Raynaud's phenomenon in children and adolescents

Škreb, Nikola

UNIVERSITY OF ZAGREB

SCHOOL OF MEDICINE

Nikola Škreb

Raynaud's phenomenon in children and adolescents

GRADUATE THESIS

Zagreb, 2020.

This graduate thesis paper was made at the Department of Pediatrics, University Hospital Centre Zagreb; School of Medicine, University of Zagreb under the mentorship of prof.dr.sc Marija Jelušić and was submitted for evaluation in the academic year 2019/2020.

Abbreviations

ACE – angiotensin converting enzyme

ANS – autonomic nervous system

AVA – arteriovenous anastomoses

CBC – complete blood count

CCTT – computerized colour telethermography

CNS – central nervous system

CTD – connective tissue disease

ERT – estrogen replacement therapy

ESR – erythrocyte sedimentation rate

IRT – infrared thermography

NO – nitric oxide

NVC – nailfold videocapillaroscopy

PDE - phosphodiesterase

PNS – peripheral nervous system

PRP – primary Raynaud's phenomenon

PVC – poly vinyl chloride

ROS – reactive oxygen species

RP – Raynaud's phenomenon

SRP – secondary Raynaud's phenomenon

SS – systemic sclerosis

SSRI – selective serotonin reuptake inhibitors

UHC – University Hospital Centre

UK – United Kingdom

VCM – vinyl chloride monomer

WHO – *World Health Organization*

List of tables

List of charts

List of figures

Table of contents

Summary

Raynaud's phenomenon (RP) is a condition characterized by increased and exaggerated vasoreactivity to cold temperatures and mental stress. Computerized colour telethermography (CCTT) is an established diagnostic procedure in clinical practice that is used for assessment and follow-up of patients with microcirculatory disorders. Objective of this study was to analyze the diagnostic value of CCTT and its use in the diagnosis of RP. CCTT results were interpreted to determine occurrence and prevalence of both primary Raynaud's phenomenon (PRP) and secondary Raynaud's phenomenon (SRP) according to sex and age. We conducted a retrospective investigation and analysis of medical records and CCTT results from 464 pediatric patients under the clinical and anamnestic suspicion of RP in a 14-year period. Prevalence of PRP and SRP was calculated in the total targeted pediatric sample as well as separately female and male prevalence of both disease variants. Patients were categorized into three different age groups to determine differences in age distribution. Pearson's chi-squared test was used to assess the correlation between sex and case distribution, and correlation between age and case distribution. Results showed prevalence of 12.93% of PRP and prevalence of 25.43% of SRP in the targeted sample. There was a clear difference in sex distribution, with males showing higher prevalence of SRP and negative cases, and females showing higher prevalence of PRP. Correlation between sex and case distribution did not prove to be statistically significant (p=0.26). Late adolescence has been demonstrated as the most affected group, showing significantly higher prevalence of both PRP and SRP and significantly lower prevalence of negative cases. This age group has also been proven as statistically significant factor (p=0.0024) for the occurrence of RP when compared to early adolescence. Based on these results, there is increased risk of RP occurrence in the female population and in late adolescence.

Key words: Raynaud's phenomenon, CCTT, children, adolescence

Sažetak

Raynaudov fenomen (RP) je poremećaj karakteriziran povećanom i pretjeranom vazoreaktivnošću na hladne temperature i mentalni stres. Kompjuterizirana kolor teletermografija (CCTT) je utemeljeni dijagnostički postupak u kliničkoj praksi koji se koristi za procjenu i praćenje bolesnika s poremećajima mikrocirkulacije. Cilj ovog istraživanja je analizirati dijagnostičku vrijednost CCTT-a i uporabu u dijagnostici RP. Rezultati CCTT-a interpretirani su kako bi se odredila pojava i prevalencija primarnog Raynaudovog fenomena (PRP) i sekundarnog Raynaudovog fenomena (SRP) prema spolu i dobi. Provedeno je retrospektivno istraživanje i analiza medicinskih nalaza i CCTT rezultata kod 464 pedijatrijskih pacijenata pod kliničkom i anamnestičkom sumnjom na RP u 14-godišnjem razdoblju. Prevalencija PRP-a i SRP-a izračunata je u ukupnom ciljanom pedijatrijskom uzorku, kao i posebno ženska i muška prevalencija obje varijante bolesti. Pacijenti su razvrstani u tri različite dobne skupine kako bi se utvrdile razlike u distribuciji po dobi. Pearsonov hi-kvadrat test je korišten za procjenu povezanosti spola i raspodjele slučajeva, te povezanosti između dobi i raspodjele slučajeva. Rezultati rada su pokazali prevalenciju PRP-a od 12,93% i prevalenciju SRP-a od 25,43% u ciljanom uzorku. Dokazana je jasna razlika u spolnoj distribuciji, pri čemu su muškarci pokazali veću prevalenciju SRP-a i negativnih slučajeva, a žene veću prevalenciju PRP-a. Povezanost između spola i raspodjele slučajeva nije se pokazala statistički značajnom ($p = 0,26$). Kasna adolescencija pokazala se kao najvulnerabilnija grupa, uz značajno veću prevalenciju i PRP-a i SRP-a i značajno nižu prevalenciju negativnih slučajeva. Ova dobna skupina se dokazala i kao statistički značajan faktor ($p = 0,0024$) za pojavu RP-a u usporedbi s ranom adolescencijom. Temeljem ovih rezultata određen je povećan rizik od pojave RP-a u ženskoj populaciji i u kasnoj adolescenciji.

Ključne riječi: Raynaudov fenomen, CCTT, djeca, adolescencija

1. Introduction

Raynaud's phenomenon, also known as Raynaud's syndrome or Raynaud's disease, is a medical condition typically characterized by periodical vasospasm of the cutaneous microcirculation occurring in response to various stimuli such as cold environment or emotional stress exposure. It can be described as an over-exaggerated central and local vascular response to a certain trigger or a stimulus, presenting itself as paroxysmal pallor or cyanosis of the digits of hands and feet, as well as tips of the nose or ears (acral body parts). It is mostly considered as an idiopathic, benign medical problem. Raynaud's phenomenon has been classified as either primary or secondary depending on certain parameters. PRP is described as an isolated disorder whereas SRP is associated with other conditions or underlying diseases, potentially explaining the symptoms, such as connective tissue diseases (CTDs), endocrine diseases, hyperviscosity syndrome and rheumatological or autoimmune disorders. SRP commonly occurs in children with systemic sclerosis and it refers to arterial changes in the extremities caused by a pre-existing condition (1). Generally speaking, PRP mostly affects children, adolescents and younger population, while SRP presents itself as a part of the clinical picture of an underlying illness, affecting mostly older adult population.

Patient's history plays a major role in the overall diagnostic algorithm, as evidence of some signs and symptoms such as xerostomia, photophobia, oral cavity ulcerations, alopecia, myalgia, joint pain and swelling, fever and rash can point towards secondary RP (2). Chronic pharmacological drug usage and smoking can also exacerbate symptoms in patients with secondary RP. Important part of the anamnesis is also data about certain social and environmental factors, such as working or living conditions (exposure to some chemicals e.g. polyvinylchloride or cold or vibrations). Clinical manifestation of the disease is sharp demarcation of skin changes on the digits of hands and feet, nose tips or earlobes described as a triphasic, tricolor event – skin pallor during vasoconstrictive phase, cyanosis due to

deoxygenated venous blood in the microcirculation during the ischemic phase and postischemic hyperemia during reperfusion phase (1). Changes most commonly occur on the digits of the hands, starting with one finger and then spreading to other, with the exception of rarely affecting the thumbs (1).

Patients usually complain of symptoms such as feeling of cold in the extremities, and skin colour changes during cold exposure. Although those changes are rarely painful, some patients do complain of uncomfort and paresthesia in their fingers, and feeling of heat during reperfusion phase. As already mentioned, course of progression of RP is usually benign, although sometimes long term effects of uncontrolled chronic ischemia can led to atrophy of the skin, subcutaneous tissues and muscles, digital ulceration and gangrenous changes (3). In addition, some rare but occasional cases show increased disability of hand function due to the intensity of symptoms (1).

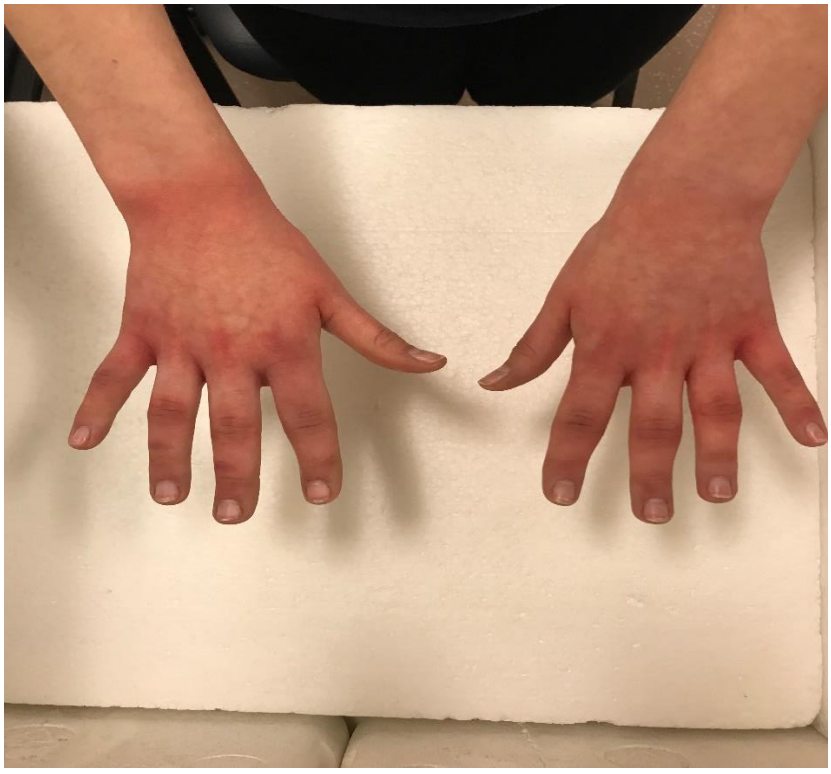


Figure 1. Bilateral pallor and cyanotic skin changes of hands and digits in a patient with SRP at room temperature (22°C). Courtesy of prim.dr.sc. Jagoda Stipić, Laboratory for CCTT, University Hospital Centre Zagreb

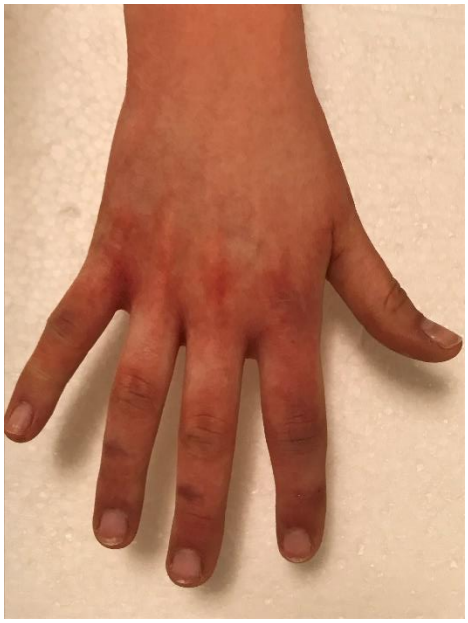


Figure 2. Cutis marmorata of the right hand in the same patient with SRP at room temperature (22°C). Courtesy of prim.dr.sc. Jagoda Stipić, Laboratory for CCTT, University Hospital Centre Zagreb.

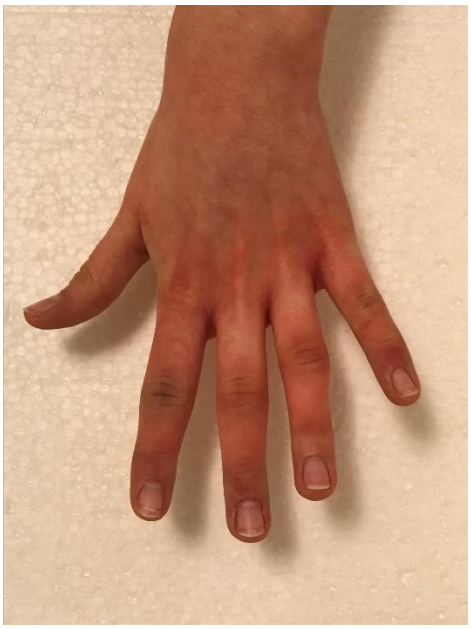


Figure 3. Cutis marmorata of the left hand in the same patient with SRP at room temperature (22°C). Courtesy of prim.dr.sc. Jagoda Stipić, Laboratory for CCTT, University Hospital Centre Zagreb.

1.1. History

Raynaud's syndrome was first mentioned by Maurice Raynaud in 1862, describing "a local asphyxia of the extremities" (4). His hypothesis was that the phenomenon occurs due to increased irritability of central parts of the spinal cord (4). Raynaud observed the first case as a medical student and defined it as episodic, symmetric, acral vasospasm characterized by pallor, cyanosis, suffusion and sense of fullness which may be painful (4). In 1930, Sir Thomas Lewis was conducting new research concerning pathogenesis of RP, and after observing that even when reflex vasodilation is produced by warming the body, vasospasm could still be induced by putting the hands in cold water, and conversely that digital vasospasm could not be induced by lowering body temperature if the hands were kept warm, he concluded that RP was due to a local defect rather than a central nervous system (CNS) problem (5). Discoveries made during that experiment ultimately helped with the classification of the condition as either primary or secondary Raynaud's disease.

Some criteria were proposed to diagnose primary rather than secondary Raynaud's phenomenon. Primary RP includes typical symptoms such as digital vasospasm, absence of peripheral vascular disease or ischemic tissue necrosis, normal nailfold capillaries, and laboratory tests showing negative antinuclear antibody test and normal erythrocyte sedimentation rate. Important clinical feature is also that primary RP typically affects all fingers, whereas secondary RP has an asymmetric distribution in most cases.

1.2. Etiopathogenesis

Normal physiological response to cold temperature is decreased blood flow to the skin, which reduces heat loss through convection and radiation and preserves core body temperature (6). Cutaneous circulation is regulated by complex neuro-immuno-vascular interactions. It involves neural signals from the autonomic and sensory nervous system, circulating hormones, as well as immunological mediators and vasoactive compounds released by endothelial cells. Psychological stress as one of the triggers of the condition points to the potential role of the CNS in the pathogenesis (1).

Thermoregulatory function is mainly conducted through the activation of autonomic nervous system (ANS) in conjunction with the direct effect of cold temperature on the cutaneous blood vessels. Release of the sympathetic neurotransmitter norepinephrine selectively amplifies vascular smooth muscle contraction. Vascular smooth muscle cells exhibit three types of adrenergic receptors: α_1, α_2 and β_2. Evidence showed primary involvement of β_2 adrenoceptors in vasodilation (7), while α_1 and α_2 adrenoceptors are mediating vasoconstriction, with α_1 adrenoceptors being widely expressed across the vascular system and α_2 adrenoceptors showing predominant expression in the arterioles and microcirculation (8).

Role of ANS occurs by virtue of a reflex response to cooling via the sympathetic nervous system as well as by local activation of α_2 adrenoceptors. Different subtypes of α_2 adrenoceptors exhibit different cold sensitivity and reactivity. Experimental work conducted on murine tail arteries demonstrated prominent role of α_{2C} adrenoceptors in thermoregulatory

function (9). Arteriovenous anastomoses (AVA) as low resistance, high flow blood conduits are major site of action for the physiological thermoregulation, since they have a high number of α_{2C} adrenoceptors and thus promptly react to both central and peripheral sympathetic outflow. Since AVAs are mainly distributed in the non-hairy, glabrous skin of the palmar surface of hands and plantar surface of feet, blood circulation reduction in response to cold is much more pronounced in the fingers of the extremities compared to other body areas such as forearms, shins or trunk. Vascular smooth muscle reactivity is also indirectly controlled by endothelial cells that release vasoactive compounds and mediators. Nitric oxide (NO) exhibits a protective action by inhibiting vascular smooth muscle contraction, proliferation and migration (10, 11) as well as thrombocyte aggregation and adhesion (12). Endothelium also produces prostaglandins such as vasodilatory prostacyclin and vasoconstrictive endothelin-1, which both play a major role in vascular remodeling (13).

There are many proposed hypotheses of the underlying mechanisms involved in the pathogenesis of RP. In the current literature, there is more recent evidence that strongly supports the mosaic theory of this disease. It is based on three basic theories that when put together, point to multi-etiology of the disease and involve all factors related to regulation of peripheral circulation. First theory is neurogenic (proposed by Maurice Raynaud in 1862) that is based on the assumed hypersensitivity of the sympathetic nervous system to the cold stimuli (1). Second theory points to changes in the blood composition and vascular endothelial walls as the main cause for the imbalance in vasoconstrictive (endothelin) and vasodilatative (NO, prostacyclin) factors, leading to vasospasm (1). Third theory puts immunological and inflammatory disorders in the centre of pathophysiology of RP (1).

1.2.1. Primary RP

In PRP, there is compelling evidence that the main defect is increased alpha-adrenergic response that is mostly mediated by α_{2C} adrenoceptors that are more prominent on the distal arteries. The role of adrenergic receptors in thermoregulation can be demonstrated by administering selective α_1 and α_2 adrenergic agonists to human volunteers, which leads to

markedly reduced cutaneous blood flow of the extremities and digits (14). Activation of those receptors causes vascular smooth muscle cells contraction and subsequently vasoconstriction. Essentially, hypersensitivity of the afferent nerve fibers to cold stimuli causes overstimulation of the sympathetic nervous system, release of norepinephrine and activation of adrenergic receptors. There is also noticeable evidence that local cooling effect on the skin induces a stronger reduction in both digital blood flow and AVAs perfusion in patients with RP compared to healthy individuals (15).

Under normal conditions at 37°C, α_{2C} adrenoceptors are dormant and stored inside the Golgi aparatus, while cold temperatures (app. 28°C) trigger translocation of adrenoceptors to the cell membrane surface and contribution to sympathetic system response (16). Exposure to cold also induces activation of Rho/Rho kinase signaling pathway responsible for increased expression of α_{2C} -adrenoceptors as well as augmented sensitivity to Ca^{2+} ions (17). Rho/Rho kinase signaling pathway activity is further aided by the formation of reactive oxygen species (ROS) in vascular smooth muscle cells following exposure to cold at approximately 28°C or below (18). RP vasospastic attacks can then initiate a cycle of ischemia and reperfusion, which produces more ROS, activates Rho/Rho kinase and provokes recurrent and prolonged episodes of vasospasm. Response of the α_2 adrenoceptors to cold stimulation can also be provoked by increased activity of tyrosine-kinase responsible for phosphorylation of tyrosine (19). Another possibility for the increased sensitivity and overstimulation of α_{2C} adrenergic receptors is increased endothelin 1 production (20), decreased sensory nerve innervation (calcitonin gene related peptide – containing nerve fibers) (21), and impaired vasodilatative effect of the endothelium (22).

1.2.2. Secondary RP

In SRP, there is a pre-existing condition, usually a rheumatic or connective tissue disease, responsible for the damage and disruption of the vascular bed. Endothelial damage leads to defective vascular function and reactivity, resulting in reduced blood flow, nutritional deprivation and tissue ischemia. In systemic sclerosis (SS), changes in the microvasculature

develop together with intimal fibrosis and endothelial dysfunction (23). Those changes occur relatively early and are associated with increased thrombocyte adhesion, decreased adenosine uptake and decreased storage of von Willenbrand factor (24-28). Anoxic conditions and ischemic reperfusion injury potentiate increased production of ROS, which alter smooth muscle receptor expression and vascular function as well (29).

Other potential changes regarding the pathophysiology of RP have been mentioned, such as enhanced endothelial cell thymidine labeling, which suggests the presence of endothelial injury and repair (30), increased blood levels of endothelin-1 and reduced activity of NO (31-33), as well as increased expression of endothelin receptors in the smooth muscle tissue of microvasculature (32).

1.3. Epidemiology

The prevalence of RP is more common among younger women and family members of RP patients (34), and based on different literature it is approximately 3-20% in the total population. Pediatric prevalence is still not well established or researched. A study of 720 schoolchildren at the age of 12-15 years in the United Kingdom (UK) reported a prevalence of 18% in females and 12% in males (35). There is a general 9:1 ratio of female to male preponderance.

Some evidence points to early manifestation of symptoms in people living in northern parts of the world experiencing low temperature climate compared to people living in areas with tropical climate due to higher and longer exposure to cold environment (36).

PRP accounts for about 80% of overall cases of Raynaud's disease (37). The underlying mechanisms of periodical vasospasm are still ill defined and inconclusive, but there are suggestions that they tend to be purely functional. Concurrently, SRP occurs mostly in patients with CTDs, although it can also be driven by physical or chemical strain, and might have a more severe progression with digital necrosis and ulceration, leading to disability of hands and feet. SRP is usually mentioned in relation to various systemic CTDs such as SS, mixed connective tissue disease and systemic lupus erythematosus, or as a localized aspect of

CREST syndrome (calcinosis, Raynaud syndrome, esophageal dysfunction, sclerodactily and teleangiectasis). Almost 70% of RP in the pediatric population is primary, while SRP is especially related to SS, being one of the primary signs of the disease in 61-70 % of patients (38, 39).

1.3.1. RP and estrogen

Epidemiological studies revealed a significantly higher incidence of RP in females compared to age-matched males, with some literature showing that 70% of patients suffering from RP in USA are females and calculating a 9:1 ratio of premenopausal females to males (40). Generated data points to a gender-based element involved in the disease prevalence and questions a potential role of sex hormones in the disease onset and pathophysiology. There is also a higher incidence of RP among premenopausal women compared to postmenopausal women, with hypothesized association between the menstrual cycle and cold modulated digital blood flow (41).

Some studies showed that post-menopausal women receiving unopposed estrogen replacement therapy (ERT) are more prone to developing RP than post-menopausal women not using ERT (42). Comparatively, post-menopausal women receiving dual therapy with the combination of estrogen and progesterone did not show a significantly higher incidence than pre-menopausal women (43). These findings emphasize the potentially significant role of estrogen in the development of RP as well as suggest a possible progesterone counter-effect on estrogen in certain conditions. Premenopausal women exhibit a higher noradrenaline-mediated cutaneous vasoconstriction at the midstage of their menstrual cycle, when the estrogen levels are higher than during the early stage of the cycle (44). Animal studies with rat females of reproductive age were also conducted in order to certify the clear role of estrogen in pathogenesis of RP. Evidence indicated that estrogen increases α_{2C} adrenoceptors expression in vascular smooth muscle cells and thus mediates cold induced vasoconstriction in rat-tail arteries (45).

1.3.2. Genetic background of RP

There have been some studies speculating about the genetic basis of RP, and the role familial predisposition of the individual might play in contribution and onset of this disease. Some candidate genes, suspected for the contributory effect in etiology of RP, such as gene for the beta subunit of the muscle acetylcholine receptor and genes for serotonin 1B and 1E receptors, were selected for genome sequencing. However, sequencing results showed no mutations (46). Case report of a 1-month male baby diagnosed with RP (47) as well as twin analysis and familial studies strengthened the belief of RP having a certain genetic background. In 2006, there was a study conducted about a potential association between polymorphisms in glutathione *S*-transferase M1 and T1 genes and RP patients exposed to vinyl chloride monomer with results showing that the combination of positive genotypes for both genes may increase susceptibility to RP (48). Another study was dealing with a two-stage whole genome screen of six extended families using 298 microsatellite markers, with the requirement that there are at least three RP patients in each family (46). Genome sequencing in that case showed five chromosomal areas of possible linkage that were corresponding to three aforemented candidate genes (β-subunit of muscle acetylcholine receptor, 1E and 1B serotonin receptors). Discovery of five possible linkages suggests potential oligogenicity of RP and provides some evidence for genetic basis in disease susceptibility and manifestation.

1.3.3. Effect of local agents on RP

Variety of mechanical and chemical stressors are classified as having a localized effect on the manifestation of RP, since they act directly on the body organ, in this case extremities or acral body parts. These “local” agents most prominently affect the digits of hands and feet. One of the examples of mechanical stress inducing RP attacks is exposure to vibrations. This hand-arm vibration syndrome (also known as vibration induced-white fingers) is indeed classified as one form of SRP occurring due to occupational hazards (49). Constant exposure to vibrations produced by vibrating machines and tools used by workers at certain jobs can sensitize their digits for increased vasospastic attacks during thermal or emotional stress. The progressive

nature of the condition along with prolonged vibrational exposure can cause increased digital vasospasm even at room temperature (49).

One of the prominent examples of chemical stressors in RP is vinyl chloride monomer (VCM). VCM is a colorless gas used in the plastic industry, during the production of poly vinyl chloride (PVC). Some conducted studies showed that almost one third of workers exposed to PVC suffer from RP (50). Angiographic and capillaroscopic examinations of these patients' hands showed vascular tone changes consistent with narrowing of the digital arteries, proving the potential toxicity of VCM for the vascular endothelium (51). There are other examples of chemical compounds showing positive correlation with the onset of RP. A study conducted in Chile showed increased prevalence of manifestation of peripheral vascular disease, including RP that was strongly associated with arsenic-contaminated drinking water (52). Further studies strengthened the evidence by showing that arsenic-exposed smelter workers exhibit heightened vasospastic reactivity in the fingers, reminiscent of RP (53). Smoking has long been considered as one of the contributing factors to the onset of RP symptoms. Nicotine, as one of the main constituents in tobacco, has been proven as a vasoconstrictive substance with the tendency of initiating and exacerbating RP attacks. Due to that fact, avoidance of nicotine abuse is considered as one of the elements in the treatment of RP.

Even some chemotherapeutic drugs have shown positive correlation with RP. A long-term study involving combined treatment with cisplatin, vinblastine, and bleomycin chemotherapy produced data showing that 35–45% of treated patients developed RP (54). Bleomycin, in particular, appears to be the key player in the development of RP in these patients. Indeed, findings of a recent large cross-sectional study showed that the only significant predictor of persistent RP at follow-up after chemotherapy was the bleomycin dose (55).

1.4. Diagnostic methods

1.4.1. Computerized colour telethermography

Computerized colour telethermography or infrared thermography (IRT) is a diagnostic functional method of determining thermal status of the skin. It involves a non-invasive, harmless modality based on visualizing, recording and analyzing physical properties of infrared radiation emanating from the surface of the skin. Infrared radiation is a part of the electromagnetic spectrum, with the wavelengths of radiation longer than visible light, and falling in the range between 700 nm and 1 mm (56). Stefan-Boltzmann law defines that the emission and strength of infrared radiation from the surface of a certain object is directly proportional to the fourth power of that object's thermodynamic temperature (57). Simply put, the warmer the object is, the more radiation it will emit. Development of integrated software programs for hardware tools in the procedure of CCTT allowed visualization and quantification of this phenomenon, providing accurate data on the temperature range of the observed object.

The average human body core temperature is estimated at approximately 37 ± 0.5°C, with surface temperature showing slightly lower values and higher variability that is dependent on ambient conditions (58). Pathological processes and concurrent biochemical changes in the human body can induce either systemic or local thermal anomalies (59). CCTT tools can observe hyperthermia as a manifestation of events such as infection, inflammation, trauma and malignancy, or hypothermia as a consequence of deficient circulatory blood flow and ischemia. Since physiological alterations, such as temperature change, precede anatomical abnormalities observed in classical medical imaging, CCTT is a potential screening tool for early detection of these conditions, and it pinpoints its role as a functional visualizing method rather than a morphological one (59).

Of special clinical relevance is the fact that CCTT represents a very safe, non-contact and non-invasive imaging modality, without any potential harmful effects or radiation exposure for both the patient and the performing physician. The image acquisition and subsequent data analysis

is relatively simple and fast, and can be repeated at short time intervals according to need. IRT has first been used during clinical experiments conducted in the field of oncology, mainly as a tool for diagnosis of breast cancer and malignant melanoma (59). During the years, IRT proved its efficacy in various fields of medicine. It showed benefits in the management and follow-up of musculoskeletal diseases such as fibromyalgia or different forms of arthritis, and as a diagnostic tool for reflex algodystrophy (complex regional pain syndrome), radicular nerve damage as well as sensory deficits of both peripheral nervous system (PNS) and CNS. It can also help with monitoring of fracture healing in trauma cases, wound healing during post-surgical care and evaluation of microcirculation in vascular diseases (59). Analysis of recorded thermograms is possible with understanding and proper evaluation of normal skin thermal values, in accordance with slight anisothermic discordances (60). Anisothermy is defined as a pathological deviation of correspondent thermal status of certain skin areas, and is classified as anisothermic hyperthermia or hypothermia (60). Modern infrared cameras and devices are equipped with the latest software capable of detecting deviations of 0.1°C.

1.4.2. Nailfold videocapillaroscopy

The Italian physician Giovanni Rasori at the start of 19th century initiated study of morphological changes of microvasculature occurring with connective tissue diseases. He was the first person to note the relationship between conjunctival inflammation and the presence of capillary disturbances, using a magnifying glass (61). In 1973, Maricq and LeRoy first described in a published paper the specific capillaroscopic patterns consistent with SS. Research data has shown that the extent of microvasculature changes detected by nailfold capillaroscopy has been shown to correlate with disease severity and prognosis. The presence of certain autoantibodies such as anticentromere antibody, older age at onset of RP, severe vasospasm, and any signs of systemic disease are the predominant risk factors for the development of a CTD, predominantly SS.

Nailfold videocapillaroscopy (NVC) is a simple, non-invasive method that enables in vivo visualisation of nail bed microvasculature. It captures present morphological appearance of

the patient's capillaries and allows tracking of potential progressive changes. Nailfold capillaries of the fingers present a good analytical sample due to their accessibility and axial position parallel with skin surface. Main indication for usage of NVC are diseases affecting the microvasculature. The procedure is usually performed with a stereomicroscope connected to a colour digital camera and a software program using high-resolution projection. Analytical sample consists of four consecutive fields extending over 1 mm in the middle of the nailfold per finger that are processed using image analysis software (62). Images are obtained due to blood flow through the lumen of the capillaries, and in normal conditions, just one column of erythrocytes passing through can be observed. NVC of the fingers of both hands should be performed following a period of acclimatization (at least 20 min) in a specialized room at a consistent ambient temperature of 20–24°C (63). Patient's hands have to be in a pronated position with palms laid down on appropriate surface (63). To obtain the best visibility and maintain proper capillaroscope-nailfold contact, drop of immersion oil or ultrasound gel on the epidermis is recommended (63). The distal rows of nailfold capillaries of 8 fingers (thumb capillaries are rarely looked at) are usually analyzed. Capillaroscopic features of normal healthy capillaries are 10–30 capillaries/1 mm, showing one capillary per one dermal papilla, with characteristic "hair-pin" appearance of loops that are arranged in parallel rows (62). The evaluation of morphological changes includes such parameters as loop density, capillary length variability, percentage of loops with architectural derangement, i.e. tortuous, meandering, enlarged/giant, ramified or bushy capillaries, irregular distribution of the capillary array and the presence of extravasations into perivascular tissue (62). Engorged, tortuous, elongated and meandered capillaries found in isolation can also be typical for healthy control groups.

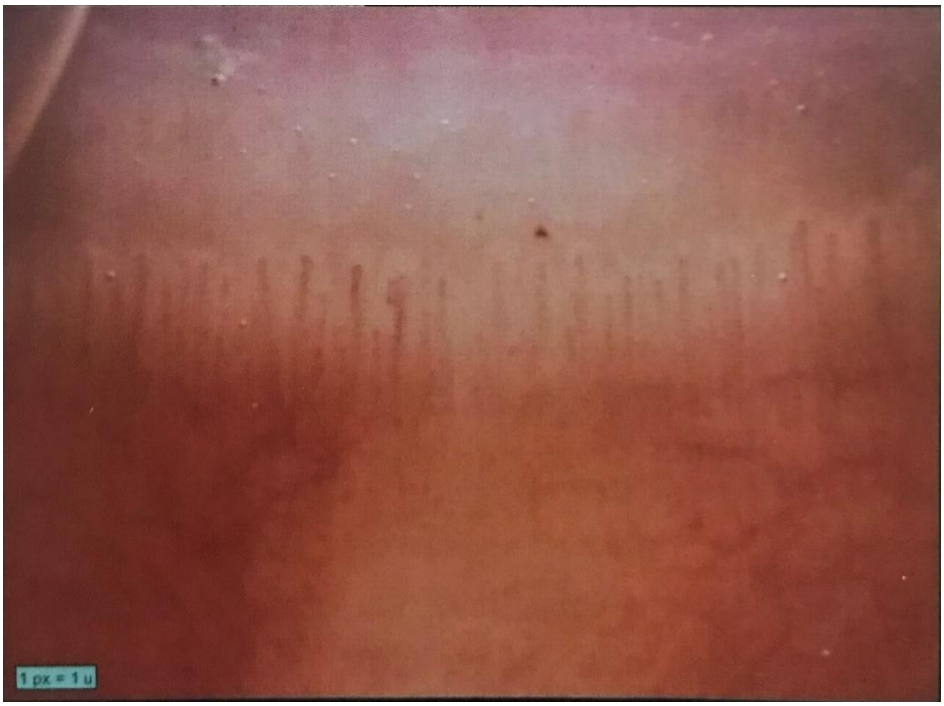


Figure 4. Normal capillaroscopy finding (magnification 50x). Within each dermal papilla there is one parallel "hair pin" shaped capillary. According to Barešić M., Anić B. Kapilaroskopija. U: Jelušić M., Malčić I. i sur. Pedijatrijska reumatologija 1.izd. Zagreb. Medicinska naklada;2014;p. 129

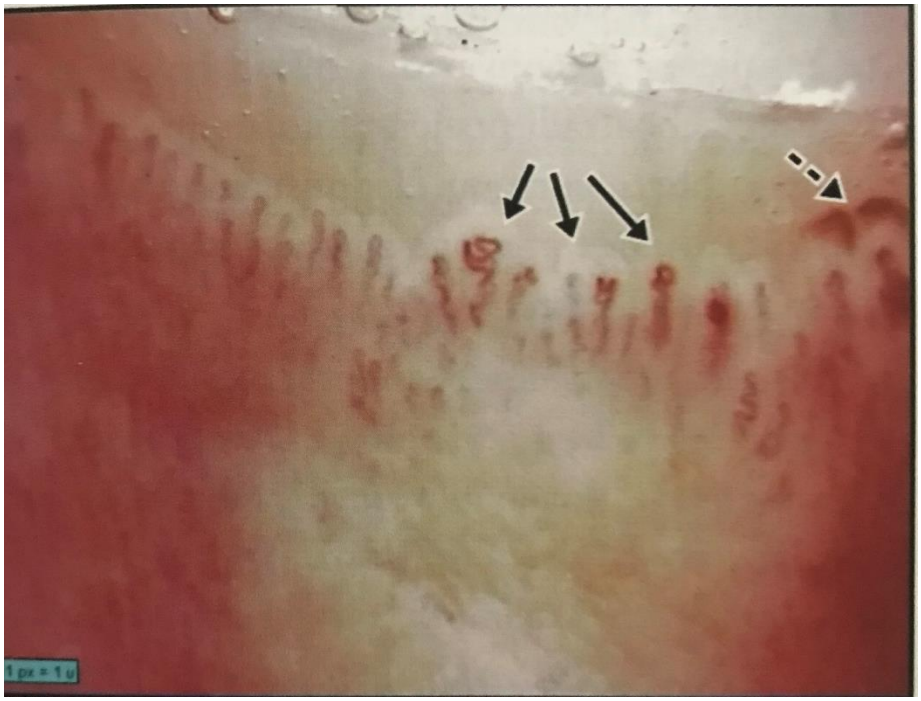


Figure 5. Engorged and tortous capillaries (shown with full line arrows) and hemorrhage (shown with streaked arrows) in systemic sclerosis (magnification 50x). According to Barešić M., Anić B. Kapilaroskopija. U: Jelušić M., Malčić I. i sur. Pedijatrijska reumatologija 1.izd. Zagreb. Medicinska naklada;2014;p. 131

1.4.3. Laboratory diagnostics

Laboratory investigation is primarily done for the purpose of establishing differential diagnosis between primary and secondary RP. Some of the tests that should be done include erythrocyte sedimentation rate (ESR), complete blood count (CBC), kidney and liver function, urinalysis and analysis of antinuclear antibodies and complement factors (C3, C4) (2). Those findings are usually negative in the case of PRP. Further investigation includes serological measurement of presence and levels of specific autoantibodies such as anti dsDNA, anti-U1RNP, anti-SSA, anti-SSB, anti-Sm, anti-topoisomerase-1, anti-centromere antibodies, and rheumatoid factor. All of those antibodies are specific for a certain rheumatological, connective tissue or autoimmune disorder. Patients should be evaluated for biochemical markers of acute inflammation (C-reactive protein - CRP) in order to assess the activity of the disease, and in some cases determination of serum proteins, cryoglobulins and cold agglutinins could be helpful (1).

1.5. Therapy

Therapeutic measures are based on individualised choice as well as on the activity, intensity and severity of the disease. First step in the management protocol would be conservative treatment. Mainstay of that type of approach is avoidance of excessive cold temperature exposure by using appropriate warm layered clothing, including gloves, shoes and head caps. Some other measures include avoiding caffeine and nicotine abuse, avoiding mental stress, careful application of vasoconstrictive drugs and maintenance of personal hygiene with warm water.

Adolescents and young adults should also be educated on professional orientation and importance of certain career choices (avoiding vibrational or cold temperature exposure). Patients not responding to conservative treatment should be considered for pharmacotherapy.

First choice medications are calcium channel blockers such as amlodipine, nifedipine etc., that provide both vasodilatative and antiaggregation effect. Dosage should be carefully titrated for the maximal efficacy and minimal side effects (hypotension, peripheral edema) (1).

Second choice treatment consists of direct acting vasodilatative agents like hidralazine and minoxidil and indirect acting vasodilatative agents such as angiotensin-converting enzyme (ACE) inhibitors, selective serotonin reuptake inhibitors (SSRIs) and angiotensin receptor antagonists. Phosphodiesterase (PDE) inhibitors (sildenafil) and endothelin receptor antagonists (bosentan) also proved their efficacy in some cases. Patients with severe secondary RP combined with chronic ischemia and digital ulcerations are candidates for parenteral administration of prostacyclin PGI2 or prostacyclin analouge iloprost (1). Surgical treatment is reserved for patients with refractory ischemic attacks with severe digital ulcerations and impending risk of gangrene and tissue necrosis. Classical sympathectomy is usually performed with mixed results - symptomatic resolution is immediate but temporary and long term results are most commonly not satisfactory (1).

2. Hypothesis

CCTT has the potential to be implemented as a quick, non-invasive, reliable and effective screening method for the diagnosis of Raynaud's phenomenon and other corresponding microcirculatory disorders.

The main diagnostic value of the method lies in its ability to detect early functional abnormalities that usually precede some anatomical or morphological findings.

3. Aim of the study

Aim of this study is to analyze the diagnostic value of CCTT for conclusion or exclusion of Raynaud's phenomenon, as well as differentiation between primary and secondary variant of the disease in the targeted pediatric population, and using the method's results, images and medical records of patients, determine the occurrence and prevalence according to sex and age.

4. Methods

This research was conducted as a retrospective study of medical records, including all the pediatric patients (defined in Republic of Croatia as all the patients up to the age of 18) that were referred to the Laboratory for CCTT, Department of Neurology at the University Hospital Centre (UHC) Zagreb, for the diagnostic procedure of CCTT imaging under the anamnestic or clinical suspicion of Raynaud's disease.

Under the referred suspected diagnosis of Raynaud's disease or other microcirculatory disturbances, there were altogether 464 patients in a 14-year time period from 2005 until 2019. Out of 464 patients, 353 were female, and 111 were male, exhibiting an approximate 3:1 female to male ratio. CCTT findings and database of hospital medical records were analyzed, interpreted, and according to reports stating the CCTT results, patients were subsequently categorized for the purpose of statistical analysis. Some patients had multiple CCTT reports with different time frames for various reasons (routine control check-ups or repeated imaging due to unclear validity of previous results), so the most recent report chronologically was taken into account. All CCTT imaging was performed using a Flir Thermacam B2 (Flir System Inc.), and the results were analyzed and interpreted by a certified telethermographist and neurologist working in the CCTT Laboratory of the Department of Neurology at the UHC Zagreb.

Figure 6. FLIR Thermacam B2 used for CCTT imaging in the CCTT Laboratory of the Department of Neurology at the UHC Zagreb

4.1. CCTT procedure

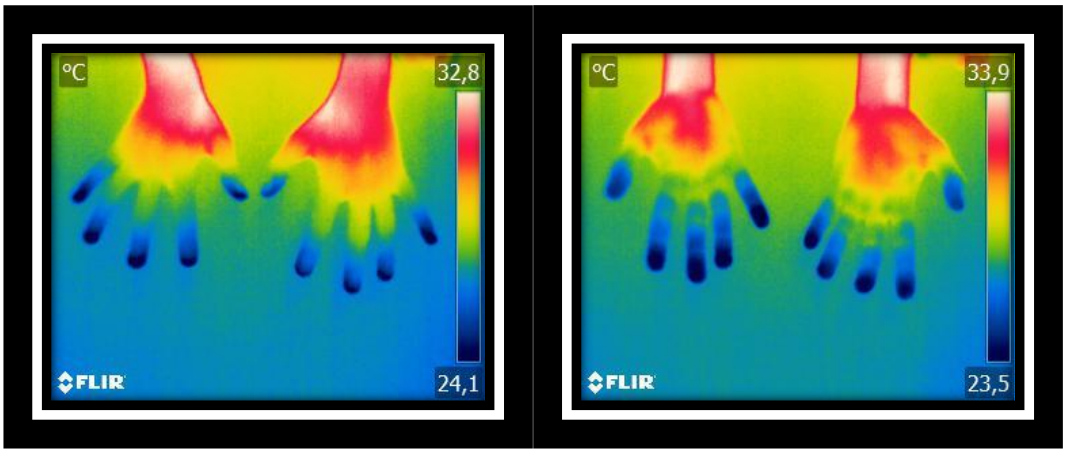
Literature usually mentions necessity of cold stress exposure as a method for investigation of microcirculation vasoreactivity. Suggested method is crioactivation by submerging hands into cold water, since heat exchange is approximately 25 times faster in liquid compared to air.

In the CCTT Laboratory of the Department of Neurology at the UHC Zagreb, the following diagnostic procedure is established. Patients are acclimatized to the ambient conditions of the laboratory environment during a 30 min period, corresponding to room temperature of 22-24°C and relative air humidity of 40-60%. Native images of both dorsal and palmar sides of hands and digits are acquired before crioactivation. Crioactivation is performed by submerging patients' hands completely (covering hands from the tips of fingers up to the radiocarpal joints) into the cold water (t=14°C) during a period of 1 minute. Hands and fingers are afterwards lightly dabbed with rolls of cellulose fibers until they are dry, avoiding rubbing motion and friction that can cause artificial thermal changes. Actual telethermographic changes and pattern of thermal restitution are then observed by sequential imaging during 20 minutes, with assessment at the 5, 10, 15 and 20-minute mark.

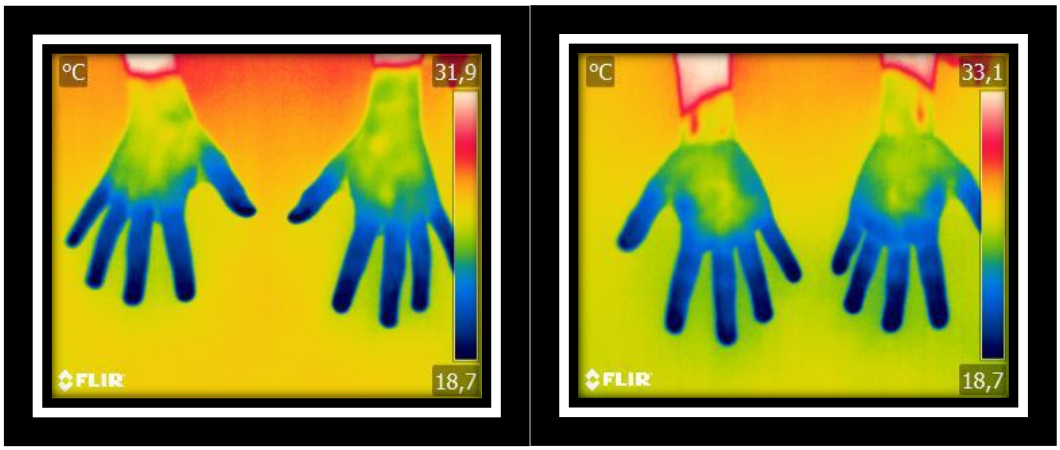
In telethermographic terms, anisothermic finding is defined as a thermal difference of 0.5°C or greater, between two corresponding registered surface areas. Thermal amputation is used as a term to describe the finding of temperatures below the ambiental values in a certain analyzed area of the skin. Critical diagnostic parameter in CCTT is thermal restitution (process of physiological return to the thermal status of the skin prior to crioactivation), of which it is important to observe both the pattern and duration. Bilateral, symmetrical thermal restitution that is completed in healthy individuals in a time period between 4 and 20 minutes, basically excludes the diagnosis of any type of vasospastic disorder. Patients with microcirculatory abnormalities or RP, exhibit prolonged thermal restitution that ranges from 15 minutes up to 1 hour.

Patients with primary RP show a characteristic, almost universal pattern of CCTT findings, with significant bilateral and symmetrical thermal amputation of all digits, present during native imaging and after the crioactivation, and persisting past the 20-minute mark during the rewarming period.

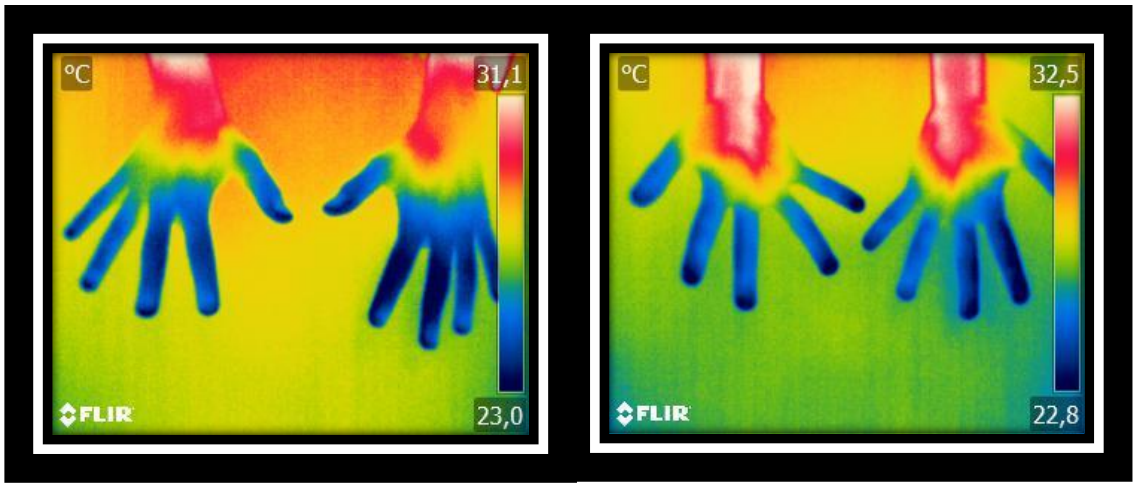
Patients with secondary RP show asymmetrical thermal amputation irregularly distributed amongst the affected digits, and commonly excluding the thumbs, in both native imaging and post crioactivation. Also present is an uneven and asymmetrical pattern of thermal restitution that is prolonged compared to healthy group, but slightly faster compared to patients with primary RP.



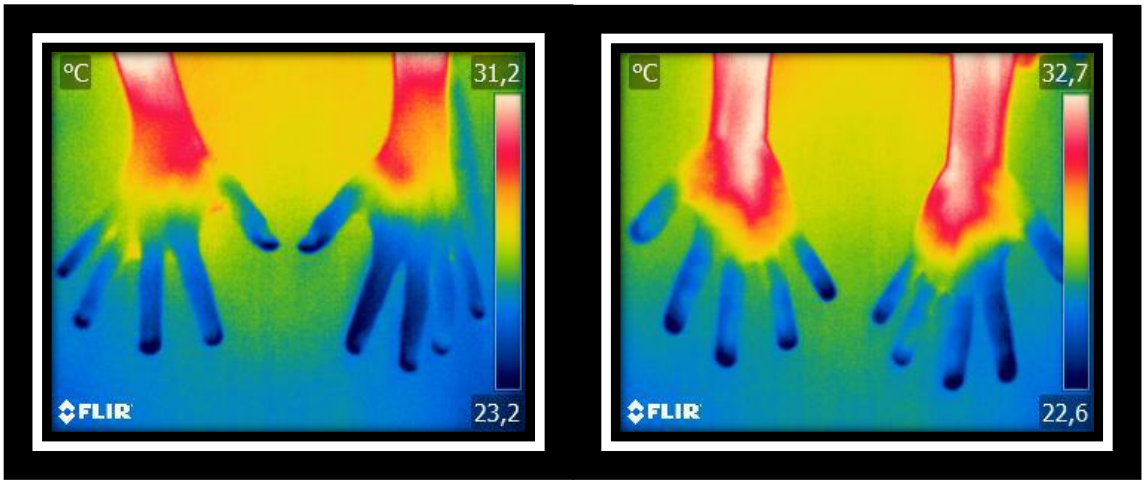
7a: Native imaging



7b: Post-crioactivation imaging at 0 min

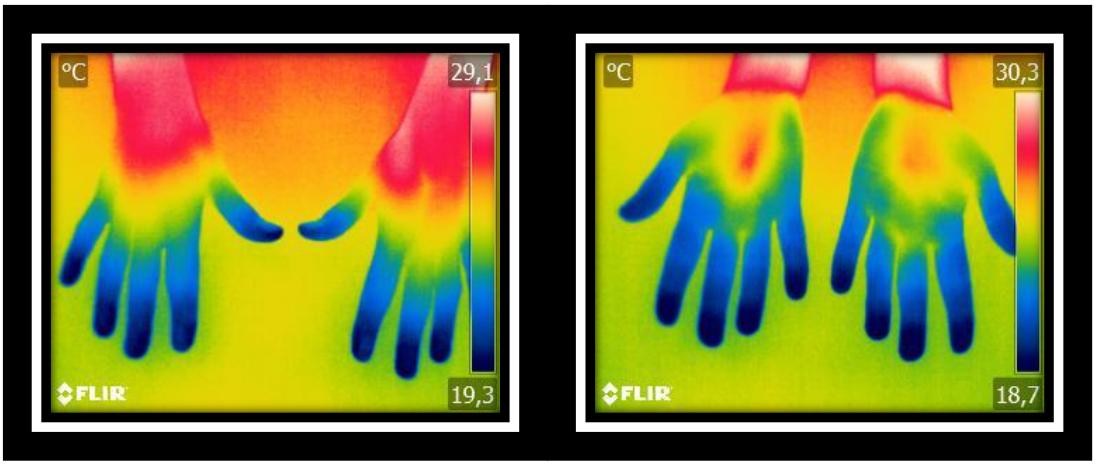


7c: Sequential imaging at 10 min

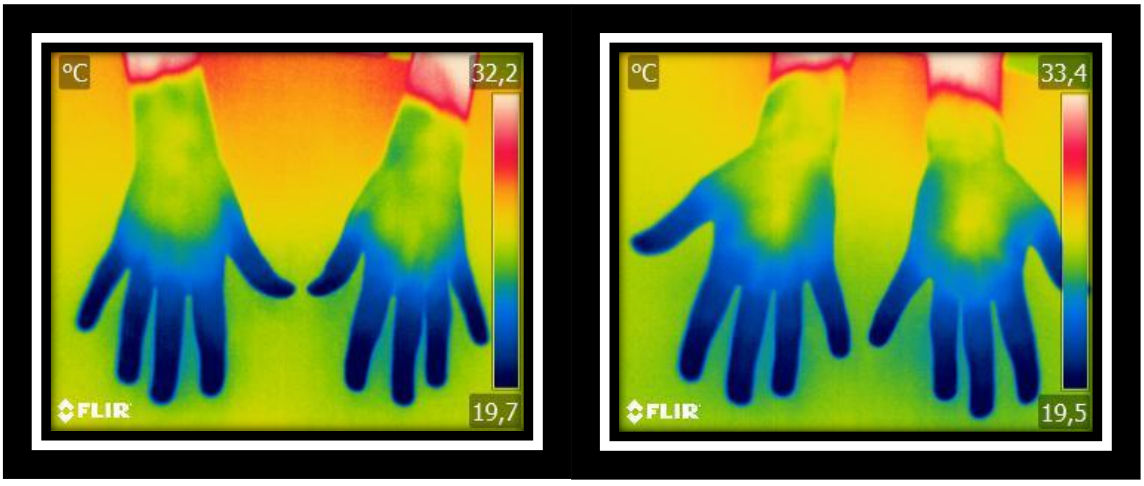


7d: Sequential imaging at 20 min

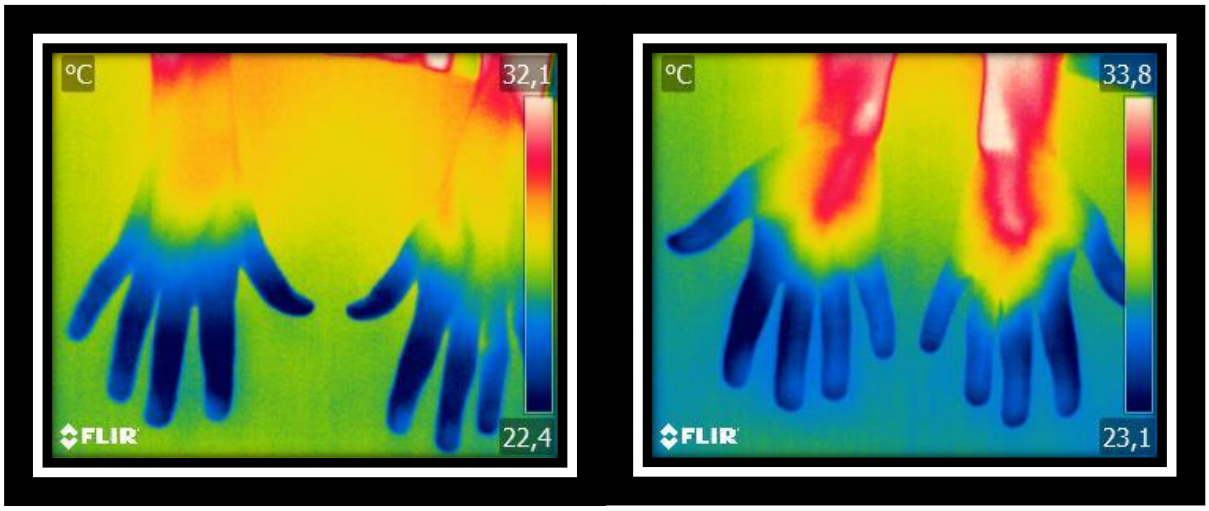
Figure 7. CCTT images of dorsal and palmar sides of both hands in a patient with PRP



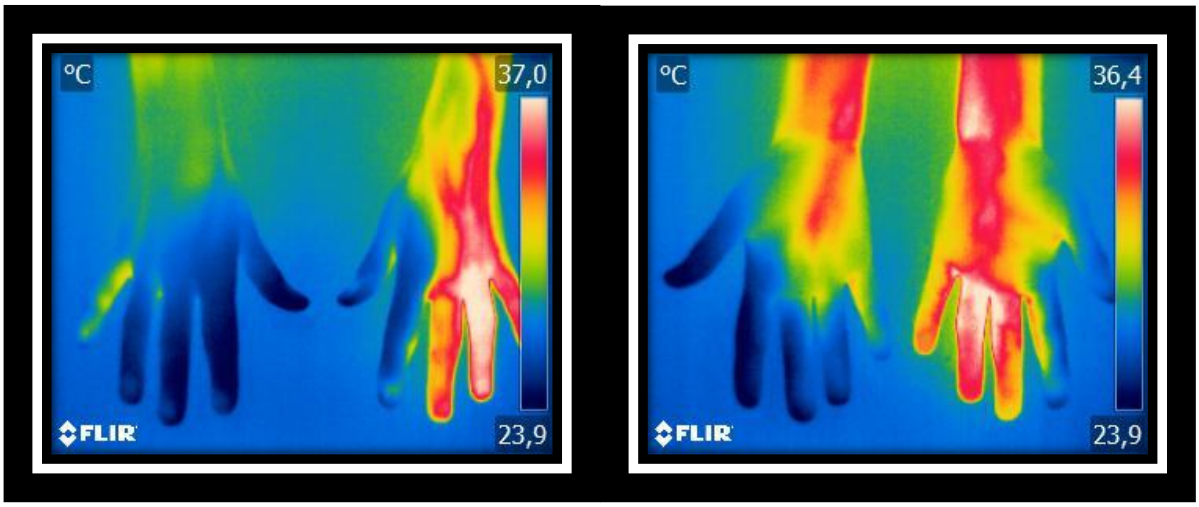
8a: Native imaging



8b: Post-crioactivation imaging at 0 min



8c: Sequential imaging at 10 min



8d: Sequential imaging at 20 min

Figure 8. CCTT images of dorsal and palmar sides of both hands in a patient with SRP

4.2. Patient categorization

For the purpose of a more efficient and conclusive statistical analysis, patients were categorized according to their age and their CCTT results.

Guiding point for the age categorization was determination of the adolescent group of patients. According to the definition by the World Health Organization (WHO), adolescence is a time period from 10-19 years of age. Therefore, the patients were divided into three groups: children (3-10 years old), and the adolescent group was subdivided into early adolescence (11-14 years old) and late adolescence (15-18 years old).

CCTT results were used to divide patients into four categories according to the findings. Category 1 were patients whose CCTT results yielded the diagnosis of primary RP, category 2 were patients whose results yielded the diagnosis of secondary RP, category 3 was reserved for patients who were negative, and category 4 of patients was classified as inconclusive, meaning that they showed a pattern of abnormal thermal restitution that was not conclusive for either primary or secondary RP, and thus they are indicated for other diagnostic procedures according to their need or clinical presentation.

5. Results

Amongst the total number of 464 patients, 353 (76.07%) were female and 111 (23.92%) were male, showing an approximate 3:1 female to male ratio. Youngest patient was 3 years old, while the oldest included was 18, with mean age ± standard deviation showing 15 ± 2.68. Systematization according to age groups showed that 323 (69.61%) patients were part of the late adolescence group, 115 (24.78%) patients were early adolescents, and only 26 (5.6%) patients were categorized in the children group, again highlighting the most vulnerable age group (15-18 years old) for the potential occurrence of RP and other microcirculatory disorders.

Table 1. Prevalence of positive, negative and inconclusive findings in the sample

	POSITIVE	NEGATIVE	INCONCLUSIVE
N (%)	178 (38.36)	227 (48.92)	59 (12.71)

CCTT yielded 12.71% of inconclusive cases, meaning that their CCTT findings were neither physiological nor representative of any type of Raynaud's disease, and those cases are candidates for further investigation. Almost half the cases were negative despite the clinical or anamnestic suspicion, showing normal thermal restitution.

Out of 178 positive cases, showing patterns indicative of PRP or SRP, 60 patients were diagnosed with PRP, showing prevalence of 33.7% in the positive sample and 12.93% in the total sample, while 118 patients were diagnosed with SRP, showing prevalence of 66.29% in the positive sample and 25.43% in the total sample.

Table 2. Occurrence and prevalence according to sex

	PRP N (%)	SRP N (%)	NEGATIVE N (%)	INCONCLUSIVE N (%)
MALE	9 (1.93)	30 (6.46)	60 (12.93)	12 (2.58)
FEMALE	51 (10.99)	88 (18.96)	167 (35.99)	47 (10.12)

Correlation between distribution of diagnostic cases and sex was assessed by using the Pearson's chi-squared test and the results (χ^2=3.97, p=0.26) showed there is not any difference in male and female case distribution at the 5% level of significance.

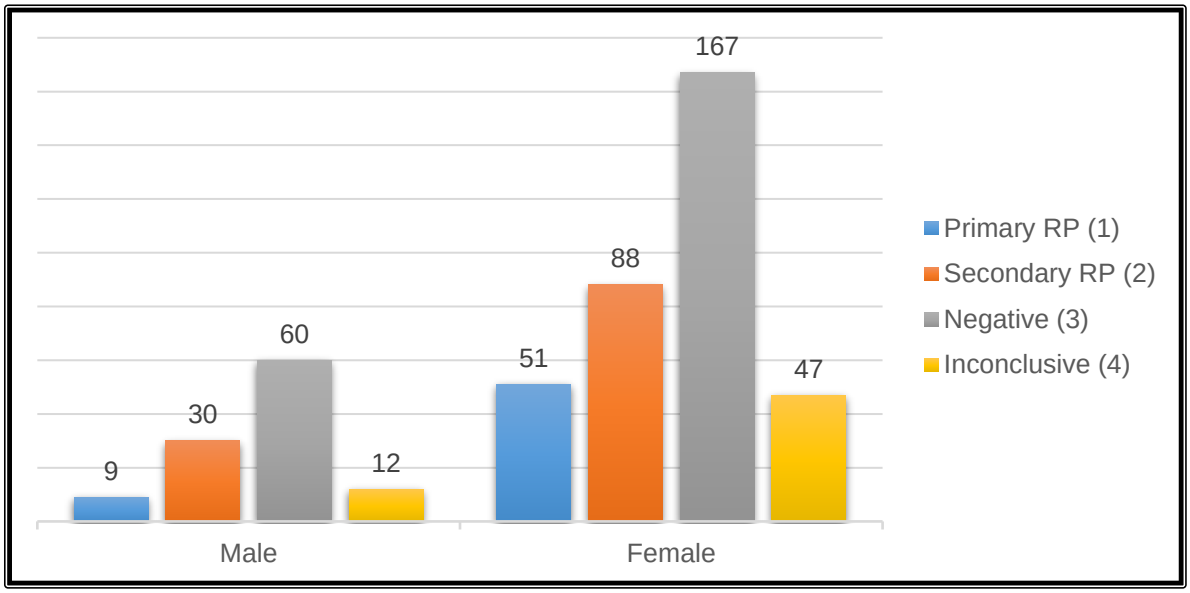
Table 3. Prevalence of positive, negative and inconclusive findings comparing males and females

	POSITIVE (PRP+SRP)	NEGATIVE	INCONCLUSIVE
MALE	35.13%	54.05%	10.81%
FEMALE	39.37%	47.3%	13.31%

Amongst male patients, out of 39 positive cases, 23.07% were diagnosed with PRP, while 76.92% were diagnosed with SRP. Female group yielded 139 positive cases, out of which 36.69% were diagnosed with PRP, while 63.3% were diagnosed with SRP.

Aforementioned diagnostic categories were used for the comparison of prevalence of primary and secondary RP between males and females as well as to compare the male to female ratio for each individual diagnostic category.

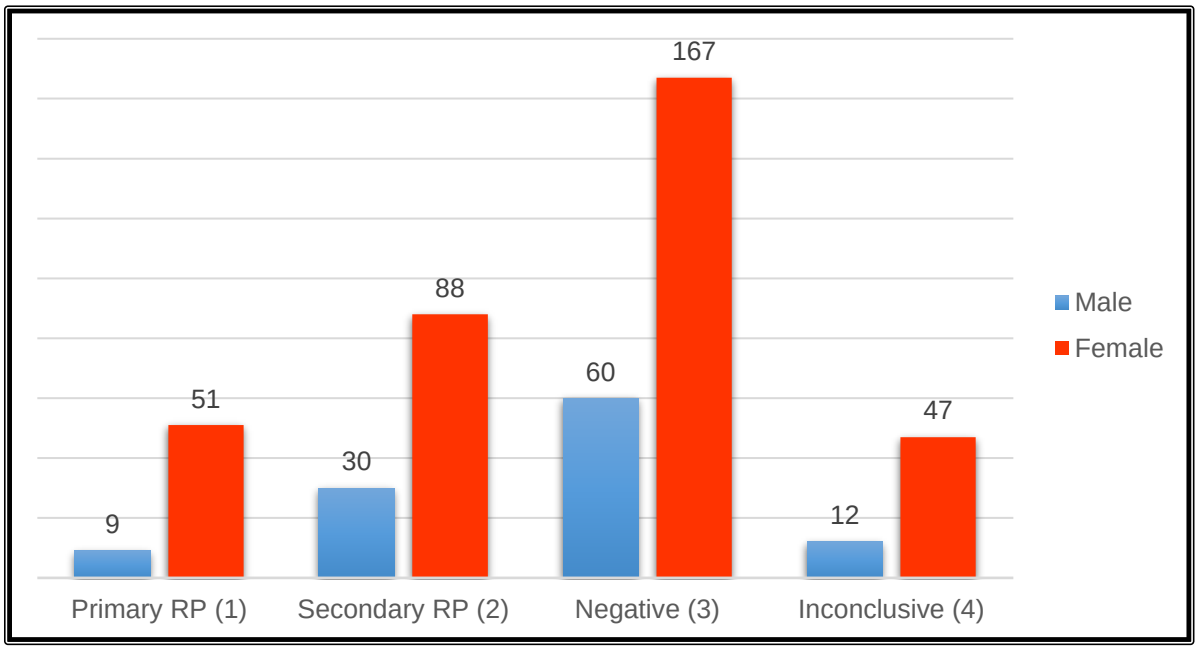
Chart 1. Number of cases of each diagnostic category according to sex



Out of 111 male patients, 54.05% were negative, 8.1% were diagnosed with PRP, 27.02% were diagnosed with SRP, while 10.81% were classified as inconclusive.

As for female patients, out of 353 cases, 47.3% were negative, 14.44% were diagnosed with PRP, 24.92% were diagnosed with SRP, while 13.31% were inconclusive.

Chart 2. Number of cases per each diagnostic category comparing males and females



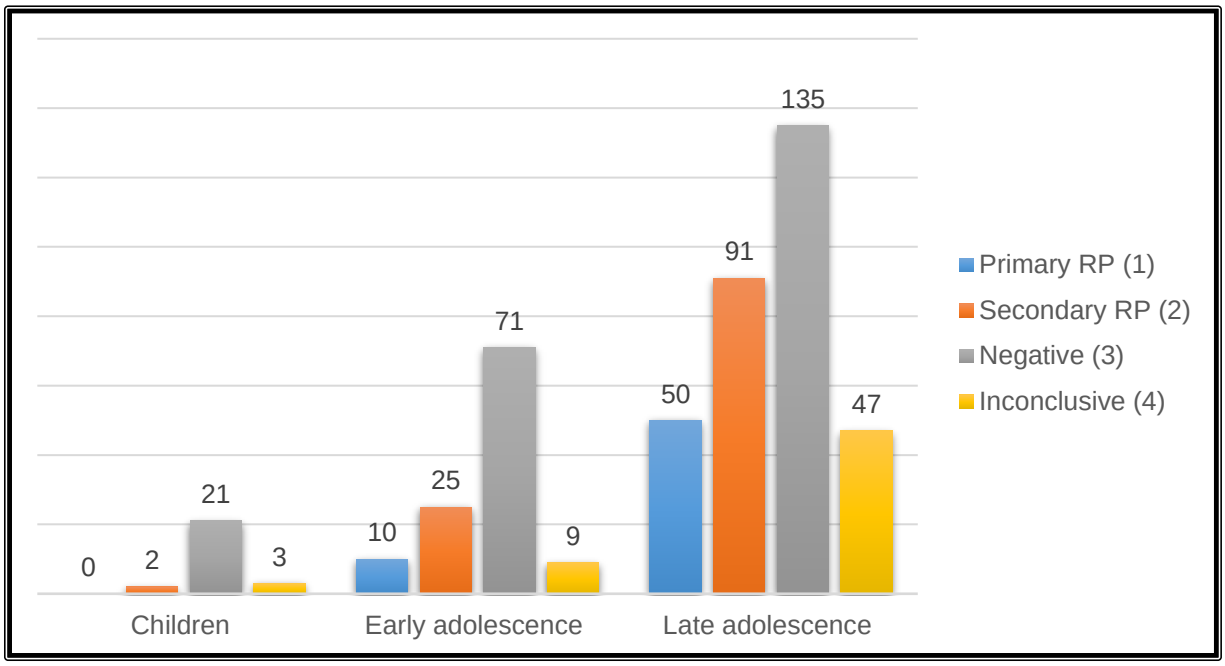
Looking at specific diagnostic category, for PRP out of total of 60 cases, 15% were male and 85% were female, for SRP out of 118 cases, 25.42 % were male and 74.57% were female, out of 227 negative cases, 26.43% were male and 73.56% were female, and for the 59 inconclusive cases, 20.33% were male and 79.66% were female.

Table 4. Female to male ratio in decimal form

	PRP	SRP	NEGATIVE	INCONCLUSIVE
F:M	**5.67**	**2.93**	**2.78**	**3.91**

This shows that for every male with PRP, there are almost 6 females with PRP, for every male with SRP, there are almost 3 females with SRP, for every male with a negative CCTT result there are almost 3 females also with negative CCTT results, and for every male with inconclusive findings there are also almost 4 females with inconclusive findings.

Chart 3. Number of cases of each diagnostic category per age group



Analysis of prevalence of diagnostic categories amongst the age groups showed the lowest representation of Raynaud's disease in children and highest representation in late adolescence.

In children (≤10 years old), out of 26 cases, vast majority of 80.76% were negative, 7.69% were diagnosed with SRP, 11.53% were inconclusive, while none of the children were diagnosed with PRP. In the early adolescence group (11-14 years old), 61.73% were negative, 8.69% were diagnosed with PRP, 21.73% were diagnosed with SRP, while 7.82% were classified as inconclusive. In the late adolescence group (15-18 years old), 41.79% were negative, 15.47% were diagnosed with PRP, 28.17% were diagnosed with SRP, while 14.55% were inconclusive.

Table 5. Prevalence of positive, negative and inconclusive findings amongst age groups

	POSITIVE (PRP+SRP)	NEGATIVE	INCONCLUSIVE
CHILDREN	7.69%	80.76%	11.53%
EARLY ADOLESCENCE	30.43%	61.73%	7.82%
LATE ADOLESCENCE	43.65%	41.79%	14.55%

Out of two positive cases in children group, all of them (100%) were diagnosed as SRP. Early adolescence showed 35 positive cases, where 28.57% were diagnosed with PRP and 71.42% were diagnosed with SRP. In the late adolescence group, out of 141 positive cases, 35.46% were diagnosed with PRP and 64.53% were diagnosed with SRP.

Table 6. Number of cases comparing two age groups

	PRP	SRP	NEGATIVE	INCONCLUSIVE
11-14 YO N (%)	10 (8.69)	25 (21.73)	71 (61.73)	9 (7.82)
15-18 YO N (%)	50 (15.47)	91 (28.17)	135 (41.79)	47 (14.55)

When observing two most represented age groups, early and late adolescence, which together comprise 94.39% of all cases, it was also important to assess any difference in case distribution. Again, Pearson's chi-squared test was used, and the results (χ^2=14.34, p=0.0024)

showed a statistically significant difference in case distribution between the two age groups at the 5% level of significance.

Since the late adolescence group comprises almost 70% of the total sample, it was significant to analyze this sample in isolation and compare gender differences.

Table 7. Comparison between male and female number of cases per diagnostic category in the late adolescence group

	PRP	SRP	NEGATIVE	INCONCLUSIVE
MALE	7	28	34	12
FEMALE	43	63	101	35

Evidence again showed clear difference between male and female prevalence. Male prevalence of PRP was 8.64% compared to female prevalence of 17.76%, male prevalence of SRP was 34.56% compared to female prevalence of 26.03%, male prevalence of negative cases was 41.97%, while female was 41.73%, and inconclusive male cases in this group comprised 14.81%, while female cases comprised 14.46%.

Pearson's chi-squared test was also used in this case to determine the difference between male and female distribution in this sample. The results (χ^2=4.84, p=0.18) did not show any statistically significant difference in distribution at the 5% level of significance.

6. Discussion

This study showed that CCTT identified 38.36 % of positive findings (PRP+SRP) in the targeted sample. That is comparable to the similar study done by Šestan and associates (64) that showed a prevalence of 38.29 % of positive findings in the sample of children that underwent the CCTT diagnostic procedure. Prevalence of secondary RP (25.43%) was almost double the prevalence of primary RP (12.93%) in the total sample.

When differentiating between sexes, results showed higher prevalence of negative cases in males (54.05%) compared to females (47.3%), and higher prevalence of positive findings in females (39.37%) compared to males (35.13%). At the same time, male sample showed higher prevalence of secondary RP (27.02%) compared to female (24.92%), while female sample showed higher prevalence of primary RP (14.44%) compared to male (8.1%). However, Pearson's chi-squared test did not show correlation between sex and case distribution.

43.65% of positive cases were recognized in the late adolescence group, showing highest prevalence and indicating vulnerability of that age group. Late adolescence group also showed significantly higher prevalence of primary RP (15.47%) compared to both early adolescence (8.69%) and children (0%), as well as with secondary RP (28.17%) compared to early adolescence (21.73%) and children (7.69%), while exhibiting much lower prevalence of negative cases (41.79%) to early adolescence (61.73%) and children (80.76%).

A study done by Jones et al. (35) in the UK showed a prevalence of 15% in the sample of children aged 12-15, with female prevalence being higher (18%) compared to male (12%). This study showed a much higher prevalence of positive cases (38.36%) in the total sample, and in both male (35.13%) and female (39.37%) sample, and that is probably indicative of both wider age range (0-18 years old) and this sample being non-randomized, unlike the study by Jones et al., since this was targeted population with clinical or anamnestic suspicion of RP. In addition, study by Jones et al. assessed children using a questionnaire, while targeted sample in this study was assessed by CCTT results. Only analyzing the late adolescence group as the most represented in the whole sample, also yielded higher prevalence of positive cases

(43.65%), and both male (43.2%) and female (43.8%) prevalence compared to study by Jones et al. due to same reasons as mentioned before (non-randomized sample, CCTT results assessment).

Pearson's chi-squared test again did not show statistically significant difference between male and female distribution of cases in the late adolescence group, although it did show statistically significant difference of case distribution between early and late adolescence.

Long-term clinical practice has shown good experience with CCTT and CCTT has been a crucial element in the diagnostic algorithm of rheumatic conditions and specifically Raynaud's disease. Study by Šestan et al. (64) also showed equal efficacy and reliability of CCTT and NVC in the diagnosis of RP and differentiation between PRP and SRP. CCTT has been proven as safe, non-invasive, reliable and highly reproducible (it can be repeated multiple times in short time intervals), which makes it very convenient for patient follow-up. The fact that it enables visualization of functional changes and abnormalities, gives it high potential for possible screening and early detection of microvasculature disorders.

7. Conclusion

1. CCTT results in this research showed a prevalence of 12.93% of PRP and 25.43% of SRP in the targeted pediatric sample.

2. Even though results of Pearson's chi-squared test (p=0.26) did not show statistically significant correlation between sex and occurrence of RP, higher female prevalence of both PRP and SRP compared to male, as well as higher male prevalence of negative cases compared to female, points to increased risk and occurrence of RP in females.

3. Age has been shown to be a significant risk factor, with late adolescence group proving to be the most vulnerable by being the most represented age group in the whole sample and showing significantly higher prevalence of both PRP and SRP, and significantly lower prevalence of negative cases when compared to two other age groups.

4. Pearson's chi-squared test showed a significant correlation between age and occurrence of RP (p=0.0024) when observing transition between early and late adolescence, that were compared as the age range that yielded almost 95% of cases in this sample.

5. CCTT managed to present conclusive results for 87.29% of patients (59 out of 464 were classified as inconclusive) which coupled with long-term effective and satisfactory clinical experience, strengthens the case for widespread use as a reliable screening tool.

6. For the establishment of a clear diagnostic algorithm and more conclusive prevalence and incidence of RP in the pediatric population, it would probably be necessary to conduct a prospective long-term study of CCTT imaging in both clinically suspicious and randomized, clinically unsuspicious pediatric sample.

Literature

1. Anić B., Barešić M. Raynaudov sindrom. U: Jelušić M., Malčić I. i sur. Pedijatrijska reumatologija 1.izd. Zagreb. Medicinska naklada; 2014; 240-245

2. Tambić Bukovac L., Šenjug Perica M. Raynaudov sindrom. Paeditr Croat. 2017;61 (Supl 1):38-42

3. Amanzi L, Braschi F, Fiori G, et al. Digital ulcers in scleroderma: staging, characteristics and sub-setting through observation of 1614 digital lesions. Rheumatology. 2010;49:1374–1382.

4. Raynaud M. Local asphyxia and symmetrical gangrene of the extremities 1862. New researches on the natureand treatment of local asphyxia of the extremities 1874. Translated by barlow London: New Syndenham Society, 1888.

5. Lewis T. Experiments relating to the peripheral mechanism involved in spasmodic arrest of the circulation of the fingers. A variety of Raynaud's disease. Heart 1929; 14:7

6. Alba BK, Castellani JW, Charkoudian N. Cold-induced cutaneous vasoconstriction in humans: Function, dysfunction and the distinctly counterproductive. Exp Physiol 2019; 104:1202.

7. McCance K. L., Huether S. E. (2013). Pathophysiology: The Biologic Basis for Disease in Adults and Children. Amsterdam: Elsevier

8. Polonia J. J., Paiva M. Q., Guimaraes S. (1985). Pharmacological characterization of postsynaptic alpha-adrenoceptor subtypes in five different dog arteries in-vitro. J. Pharm. Pharmacol. 37 205–208. 10.1111/j.2042-7158.1985.tb05043.x

9. Freedman RR, Moten M, Migály P, Mayes M. Cold-induced potentiation of alpha 2-adrenergic vasoconstriction in primary Raynaud's disease. Arthritis Rheum 1993; 36:685.

10. Flavahan NA, Vanhoutte PM. Endothelial cell signaling and endothelial dysfunction. Am J Hypertens 1995; 8:28S.

11. Garg UC, Hassid A. Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. J Clin Invest 1989; 83:1774.

12. Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 1991; 43:109.

13. Kirchengast M, Münter K. Endothelin-1 and endothelin receptor antagonists in cardiovascular remodeling. Proc Soc Exp Biol Med 1999; 221:312.

14. Coffman JD, Cohen RA. Role of alpha-adrenoceptor subtypes mediating sympathetic vasoconstriction in human digits. Eur J Clin Invest 1988; 18:309.

15. Lindblad LE, Ekenvall L, Etzell BM, Bevegård S. Adrenoceptors in Raynaud's disease. J Cardiovasc Pharmacol 1989; 14:881.

16. Bailey S. R., Eid A. H., Mitra S., Flavahan S., Flavahan N. A. (2004). Rho kinase mediates cold-induced constriction of cutaneous arteries: role of alpha2C-adrenoceptor translocation. Circ. Res. 94 1367–1374. 10.1161/01.RES.0000128407.45014.58

17. Bailey SR, Eid AH, Mitra S, et al. Rho kinase mediates cold-induced constriction of cutaneous arteries: role of alpha2C-adrenoceptor translocation. Circ Res 2004; 94:1367.

18. Bailey SR, Mitra S, Flavahan S, Flavahan NA. Reactive oxygen species from smooth muscle mitochondria initiate cold-induced constriction of cutaneous arteries. Am J Physiol Heart Circ Physiol 2005; 289:H243.

19. Furspan PB, Chatterjee S, Mayes MD, Freedman RR. Cooling induced contraction and protein tyrosine kinase activity of isolated arterioles in secondary Raynaud's phenomenon. Rheumatology (Oxford) 2005; 44;488-94.

20. Zamora MR, O'Brien RF, Rutherford RB, Weil JV. Serum endothelin-1 concentrations and cold provocation in primary Raynaud's phenomenon. Lancet 1990;336:1144

21. Bunker CB, Terenghi G, Springall DR, et al. Deficiency of calcitonin gene-related peptide in Raynaud's phenomenon. Lancet 1990; 336:1530

22. Knock GA, Terenghi G, Bunker CB, et al. Characterization of endothelin-binding sites in human skin and their regulation in primary Raynaud's phenomenon and systemic sclerosis. J Invest Dermatol 1993; 101:73

23. Wigley FM. Vascular disease in scleroderma. Clin Rev Allergy Immunol 2009; 36:150.

24. Blann AD, Illingworth K, Jayson MI. Mechanisms of endothelial cell damage in systemic sclerosis and Raynaud's phenomenon. J Rheumatol 1993; 20:1325.

25. Freemont AJ, Hoyland J, Fielding P, et al. Studies of the microvascular endothelium in uninvolved skin of patients with systemic sclerosis: direct evidence for a generalized microangiopathy. Br J Dermatol 1992; 126:561.

26. Kazandjian S, Fiessinger JN, Camilleri JP, et al. Endothelial cell renewal in skin of patients with progressive systemic sclerosis (PSS): an in vitro autoradiographic study. Acta Derm Venereol 1982; 62:425.

27. Pearson JD. The endothelium: its role in scleroderma. Ann Rheum Dis 1991; 50 Suppl 4:866.

28. Prescott RJ, Freemont AJ, Jones CJ, et al. Sequential dermal microvascular and perivascular changes in the development of scleroderma. J Pathol 1992; 166:255.

29. Matucci Cerinic M, Kahaleh MB. Beauty and the beast. The nitric oxide paradox in systemic sclerosis. Rheumatology (Oxford) 2002; 41:843.

30. Fleischmajer R, Perlish JS. [3H]Thymidine labeling of dermal endothelial cells in scleroderma. J Invest Dermatol 1977; 69:379.

31. Kahaleh MB. Endothelin, an endothelial-dependent vasoconstrictor in scleroderma. Enhanced production and profibrotic action. Arthritis Rheum 1991; 34:978.

32. Vancheeswaran R, Azam A, Black C, Dashwood MR. Localization of endothelin-1 and its binding sites in scleroderma skin. J Rheumatol 1994; 21:1268.

33. Freedman RR, Girgis R, Mayes MD. Acute effect of nitric oxide on Raynaud's phenomenon in scleroderma. Lancet 1999; 354:739.

34. Suter LG, Murabito JM, Felson DT, Fraenkel L. The incidence and natural history of Raynaud's phenomenon in the community. Arthritis Rheum. 2005; 52:1259-63

35. Jones GT, Herick AL, Woodham SE, Baildam EM, Macfarlane GJ, Silman AJ. Occurrence of Raynaud's phenomenon in children ages 12-15 years: prevalence and associtaion with other common symptoms. Arthritis Rheum. 2003; 48:3518-21

36. Maricq HE, Carpentier PH, Weinrich MC et al. Geographic variation in the prevalence of Raynaud Phenomenon: a 5-region comparison. J Rheumatol 1997; 24: 879-89.

37. Prete M, Fatone MC, Favoino E, Perosa F. Raynaud’s phenomenon: from molecular pathogenesis to therapy. Autoimmun Rev. 2014; 13:655–67.

38. Nigrovic PA, Fuhlbrigge RC, Sundel RP. Raynaud's phenomenon in children: a retrospective review of 123 patients. Pediatrics. 2003; 111:715–21.

39. Martini G, Foeldvari I, Russo R, Cuttica R, Eberhard A, Ravelli A, et al. Systemic sclerosis in childhood: clinical and immunologic features of 153 patients in an international database. Arthritis Rheum. 2006; 54(12):3971–8.

40. Maricq H. R., Carpentier P. H., Weinrich M. C., Keil J. E., Franco A., Drouet P., et al. (1993). Geographic variation in the prevalence of Raynaud's phenomenon: Charleston, SC, USA, vs Tarentaise, Savoie, France. J. Rheumatol. 20 70–76.

41. Greenstein D., Jeffcote N., Ilsley D., Kester R. C. The menstrual cycle and Raynaud's phenomenon. 1996 Angiology 47 427–436.

42. Mayes M. D. (1999). Epidemiologic studies of environmental agents and systemic autoimmune diseases. Environ. Health Perspect. 107(Suppl. 5), 743–748.

43. Fraenkel L., Zhang Y., Chaisson C. E., Evans S. R., Wilson P. W., Felson D. T. (1998). The association of estrogen replacement therapy and the Raynaud phenomenon in postmenopausal women. Ann. Intern. Med. 129 208–211

44. Chan N. N., MacAllister R. J., Colhoun H. M., Vallance P., Hingorani A. D. (2001). Changes in endothelium-dependent vasodilatation and alpha-adrenergic responses in resistance vessels during the menstrual cycle in healthy women. J. Clin. Endocrinol. Metab. 86 2499–2504

45. Eid A. H., Maiti K., Mitra S., Chotani M. A., Flavahan S., Bailey S. R., et al. (2007). Estrogen increases smooth muscle expression of alpha2C-adrenoceptors and cold-induced constriction of cutaneous arteries. Am. J. Physiol. Heart Circ. Physiol. 293 H1955–H1961

46. Susol E., MacGregor A. J., Barrett J. H., Wilson H., Black C., Welsh K., et al. (2000). A two-stage, genome-wide screen for susceptibility loci in primary Raynaud's phenomenon. Arthritis Rheum. 43 1641–1646

47. Sharathkumar A. A., Castillo-Caro P. (2011). Primary Raynaud's phenomenon in an infant: a case report and review of literature. Pediatr. Rheumatol. Online J. 9:16

48. Fontana L., Marion M. J., Ughetto S., Catilina P. (2006). Glutathione S-transferase M1 and GST T1 genetic polymorphisms and Raynaud's phenomenon in French vinyl chloride monomer-exposed workers. J. Hum. Genet. 51 879–886

49. White C. R., Haidekker M. A., Stevens H. Y., Frangos J. A. (2004). Extracellular signal-regulated kinase activation and endothelin-1 production in human endothelial cells exposed to vibration. J. Physiol. 555

50. Maricq H. R., Darke C. S., Archibald R. M., Leroy E. C. (1978). In vivo observations of skin capillaries in workers exposed to vinyl chloride. An English- American comparison. Br. J. Ind. Med. 35 1–7.

51. Falappa P., Magnavita N., Bergamaschi A., Colavita N. (1982). Angiographic study of digital arteries in workers exposed to vinyl chloride. Br. J. Ind. Med. 39 169–172.

52. Nordberg G. F., Fowler B. A., Nordberg M., Friberg L. (2014). Handbook on the Toxicology of Metals. Amsterdam: Academic Press

53. Lagerkvist B., Linderholm H., Nordberg G. F. (1986). Vasospastic tendency and Raynaud's phenomenon in smelter workers exposed to arsenic. Environ. Res. 39 465–474.

54. Hansen S. W., Olsen N. (1989). Raynaud's phenomenon in patients treated with cisplatin, vinblastine, and bleomycin for germ cell cancer: measurement of vasoconstrictor response to cold. J. Clin. Oncol. 7 940–942.

55. Glendenning J. L., Barbachano Y., Norman A. R., Dearnaley D. P., Horwich A., Huddart R. A. (2010). Long-term neurologic and peripheral vascular toxicity after chemotherapy treatment of testicular cancer. Cancer

56. Liew SC. Electromagnetic Waves. Centre for Remote Imaging, Sensing and Processing. http://www.crisp.nus.edu.sg/~research/tutorial/em.htm

57. Formenti D, Ludwig N, Gargano M, et al. Thermal imaging of exercise-associated skin temperature changes in trained and untrained female subjects. Ann Biomed Eng. 2013; 41:863–871

58. Kelly G. Body temperature variability (Part 1): a review of the history of body temperature and its variability due to site selection, biological rhythms, fitness, and aging. Altern Med Rev. 2006; 11:278–293

59. Chojnowski M. Infrared thermal imaging in connective tissue diseases. Reumatologia. 2017; 55(1): 38-43

60. Stipić J. Kompjutorizirana kolor-teletermografija. U: Jelušić M., Malčić I. i sur. Pedijatrijska reumatologija 1.izd. Zagreb. Medicinska naklada; 2014; 123-128

61. Cutolo M, Grassi W, Matucci Cernic M. Raynaud's phenomenon and the role of capillaroscopy. Arthritis Rheum. 2003; 48:3023–3030.

62. Kuryliszyn-Moskal A, Kita J, Hryniewicz A. Raynaud's phenomenon: new aspects of pathogenesis and the role of nailfold videocapillaroscopy. Reumatologia. 2015;53(2):87–93.

63. Barešić M., Anić B. Kapilaroskopija. U: Jelušić M., Malčić I. i sur. Pedijatrijska reumatologija 1.izd. Zagreb. Medicinska naklada; 2014; 129-132

64. Sestan M, Cekada N, Turudic D, Batnozic Varga M, Frkovic M, Stipic J, Baresic M,

Kifer D, Jelusic M. Nailfold capillaroscopy, computerized color telethermography and immunological findings in 224 children with Raynaud's phenomenon. Pediatric Rheumatology 2018, 16(Suppl 2):52

Acknowledgments

First, I would like to thank my mentor professor Marija Jelušić for her guidance, patience and trust.

I would like to thank my girlfriend and my friends for providing joy and fun during hard work and dedication.

Lastly, I would like to thank my family, especially my mother and sister who are my biggest motivation, for their unconditional love and support.

Biography

I was born on October 1st 1995 in Zagreb, Croatia. I finished both primary and secondary education in my hometown with excellent success. Coming from a family of physicians, I was drawn to medicine from my early days so naturally I enrolled in School of Medicine, University of Zagreb in 2014. During my studies, I was an active participant in teaching as a student demonstrator at the Department of Pathophysiology, UHC Zagreb for one year, as a student demonstrator at the Department of Pediatrics, UHC Zagreb for one year, and as a student demonstrator at the Department of Internal medicine, UHC Zagreb for two years. During my last year of study, I actively participated as a co-author of two scientific abstracts at two different medical congresses with international participation, Annual meeting of the Croatian Immunological Society in 2019 and 6th Croatian Rhinologic Congress in 2020. I am proficient in English and German language. I will graduate in July 2020 with excellent success.