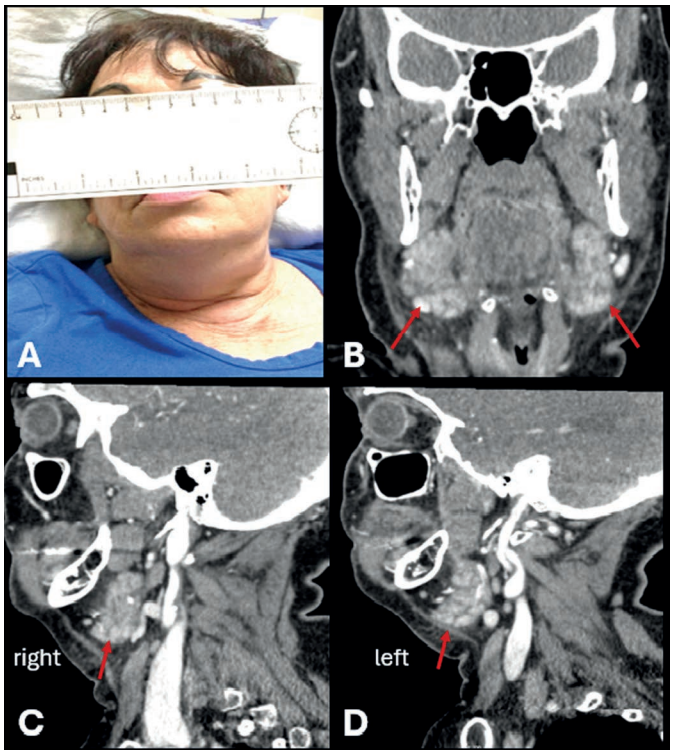

Fig. 2 Frontal photograph (A) showing cervical swelling, predominantly in the submandibular regions. Contrast-enhanced CT of the neck, coronal (B) and sagittal (C, D) images. Swelling of the submandibular glands (red arrows) with heterogeneous hyperenhancement, associated with fat stranding in the surrounding planes and reactive thickening of the platysma muscles.

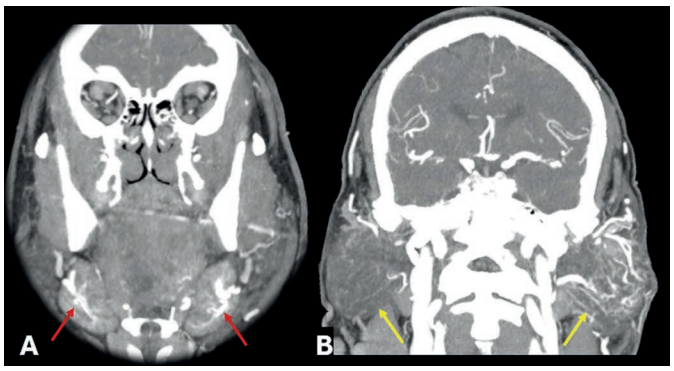

Fig. 3 Craniocervical CT angiography (CTA), coronal maximum intensity projection (MIP) images. Marked enlargement with diffuse vascular prominence of the submandibular glands (A, red arrows) and parotid glands (B, yellow arrow), particularly pronounced in the left parotid gland, with findings consistent with an acute inflammatory process.

DISCUSSION

Contrast-induced sialadenitis is a self-limiting inflammatory reaction resulting caused by iodinated contrast media accumulation in the salivary glands (7, 8). First described in 1956, it remains underreported despite the widespread use of ICM. Its pathogenesis may involve ductal obstruction, local inflammatory edema, or pseudoallergic reactions (9). Iodine accumulation in the salivary gland ducts may lead to obstruction and inflammation, which underpins the pathological basis of the condition (8). Additionally, idiosyncratic or pseudoallergic mechanisms have been proposed, with evidence suggesting that impaired iodine clearance in individuals with renal dysfunction may contribute to its development (6). Pseudoallergic reactions mimic true allergic responses but occur through non-IgE-mediated pathways, including direct mast cell degranulation or complement activation. Idiosyncratic reactions, in contrast, are unpredictable and patient-specific, not following typical immunological hypersensitivity mechanisms. These distinctions are essential for understanding contrast-induced sialadenitis and optimizing management strategies.

Non-ionic, low-osmolar iodinated contrast agents, including Iohexol (Omnipaque), Iodixanol (Visipaque), and Iopromide (Ultravist), have been more frequently associated with sialadenitis (3). These agents, although safer than high-osmolar contrast media, can still lead to iodine accumulation and subsequent inflammation (7). Symptoms typically appear within hours to days after ICM administration, ranging from painless glandular swelling to tenderness and erythema. Although bilateral submandibular and parotid gland involvement is common, unilateral cases have also been reported (3). This variability highlights the importance of maintaining a high index of suspicion in the appropriate clinical context.

CT and ultrasound imaging crucial for diagnosis. CT findings include glandular enlargement, fat stranding, and heterogeneous enhancement, while Doppler ultrasound can demonstrate increased vascularity and ductal dilation. These modalities aid in confirming the diagnosis and ruling out other conditions, such as neoplasms, obstructive sialadenitis, or abscesses (1).

The European Society of Urogenital Radiology (ESUR) guidelines (10) recommend premedication with corticosteroids and antihistamines for patients at risk of hypersensitivity reactions to ICM; however, they acknowledge that premedication does not completely eliminate the risk of such reactions, including CIS.

Furthermore, no formally established corticosteroid regimen exists for the prevention of CIS. Current premedication strategies are primarily derived from protocols used for general contrast hypersensitivity reactions. To address this gap, further research is needed to determine the most effective corticosteroid regimen specifically for CIS prevention.

In accordance with ESUR guidelines, even full premedication does not completely prevent adverse reactions. Therefore, preventive strategies should focus on risk minimization, particularly in patients with a history of hypersensitivity reactions. These strategies include premedication with corticosteroids and antihistamines to mitigate pseudoallergic reactions, maintaining adequate hydration before and after contrast administration to enhance iodine clearance from salivary tissues, and reducing the contrast dose to the lowest amount necessary for diagnostic accuracy.

The efficacy of corticosteroids in preventing CIS remains uncertain, as their use is primarily supported by clinical experience and indirect evidence from hypersensitivity reactions to iodinated contrast agents. Prospective studies are needed to determine an optimal preventive approach for CIS.

Some authors suggest that these complications may represent clinical manifestations of an underlying iodine or iodide allergy. Considering this possibility, laboratory testing for hypersensitivity in individuals without a known history of iodine allergy could be valuable. Detecting latent hypersensitivity could guide preventive measures and enable tailored risk mitigation strategies for patients undergoing contrast-enhanced imaging.

Furthermore, increased iodine uptake in major salivary glands has been observed in individuals treated with radioactive iodine (^{131}I), and a potential link between thyroid function and iodine uptake has been suggested (11). Hypothyroidism is known to alter iodine metabolism and may lead to increased iodine retention in various tissues, including the salivary glands. This raises the question of whether hypothyroidism could serve as a precipitating factor for contrast-induced sialadenitis, also referred to as "iodine mumps". Although direct evidence remains limited, this possibility warrants further investigation. Given these potential implications, screening for thyroid dysfunction in patients with a history of iodine-related reactions could be incorporated into a comprehensive risk assessment strategy.

Management is generally supportive, as the condition resolves spontaneously within days to weeks. Symptomatic management includes corticosteroids, antihistamines, and nonsteroidal anti-inflammatory drugs (NSAIDs). In severe cases, particularly those with airway compromise, hospitalization and close monitoring may be necessary. Early recognition and intervention are essential to alleviating symptoms and prevent complications (4).

CONCLUSION

These cases emphasize the importance of recognizing contrast-induced sialadenitis as a potential adverse reaction to iodinated contrast administration. Radiologists play a critical role in its early diagnosis and in assisting clinicians with effective management. Although self-limiting, contrast-induced sialadenitis can cause significant patient distress and diagnostic uncertainty due to its under-recognized nature. By increasing awareness, this article underscores the need for further research to improve our understanding of its pathophysiology and to optimize preventive and therapeutic strategies.

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