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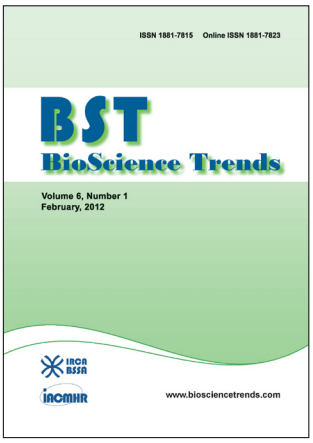

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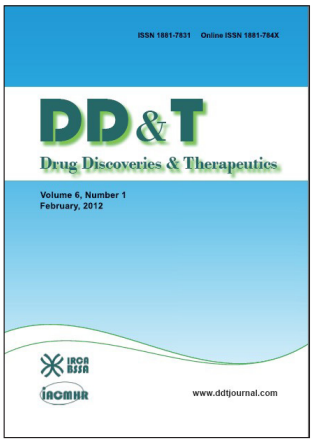

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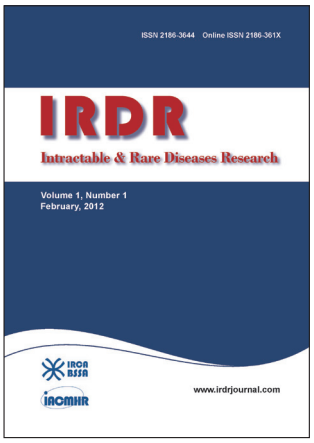

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Review

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Granulomatosis with polyangiitis (Wegener's disease): An updated review of ocular disease manifestations

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Summary **Granulomatosis with polyangiitis (GPA) is a potentially lethal systemic disorder that is characterized by necrotizing vasculitis of small arteries and veins. The respiratory system is most commonly affected in limited forms of the disease, however upper and lower respiratory system, systemic vasculitis, and necrotizing glomerulonephritis are the characteristic components of the disease triad. The peak incidence is observed at 64-75 years of age, with a prevalence of 8-10 per million depending on geographic location. In this review we focus on the ocular manifestations of the disease which occur in nearly in one third of the patients. In addition we describe the neuro-ophthalmic complications which occur in up to half of cases. We also discuss the current systemic treatment options including corticosteroids, cyclophosphamide, azathioprine, and the available biologic response modifiers including rituximab. The disease remains difficult to diagnose due to the generalized symptomatic presentation of patients with GPA. As a result, several sets of diagnostic criteria have been developed which include clinical, serological, and histopathological findings to varying extents. Early diagnosis and multi-specialty collaboration among physicians is necessary to adequately manage the disease and the potential complications that may result from drugs used in the treatment of the disease. Despite recent advances, more research is necessary to prevent the high rates of mortality from the disease itself and from therapeutic side effects.**

Keywords: Granulomatosis with polyangiitis, Wegener's, vasculitis, ocular complications, granuloma

1. Definition

Granulomatosis with polyangiitis (GPA) is a systemic disorder that is characterized by necrotizing vasculitis of small arteries and veins (*1,2*). The classic diagnostic criteria for GPA were based on the initial detailed clinical and pathologic findings as described by Godman and Churg in 1954 (*3,4*). This includes a triad of necrotizing granulomas of upper and lower respiratory system, systemic vasculitis, and necrotizing glomerulonephritis. An incomplete or limited form of GPA in which the kidneys are usually spared has been reported (*5-8*). The respiratory system is the most common organ to be involved in limited GPA, although any other organ system can be involved. A very limited form of the disease, with clinical involvement of a single organ such as the eye, has also been described with any ocular structure being affected (*9*). GPA is a complex and potentially lethal disease with high mortality rate if left untreated. Eearly detection of the disease and the introduction of immunosuppressive therapy has resulted in improved prognosis and decreased mortality rate.

2. History

Although GPA was first described by Klinger as a form of polyarteritis nodosa (PAN) (*10*), the unique nature of the disease was recognized earlier by Wegener (*11*).

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The term GPA was first introduced into the English literature in 1954 by Godman and Churg (*4*) This term, as opposed to Wegener's granulomatosis, describes the main pathologic feature (granulomatous inflammation) and reflects the vasculitic involvement of multiple types of vessels (polyangiitis).

3. Epidemiology

The incidence of GPA is estimated to be 8-10 cases per one million depending on geographic location (*12*). It has been suggested that the incidence of GPA is increasing, however this may simply reflect the availability of new diagnostic modalities and serologic tests such as anti-neutrophil cytoplasmic antibodies (ANCAs) that allows a more frequent diagnosis (*13*). The age of symptoms onset has a wide distribution with a peak incidence at 64-75 years of age (*12,14,15*).

Previous studies showed that GPA can occur in children with 8-15% of cases occurring in patients age 19 or younger (*14-16*). Although slight male predominance has been reported in few case series (*17,18*) a recent study including 158 patients showed no sex predilection (*19*). GPA is most frequently reported in white Caucasian patients but can be seen in all racial and ethnic groups (*12,15,18,19*).

4. Systemic manifestations

Classic GPA, as detailed by Godman and Churg in 1954 (*4*), includes the triad of necrotizing granuloma of upper and lower respiratory system, systemic vasculitis, and necrotizing glomerulonephritis. The kidneys are usually spared in the limited form of GPA. Classic GPA can sometimes begin with limited organ involvement and then convert to a more generalized form with nose, lung and kidney being affected (*20*). Patients with GPA usually present with nonspecific symptoms of generalized systemic illness including fever, malaise, weight loss, arthralgia, and myalgia (*18*) .

The earliest complaints, which are also the most common reasons for seeking medical attention, are usually related to upper respiratory tract problems including sinus pain, purulent nasal discharge, epistaxis, nasal ulceration, and serous otitis media. The presence of clinical signs such as suppurative otitis, mastoiditis, a saddle-nose defect, and hearing loss should alert the physician for GPA (*7*). It has been shown that over 90% of patients with GPA have upper respiratory tract involvement (*19*) A large number of patients present with pulmonary symptoms (cough, hemoptysis, dyspnea and less commonly, pleuritic chest pain and tracheal obstruction). Bilateral or unilateral pulmonary infiltrates are present in nearly 50% of patients initially, with lung disease eventually developing in 85-90% of patients. Pleural effusion has also been reported in 12% of cases (*21*). GPA can cause significant morbidity and mortality secondary to diffuse pulmonary hemorrhage (*22*).

Although renal involvement is clinically evident in only 11–20% of cases at presentation, glomerulonephritis eventually develops in 77–85% of patients, usually within the first two years of disease onset (*18,19*). Dermatologic involvement has been reported in about 50 % of patients with GPA with purpura involving the lower extremities being the most common finding (*19*). Less commonly, ulcers, vesicles, papules, subcutaneous nodules and lesions resembling those of pyoderma may be seen. Arthralgia and myalgia are seen in 70% of patients (*19*). Nervous system involvement is seen in about one-third of patients with peripheral neuropathies being the most common (*23*). Cranial neuropathies, external ophthalmoplegia, seizures, cerebritis and stroke syndromes are also important findings. Diabetes insipidus may occur when granulomas extend from the sinuses into the pituitary gland (*23*). Cardiac involvement is rare, with pericarditis being the most frequent complications (6%).

5. Ocular manifestations

In a survey of 701 North American patients with GPA, 30% of patients were reported to have ocular involvement (*15*). Other studies have reported similar findings, with ocular involvement in about 50% of the patients (*19,24*). Ocular disease can be the presenting or even the only clinically apparent manifestation of GPA (*25*). Straatsma classified the ocular involvement as contiguous or noncontiguous based on the presence or absence of direct extension from the adjacent involved sinuses (*26*).

Severe ocular morbidity with vision loss or total blindness may be seen in 8-37% of patients, especially if there has been a delay in diagnosis, or if the disease has been inadequately treated (*24*).

6. The orbit

The orbit is one of the most frequently involved ocular structure in GPA, and is more often secondary to extension of sinus pathology (*19,24,27*). Manifestations of orbital disease include proptosis, lid edema, diplopia, and decreased vision. Orbital pain was present in only 30% of patients in an Australian cohort (*28*). Of patients with orbital involvement, 14-30% have bilateral disease (*27*). Damage to ocular structures may result from mass compression, vascular occlusion or spread of an orbital cellulitis. Proptosis occurs in up to one-third of cases (*19*). GPA can present as an orbital mass leading to cranial nerve involvement and entrapment of extraocular muscle resulting in diplopia (*29*). Also, orbital involvement may result in blindness from a compressive ischemic optic neuropathy (*19*). In a recently published National Institutes of Health (NIH) report, a group of 158 patients with GPA were evaluated and about one-half of patients

with retro-orbital involvement lost vision (*19*). Orbital involvement has also been reported in children (*30*).

7. The eyelids

Eyelid changes in GPA may include edema, entropion, trichiasis, and xanthelasma. Woo *et al.* found that some of their patients with lid edema had an orbital mass and recommended consideration of GPA as a potential diagnosis in atypical lid edema (*28*).

8. The lacrimal system

Inflammation of the lacrimal gland (dacryoadenitis) has been reported as a presenting sign of GPA (*31*). This presents with pain and edema of the anterior orbit in the superior-temporal region with swelling of the eyelid and discomfort with eye movement. Nasolacrimal duct obstruction is a late finding and is usually associated with nasal involvement (*32*). Sicca syndrome with positive single strand A/ single strand B (SS-A/SS-B) auto-antibodies has also been reported.

9. The conjunctiva

Conjunctival involvement includes chronic inflammation, sometimes with granuloma formation or ulceration (*33*). Ulcerative conjunctivitis may result in conjunctival cicatrization. The conjunctiva serves as a useful biopsy site if a granuloma is present or as a proxy in those with scleritis or peripheral ulcerative keratitis (PUK) (*34*).

10. The episclera and sclera

Both scleritis and episcleritis have been previously reported in patients with GPA (*35*). GPA can result in nodular, diffuse, or necrotizing scleritis with tendency toward a more severe scleritis compared to other etiologies (*36*). Necrotizing scleritis can lead to significant ocular morbidity with severe vision loss and blindness if not adequately treated. Complications include globe perforation requiring enucleation (*37*). In necrotizing scleritis, an area of the inflamed sclera becomes avascular and ischemic, often secondary to occlusive vasculitis. Hoffman *et al.* (*16*) reported scleritis to be the third most common ocular manifestation of GPA following orbital and nasolacrimal involvement. Necrotizing scleritis has been reported following routine cataract surgery in patients with GPA. In some patients, it has been the presenting sign of GPA, while in others, it occurred despite being in remission (*38*).

11. The cornea

PUK is the most significant corneal complication of GPA. On histopathology, there is an immune-mediated occlusive necrotizing vasculitis of the anterior ciliary arteries. These arteries supply the anterior segment of the eye including the sclera, conjunctiva and the peripheral cornea. Concentration of this hematologic inflammatory milieu in the peripheral cornea leads to ulceration of the peripheral corneal proteoglycans and collagen. This can progress concentrically and/ or centrally and is often bilateral. Owing to the shared blood supply, PUK is often accompanied by scleritis (usually necrotizing) (*39*). It has been proposed that necrotizing scleritis with PUK may characterize systemic vasculitis (*40*). While PUK is the prototypical corneal complication in GPA, many other corneal manifestations have also been described. In some cases, the adjacent scleral inflammation leads to an exudative peripheral keratitis without ulceration. Stromal (interstitial) keratitis, is a rarely described feature of GPA (*41*).

12. The uvea

Although uncommon in isolation, intraocular inflammation has been described in patients with GPA. The uveitis associated with GPA is nonspecific, unilateral or bilateral and can be anterior, intermediate, or posterior with or without vitritis (*8,24,26*). An analysis of a large cohort of patients with anti-neutrophil cytoplasmic antibodies (ANCA) positive vasculitis found an incidence of 17.9% for uveitis: 70% anterior uveitis, 10% intermediate uveitis, and 20% posterior uveitis. The authors noted that 50% of patients with anterior uveitis had a coexisting scleritis (sclera-uveitis), suggesting that often uveitis was a secondary phenomenon (*42*). Also a granulomatous panuveitis has been described as the initial manifestation of GPA (*43*).

13. The retina and choroid

Retinal and choroidal involvement are uncommon manifestations of GPA with vessel involvement (with or without clear vasculitis) being the most common manifestation. Bilateral arterial occlusions of the retinal and choroidal circulations as well as vitreous hemorrhage have been previously reported (*8,44,45*). Bullen and colleagues identified four patients with retinal vasculitis manifesting as retinal hemorrhages and edema, cotton-wool exudates and choroidal thickening (*46*). Choroidal folds with uveal thickening and chorioretinal ischemia with infarction presenting clinically as single or multiple, white or creamy lesions at the level of the retinal pigment epithelium have also been reported (*47*). Central retinal vein occlusion has also been reported in younger people with GPA, although the mechanism remains unclear (*48*). Significant angiopathy and retinal hemorrhages could be the presenting sign of GPA (*49*). Although many chorioretinal manifestations are of vasculitic origin,

there are reports of choroidal granulomatous lesions. Inflammatory sclero-choroidal masses have simulated ocular neoplasms (*50*) .

14. Neuro-ophthalmic

In a large Australian cohort with orbital GPA, binocular diplopia occurred in half of the patients at some point during the study (*28*). Granulomatosis can cause an adjacent mass effect, or directly involve the oculomotor nerves or extraocular muscles. In addition vasculitis can interrupt the nerve's blood supply (28). In a report by Hoffman *et al.*, 50 % of their GPA patients lost vision due to compressive optic neuropathy secondary to orbital granuloma. In another study, Holle *et al.* (*27*) found that of the 25 optic nerves encircled by granulomatous tissue, only six showed signs of compressive optic neuropathy. Retro-bulbar optic neuritis due to adjacent granulomatosis has been described (*51*). Takazawa reported optic perineuritis that caused by granulomatous infiltration in two patients (*52*). Both anterior ischemic optic neuropathy (AION) and posterior ischemic optic neuropathy (PION) have been described in patients with GPA secondary to vasculitis disrupting the blood supply to the optic nerve (*31,53*).

15. Diagnosis

The diagnosis of GPA is difficult and often delayed due to the wide range of clinical presentations. Historically, the diagnosis of GPA has been made following the criteria of granulomatous involvement of upper and lower respiratory tract, glomerulonephritis and varying degrees of systemic vasculitis (*4*). In an effort to diagnose GPA, Fauci and colleagues at the NIH came up with definitive diagnostic criteria for GPA. According to these criteria, a patient should have clinical evidence of disease in at least two of three areas (upper airways, lung and kidney), and biopsy results that show disease in at least one and preferably two of these organ systems (*18*).

The American College of Rheumatology has established the following criteria for the diagnosis of GPA in order to distinguish the disease from other vasculitides: *i*) a urinary sediment containing red blood cell casts or more than five red blood cells per high-power field, *ii*) abnormal findings on the chest radiograph, *iii*) oral ulcers or nasal discharge and *iv*) granulomatous inflammation on biopsy (*22*). The presence of two or more of these four criteria was associated with an 88% sensitivity and 92% specificity.

Another diagnostic system known as the ELK (E for ears, nose and throat or upper respiratory tract; L for lung; and K for kidney) classification system proposed by DeRemee and colleagues utilizes ANCA results (*54*). According to this system, any typical manifestation in the E, L or K supported by typical histopathology or a positive cytoplasmic ANCA (c-ANCA) test qualifies for the diagnosis of GPA (*55*). ANCA has been recognized to be both sensitive and specific for GPA (*56*) and is highly associated with GPA, being present in 80-90% of patients with systemic disease. Still, there are some cases of the disease where ANCA is negative (*57*). Off all the ANCA associated with GPA, 80-95% of cases are associated with c-ANCA with autoantibodies directed against proteinase 3 antibodies (PR3) the remainder are p-ANCA directed against myeloperoxidase antibodies (MPO) (*58,59*).

In patients with limited form of the disease, ANCA is found in 50-80% of cases (*60,28,34*) . In both limited and systemic GPA cases, PR3-ANCA is more common than MPO-ANCA (*57*). Whether ANCA-negative GPA represents a detection issue or different mechanisms that do not involve ANCA is yet to be known (*25*). GPA patients with MPO-ANCA tend to have less severe disease and a more favorable course (*57*).

Despite clinical remission, elevated ANCA titers may still persist in up to 40% of patients, and ANCA titer changes with disease activity in only 64% of patients (*19,61*). A recent study suggested that the presence of MPO-ANCA may be associated with more treatment resistance, and the presence of PR3-ANCA might be a predictor of disease relapse (*62*).

Other Laboratory findings at the time of diagnosis such as leukocytosis, anemia, and thrombocytosis are generally nonspecific (*16*). Although both erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are elevated in patients with GPA, ESR correlates better with disease activity than does CRP (*19*). All immunoglobulins levels may be elevated, especially IgE (*63*).

Rheumatoid factor (RF) has been reported to be elevated in more than 50% of patients (*19*). The presence of abnormal urinary sediment, proteinuria, and abnormal creatinine clearance should raise the suspicion of glomerular involvement.

Biopsy of orbital or ocular tissue can be performed. Perry *et al*. (*64*) reviewed orbital biopsies in patients with GPA and found that common elements included changes in orbital fat (necrosis, lipid-laden macrophages and giant cells), micro abscesses, and granulomatous inflammation. Necrotizing vasculitis was uncommon (*64*). Typical findings on scleral biopsy include: granulomatous foci, polymorphous inflammation (plasma cells, lymphocytes and neutrophils), collagen necrosis and vasculitis (*34*).

Lung tissue is the most commonly biopsied site. A renal biopsy, may show a necrotizing glomerulonephritis (*60*).

Radiologic evaluation of the lungs, trachea, sinuses and orbits can be used to identify additional areas of involvement (and potential biopsy sites).

16. Etiology/pathophysiology

Although the exact etiology of GPA is unknown. It

is believed that the onset of the disease is triggered by an initial insult (infectious or environmental) in a genetically susceptible individual. Furthermore, various allergies are reported to be more common in patients with GPA (*65*). It is possible that infectious agents, such as parvovirus B19 and Staphylococcus aureus, may play a role through providing an antigenic primer especially since some relapses are associated with a preceding or concurrent infection (*66,67*). GPA has been observed in siblings and a higher frequency of certain human leukocyte antigen (HLA) markers (B2, B8, DR1, DR2 and DqW7) has been reported without a consistent relationship to the disease (*68,69*).

The granulomatous and vasculitis features of the disease appear to have separate mechanisms that are being unraveled. There are contributions from both cell-mediated and humoral arms of the immune system where granuloma formation is felt to be a cell-mediated process that could represent an early manifestation of the disease (*70,71*). The vasculitis in GPA is pauci-immune with a predilection for small vessels. Proposed mechanisms for the vasculitis include pathogenic B and T lymphocytes and possibly ANCA autoantibodies themselves which may be compounded by an increase in regulatory T cells (*71*). Presently, GPA-associated vasculitis cannot be fully explained by ANCA alone. Evidence for a direct pathogenic role of ANCA is strongest for MPO-ANCA where animal models for MPO-ANCA induced vasculitis exist in literature (*72*). Additionally, there are two case reports of human infants being born with circulating maternal MPO-ANCA antibodies who developed glomerulonephritis and alveolar hemorrhage that improved with plasma exchange (*73*). The evidence for a direct pathogenic role of PR3-ANCA is less conclusive, and the presence of PR3-ANCA itself has not been found to be pathogenic in mice models. However, the combination of PR3-ANCA and a genetic susceptibility (NOD gene mutation) does lead to vasculitis in mice (*72*). Until today, there is no single animal model that has reproduced both the vasculitic and granulomatous features of GPA (*74*). Given the different mechanisms by which MPO-ANCA and PR3-ANCA incite vasculitis, it is likely that there is more than one pathway leading to the GPA clinical phenotype (*70,72*).

17. Treatment

17.1. *Systemic Treatment*

The average life expectancy for a patient with GPA without treatment is only 5 months, with a 1-year survival rate of less than 20% (*2,17-19*). It is a common misconception that the presence of an ocular manifestation in the absence of systemic manifestations represents a quiet disease. It should be noticed that ocular manifestations, particularly necrotizing scleritis, can be an indicator for both morbidity and mortality unless appropriate systemic treatment is initiated (*37,75*).

The best treatment approach requires team collaboration between different medical specialties in order to cover the different organs involved by the disease. Because of the importance of systemic therapy, a brief overview of current regimens will be discussed. Historical systemic treatment included a variety of modalities such as antibiotics, chelating agents and local irradiation (*17,76*). None of these modalities were successful. Corticosteroid treatment was also tried and it has been shown that corticosteroid alone doubled the life expectancy to about 12 months with a 1-year survival of 34% (*77*). Adding cyclophosphamide to corticosteroid therapy altered the prognosis of the disease and resulted in remission and extension of the survival rate (*33*). Once remission has been achieved, it is recommended that cyclophosphamide treatment to be continued for at least another year before tapering the medication. Both oral and intravenous cyclophosphamide, in combination with corticosteroids, have been used successfully with equal effectiveness (*61,78,79*). Because of the significant toxicity associated with cyclophosphamide therapy, alternative maintenance therapies have been used. Azathioprine has shown some success, but it is less effective than cyclophosphamide and should only be considered in patients experiencing adverse side effects or when fertility concerns arise (*80*). Methotrexate has been used in patients with limited GPA, though it is less likely to achieve and sustain remission (*81,82*). Recently, trials confirmed that B cell depletion with rituximab were comparable to cyclophosphamide as part of induction therapy for active ANCA-associated vasculitis and with possibly superior performance in relapsing disease (*83,84*). Rituximab is a monoclonal antibody that targets the CD20 antigen on B cells and clears circulating B cells from the circulation, without affecting plasma cells which may be important in disease relapse (*84,85*). Neutropenia may develop, for which the patient should be monitored. Studies on the long-term effectiveness of rituximab are still in progress. The rituximab in ANCA-Associated Vasculitis (RAVE study) (*83*), a double-blind, randomized, multicenter trial, showed that rituximab is a non-inferior alternative to cyclophosphamide for induction. Following this, Holle *et al*. (*86*) reported that rituximab was effective for vasculitic manifestations refractory to cyclophosphamide but was less effective for granulomatous manifestations. There are case reports of success treatment with infliximab as an adjuvant therapy to cyclophosphamide and methotrexate in the treatment of two adults with necrotizing scleritis and one child with non-necrotizing scleritis (*87,88*).

Other therapies demonstrating some efficacy for induction and/or maintenance of remission have included mycophenolate mofetil, plasmaphereses, cyclosporine, intravenous immunoglobulin (IVIG), and protein A immunoadsorption (*89*). Trimethoprim-

sulfamethoxazole has been reported to be beneficial in patients with the limited form of GPA, where there is no renal involvement (*90*).

Given the success of rituximab in GPA, other B lymphocyte targeting medications are being under investigations. There is currently a clinical trial underway to determine if a monoclonal antibody against B Cell Activating Factor can prevent relapse (*91*). Therapies to modulate the memory T cell (Th17) pathway are in development, though no studies specific to GPA are yet in progress (*91*). Given the presumed role of T lymphocytes in granuloma formation, it remains hopeful that therapies directed against T lymphocytes may benefit patients with refractory orbital granulomatous inflammation (*74*). An inhibitor to the activated complement molecule c5a is being studied as an induction agent in patients with GPA (*81,91*).

17.2. *Local treatment (medical/surgical)*

Local corticosteroid therapy can be used for the treatment of some non-vision threatening ocular manifestation of GPA such as conjunctivitis and episcleritis, with careful monitoring of severe ocular complications that might appear during the course of the disease. These complications such as scleritis, PUK, uveitis and retinal and optic nerve vasculitis usually fail to respond to local therapy alone and require the use of systemic immunosuppressive therapy as soon as possible (*92*). Of note, orbital granulomas are more prone to relapse and many authors have found that these granulomas may only be partially responsive to cyclophosphamide or rituximab (*86,93*). Of the 40 patients with orbital granulomas reported by Holle *et al.*, 41% of cases were refractory to cyclophosphamide induction (*27*). Despite intensive immunosuppression, 72% developed some form of visual impairment and 19% suffered blindness secondary to optic nerve compression. Surgery is of limited benefit and is reserved for grave situations. Orbital decompression with de-bulking of the granuloma can be done in cases of compressive optic neuropathy. In cases of severe pain and complete blindness, retro-bulbar alcohol injection or enucleation can be used as palliative measures (*27,94*). In cases of nasolacrimal duct obstruction, surgical creation of a new outflow (dacryocystorhinostomy) is required to bypass obstruction and relieve epiphoria for resolution of these symptoms, though it is not without risk. Postoperative wound necrosis and naso-cutaneous fistula have been reported (*95*). The rate of adverse events is improved with preoperative and postoperative control of the underlying disease (*28,96*).

Necrotizing scleritis and PUK can progress with resultant globe perforation. At this point, conjunctival resection, Tectonic scleral grafting, and cyanoacrylate glue are all used as temporizing measures while the systemic disease is brought under control (*39*). Even after remission is achieved, patients with corneal or scleral thinning can perforate with minor trauma and the use of polycarbonate safety glasses are highly recommended.

Cataract and glaucoma are common in patients with GPA and are secondary to the chronic inflammation and corticosteroid treatment rather than the GPA itself. It is recommended that no surgery should be done during active disease, and even when the patient is in remission, the patient needs to be monitored closely during the post-operative period.

18. Prognosis

Although the prognosis of GPA has dramatically improved with the introduction of immunotherapy, there is still significant morbidity from the disease itself (86%) or side effects from the therapy (42%) (*19*). It has been shown that the presence of prior relapses is a predictor of future relapses (*72*).

The visual prognosis depends on severity and chronicity of the eye disease and, in general, is good when treated appropriately with systemic immunotherapy. Vision loss or total blindness may be seen in 8-37% of patients, especially if the disease has been long-standing or inadequately treated, or when there has been a delay in diagnosis (*19,24*).

Major causes of vision loss in the setting of GPA are compressive optic neuropathy, retinal and optic nerve vasculitis, and globe perforation from necrotizing scleritis and peripheral ulcerative keratitis. Holle *et al.* (*27*) showed that the risk of blindness is higher with longer time to remission, higher number of relapses or the presence of refractory disease. In general, the prognosis for limited GPA is better than for the complete form. Despite systemic immunotherapy, patients with severe renal disease have a guarded prognosis with higher mortality rate (*97*).

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Review

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Cardiac manifestations of idiopathic pulmonary fibrosis

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Summary **Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, parenchymal disease of the lung with an estimated prevalence of 14-43 per 100,000. Patient usually presents with coughing and exertional dyspnea, which can lead to acute respiratory failure. IPF has been associated with various co-morbidities such as lung cancer, emphysema, obstructive sleep apnea (OSA), GERD and multiple cardiovascular consequences. The cardiovascular manifestations of IPF include pulmonary hypertension, heart failure, coronary artery disease, cardiac arrhythmias & cardiac manifestations of drugs used to treat IPF. This review will outline evidence of the association between IPF and cardiovascular conditions and attempt to provide insights into the underlying pathophysiology. We also discuss the impact of these cardiovascular diseases on patients with IPF including increased morbidity and mortality.**

Keywords: Idiopathic pulmonary fibrosis, pulmonary hypertension, right heart failure, atrial fibrillation, arrhythmias, coronary artery disease

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, parenchymal disease of the lung with a median survival of 3-5 years after diagnosis. The course can be variable ranging from slow progression of the disease over years to rapid decline and death over a period of months (*1*). IPF has an estimated prevalence of 14-43 per 100,000 and is the most common idiopathic interstitial pneumonia (*2*). The incidence of IPF is increasing given the aging population and an increase in the diagnosis due to prevalent use of chest imaging (*3*).

The patient usually presents with coughing and exertional dyspnea, which can lead to acute respiratory failure. Findings on exam include "Velcro" like rales on lung exam and other signs of chronic lung disease. According to the American Thoracic Society/the European Respiratory Society (ATS/ERS) statement on IPF (*4*), diagnosis is based on the presence of usual interstitial pneumonia (UIP) pattern on high-resolution computed tomography (HRCT), histology patterns and the exclusion of other known causes of interstitial lung disease (ILD). Thus a multi-disciplinary approach is involved in diagnosing these patients (*4*).

IPF has been associated with various co-morbidities such as lung cancer, emphysema, obstructive sleep apnea (OSA), GERD and multiple cardiovascular consequences. The cardiovascular manifestations of IPF include pulmonary hypertension, heart failure, coronary artery disease, cardiac arrhythmias & cardiac manifestations of drugs used to treat IPF. This review will outline evidence of the association between IPF and cardiovascular conditions and attempt to provide insights into the underlying pathophysiology.

2. Pulmonary hypertension & right heart failure in IPF

Pulmonary Hypertension (PH) and Right heart failure (RHF) are very severe complications of IPF and contribute significantly to morbidity and mortality in IPF (*5*). Despite this, PH in IPF is not well studied

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except in severe IPF. PH is now defined as mean pulmonary arterial pressures (mPAP) more than or equal to 25mm Hg at rest, confirmed by right heart catheterization (RHC) (*6*). According to the updated Venice clinical classification of PH, PH associated with IPF is categorized as group 3 PH, which includes PH in chronic lung disease and/or hypoxemia (*7*).

It has been very difficult to estimate the epidemiology of PH in IPF (PH-IPF). IPF is usually an insidious disease and usually diagnosed later in its course, which might lead to overestimation of incidence of PH in these patients. Also the accuracy of diagnosis of PH in IPF with transthoracic echocardiogram has been seriously questioned (*8*). Right heart catheterization (RHC), considered to be the gold standard in diagnosis of PH, is invasive and inconvenient which has made it hard to study PH-IPF in longitudinal studies. Lastly, most of the data about PH-IPF comes from small cohort of patients who are referred for lung transplantation evaluation, which does not represent all patients with IPF. Lettieri *et al*. studied IPF patients who underwent RHC for lung transplant evaluation and found that 32% patients had PH (*9*). Patel *et al*. studied a similar population in their center and found that 22% patients had PH (*10*). More recently in the multicenter ARTEMIS-IPF trial of ambrisentan, RHC done to risk stratify patients showed that 10% of patients had PH. This study was unique because it excluded the severely sick patients (FVC < 50 % baseline and extensive honeycombing), which gives us insight into prevalence of PH in mild IPF. Only 5% of the patient population developed PH during the follow up RHC at 48 weeks indicating that PH progresses slowly in mild, clinically stable patients with IPF (*11*).

Pulmonary vasculopathy in IPF has partly been attributed to hypoxia induced smooth muscle hypertrophy and collagen deposition in small pulmonary arteries, muscularization of arterioles and intimal proliferation of venules. Vascular destruction and obstruction due to progressive parenchymal fibrosis also contributes to this process. Thrombosis in situ in pulmonary arteries has also been observed. Increase in levels of cytokines like endothelin-1, Fibroblast growth factor beta, platelet derived growth factor lead to vascular remodeling contributing to PH (*12*).

Most of the symptoms of PH overlap with IPF making it difficult to diagnose PH-IPF unless there is high clinical suspicion. Physical signs like accentuated pulmonary heart sound, tricuspid regurgitation murmur and fixed splitting of S2 can bee seen in PH. Signs of right ventricular failure like elevated jugular venous pulse, peripheral edema and hepatomegaly can be seen in advanced cases. Signs of pulmonary hypertension on Computed tomography (CT) of the chest include right ventricular dilation (RVD, increased main pulmonary artery diameter greater than 29 mm and pulmonary artery diameter greater than that of aorta (*13*). The electrocardiogram (EKG) might show signs of RVD. These include ST wave depression or T wave inversions in leads associated with the RV. But neither CT scan nor EKG has enough accuracy for diagnosing PH-IPF (*14,15*). Pulmonary function parameters have poor association with PH (*5*). A combination of decreased DLCO (< 40%) and need for supplemental oxygen is very specific (94%) but not sensitive (65%) in predicting PH (*9*). Transthoracic Echocardiogram (TTE) is a very useful non-invasive test to diagnose PH with sensitivity of 80-100% and specificity of 60-98%. But in presence of chronic lung disease, TTE has significant limitations. Arcasoy *et al*. demonstrated that right ventricular systolic pressure (RVSP) could be estimated in less than 50% of patients with advanced lung diseases. In this study less than 37% of people who had PH on TTE were found to have PH on RHC (*8*). Plasma B type natriuretic peptide (BNP) may serve as a screening tool as it has more than 85% sensitivity and specificity in identifying PH in patients with chronic lung disease (*16*). But for now, RHC remains the gold standard in diagnosis of PH in IPF patients.

3. Cardiac arrhythmias and IPF

Atrial arrhythmias (AA) are the most commonly seen arrhythmias in patients with IPF. Amongst them, increased incidence of Atrial Fibrillation (AF) and Atrial Flutter (AFL) has been the most commonly reported arrhythmias in patients with IPF. Nielsen *et al*. did a systematic analysis in 2004 where increase incidence of atrial fibrillation was found post-operatively in patients who underwent lung transplant (*17*). The highest incidence of AF (55.9%) was found in patients who underwent lung transplantation for IPF and IPF was found to be a significant predictor for AF (OR, 2.3; CI, 1.1 to 4.8; $p = 0.03$). Exclusion of the patients with previous history of AF did not lead to a significant change in the results. Azadani *et al*. did a retrospective analysis of the lung transplant patients to assess the predictors for atrial flutter (AFL) (*18*). Out of 269 patients who underwent lung transplant, 35 (13%) patients developed AFL. Again, the incidence of AFL was found to be higher in patients with IPF at 65.7% (OR, 2.6, 95% confidence interval 1.1 to 6.1, $p = 0.03$). A second multivariate analysis showed IPF as an independent predictor for AFL (OR, 2.94; CI, 1.31-6.62; $p = 0.009$) (4) (*19*). Orrego *et al*. also performed a retrospective analysis to determine the predictive factors of arrhythmias post-operatively in patients undergoing lung transplant (*20*). The incidence of AA was found to be 25.4% in 366 patients (AF - 17.8% and AFL/SVT - 7.6%). Multivariate analysis to determine the risk factors showed IPF as one of the significant causes (OR, 1.98, 95% CI, 1.15 to 3.40; $p = 0.013$). The possible explanations for this increased incidence include native fibrosis from IPF making

surgery difficult, increased age and the increased risk of coronary artery disease in patients with IPF. Shibata *et al*. studied patients with IPF and COPD over 5 years to assess the causal relationship between pulmonary function tests and the development of AF (*19*). They reported that both decrease in FEV% (forced expiratory volume) (OR, 0.982; CI, 0.965 to 0.999; $p < 0.05$) and FVC% (functional vital capacity) (OR, 0.977; CI, 0.956 to 0.998; $p < 0.05$) were independent risk factors for development of AF.

The postulated mechanisms for arrhythmias in these patients include the presence of hypoxia, elevation of pulmonary pressures, the risk of coronary artery disease and the presence chronic inflammation (*19,21*). Hypoxia results in increased sympathetic activity thereby increasing the risk for arrhythmias. Elevated pulmonary pressures (pulmonary hypertension) can lead to increased incidence of arrhythmias. The trigger for AF is around pulmonary veins, so alteration of these hemodynamics might explain the increased risk. The increased risk of coronary artery disease and associated heart failure also leads to an increased of arrhythmias (*22*). Lastly, chronic inflammation associated with IPF leads to an increased level of cytokines such as Il-6 and TNF-a, which could act as a trigger for various arrhythmias (*23*).

4. Coronary artery disease and IPF

A number of studies have demonstrated a relation between coronary artery disease (CAD) and IPF. Kizer *et al*. showed that pulmonary fibrosis was associated with an increased incidence of CAD when compared to non-fibrotic lung diseases (OR, 2.18; 95% CI, 1.17 to 4.06) (*22*). They hypothesized a causal relation wherein pulmonary fibrosis promotes atherosclerosis as evidenced by the increasing serum levels of interleukins, cytokines, circulating immune complexes and development of fibrosis in extra-pulmonary organs like the digits and mediastinum. Of the immune mediators, Interleukin-4 (IL-4), Interleukin-8 (IL-8) and tumor necrosis factor-α (TNF- α) are common elements, responsible for angiogenesis, which are elevated in IPF. Clubbing, a common finding in IPF, involves neovascularization and fibroplasia, which is observed in both IPF and atherosclerosis. Hence the presence of fibro-proliferative process beyond the lungs and the presence of mediator molecules produced in these disorders might explain the possible mechanisms of increased atherogenesis in these patients. Other possible mechanism includes hypoxia causing worsening angina. It is also possible that the presence of a severe lung disease might lead the attention away from routine cardiovascular care with decreased primary and secondary prevention. This hypothesis is supported by the fact that people with IPF were less likely to receive statins and beta-blockers than the general population (*24*).

Izbicki *et al*. in their analysis showed that CAD was present in 28.6% of patients with lung fibrosis in comparison to 9.8% patients with emphysema (*25*). This was remarkable given the fact that almost 98% of patients in the emphysema group were smokers versus only 31% in the fibrosis group, thus eliminating smoking as a confounding factor. In a population based study, Hubbard *et al*. compared 920 cases of IPF with 3593 matched controls and found an increased risk of acute coronary syndrome (OR, 1.53; 95% CI, 1.15 to 2.03) and angina (OR, 1.84; 95% CI, 1.48 to 2.29) (*24*). During the follow up period, again there was an increased risk of acute coronary syndrome (RR, 3.14; 95% CI, 2.02 to 4.87) with the mean follow up of 2.7 years from the date of diagnosis of IPF. In a comparative study between transplant candidates for COPD and IPF, Nathan *et al*. showed that the prevalence of CAD was found to be 65.8% in the IPF group compared to 46.1% in patients with COPD ($p < 0.028$) (*26*).

Cicchitto *et al*. suggest performing an extensive Cardiopulmonary Exercise Testing (CPET) in patients with IPF not only for identifying useful prognostic parameters, but also to detect potentially treatable cardiovascular alterations. However further studies are needed to better assess the cost and benefits of routine CPET testing in all IPF patients (*27*). The value of coronary calcification on chest computed tomography (CT) as a marker for CAD in patients with IPF was evaluated by Nathan *et al*. which showed that the sensitivity of moderate to severe calcification for significant coronary artery disease was 81% while the specificity was 85% with an associated odds ratio of 25.2 (4.64 to 166, $p < 0.005$) (*28*). Thus the presence of coronary calcification on chest CT's in patients with IPF can indicate the need for further cardiac workup to assess for the presence of CAD.

5. Drugs to treat IPF and their effects on the heart

Evidence-based guidelines for the diagnosis and management of IPF were published in 2011 (*29*) and were recently updated in 2015 based on a multidisciplinary panel review of articles published in the interim (*4*). No medication till date has been shown to cure IPF but two new drugs; Pirfenidone and Nintedanib have shown to slow disease progression in clinical trials.

The ASCEND study group studied Pirfenidone in 555 patients with IPF in a phase 3 randomized controlled trial (RCT) and when compared with placebo, Pirfenidone reduced disease progression, as reflected by lung function, exercise tolerance, and progression-free survival, in patients with idiopathic pulmonary fibrosis. The side effect profile of Pirfenidone mainly included gastrointestinal side effects but no cardiac side effects were reported (*30*) and the same results were found in the CAPACITY trial, which was performed in 113

centers in 13 countries (*31*). Patients who completed the CAPACITY studies were eligible to enter the ongoing open-label, long-term, follow-up extension study, RECAP and an interim analysis done in August 2013 showed a similar side effect profile to the CAPACITY trials and again without cardiac side effects (*32*). A comprehensive analysis of safety outcomes performed by Lancaster *et al*. in a large and well-defined cohort of 1299 patients with IPF who were followed prospectively for up to 9.9 years demonstrated that long-term treatment with Pirfenidone is safe and generally well tolerated and again without any cardiac side effects (*33*). Pirfenidone when studied in animal models has in fact been found to have a beneficial effect on myocardial infarction, atrial fibrillation and diabetic cardiomyopathy. This is believed to be due to a 2 prong mechanism in which it not only reduces the myocardial fibrosis and stiffness but it also stimulates the L-Type voltage gated Ca^{2+} channels which are pivotal in the excitation contraction coupling and thus helping systolic function (*34*).

Nintedanib, studied in the INPULSIS® 1&2 and also in the TOMORROW trial, was associated with a trend toward decline in lung function with fewer exacerbations (*35*). The adverse effect profile of Nintedanib was also primarily gastrointestinal but cardiac events were also reported. 9.7% of patients in the INPULSIS®-1 study group were found to have a cardiac adverse event while 10.3% of patients were found to a cardiac adverse event in the INPULSIS®-2 study group. These events were fatal in 0.3% (1/30) and 0.6% (2/34) of patients in the INPULSIS® 1&2 study groups respectively. Ischemic heart disease was also reported in 4.2% and 4.3% of patients in the INPULSIS® 1&2 study groups respectively (*36)*.

Other medications studied for the treatment of IPF include a RCT comparing a combination therapy of prednisone, azathioprine and N-Acetylcysteine versus placebo. It showed an increased risk of death and hospitalizations thus recommending against the use of this combination for the treatment of IPF. It also showed cardiac adverse events in 3/77 patients in the treatment arm (*37*).

6. Impact of cardiac manifestations of IPF

IPF is a life threatening disease. The median survival of patients with IPF is 2.5 to 3.5 years (*38*). Age, sex, ethnicity, smoking are the common predictors of mortality in IPF. Although, the most common cause of death in IPF patients is respiratory failure from progression of the disease, death due to cardiac disease have been reported in the patients (*26)*. It has a significant impact on the mortality and hospitalizations related to IPF (Table 1).

Presence of PH in IPF is associated with increased morbidity and mortality. Lettieri *et al*. showed presence of PH in IPF was associated with a significant decrease in exercise capacity, as measured by a 6 minute walking test (6MWD) (*9*). Patel *et al*. found similar results in patients undergoing evaluation for transplant (*10*). Nadrous *et al*. showed that patients with RVSP > 50 mm hg had significantly worse survival (*39*). Lettieri *et al*. also showed that presence of PH was associated with significantly higher 1 year mortality rates (*9*). Presence of PH also has an impact on transplant outcomes in patients with advanced lung disease. Whelan *et al*. showed that increased PAP is associated with increased 90-day mortality post single-lung transplantation and 30-day mortality post double-lung transplant in patients with IPF (*40*).

Table 1. Cardiac Manifestations of Idiopathic Pulmonary Fibrosis

Pulmonary hypertension
Right sided heart failure
Arrhythmias
- Atrial Fibrillation
- Atrial Flutter
- Supraventricular tachycardia
- Multifocal atrial tachycardia
- Other atrial arrhythmias
Coronary artery disease
Cardiac manifestations of drugs used to treat IPF

The presence of associated cardiovascular co-morbidity can significantly reduce the survival and outcomes in the IPF patients who undergo lung transplantation. Orrego *et al*. studied the effect of atrial arrhythmias on outcomes and mortality of IPF patients who underwent lung transplant (*20*). The one-year mortality rate was found to be higher (21.5%) in AA group compared to the one without AA (15.7%) ($p < 0.05$). Also, length of the stay in the hospital (morbidity) was increased with presence of AA (median: 20 days) compared to the patients with AA (median: 15 days) ($p < 0.0001$). Nielsen *et al*. demonstrated a similar increase in morbidity and mortality in patients with atrial arrhythmias amongst IPF patients after lung transplantation (*17*). The presence of AF led to increased the length of stay, (32.4 ± 60.0 days *vs*. 17.5 ± 24.1 days, $p = 0.04$) increased in-hospital deaths (OR, 5.7; CI, 2.1 to 15.1; $p = 0.0005$) and an increase in the chances of tracheostomy (OR, 3.6; CI, 1.8 to 7.3; $p = 0.0003$) in these patients.

Presence of CAD also has a significant effect on the outcomes of IPF patients who undergo lung transplantation. Nathan *et al*. showed that outcomes of IPF patients with significant CAD was worse than those with no or non-significant disease ($p < 0.003$) with a median survival of 572 days from the time of left heart catheterization (*26*).

7. Conclusion

Cardiac disease has a significant effect on the mortality of IPF patients. Dyspnea and hypoxia from

cardiovascular disease can be masked in these patients. On the other hand, cardiac disease can be worsened by hypoxia and pulmonary hypertension from the IPF. It is prudent to monitor these patients for cardiac manifestations and cardiac events to reduce the overall morbidity and mortality.

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Review

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The progress of early growth response factor 1 and leukemia

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Summary **Early growth response gene-1 (*EGR1*) widely exists in the cell nucleus of such as, zebrafish, mice, chimpanzees and humans, and it also can be observed in the cytoplasm of some tumors. *EGR1* was named just after its brief and rapid expression of different stimuli. Accumulating studies have extensively demonstrated that the widespread dysregulation of *EGR1* is involved in hematological malignancies such as human acute myeloid leukemia (AML), chronic myelogenous leukemia, chronic lymphocytic leukemia, multiple myeloma, and B cell lymphoma. With the deep research on *EGR1*, its expression, function and regulatory mechanism has been gradually elucidated, and provides more possibilities for treatment strategies of patients with leukemia. Herein, we summarize the roles of *EGR1* in its biological function and relationship with leukemia.**

Keywords: Early growth response gene-1 (*EGR1*), acute myeloid leukemia, tumor

1. Introduction

Early growth response gene-1 (*EGR1*), also known as *NGFI-A*, *krox-24*, *ZIF268* and *TIS8*, is an immediate early gene which encodes a Cys2-His2-type zinc finger transcription factor widely expressed in eukaryotic cells from yeast to humans (*1-3*). It is one of the largest studies of tumor-specific proteins, which are located in the 5q31 region (*4,5*). It has an important role in controlling synaptic plasticity, wound repair, female reproductive capacity, inflammation, growth control, differentiation, apoptosis and tumor progression (*6*). Experiments have also proved that acute myeloid leukemia and myelodysplastic syndromes are associated with heterozygous loss of *EGR1* (*7*). Here, we focus on the relationship of *EGR1* with acute myeloid leukemia.

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2. The summarization of EGR1's discovery and function

EGR1 was first discovered in the mid-1980s (*8*). The EGR family includes *EGR1*, *EGR2*, *EGR3*, *EGR4* four related members, that can quickly and briefly be up-regulated through a variety of external stimuli, including activation, proliferation and differentiation signals, tissue damage and apoptosis signals (*9*). *EGR1*, *EGR2*, *EGR3* and *EGR4* share a highly conserved DNA binding domain, composed of three zinc finger motifs that together bind to a 9-bp G/C-rich consensus sequence (GCGGGGGCG) (*10*). It has been used extensively as a model system for detecting how TFIIIA-like zinc fingers recognize DNA, and how it has served as a basis for engineering some types of artificial DNA-binding proteins (*11*). EGRs are involved in regulating the immune response by means of the induction of differentiation of lymphocyte precursors, and activation of B and T cells (*12*). *EGR1* binds to DNA G/C-rich sequences through 3 zinc-finger motifs in its carboxyl terminal and regulates gene transcription through co-operation with other activating or repressing factors (*13*). It may be divided into three zones. The N-terminal portion (amino acids 1-331) is rich in proline (14.2%) and serine (16%) and has 7.9% alanine and 7.9%, threonine. The C-terminal region

(residues 417-533) also contains a very high proportion of proline and serine (15.4% and 26.5%, respectively) as well as 10.3% alanine and 11.1% threonine (*14*).

3. Biological function and role in tumors

The *EGR1* gene encodes a zinc finger protein and its expression is modulated in diverse biological systems with kinetics resembling those of c-fos (*14*). *EGR1* together with c-fos is crucial for normal myeloid cell differentiation through transcriptional regulation (*15*). Gene expression analysis revealed that *EGR1* and *c-fos* were down-regulated in hematopoietic primitive cells (*16*). *C-fos* and *EGR1* represent the key transcription factors that are differentially activated by macrophage colony-stimulating factor (M-CSF) and granulocyte colony-stimulating factor (G-CSF) to resolve neutrophil versus monocyte cell fate (*17*). However, *EGR1* has more of an advantage than c-fos because of different structure, which increases its expression and decreases sensitivity to stimulation (*18*). *EGR1* can regulate cell growth, differentiation, growth inhibition, and apoptosis in various kinds of cells (*19*). Many factors can regulate expression of *EGR1*, including *miR-424*, *miR-146a*, *miR-181a*, *E2h2*, wilms tumor suppressor 1 (WT1), and Iron (*9,20-25*). It's also reported in the literature that *EGR1* can be regulated by erythropoietin (EPO) (*26,27*). *MiR675* upregulates long noncoding RNA H19 through activating *EGR1* in human liver cancer (*28*). More importantly, *EGR1* can regulate some signaling such as p53, transforming growth factor beta 1 (TGFβ1), phosphatase and tensin homolog deleted on chromosome ten (PTEN), Fibronectin, and enterovirus 71 (EV71) (*29-32*). The promoter of the human TGFβ1, p53, and the fibronectin gene contains at least two EGR1-binding sites, both of which can bind *EGR1* to activate transcription. The proximal promoter of PTEN is GC rich and contains one functional *EGR1*-binding site (*29*). Moreover, it plays important roles in decidualization, megakaryocyte differentiation, apoptosis, tendon development, lung injury, liver injury, kidney diseases, chronic obstructive pulmonary disease (COPD), angiogenesis, fibrosis, atherosclerosis, cell cycle and other biological functions (*33-52*). *EGR1* has a critical role in promoting autophagy and apoptosis in response to cigarette smoke exposure *in vitro* and *in vivo* (*53*). *EGR1* controls metabolism, especially its suppression of lipolysis and promotes fat accumulation by inhibiting the expression of triglyceride lipase (*54*). Although the expression of *EGR1* is low in most tissues, it is high in islets. *EGR1* regulates insulin gene expression by up-regulating Pdx1 (*55*). *EGR1* gene expression may contribute to the decrease of B-cell proliferation and the consequent cell failure observed in the later stages of type 2 diabetes (*56*). The increase of *EGR1* expression in the brain is associated with formation of emotional memory and schizophrenia (*57*). It has been proved that *EGR1* mutant mice had no changes in short-term memory, but long-term memory was severely damaged (*58*). Ischemia-induced *EGR1* expression may exaggerate brain injury by reducing brain-derived neurotrophic factor (BDNF) expression (*59*). *EGR1* exhibited a biphasic expression behavior. It was previously described to be down-regulated in many breast carcinoma tissues while it was upregulated in highly invasive inflammatory breast carcinoma. It started to be upregulated 4 h after SNAI1 induction, and was repressed after 24 h (*6*). Interestingly, in prostate cancer, kidney cancer and stomach cancer *EGR1* stimulates the growth of tumor cells, and is associated with poor prognosis. In contrast, *EGR1* is a tumor suppressor in fibrosarcoma, glioblastoma, melanoma, esophageal cancer, lung cancer and breast cancer (*60-64*).

4. Pathogenesis mechanism of AML by *EGR1*

In the absence of *EGR1*, a significant increase in cell cycling occurs in hematopoietic stem cells (HSCs), culminating in an increased number of HSCs and an increased frequency of primary reconstitution under limiting dilution conditions. Most interestingly, loss of *EGR1* causes efficient mobilization of HSCs out of their niches (*65*). Abnormalities of chromosome 5 are common aberrations in acute myeloid leukemia (AML), with del(5q) the most frequent (*66,67*). There is also literature, which shows that *EGR-1* was related to recurrent disease following high-dose chemotherapy (*68*). Nevertheless, *EGR1* haploinsufficiency alone *in vivo* does not lead to expansion of HSCs or abnormalities in adult hematopoiesis. It has been proven that loss of a single allele of more than one gene on 5q contributes to the pathogenesis of AML (*69-71*). A number of genes and several microRNAs (miRNAs) located on 5q, including *miRNA-145*, *miRNA-146a*, the ribosomal protein S14 (RPS14), the cell division cycle 25 (CDC25), the adenomatous polyposis coli gene (*APC*) have been implicated in the development of myeloid disorders caused by a gene dosage effect (*72,73*). (Figure 1) *EGR1* may play a functional role in the pathogenesis of AML in patients with del(5q) (*74,75*). The loss of *EGR1* or inactivation increases risk of AML (*76*). Using locus-specific probes, a deletion of the *EGR1* locus 5q31, 7q31 and the *TP53* gene was observed in 103 (82%), in 57 (46%) and in 66 (53%) patients respectively. Thirty patients (24%) showed a deletion of all three loci, and in only 13 cases (10%), 5q31, 7q31, or 17p13 was not deleted. An EGR1 deletion alone was observed in 19 cases (15%) in only five and four AMLs respectively (*77*). In an attempt to define the loss of the 5q31.1 region, fluorescence in situ hybridization analysis was performed in HL-60 cells, which spanned the *EGR1* and *IL9* gene interval, which was previously shown to be a critical region of loss in AML (*78*). Loss of the *EGR1* gene with deletions of 7q31 or TP53 alone played a role in at least two

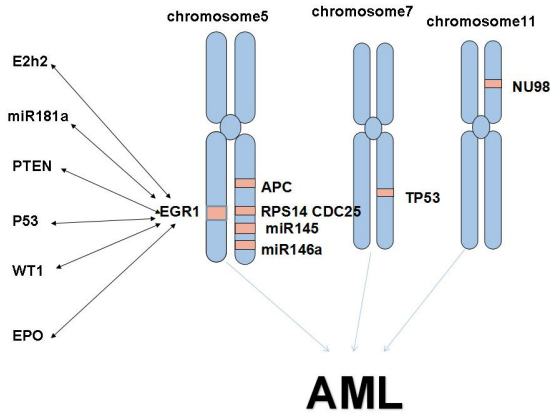

Figure 1. E2h2, miR181a, PTEN, P53, WT1, EPO and EGR1 can regulate each other. The cooperation of EGR1, APC, RPS14, CDC25, miR145, miR146a, TP53 and NU98 may lead to the formation of AML.

aspects. First, *EGR1* directly controls the expression of fibronectin (FN1) through pathways that involve GFB1 and plasminogen activator-1 (PAI1). Thus, FN1 and PAI1 act together to inhibit the growth of cancer cells. Second, *EGR1* is required for p53-dependent apoptosis through the mediation of retinoblastoma (*79*). To examine the role of *EGR1* in hematopoiesis, $EGR1^{+/-}$ and $EGR1^{-/-}$ mice was characterized, and found that $EGR1^{+/-}$ and $EGR1^{-/-}$ mice develop T-cell lymphoma or a myeloproliferative disorder (MPD) at an increased rate and a reduced latency over that observed in wild-type littermates. $EGR1^{+/-}$ and $EGR1^{-/-}$ mice develop T-cell lymphoma or MPD at the same rate and latency, suggesting that loss of a single allele of EGR1 is sufficient for disease predisposition. This is consistent with observations in patients with AML characterized by abnormalities of chromosome 5, in that only 1 EGR1 allele is affected (*80*). Interestingly, *EGR1* is regulated by multiple factors in AML. The cyclin-dependent kinases (CDK) CDK6 and Src family kinases (SFKs) inhibit expression of *EGR1* (*81,82*). On the contrary, Llgl1 (lethal giant larvae homolog 1) and PMA (Phorbol 12-myristate 13-acetate) contribute to the differentiation of hematopoietic stem cells (*83,84*). Andra Schaefer *et al*. found that the expression of EGR-1 had a regulatory role in Epo signal transduction in leukemia cells (*85*).

5. The possibility of *EGR1* as therapy target of patients with AML

The primary structure of the EGR1 protein suggests that it is a DNA-binding protein with transcriptional regulatory activity, and it may function as a tumor suppressor locus whose absence or loss of function could lead to deregulated cell growth (*86*). This gives us an inclination that *EGR1* or *EGR1* target gene is useful for treatment of blood malignant tumors (*87*). One study mentioned that *EGR1* and p21 are key signaling molecules of genipin-induced apoptosis in gastric cancer cells (*88*). Another article revealed that the down-regulation of EGR1-p21 expression provides a mechanism for improved hematopoiesis (*89*). Histone deacetylase (HDAC) inhibitors can reactivate EGR1 in various cell types, leading to decreased cell proliferation and increased cell apoptosis (*90*). HDAC recruitment may participate in the repressive mechanism that EGR1 directly represses myocyte enhancer factor 2 (MEF2) activity for treatment of cardiac disease (*91*). Experimental evidence has demonstrated that EGR1 diminished the aggressiveness of M1myc leukemia and abrogated the leukemic potential of IL-6-treated M1myc cells. Altered EGR1 expression can work together with deregulated c-Myc in exacerbating the leukemic phenotype (*92*). It is also reported that *EGR*-1 plays an indispensable role in the regulation of platycodon D-induced cell death and the 1, 25D3-induced cessation of cell proliferation, which is characteristic of the terminal stage of differentiation of these cells (*93,94*). *EGR1* and WT1 are structurally related transcription factors and bound to quite similar DNA sequences (*95*). This gives us a revelation that down-regulating the expression of WT1 can up-regulate the expression of *EGR1*. In this way, inhibition of proliferation and differentiation of leukemia cells is no longer a problem. *EGR1* is also important for development of the macrophage lineage (*96*). It is interesting to note that *EGR-1* abrogates the block in M1 terminal differentiation imparted by oncogenic c-Myc or E2F-1, suppressing their leukemia promoting function in nude mice (*97*). A novel mechanism of thalidomide in the treatment of leukemia is that thalidomide could suppress leukemia cell invasion and migration by upregulation of *EGR*-1 (*98*). Also that paeoniflorin (PF) playing a role in human leukemia U937 cells is based on the regulation of *EGR1* (*99*). LY294002 (LY29) is a commonly used pharmacologic inhibitor of phosphatidylinositol 3-kinase (PI3 K) and has shown an antitumorigenic effect. It could suppress leukemia cell invasion and migration at least in part through up-regulation of *EGR-1*, independent of its PI3 K-Akt inhibitory activity (*100*). In summary, we believe that *EGR* is likely to be a target for treatment of AML.

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Review

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Evaluation of quality of life in individuals with severe chronic motor disability: A major challenge

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Summary **Diverse conditions causing a very heavy and chronic motor disability, such as an advanced amyotrophic lateral, advanced form of multiple sclerosis, high spinal cord injury or a locked-in syndrom, are now getting better medical care and benefit of life support technology with consequent prolonged survival. Quality of life (QoL) assessment is being considered increasingly important to globally apprehend their general well-being. However, the motor disability that affects them appears as a substantial limitation for the assessment of their QoL and consequently a major challenge for all the community that carries an interest for them. This review discussed several avenues to provide to patients and caregivers, clinicians and researchers, and health decision making authority: *i*) elements to determine the most appropriate QoL measure with regard to the interest of patient's point of view, the QoL instruments suitable for this category of patients and their acceptability, *ii*) some arguments of the clinical relevance and accuracy of QoL assessment: interpretations of the questionnaires, QoL determinants, particularity of QoL evaluation for individuals with cognitive impairment and the caregivers perceptions of patients QoL. In conclusion, evaluation of QoL in patients with severe chronic motor handicap is a challenge of major interest, with major ethical issues. It needs to use adapted QoL scales and longitudinal following because of adaptive phenomena to the degree of handicap.**

Keywords: Motor handicap, quality of life, evaluation, death, caregivers

1. Introduction

Diverse conditions causing a very heavy and chronic motor disability, such as an advanced amyotrophic lateral (ALS), advanced form of multiple sclerosis (MS), high spinal cord injury (SCI) with subsequent tetraplegia or a locked-in syndrom (LIS), are now getting better medical care and benefit of life support technology with consequent prolonged survival. These medical advances do not prevent against collateral and important consequences on the everyday life of patients but also their caregivers, both on institutional and natural (family) caregivers. Similarly to other less severe chronic conditions, evaluation of disease course and management of care, identification of specific supports may not rely only on the physical and functional disability that does not reflect all the facets that individuals consider important in their life. Many studies demonstrate that in patients with severe chronic motor disability, quality of life (QoL) does not correlate with physical function (*1-4*). In this context, QoL assessment is being considered increasingly important to globally apprehend the general well-being of these individuals. However, the motor disability that affects them appears as a substantial limitation for the assessment of their QoL and consequently a major challenge for all the community that carries an interest for them. This review discusses several avenues to provide to patients and caregivers, clinicians and researchers, and health decision making authorities: *i*) elements to determine the most appropriate QoL measure and *ii*) some arguments of the clinical

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relevance and accuracy of QoL assessment.

2. Quality of life assessment in individuals with severe chronic motor disability

2.1. *The major interest of patient's point of view*

Quality of life may be assessed from individual face-to-face interviews (unstructured or semi-structured) performed by experienced professionals. For this specific category of patients, this approach can be considered as inappropriate because of a very limited verbal communication and a major motor handicap. For these patients, communication may imply the use of alternative communication as eye blinks or eye movements or communication boards, and upper limbs disability may imply the help of electronic communication devices or the help of someone else. While these particularities prevent the implementation of any types of interviews, using measures as standardized and self-reported questionnaires may be an interesting alternative approach that is commonly used for individuals without severe chronic motor disability.

Quality of life may be assessed by a proxy or a caregiver in specific situations, as parents for children too young for answering a questionnaire or natural caregivers for patients with important cognitive dysfunction. For patients with severe chronic motor disability, the QoL was often assessed by their families and by caregivers in the majority of studies. They often felt that the patient presents a very degraded QoL. Several studies indicate that patients with chronical severe motor handicap and their caregivers or families do not always hold the same ideas and beliefs on patient's treatment course and end-of-life decisions (*4*). They may consider that patient's QoL is so poor their lives are not worth sustaining. This point is of major interest because decisions of life terminating measures are often influenced by professional or natural caregivers. The QoL of people in heavy motor disabilities is often subject to representations of caregivers whose patients depend: healthy individuals, caregivers, family support and health professionals frequently underestimate the QoL of the patients assuming that QoL in severely handicapped people is very poor (*4-7*). But some studies showed that these persons may report relatively satisfactory QoL levels that are stable over time (*8,9*). The QoL is sometimes better than patients presented other disabilities, such as patients with new diagnoses of Alzheimer's disease (*10*) and patients with facial prostheses (*11*). These findings, suggesting that life with severe chronic motor disability is worth living in contrast to the general and widespread opinions, highlight the importance to directly assess the report of the patients. Indeed, the management of these diseases, raising ethical questions, should be supported by the QoL assessment of the individuals themselves.

2.2. *A large variety of quality of life instruments*

It is important to have robust, valid, reliable, and widespread measures. Many questionnaires, specific and generic, are proposed to assess QoL. Generic instruments are generally used to compare QoL across different populations, while disease-specific instruments focus on particular health problem and seem more sensitive for detecting changes (*12,13*).

Many studies described QoL for severe chronic motor disability using generic QoL questionnaires:

- The 36-item short form (SF36) is a generic questionnaire used worldwide (*14*) for which norms are available (*15*). SF36 was used among patients with severe MS (*16*), LIS (*17*), ALS or SCI (*18,19*).
- The Sickness Impact Profile (SIP) (*20*) may be used in patients with chronic motor handicap (*21,22*) but this scale is less effective in assessing psychosocial wellbeing than physical status. Trail *et al*. demonstrated for patients with ALS that important domains of QoL do not correlate with physical functional abilities as measured by generic and function-based instruments such as SIP (*4*).
- The World Health Organization Quality of Life (WHOQOL-BREF) questionnaire is a generic questionnaire used worldwide (*23,24*) and has been used in several studies concerning motor disabled patients (*25,26*).
- The Anamnestic Comparative Self-Assessment (ACSA) provides an overall assessment of QoL based on the patient's memories of the best period in their life before the disease and their worst period (*27*). It is an instrument particularly adapted to populations with severe chronic motor disability and very limited verbal communication due to the rapid passation time (less than 5 minutes) (*27,28*). ACSA has demonstrated its feasibility among LIS patients (*9,29*).

Two other generic instruments are largely used in these specific populations: the McGill scale and the Schedule for the Evaluation of Individual QoL-Direct Weighting (SEIQoL-DW). These two instruments are individualized QoL measures investigating existential and psychological factors. These factors appear to play a significant role in the QoL of these patients such as faith, dignity, maintain of identity, and spirituality. It demonstrated improvements in QoL areas despite loss of physical function; they are thus very fitted for patients with heavy motor handicap.

- The SEIQoL-DW is derived from the original SEIQoL (*30-32*). The SEIQoL-DW is an interesting QoL generic instrument using a semi-structured interview to collect data allowing patients to spontaneously and freely nominate areas that appear important in their life. SEIQoL-DW is very fitted and useful for patients with heavy motor handicap (*3,21,33-36*).

- The McGill scale includes physical and psychological aspects. But the physical and functional aspects are less pronounced, while existential domains are emphasized. It has been used in patients with ALS and has a good validity (*1,25,37*).

Several authors reported that generic instrument accurate not well estimation of patient's QoL (*2,38*). Some disease-specific questionnaires were used on subgroups of severe chronic motor disability. The Amyotrophic Lateral Sclerosis Assessment Questionnaire (ALSAQ-40) was designed for ALS patients (*39*) but is rather heavily weighted toward physical function. A large number of MS-specific QoL instruments are available (the Multiple Sclerosis Quality of Life Index (MSQLI) (*40*), the Multiple Sclerosis Impact Scale (MSIS-29) (*41*), the Multiple Sclerosis International Quality of Life questionnaire (MusiQoL) (*42*) but not really adapted for advanced form of MS presenting severe chronic motor disability.

To our knowledge no specific questionnaire designed for severe chronic motor disability is available. The content of a specific questionnaire relies in general on either the literature or experts to determine the domains and concerns that are important for the individuals, although it is now generally accepted that the content of QoL measures should be directly derived from affected individuals (*43*). The development of this kind of QoL questionnaire should be a major project for researchers, health care workers, patients, and families.

2.3. *The acceptability of the questionnaire*

A great asset of a QoL questionnaire is its acceptability. It concerns the ergonomics of the questionnaire, the length of the questionnaire, and the content of the questionnaire. Due to severe chronic motor disability (leading to communication and movements' limitation), questionnaires should have specific attributes to be used among patients with:

i) The need of availability of e-form QoL validated questionnaires: a potential opportunity for questionnaire development exists in the growing use of electronic measures. For patients with severe chronic motor disability it's a really challenge to provide e-forms that can be used with computer stations and hand-held devices. In cases where patients are equiped with computer interface systems, this allows them to dispense with the assistance of a third person. It is well documented that the presence and assistance of a third person may influence the responses of patients who over- or under-estimates the QoL compared to questionnaires completed alone.

ii) The need of availability of short questionnaires to take account the difficulties of concentration, or tiredness, or other cognitive dysfunction that may affect the individuals. In our personal experience, a quadriplegic (C1) patient or a LIS patient needs about 45 minutes to fullfill the SF36 scale (unpublished data) that it is not apropriate with a clinical routine evaluation. Questionnaires intended for use should be as brief as possible. It highlights the interest of using uniscales giving one overall QoL score, easier to use rather than longer multi-items scales. Future challenges now focus on the concept of computer adaptative testing. The number of items can be reduced substantially to target questions through an iterative process in which responses determine which items are subsequently presented. This approach requires development and validation of algorithms in addition to development and validation of the original questionnaire (*44*).

iii) The specificities of some questions could make the person feel bad about his/her physical restriction and may suggest a QoL-assessment nocebo effect (*i.e.*, negative expectations that derived from the clinical encounter and led to poor health outcomes (*45*). Measuring QoL may cause 'side effects' through the exploration of sensitive subjects, thereby generating new expectations for the clinicians on the part of the patients (*46*).

3. Arguments of the clinical relevance and accuracy of quality of life assessment

3.1. *Assistance to interpret quality of life scores*

In some specific situations, clinicians can be perplexed when interpreting QoL scores.

The first difficulty encountered when interpreting a QoL score for clinicians is the lack of norms values. The SF36 or the WHOQOL-BREF, generic instruments, are commonly used due to the availability of normative data from healthy adults (*47*). It is rare to have scores according to sex and gender. Additionally, it becomes imperative to produce norms for the most popular instruments. Aggregating datasets may contribute to produce valid and robust norms. Each patient would be compared to norms.

A second difficulty expressed by clinicians is the interpretation of QoL measures in longitudinal studies because QoL, self-reported by the patient, might be influenced by psychological phenomena such as adaptation to illness. It has been previously observed that patients' subjective QoL is not related to physical impairments; this observation agrees with previous studies of different motor neuron disorders (*1,2,25,34,38,48-53*), SCI patients (*54*), and LIS patients (*5,9,49,53,55*). This illustrates the "disability paradox" reported by Albrecht and Devlieger (*51,56*).

Albrecht & Devliger stated that QoL is dependent on establishing and monitoring a harmonious set of relationships with the person's social context and external environment (*56*). Most people with long-term chronic condition do not mention physical disability

as their primary concern but rather their psychological and emotional well-being (*57*). This lack of association between objective health/handicap change and QoL could also be explained by the presence of the well-known 'response shift phenomena' (*58*). The presence of a response shift may result in the over- or under-estimation of the true changes and lead to challenges in interpreting QoL measures, especially in longitudinal studies (*52*). The three classical components of the response shift are reconceptualization defined by as a redefinition of QoL, reprioritization defined as a change in the importance attributed to the component domains that constitute QoL, and recalibration defined as a change in a patient's internal measurement standard. Methods of response shift identification are now well-established. However, determining how to integrate the response shift in the interpretation of QoL scores is a true challenge for the next years.

3.2. *Knowledge of quality of life determinants and predictive role of quality of life on health status*

Evidence regarding the determinants of QoL and predictors of mild- and long-term QoL are lacking. Knowledge of which factors are determinants of QoL in this category of patients would assist clinicians in choosing appropriate care intervention. Number of these determinants might be amenable to specific treatment interventions, which may be expected to improve QoL: depression, cognition, access to compensatory techniques, and equipment, *etc*.

In the same way, predictive factors of long-term disability were also reported in patients presenting severe and chronic diseases: cancer patients (*59,60*), cardiovascular diseases (*61*), and MS (*62*). We can hypothesize that QoL level may provide prognostic information about the evolution of disability in patients with sever motor chronic disability. The identification of early predictors of the evolution of disability status may be useful to identify high-risk patients who require early and more aggressive therapies.

Patient-reported QoL provide additional prognostic information beyond traditional clinical or sociodemographic factors. These findings provide strong support for the integration of QoL into clinical practice, in addition to other standard assessments, and reinforce the importance of incorporating a patient's evaluation of their own QoL level during patient monitoring and the assessment of therapeutic effects (*63*). Future studies should provide data from longer follow-up times.

3.3. *Quality of life evaluation for individuals with cognitive impairment*

Patients with advanced MS, patients with ALS associated with fronto temporal dementia, and some LIS patients with extended stroke present cognitive impairment (*64*). One may question the relevance of QoL evaluation results using self reported questionnaires in patients with cognitive impairment. Although, recent studies reported data providing strong arguments to support the conclusion that patients with cognitive dysfunction are reliable and consistent when answering a QoL questionnaire. These works suggested that cognitive dysfunction did not compromise the reliability or validity of the self-reported QoL questionnaires among subjects with cognitive dysfunction and clarify the relevance of using self-reported QoL assessments in clinical practice (*12,65,66*).

4. The quality of life of the caregivers

It is now well-known that caregiving negatively impacts the life of the caregiver (*67-69*).Caregiving leads to a higher risk of mortality (*70*) and resulted in a significant and substantial burden, restricted roles and activities, and increased psychosomatic (*71*), anxious, or depressive symptoms (*72*), and lower QoL (*73*).

However, while caregiving was most often thought to be a negative phenomenon, it is increasingly recognized that caregivers also experience subjective gains and satisfaction (*74*). The caregiving experience can promote a sense of accomplishment, companionship, fulfillment, enjoyment and improved self-esteem. Some families can be brought closer together when someone is in need of care.

Additionally, caregivers' experience, which can be positive or negative, may affect their ability to care and support for the patients. Caregivers have been highlighted as key-actors in the provision of health care, especially regarding their ability to support patients. Caregivers may contribute to the patients' acceptance of treatments. So, considering the caregivers' experience is a noteworthy issue both for the caregivers themselves and indirectly for patients' health. The assessment of caregiver experiences is considered increasingly important with regard to evaluating disease progression, treatment and the management of care provided to patients and evaluating his/her own mental and physical health status. Several groups have published detailed recommendations for QoL assessment that is now being considered increasingly important with regard to evaluating the management of care provided to the caregivers (*75,76*). Despite the acknowledged need to consider caregiver experience issues, their assessment remains routinely under-utilized. The QoL of relatives and careers is also important and is a potential target for intervention: human aid, technical aid, respite care, and psychological support. The feed-back to caregivers of patient's QoL may help caregivers to cope better with the situation.

There are almost no data about caregivers of patients with severe chronic motor disability (*77*).

5. Conclusion

Evaluation of QoL in patients with heavy motor handicap is a challenge of major interest, with considerable ethical issues. It needs to use appropriate QoL scales and longitudinal design due to presence of adaptive phenomena to the degree of handicap. Evaluation and longitudinal monitoring of the QoL of people with severe chronic motor disabilities can help to maximize the social and health policies.

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Review

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Role of metabolism during viral infections, and crosstalk with the innate immune system

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Summary **Viruses have been for long polemic biological particles which stand in the twilight of being living entities or not. As their genome is reduced, they rely on the metabolic machinery of their host in order to replicate and be able to continue with their infection process. The understanding of their metabolic requirements is thus of paramount importance in order to develop tailored drugs to control their population, without affecting the normal functioning of their host. New advancements in high throughput technologies, especially metabolomics are allowing researchers to uncover the metabolic mechanisms of viral replication. In this short review, we present the latest discoveries that have been made in the field and an overview of the intrinsic relationship between metabolism and innate immunity as an important part of the immune system.**

Keywords: Metabolism, viral infections, metabolomics, innate immune system, virus

1. Introduction

Recently, a number of studies have highlighted the importance of studying metabolism for a better understanding of the infection process caused by many pathogens (*1-6*). In particular, viruses are infective particles that need to take advantage of the host metabolism, hijacking the cellular machinery in order to replicate (*4,6,7*), and that has also been demonstrated for viruses in other species (*8,9*). These new approaches have uncovered different mechanisms used by the viruses to continue their life cycle, and raise the possibility that these altered pathways could become new therapeutic targets in order to treat viral infections (*4,7,10*).

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Central metabolism is a key element for the immune system as well (*11*). One of the branches of the immune system, innate immunity, is one of the first lines of defence in case of an infection process, and for example, metabolic programmes for polarized macrophages are completely different (*12*). Given the importance of understanding the players involved during infection, a broad perspective is presented here.

The focus of this review will be first over the described perturbations that viral infections have over the host metabolism, and second will describe the recognized importance of metabolism for the innate immune system.

2. Metabolomic effects of viral infection

The complexity of viruses still fuels the debate about if they are living entities or not (*13*), due to their differences with other types of unicellular or multicellular organisms. One of the confounding features, is the need of an ordered enzymatic environment (*14*), as viruses depend on the host metabolism in order to perform the necessary events

Table 1. Metabolic effects of viral infections

Virus	Metabolic effect	*Ref.*
VACV, DENV	Alteration of glycolysis, FAS, and glutaminolysis	(*15*)
HCMV	*de novo* pyrimidine biosynthetic flux in host cells	(*4*)
HCMV, influenza A	Inhibition of FAS, suppression of replication HCMV and influenza A	(*27*)
HCMV	Infection requirement of long chain FA metabolism: acyl-CoA synthetases and fatty acid elongases	(*18*)
KSHV	Inhibition of FAS and decrease in number of lytic enveloped viruses, induced apoptosis infected cells	(*10*)
EBV, VZV, poliovirus, HCV	Inhibition of FAS, lower number of infectious virus	(*2,32-35*)

This table summarizes the main effects that viral infections have over metabolism. VACV: vaccinia virus; DENV: Dengue virus; HCMV: Human Cytomegalovirus; KSHV: Kaposi's Sarcoma-associated herpesvirus; EBV: Epstein-Barr virus; HCV: Hepatitis C virus; FA: Fatty Acids; FAS: Fatty Acids Synthesis; TCA: tricarboxylic acid cycle.

leading to their replication (*15-19*), while that process by itself requires high amounts of energy in a very short period of time (*3*). The energy requirement and dependence on the host metabolism, is especially important in viruses with a limited coding capacity, *e.g*. Dengue Virus (DENV) (*20*). The latter has been reported for several viruses, in a number of studies reviewed by Sánchez and collaborators (*3*). Among others, the main altered metabolic pathways are glycolysis, fatty acid synthesis (FAS), and glutaminolysis (*3*).

These ideas have been previously summarized by Fontaine (*15*) (Table 1), where she performed different analysis of the effect of the infection of vaccinia virus (VACV) and DENV over the human cellular metabolome, using high-throughput metabolomic approaches. The Warburg effect (aerobic glycolysis), has also been reported upon viral infections (*21,22*). Similarly to what happens in cancer cells, the system carries out a higher rate of glycolysis, instead of using the more cost-effective tricarboxylic acid (TCA) cycle for the generation of energy. Although TCA is not shut-down, it works at a lower rate than in normal conditions, resulting in increased levels of lactate. On the exploration of metabolomic alterations upon viral infections, DeVito and collaborators (*4*) (Table 1) have studied human fibroblasts infected with Human Cytomegalovirus (HCMV), finding that infection induced a *de novo* pyrimidine biosynthetic flux in the host cells, which is required to maintain uridine diphosphate glucose (UDP-glucose), and glycosylation of a virion protein. Interestingly, when inhibiting pyrimidine biosynthesis they observed decreased levels of viral DNA accumulation. As they have addressed, membranes of coated virus include critical glycoproteins for the infection, as occurs for example in infections with hantavirus (HTV). The Gn (or G1 (of 68-76 kDa)) (*23*) and Gc (or G2 (of 52-58 kDa)) (*23*) glycoproteins of HTV mediate the infection and entry into the hosts' cells (*24-26*). This metabolic link is important in diverse viral families, as DeVito and colleagues have pointed out according to their observations. This is a main idea that has been highlighted by other authors as well, for example in the review of Miyake-Stoner (*16*). Sánchez and collaborators (*3*) have additionally suggested that the shift in metabolism could help infected cells to survive, and that can ultimately become an advantage for the virus.

DeVito (*4*) has as well reported an elevated metabolism of lipids of the infected cells within their assays. A similar observation of up-regulation in FAS was reported by Munger (*27*) (Table 1) and Koyuncu (*18*) (Table 1). According to Kelly (*28*), the reduced activity of the TCA cycle (when it is under-regulated) drives citrate into the *de novo* synthesis of fatty acids (FA). Consequently with cell homeostasis, the TCA cycle cannot be completely shut down, and because of that, glutamine is used to replenish the TCA intermediates in an anaplerotic way (*29*). As an example of the shortage of development in this field in viruses responsible for infectious diseases, by the end of 2015 there were no metabolomics studies on the influence of HTV either on the human or on the reservoir (*30*).

There is an evident translational potential for the findings made in this field (*31*), as the modified metabolic pathways could be targeted to decrease the expansion of infection. Very interestingly, there have been reported clinical trials for drugs that target metabolism in cancer (*22*). Some of the experimental results for viruses are those of Delgado and collaborators (*10*) (Table 1), where after inhibition of FAS they describe a decrease in the number of lytic enveloped viruses through induced apoptosis of Kaposi's Sarcoma-associated herpes virus (KSHV) infected cells. They inhibited FAS using two drugs: 5-(Tetradecyloxy)-2-Furoic Acid (TOFA, which inhibits acetyl-CoA carboxylase (ACC1) enzyme) or C75 (which inhibits fatty acid synthase (FASN) enzyme). Interestingly, they further demonstrated that this apoptotic effect induced by inhibition of FAS was specific for infected cells, but not for the control. In the same direction, inhibition of FAS has led to a lower number of infectious viruses (Table 1) in cases of HCMV and influenza virus (*27*), Epstein-Barr virus (EBV) (*32*), varicella-zoster virus (VZV) (*33*), poliovirus (*34*), Hepatitis C virus (HCV) (*2,35*), yellow fever virus, West Nile virus and DENV (*36*).

Table 2. Metabolism of innate immunity cells as well as the main metabolic reactions in the main innate immunity cells

Cell line	Metabolic effect	*Ref.*
Macrophages/DCs	Reduced activity of TCA cycle drives citrate into the de novo synthesis of FA	(*28*)
M1 macrophages	Glycolysis based metabolism, higher glycolytic activity	(*12*,*46*)
M2 macrophages	TCA cycle based metabolism,	(*12*,*46*)
moDCs	Decrease in OXPHOS activity with immunogenicity	(*60*,*28*)
NK	mTOR central role in activation, no increase in glycolysis or OXPHOS after short-term activation	(*66*,*68*)

DCs: dendritic cells; NK: natural killer cells; moDCs: monocyte derived DCs; OXPHOS: oxidative phosphorylation; mTOR: mammalian target of rapamycin.

If we consider again another type of viral infection, that produced by HTV, and that according to CDC (*www.cdc.gov*), there is no current treatment for HTV infections, these findings are quite relevant if this virus disturbs the host metabolism in a similar basis as reported in the mentioned studies. Besides, the fact that those tested drugs are already approved for cancer treatment and the dosage per patient has already been defined, make their use to treat HTV infections more feasible than in the case of developing a totally new drug from scratch.

It is puzzling to find the dramatic increase in lipogenesis during viral infections. One of the first thoughts is that FAS is driven towards the biosynthesis of the viral lipid membrane. By extrapolation we could assume the same for HTV or other similar viruses, which also have a lipid bilayer (*37*). Nevertheless, despite the above mentioned findings and the initial conclusions drawn from some authors, Delgado *et al*. (*10*) have raised questions regarding the decrease of viral numbers, pointing out that it is not consistent with only a block in the viral envelope. According to them, FA molecules are not only restricted to the construction of the viral membrane when the virus is present in the cells. They are also required during membrane production at the Golgi apparatus and endoplasmic reticulum, when protein production increases due to higher metabolic requirements from the virus (*28*). A very interesting contribution from Perera and collaborators (*38*) appeared as an abstract, raising the idea that lipids in DENV infection, are used for modification of physical properties of the lipid bilayer, such as curvature, permeability, or recruitment and assembly of membrane protein complexes. These mechanisms may act in a similar way in the described viruses that alter lipid pathways. If we have in mind that, as indicated by Schountz (*30*), metabolic products (*e.g*. lipids, carbohydrates, among others) are identical between different vertebrate species, it could be extrapolated and suggested that different viral species are using metabolites or altering metabolic pathways in a similar fashion. We can support this hypothesis according to what Emini and Fa (*39*) previously indicated, regarding the necessity of the virus for using host-cell machinery to carry out replication (*40*). Besides, a number of viruses in different organisms depend on the host cellular processes for carrying out their life cycles (*9,41*) or altering them for their own advantage (*42,43*). It is important though, to take into account the observations summarized by Fontaine (*15*): *i*) closely related viruses can perform very divergent metabolic programs; *ii*) the same metabolic perturbations (*e.g*. Fatty Acid Synthesis alteration) may be needed by divergent viruses; *iii*) the metabolic programs induced by viruses are cell specific, according to the capability of viruses to adapt to an unique host environment.

Not only does metabolomics serve as a way to characterize the infection process, or to evaluate the possibility to tailor therapies, but additionally can be used for diagnostic purposes due to its specific profiles for each condition (*44*). It cannot be forgotten that the metabolism is the end point of the system, and thus a reflection of the phenotype (*45*).

3. Crosstalk between metabolism and the innate immune system

Besides what has been summarized above for the different studies on viral alteration of metabolism, we cannot forget that there is crosstalk between the immune system and central metabolism (*46,47*), further reviewed by Cheng and collaborators (*46*). Among the two branches of the immune system, innate immunity represents the first line of defence against external infections (*48*). One of the main cell components of this system are macrophages, which in their polarized state are broadly classified into two types (*49,50*): M1 or classical (pro-inflammatory), or M2 or alternative (anti-inflammatory) macrophages (*28,51-53*). They both need fatty acids for production of cytokines, as those are proteins that have to go through the Golgi apparatus for modifications. Regarding these two types of cells, after activation, M1 or M2 macrophages display a different metabolism, relying heavily on the M1 type in glycolysis (Warburg effect), while M2 macrophages rely on the TCA cycle (*12*) (Table 2). Moreover, immune cells can shift their metabolism depending on the given conditions (*54,55*). In detail, M1 polarization is induced by intracellular pathogens, bacterial cell wall

components, lipoproteins, and cytokines such as IFNγ or TNFα, and a shift into glycolysis would be required for their activation (*28*) (Table 2). According to Kelly M1 macrophages are implicated in inflammatory cytokine secretion and NO production, that leads to effective pathogen killing. M2 on the other hand are activated by fungal cells, parasites, immune complexes, complement, apoptotic cells, macrophage colony-stimulating factor (MCSF), IL4 and IL13 (those two especially), IL10, or TGF-β. Following the paper from Kelly (*28*), in this case oxidative phosphorilation (OXPHOS) is the most representative pathway for M2 activation. These macrophages have high phagocytosis capacity, produce extracellular matrix (ECM), participate in wound healing, as well as in clearance of apoptotic cells. Roszer (*52*) indicates that macrophages synthesize lipid derivatives with anti-inflammatory effects, most probably in the M2 population.

As indicated by Cheng (*46*) a Warburg effect can be observed in active state macrophages, and according to Zhu (*12*) this is nothing other than the M1 macrophage state. Cheng (*46*) (Table 2) has also addressed that M1 macrophages are pro-inflammatory and posses higher glycolytic activity, while M2 are anti-inflammatory, and use oxidative glucose metabolism as the principal metabolic pathway to obtain energy in a sustained way. One of the measurable metabolites in the state of frenzy energy is Lactate, which is expected to be up-regulated and in higher concentrations in the M1 active state, according to the above mentioned references.

Although there are some studies with results that do not follow the main stream. For example in the work of Hollenbaugh and collaborators (*56*) they used a macrophage model (although not macrophages derived from human primary monocytes; *i.e*. U1 and U937 cells), and found significant reductions in the levels of some glycolytic intermediates such as hexose-P, FBP, and G3P, coupled with a decrease in glucose uptake, suggesting a down-regulation or suppression of glycolysis in HIV infected macrophages. However, they found an increase in the levels of pyruvate, suggesting that either this comes as an effect of a down-regulation of the TCA cycle, or is a *de novo* synthesis of pyruvate through other sources (amino acid oxidation). Although it does not exactly follow the model of the Warburg effect in M1 macrophages, or the TCA cycle in M2 macrophages (*12*), the different source of cells has to be taken in account, and the use of another type of virus (HIV).

Dendritic cells (DC) play a key role within both branches of the immune system (*57*), especially by capturing antigens and presenting them to T-cells of the adaptive immune system (*58,59*). Malinarich *et al*. (*60*) (Table 2) evaluated the different metabolic profiles of monocyte derived DC (moDC) in several differentiation stages. After an analysis of the differentially expressed genes (DEGs) and their functional annotation enrichment, they observed a distinct profile in immature or tolerized moDCs against mature moDCs. Importantly, they have described that there is a OXPHOS activity decrease with immunogenicity, which is highest in mature moDCs, as indicated in the review by Kelly and O'Neill (*28*) (Table 2).

Natural Killer (NK) cells are another set of important players for the immune system (*61,62*), belonging to the innate immune branch. Among their functions NKs kill infected cells after recognition (*63*), for example those infected by virus without prior immunization (*64*). Metabolism of this type of cells has been also described, where the mammalian target of rapamycin (mTOR) (*65*) plays a significant and central role for their activation (*66*) (Table 2). It has been shown recently that their anti-viral activity is severely affected by hypoxia (*64*), a situation that can in general disturb the first stage of the TCA cycle (*67*). Murine NK cells have been reported to display a rather metabolically inactive state prior to activation, and no significant increase in glycolysis or OXPHOS after short-term activation (*68*) (Table 2), changing into an increased overall metabolism and especially glycolysis after high-dose prolonged culture with IL15. Additionally, the external environment is capable of influencing NKs, as dietary high-fat intake has also been related to a decrease in the cytotoxicity of NK cells (*69*). In keeping with those results, Viel *et al*. (*70*) have shown that NK cells in obese subjects were less responsive to stimulation, and that could lead to a higher susceptibility to viral infections in this study group. Lastly, it has been addressed that during bacterial infections in acute phases there is a subchronic inflammation state that leads to insulin resistance (*71*). That has the effect of more available energy for the immune system (synthesis of glucose, increased circulating levels of glucose) for fuelling the immune system, in keeping with the reported observations of the Warburg effect in some immune cell lines. Interestingly enough, this subchronic inflammation state is similar to what has been reported in obesity studies (*72,73*), and as reviewed here has a direct impact on the phenotype of NK cells.

4. Conclusion and research directions

It is clear that there is a metabolic component of the immune system that is important for the immune response, involving cells of innate immunity, and that these cells (among others) can shift their activities depending on the conditions in the human body, *e.g*. a viral infection. Viruses can as well modify the metabolic state of the cells, due to their synthetic genome, especially those with really small and reduced ones. Metabolomics is a useful approach to systematically and quantitatively study viral cellular regulation and control, through the screening of

metabolites, through the use of Gas Chromatography-Mass Spectrometry (GC-MS) or Nuclear Magnetic Resonance (NMR) among other platforms. It is also very interesting to profile metabolites, as they are the end point of the interaction between genes, transcripts and proteins, a reflection of the phenotype of a cell or given organism (*74*). Nevertheless, a main complication in metabolomics analyses is the variation that can be observed among individuals (*27,75*). Although a global perspective could be sought, comparing healthy individuals and infected patients, the expected variation emerging from several cell lines present in the body and response to the infection in a systemic way, would increase the difficulty in the analysis. A more concrete study would be to restrict this metabolite profiling to single cell lines, because this approach would decrease variability.

Metabolism is also an important part of the innate immune system, and there is an overall association of immunogenic phenotypes with an increased rate in OXPHOS. Importantly, the metabolic state of the organism has a direct impact over the function of immune cells, such as NK cells. Obesity has been shown to impair their functions, and could be an explanation for higher susceptibility to viral infections in obese subjects. It is interesting to try to address if metabolism is an alternative pathway for the recognition of pathogens, in another level of innate immunity.

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Review

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Fever as an important resource for infectious diseases research

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Summary **Fever or pyrexia is a process where normal body temperature is raised over homeostasis conditions. Although many effects of fever over the immune system have been known for a long time, it has not been until recent studies when these effects have been evaluated in several infection processes. Results have been promising, as they have reported new ways of regulation, especially in RNA molecules. In light of these new studies, it seems important to start to evaluate the effects of pyrexia in current research efforts in host-pathogen interactions. Viruses and bacteria are responsible for different types of infectious diseases, and while it is of paramount importance to understand the mechanisms of infection, potential effects of fever on this process may have been overlooked. This is especially relevant because during the course of many infectious diseases the organism develops fever. Due to the lack of specific treatments for many of those afflictions, experimental evaluation in fever-like conditions can potentially bring new insights into the infection process and can ultimately help to develop treatments. The aim of this review is to present evidence that the temperature increase during fever affects the way the infection takes place, for both the pathogen and the host.**

Keywords: Fever, infectious diseases, immune system, computational tools, RNA structure

1. Introduction

Fever is one of the usual clinical features that appear during the course of several infectious diseases, such as chikungunya fever caused by Chikungunya virus (CHIKV) (*1*), Hantavirus Pulmonary Syndrome (HPS) (*2*) or Hemorrhagic Fever with Renal Syndrome (HFRS) (*3,4*) caused by several members of the Hantavirus (HTV) genus, uncomplicated malaria fever caused by *Plasmodium falciparum* infection (*5*), enteric fever caused by *Salmonella* serovars (*6*), cat scratch fever caused by *Bartonella* spp. (7), or is a common symptom in infants or adolescents affected by pulmonary tuberculosis due to *Mycobacterium tuberculosis* infection (*8*), among other type of infectious diseases. It is also a symptom that helps to recognize disease (*9*).

In order to develop new applications which could potentially serve as treatments, we need to increase our knowledge on the way that host-pathogen interaction works. Although there are many excellent efforts in this regard, an aspect that has not been broadly experimentally addressed has been the potential implications of fever on the interaction with the host and the development of the infection. Some studies have shown the importance of these types of considerations when evaluating hyperthermic incubation conditions compared with the homeostasis temperature of 37°C (*10*). Therefore this review will focus on fever as an important variable to be considered in infectious diseases research, focusing especially on studies that reported how pyrexia can affect on the one hand the host immune response (*11*), and on the other hand the pathogen response.

2. Acquisition of fever during evolution

The acquisition of mechanisms to regulate body

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temperature has been an evolutionary achievement for homeothermic and later endothermic organisms (*12*), in order to gain freedom and occupy different environmental niches, and it is translated as a notable degree of independence from environmental fluctuations (*13*). Within the regulation of body temperature, a further specialization has been the development of fever during certain conditions, among them infections caused by pathogens. Although it is still a matter of debate if this response represents an advantage for the host or is deleterious (*9*), the fact that fever is a costly process for metabolism indicates that it may have been conserved due to an evolutionary advantage (*14*). As indicated by Harden *et al*. (*9*), there is a growing body of evidence to sustain the view that fever is beneficial for the host (*15,16*). Recently, Earn *et al*. (*17*) have found that suppression of fever in normal clinical settings can potentially lead to negative effects at the population-level, due to a possible increase in the spread of associated infections.

Fever is a process in which the body temperature rises, deviating from normal values (*7,18*), and according to the excellent scientific text of Saladin (*18*), it is a beneficial process as long as it does not persist, or reaches 44ºC to 46ºC, where it could be fatal or lead to irreversible brain damage. For further information about its origin, control, and implications on the immune response we strongly encourage the reader to consult the above mentioned source (*18*).

2.1. *Effect of fever over the pathogen*

As well as human microbiota (*19*), pathogens have coexisted with their hosts for a long time during evolution, and they have developed mechanisms especially adapted to circumvent immunological barriers (*20*). Regarding temperature changes, bacteria have developed strategies to adapt to these variations in the environment (*21*), and similar strategies could be found in pathogenic ones (*22*). One of the first phases for a pathogen during the infection process would be to sense when they are within the host (*22*). Body temperature (37ºC) acts as a signal for transcriptomic changes in *Histoplasma capsulatum* (*Hc*) (*23*). As these authors have demonstrated that homeostatic temperature of the host is capable of inducing changes in the transcriptomic landscape of the pathogen, it could be consequently thought that infectious agents have probably adapted to develop a response when fever appears. Morita *et al*. (*24*) highlighted a mechanism of temperature sensing in *Escherichia coli*, through the secondary structure of mRNA, where ribosome binding is facilitated at higher temperatures. In keeping with these suggestions Loh *et al*. (*25*) described an effect of temperature (such as pyrexia) in immune evasion by the pathogen *Neisseria meningitidis*. The pathogen is able to detect increases in temperature through RNA thermosensors, that leads to expression of mechanisms to avoid the host immune responses that are triggered during fever. In another set of experiments, Eldahad *et al*. (*6*) have demonstrated that 3 different typhoidal serovars from *Salmonella enterica* are affected at fever-like temperatures, several infective features are impaired, and that fever could be used by the pathogen as a signal to enter into a phase of persistence in the organism. Moreover, fever temperatures *in vitro* inhibited the growth of the protozoan parasite *Plasmodium falciparum* (*5*). In the case of viruses, high temperature have been demonstrated to destabilize viral RNA polymerase in highly pathogenic avian influenza (HPAI) (*26,27*), or can exert an inhibitory effect over the synthesis of the viral RNA genome of Human Influenza A virus (*28*).

These studies open the door for the evaluation of molecular changes on the pathogen in an environment where temperature increases, especially over gene expression, and conformational changes that may affect RNA structures (as shown for thermosensors), or efficiency of the viral replication machinery.

2.2. *Effects of fever on the immune system*

Increase of temperature within the host has also an impact over the immune system (*29*), demonstrated by Murdock *et al*. (*30*), where they report that temperature can impact mosquito (*Anopheles stephensi* Liston) immunity. In humans, Tomiyama *et al*. (*11*) have evaluated the effect of mild hyperthermia treatment on healthy adults, reporting an enhancement of innate and adaptive immunity. Another study focused on the characterization of the response of Dendritic Cells (DCs) to *Aspergillus fumigatus* at two different temperatures (homeostasis at 37ºC, and fever-like temperature at 40ºC), having found no differences in DC viability or cytokine release, but a higher maturation rate for DCs and a lower phagocytic capacity at 40ºC (*31*). Further characterization of the influence of fever-like temperatures on mice DCs (*15*) found an effect of higher temperatures on the maturation of these cells. DCs are precisely one cell type, with macrophages, that are activated to unchain a series of events that leads to a systemic induction of fever when infection occurs (*29*). It is then that the same cells, which are key to the febrile process, are ultimately affected by fever in their immunological features.

Fever has been demonstrated to affect other immune cells as reflected by Harden *et al*. (*9,32*), including different types as neutrophils, monocytes or T-cells, or Natural Killer cells (NK) (*29*) as well.

It has been highlighted (*18*) that enzymatic reactions may race ahead during a period with higher temperatures, further explained by Fields *et al*. (*33*) (view section 3). In the sense indicated in (*29*), fever is a product of one or several biological processes, where the detection of pathogen unchains a set of events that end up in a fever

process, that plays in favor of the host.

3. Molecular consequences during fever: a theory

Increases of temperature have an impact on biological processes in living systems, with several well documented examples. It has been demonstrated in plants that heat shock is responsible for blocking mature ribosome production (*34,35*), which are key organelles for the normal performance of the entire cell and ultimately the whole organism (*36*). Higher temperatures induce physiological processes in the body such as sweating (that essentially have a molecular origin/response), as one of the mechanisms to dissipate heat (*37*).

At a deeper molecular level, heat-shock proteins (HSPs) (*38,39*), are a set of genetically conserved stress sensitive proteins (*39*) that accumulate in cases of exposure to high temperatures (*35,40-43*), among other sets of stressful conditions, in order to protect the cell (*44*), potentially by aiding in correct folding of proteins denatured by heat shock (*35,45*). They have been described in a range of species (*40,44,46*), including vertebrates (*41*) and among those, humans (47). Lack of the 70-kilodalton family of HSPs (HSP70), a highly conserved protein promoter of heat tolerance (*46*), induces heat shock sensitivity in *E. coli* strains that would be otherwise resistant to that type of stress. For humans and rodents, short *in vitro* exposure to the range of 41-43°C induces heat shock, leading to induction of HSPs synthesis (*48*). Heat shock is connected with immune response mediators, such as cytokines (*48*). HSPs have an important role for the host immune response as well as for the pathogen mechanisms of evasion. From the point of view of the pathogen, overexpression of HSP70 and HSP60 are fundamental for the survival of *Salmonella typhimurium* during macrophage infection (*46,49,50*). Besides, within the host they can act as antigen carriers, although not as direct activators of the innate immune response (*51*).

Temperature has the capability to influence membrane fluidity, as it has been described in plants through the modification of the saturation pattern of the fatty acid component of membrane lipids (*52*). The effect of a temperature decrease will lead to lowered fluidity (*e.g.* oils in nature get solid when reaching a certain temperature), while higher temperatures increase lipid fluidity (*53-55*); and temperature also has a direct impact on the ion transport systems in frog erythrocytes (*56*). Because fluidization can have negative consequences for the cell at very high temperatures (*57*), there is a higher content of saturated fatty acids displayed in that set of stressful conditions to maintain cell function and integrity (*55*). Further stabilization of the membrane structure is achieved through participation of cholesterol (*58*). All of the above has important consequences for infectious diseases, as the cell membrane is one of the first contact surfaces with the external environment. Cholesterol is also one of the key components of membrane lipid rafts (*59,60*), which are used by a number of pathogens as an entry point (*58*) due to the abundance of potential receptors for the pathogen. Amini-Bavil-Olyaee have found that an indirect disturbance of intracellular cholesterol homeostasis is capable of interfering with viral entry (*60*). In keeping with those results, Kapadia and Chisari showed that Hepatitis C virus (HCV) RNA replication relies on the host biosynthetic pathways of cholesterol and fatty acids. Phenothiazines have been used to target and increase fluidity leading to inhibition of HCV entry (*61*); while drugs that inhibited host-membrane fluidity elevated mortality in mice treated with normally mild influenza A virus (IAV) infection (*62*). These and other findings are pointing to lipid membranes as very interesting targets for treatment of infectious diseases, or to tackle antimicrobial resistance (*63*). Although we are considering an increase of temperature from the homeostasis point of 37ºC, to 40-41ºC, that deviation from normality can lead to the above mentioned perturbations in terms of HSPs expression or in membrane fluidity through fatty acid composition or permeability, and that can have consequences for the infection process, as has been previously highlighted. The remaining question is if this deviation of a few degrees would translate into changes in the structure or the activity of proteins, such as enzymes (*64*), in a way that they cannot perform their normal functions, or push ahead cellular metabolism (*33*). Enzymes can display sensitivity to temperature, and this can be transferred to the biochemical process in which they take part (*33*). Additionally, the rate of a reaction has an exponential relationship with the temperature (*33*). In this scenario recent efforts have evaluated the metabolomic profile during a transitional fever process in order to understand better the biochemical basis in rats (*65*). These type of experimental approaches are appropriate as many metabolic reactions are affected by temperature (*33*), and the generated information has great potential to aid in understanding the overall infection process.

4. Computational tools to analyze fever effects

In order to evaluate potential effects of fever on the pathogen RNA secondary structure, computational approaches can be used. It can be hypothesized that some pathogens may be changing their behaviour during pyrexia due to different properties of their RNA molecules, or that bacterial pathogens may have some type of RNA thermosensors which have not been described yet. It is known that RNA are not linear molecules, and have complex tridimensional structures that may be affected by temperature, as shown for RNA thermosensors/thermometers (*66,67*). RNA thermometers follow changes in their structure upon temperature variation (*68*), with the capability of even

sensing changes down to 1ºC (*69*). They importantly control virulence factors in bacteria (*70,71*).

The use of computational tools to evaluate in a first step potential temperature-induced perturbations of the secondary structure of RNA in different viruses or bacteria, seems like a reasonable approach prior to experimental evaluation. These changes in the structure would appear as a consequence of the increase of temperature that occurs in inflammation processes upon infection. Potential changes can be evaluated using RNAtips (*72*), a user-friendly tool for the evaluation of temperature-driven perturbations in RNA structure, and which was developed by Chursov and colleagues (*72*). For an evaluation of the fever, the temperature range can be set from 37ºC to 41ºC. Another type of analysis can be performed with the RNAfold web server (*73,74*) in order to generate plots for the Minimum Free Energy (MFE) or the centroid secondary structure, as an example, to evaluate the influence of temperature over the structure, at either 37ºC or 40ºC.

These are two types of computational tools that can be used to carry out *in silico* simulations in order to evaluate how feasible it would be to perform this experimental approach. Due to the current advances in sequence information for many infective agents, we strongly encourage the scientific community to evaluate potential changes in the secondary structure of RNA as a first step towards further experimental studies.

5. Conclusions

The usual growth temperature when carrying out *in vitro* cultures using cells from human, mouse, or other mammals, is normally 37ºC, in order to mimic the biological conditions of these species (regarding their body temperature). In the field of infectious diseases, the *in vitro* interaction between the host and the pathogen is also evaluated at 37ºC. This information is of paramount importance to better understand the interaction process. However, as *in vitro* interaction experiments are not performed at fever-like temperatures (from 39ºC to 41ºC), we are losing valuable data. Fever is an overall beneficial process for the host although it is energetically costly. Pathogens have developed an array of tools that allow them to adapt to the host, such as RNA thermometers, to sense when they are within the host in order to express virulence genes, or to enter in a state of dormancy. Fever has an impact over diverse types of pathogens, such as bacteria, viruses, or parasites. As well, the host is affected by febrile processes in its immune system, as shown for different host species such as moquitoes or humans. Those increases in temperature in the range of a few degrees induce changes at different biological levels: in the behaviour of DCs, in the developmental programmes of parasites, in the virulence factors of bacteria, and in the tridimensional structure of RNAs (a set of them, called RNA thermometers) (*67,69,75,76*).

At a molecular level, fever affects cells by the induction of HSPs expression, a set of evolutionarily conserved proteins. Cellular membranes are key elements in the entry of several pathogens, as demonstrated for viruses. In that process key aspects such as lipid composition or fluidity can be directly affected by pyrexia. Enzymatic reactions are also dependent on temperature, and there is a potential impact of fever on normal metabolic flux. Taking into account all of the discoveries in the field until now, infectious disease research can benefit enormously by considering fever as a key element for the development of the infection process. The value of these kind of experiments at fever-like temperatures is that a better understanding of the infection process can be achieved, observing if fever has a negative impact over the pathogen, or if it increases its virulence. Ultimately, this information can help in the development of new treatments for intractable diseases, or to manage fever differently in clinical settings.

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Original Article

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Inflammageing assessed by MMP9 in normal Japanese individuals and the patients with Werner syndrome

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Summary **Age-associated minor inflammation: inflammageing may explain human ageing mechanism(s). Our previous study reported a significant increase in the serum level of highly sensitive C-reactive protein (hsCRP) with normal ageing and the patients with Werner syndrome (WS). To further study the minor inflammatory condition associated with ageing, another possible ageing biomarker: matrix metalloproteinase-9 (MMP9) was examined in the sera from 217 normal Japanese individuals aged between 1 and 100 years and 41 mutation-proven Japanese WS aged between 32 and 70 years. MMP9 was assayed by ELISA. The serum level of MMP9 was elevated significantly ($p < 0.001$) with normal ageing from both sexes as hsCRP. In contrast to normal ageing, the serum MMP9 level in WS decreased significantly with calendar age ($p < 0.05$). The MMP9 level (ng/mL) in WS (147.2 ± 28.5) was not significantly different in comparison with those from age-matched normal adult population aged between 25 and 70 years (109.1 ± 9.4), nor normal elderly population aged between 71 and 100 years (179.9 ± 16.1). Although both normal ageing and WS were associated with minor inflammation, the inflammatory parameters such as serum MMP9 and hsCRP changed differently between normal ageing and WS. The WS-specific chronic inflammation including skin ulcer and diabetes mellitus may contribute the different behavior of both ageing biomarkers from normal ageing.**

Keywords: Aging, C-reactive protein (CRP), inflammageing, matrix metalloproteinase-9 (MMP9), Werner syndrome

1. Introduction

The significant contribution of low-grade, and systemic inflammation to normal ageing: inflammaging has been proposed to explain pathophysiology of human ageing and age-associated diseases (*1,2*). The minimal, but significant elevation of highly sensitive C-reactive protein (hsCRP) has been frequently reported in the normal elderly population that may follow the ageing-related conditions including skin atrophy, cataract, arthritis, osteoporosis, hypogonadism, metabolic syndrome, obesity, diabetes mellitus (DM), immune dysfunctions, sarcopenia, cancer, atherosclerosis, cognitive decline and finally death (*1-4*).

Most Japanese studies on the chronological changes of inflammatory conditions in normal ageing indicated a similar age-associated increase of low grade inflammation to the western studies, though the level of inflammation assessed by hsCRP was several times lower than that in the western populations (*3,5*).

C-reactive protein (CRP): the representative acute phase protein of pentraxin family, primarily produced by the hepatocytes was also produced by smooth muscle cells and adipocytes (*6*). Matrix metalloproteinases

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(MMPs) produced by macrophages, T helper type 2 lymphocytes, fibroblasts, smooth muscle cells and endothelial cells belong to a family of catalytic enzymes for the long-lived extracellular matrix proteins including collagen, gelatin, fibronectin, laminin, elastin and proteoglycans (*7,8*). MMPs, especially MMP2 (72-kDa gelatinase A) and MMP9 (92-kDa gelatinase B) both in the circulation and the affected tissues have been reported to increase with inflammation, normal ageing and age-associated diseases like atherosclerosis, coronary artery disease and DM in response to interleukin (IL)-13 (*6-9*).

Elastin fragment degraded by MMP9 produces a large amount of pro-inflammatory cytokines leading to chronic inflammation (*10*). A strong relationship between serum levels of MMP9 and the process of inflammation characterized by hsCRP has been shown in acute coronary syndromes (*9,11*).

Werner syndrome (WS; MIM#27770), the representative progeroid syndrome, has been extensively studied as the natural model of human ageing (*12*). The patients with WS show a wide variety of ageing-associated clinical manifestations such as gray hair/alopecia, hoarseness, cataracts, skin atrophy, skin hyper-/hypo-pigmentation, sarcopenia, DM, hypogonadism, hyperlipidemia, atherosclerosis, osteoporosis, and malignancy at a relatively early stage of their life followed by death at around 50 y.o. due to atherosclerosis-related diseases or malignancy (*13*). Surprisingly, majority of the WS patients are of Japanese origin, probably because of the relatively high frequency of consanguineous marriage in the rural area of Japan and the extremely high frequency of heterozygous careers in Japan (*12*). We have reported the inflammatory conditions observed in WS in a series of publications (*12*).

The aim of this study was to clarify the contribution of minor inflammation to ageing by investigating serum MMP9 levels using the serum samples from apparently normal Japanese volunteers and the mutation-proven Japanese WS patients.

2. Materials and Methods

2.1. *Study population*

A total of 217 normal serum samples (M = 91, F = 126) between 1 to 100 years old were the same sera as were studied in the previous study (*5*). The normal individuals, enjoying the usual daily life at home or nursing home, had neither apparent inflammatory diseases including infection, cancer, lymphoproliferative disorders, DM, Alzheimer disease, autoimmune diseases and arthritis at the time of serum sampling, nor history of cardio-/cerebro-vascular accidents. Exclusion protocol for elderly individuals met the SENIEUR criteria (*14*).

Serum samples were also obtained from untreated 41 mutation-proven WS (M = 24, F = 17; between 32 and 70 years old); a part of "Goto collection of Werner syndrome" (*http://www.brc.riken.jp/lab/cell/english/index_gmc.shtml*). As indicated in Table 1, nine patients with WS were free from skin ulecers SU[SU(-)], while 32 had SU[SU(+)]. Twenty four had DM [DM(+)], but 17 did not [DM(-)]. WS patients were sub-grouped into 1) SU(+)DM(+) (n = 20), 2) SU(+)DM(-) (n = 12), 3) SU(-)DM(+) (n = 4) and 4) SU(-)DM(-) (n = 5). For statistical comparison with WS, normal individuals were divided into two groups according to their age: normal adult aged between 25 and 70 years (NA, n = 86) and normal elderly aged between 71 and 100 years (NE, n = 85).

All of the individuals provided written informed consent for this study, which was approved by the Ethics Committee of Toin University of Yokohama. All of the samples were stored at -80°C until use.

2.2. *Determination of MMP9 and hsCRP*

The concentration of MMP9 (ng/mL) in the sera was determined by specific sandwich ELISA using a Human MMP9 ELISA kit (Fuji Chemical Industries, Toyama, Japan) as described before (*15*). hsCRP was assayed by Circulex high-sensitivity CRP ELISA kit (Cyclex Co., Nagano, Japan) (*5*).

2.3. *Statistical Analysis*

The multiple liner regression model was used to examine relationship between aging and serum level of MMP9 with adjustment of sex effect on the serum levels and to examine relationship among WS, NE and NA. We coded one for male and zero for female in Sex variable.

Differences of serum levels of MMP9 between two groups (NE *vs*. NA, WS *vs*. NA, WS *vs*. NE or subgroups defined by the presence/absence of SU or DM, respectively) were tested by two-sample t-test with unequal variances. Multiple regression analyses were performed to explain the relationship between age and serum levels of MMP9 with adjustment of sex effect on the serum levels and to examine relationship among WS, NE and NA. These models were selected based on the Akaike's information Criterion (AIC) (*16*). Statistical Language R (*17*) was used for these analyses. p values < 0.05 were considered to be statistically significant.

3. Results

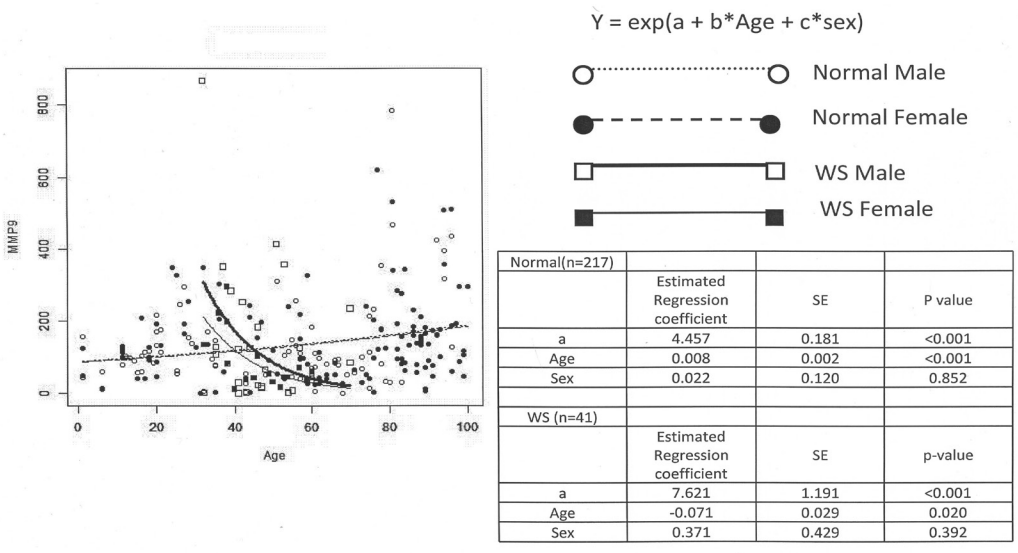
3.1. *Charcteristics of MMP9 in normal individuals*

Using a non-linear regression model, statistically significant temporal effect of age on the serum level of MMP9 was observed ($p < 0.001$) with adjustment of sex effect on the serum levels as indicated in Figure 1.

Table 1. Clinical characteristics in Werner syndrome patients

Subgroups	SU	DM	ID	Age	Sex	MMP-9 (ng/mL)	hsCRP (ug/mL)
1	+	+	WS12901	32	F	134	2.61
1	+	+	WS57201	37	M	351	22.8
1	+	+	WS19201	38	M	82.2	7.03
1	+	+	WS56301	39	M	284	0.79
1	+	+	WS5501	40	F	10.2	11.1
1	+	+	WS57801	41	M	122	1.04
1	+	+	WS51301	42	M	252	17.4
1	+	+	WS19201	44	M	124	18.7
1	+	+	WS4705	45	F	224.3	42.4
1	+	+	WS6301	46	M	184	27.2
1	+	+	WS53601	46	M	24	16.3
1	+	+	WS0101	47	M	49.3	15
1	+	+	WS58501	51	M	414	4.02
1	+	+	WS58301	53	M	357	3.21
1	+	+	WS4704	54	M	32.1	3.42
1	+	+	WS17201	54	F	198.5	2.26
1	+	+	WS0801	55	F	167	8.99
1	+	+	WS54801	57	M	127	5.88
1	+	+	WS56201	70	M	85.8	10.3
1	+	+	WS1801	70	M	235.5	28.3
2	+	-	WS6103	32	M	2	1.62
2	+	-	WS6104	32	M	867.6	25
2	+	-	WS14501	35	M	129	4.55
2	+	-	WS51601	36	F	222	0.98
2	+	-	WS53101	38	F	294	22.9
2	+	-	WS53901	43	F	38.4	1.28
2	+	-	WS53801	46	F	103	0.98
2	+	-	WS2101	50	F	30.8	8.66
2	+	-	WS55801	53	F	45.4	18.2
2	+	-	WS52901	54	F	45.8	10.8
2	+	-	WS54001	57	F	70.3	7.04
2	+	-	WS4701	59	F	33.6	11.8
3	-	+	WS58701	35	M	108	2.93
3	-	+	WS57701	38	F	197	2.07
3	-	+	WS57401	41	M	0	26.9
3	-	+	WS4401	41	M	30	24.9
4	-	-	WS5801	43	M	2.9	1.28
4	-	-	WS0402	47	M	19.5	1.15
4	-	-	WS7501	48	M	66.7	22.3
4	-	-	WS0401	49	F	52.9	4.76
4	-	-	WS10501	52	F	16.7	3.86

Note: 32 had skin ulcers (SU) and 24 DM at blood sampling. Subgroups: 1) SU(+)DM(+); *n* = 20, 2) SU(+)DM(-); *n* = 12, 3) SU(-)DM(+); *n* = 4, 4) SU(-)DM(-); *n* = 5. SU: skin ulcer, DM: diabetes mellitus, MMP9: matrix metalloproteinase 9, hsCRP: high sensitivity CRP



Normal(n=217)			
	Estimated Regression coefficient	SE	P value
a	4.457	0.181	<0.001
Age	0.008	0.002	<0.001
Sex	0.022	0.120	0.852
WS (n=41)			
	Estimated Regression coefficient	SE	p-value
a	7.621	1.191	<0.001
Age	-0.071	0.029	0.020
Sex	0.371	0.429	0.392

Figure 1. Serum MMP9 in normal ageing and Werner syndrome from both sexes. Non-linear regression model used in this analysis was expressed as MMP9 = exp (a + b*Age + c*Sex), where a, b, and c are estimated regression coefficients. Estimated regression coefficient and *p* values for age and sex are inserted in the right table next to the figure for comparison. ○: Normal Male (dotted line; *n* = 91), ●: Normal Female (dotted line; *n* = 126), □: WS Male (solid line; *n* = 24), ■: WS Female (solid line; *n* = 17).

Non-linear regression model expressed as MMP9 = exp (a + b*Age + c*Sex) was selected based on the AIC, where a, b and c are estimated regression coefficients. The serum level of MMP9 significantly increased 1.008 (= exp (0.008)) times a year, as shown in the coefficient of Age in the right table of Figure 1. Neither significant gender difference of serum level of MMP9 was observed concerning to the age-associated increase in MMP9 level as indicated in the right table of Figure 1, nor concerning to the mean ± SE level of MMP9 (ng/mL) throughout all ages between male (127.6 ± 12.7; n = 91, solid line for regression model) and female (143.8 ± 10.1; n = 126, dotted line for regression model), as of the result of hsCRP (*5*).

The serum level of MMP9 in the NE (179.9 ± 16.1; n = 85) was significantly elevated in comparison with the NA (109.1 ± 9.4; n = 86) from both sexes ($p < 0.001$) as indicated in Table 2.

3.2. *Characteristics of MMP9 in WS patients*

The serum level of MMP9 in WS patients (142.3 ± 24.9 ng/mL) was insignificantly increased compared with the age-matched NA from both sexes (109.1 ± 9.4), but insignificantly decreased compared with the NE (179.9 ± 16.1) (Table.2).

The serum levels of MMP9 in WS patients was significantly decreased ($p < 0.02$) with ageing as indicated in Figure1.

The serum MMP9 was significantly different between SU(+) (166.9 ± 5.1; n = 32) and SU(-) groups (54.9 ± 7.0; n = 9) ($p < 0.001$) or DM(+) (158 ± 4.8; n = 24) and DM(-) groups (120 ± 12.2; n = 17) ($p < 0.01$), respectively. The MMP9 level in Group 1 SU(+)DM(+) subgroup (172.9 ± 26.1; n = 20) was significantly increased compared with that in Group 4 SU(-)DM(-) subgroup (31.7 ± 12; n = 5) ($p < 0.001$). However, the MMP9 level was not significantly different between Group 1 SU(+)DM(+), Group 2 SU(+)DM(-) and Group 3 SU(-)DM(+) subgroups, respectively. The comparisons between the rest of the subgroup combination were also statistically insignificant as shown in Table 3.

Table 2. Serum MMP9 in Werner syndrome and normal individuals from different age groups of both sexes

Group	Mean	SE	p value
NA (n = 86)	109.1	9.4	-
NE (n = 85)	179.9	16.1	< 0.001
WS (n = 41)	142.3	24.9	0.21

Note: Reference group was normal adult 25-70 years old group (NA). The p value between reference group and normal elderly 71-100 years old group (NE) was $p < 0.001$. The p value between WS and NA or NE were statistically insignificant ($p > 0.05$).

4. Discussion

Although CRP is the prototypical acute-phase reactant in man, both serum hsCRP and MMP9 have been proposed as biomarkers of atherosclerosis-associated diseases including coronary heart disease and cerebrovascular accidents (*18-20*). CRP can act as pro-inflammatory by inducing the expression of tumor necrosis factor α and IL-1 (*21*).

However, CRP as a component of the innate immune system can function as a protective machinery against a variety of inflammatory conditions and autoimmunity by activating the classical complement pathway (*22*), enhancing phagocytosis, and binding to the Fcγreceptors on leukocytes, leading to the anti-inflammatory cytokine production such as IL-10, transforming growth factor β and IL-12 (*23-28*).

Similarly, MMP9 can degrade gelatin, leading to the activation of potent signals for cell survival and tissue repair on one-hand, but the degraded fragments can also promote the production of pro-inflammatory mediators on the other hand (*7*). Both CRP and MMP9 may have beneficial and deleterious effects for an organism, coining antagonistic pleiotropy (*1,2*).

In the present study, we have reported significantly increasing levels of serum MMP9 with normal ageing, resulting from the sum of tissue degradation and tissue repair. However, the level of serum MMP9 in Japanese population was several times lower than that in the western populations, the situation being similar as of hsCRP as already reported (*5*). The sharp contrast of serum hsCRP and MMP9 levels between Japanese and the western populations is not still clear, but has been presumed by the difference in diet, lifestyle and body mass index (*3,7*).

WS has been nominated as a human model of natural ageing, ageing twice faster than normal (*12*). Although the serum level of hsCRP in WS was several

Table 3. Serum MMP9 in Werner syndrome from different subgroups

Subgroups	Mean	SE	p value matrix		
			Group 2	Group 3	Group 4
Group 1: SU(+)DM(+) (n = 20)	172.9	26.1	0.83	0.14	< 0.001
Group 2: SU(+)DM(-) (n = 12)	156.8	69.3	-	0.39	0.10
Group 3: SU(-)DM(+) (n = 4)	83.8	44.1	0.39	-	0.33
Group 4: SU(-)DM(-) (n = 5)	31.7	12.0	0.10	0.33	-

Note: Serum MMP9 in group 1 was significantly elevated ($p < 0.001$) compared with group4. No significant differences were between the rest of any subgroup combinations. SU: skin ulcer, DM: diabetes mellitus.

times higher than the normal control and tended to increase with calendar age, another inflammation biomarker: MMP9 in WS was significantly decreased with ageing and neither significantly changed compared with age-matched NA, nor NE.

The contrasting profile of hsCRP and MMP9 between normal ageing and WS may suggest that *i*) the ageing in WS may not progress in accordance with normal calendar ageing, but progress in the genetically-determined unique fashion as the patients with WS age faster than normal, and age prematurely even at a relatively younger stage; *ii*) as was described in the Introduction, the inflammatory proteins: hsCRP and MMP9 are produced by different cell types, repectively and WS patients with SU and DM produced more MMP9, but not hsCRP by hidden inflammation/infection than those without; *iii*) the limited number of the subgroups in WS may affect the statistics, though our Goto collection is unquestionably the world largest (*12*).

We have shown the ageing-associated increasing level of inflammation markers such as hsCRP and MMP9 by using serum samples from carefully selected normal Japanese ages between 1 and 100 y.o. from both sexes. There was no gender difference concerning to the degree and the trend of inflammation.

As shown in the previous publication on WS (*5*), we did not find a significant difference between SU(+) DM(+) group and SU(-)DM(-) group concerning to the serum level of hsCRP. However, we have found a significant difference between SU(+)DM(+) group and SU(-)DM(-) group in the present MMP9 study. As hsCRP and MMP9 may change differently in response to inflammation possibly induced by SU and DM, we should bear the different behavior of hsCRP and MMP9 as aging markers in mind.

Further study may clarify the effect of mild inflammation:inflammageing on the process of normal ageing and the patients with progeroid syndrome such as WS.

5. Conclusion

Although minor inflammation evaluated by MMP9 may be associated with normal aging and the patients with WS, the serum levels of MMP9 and hsCRP may change differently between two conditions.

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Brief Report

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Dietary supplement use and nosebleeds in hereditary haemorrhagic telangiectasia – an observational study

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Summary **Understanding potential provocations of haemorrhage is important in a range of clinical settings, and particularly for people with abnormal vasculature. Patients with hereditary haemorrhagic telangiectasia (HHT) can report haemorrhage from nasal telangiectasia in real time, and suggested dietary factors may precipitate nosebleeds. To examine further, nosebleed severity, dietary supplement use, and blood indices were evaluated in an unselected group of 50 HHT patients recruited from a specialist UK service. Using the validated Epistaxis Severity Score, nosebleed severity ranged from 0 to 9.1 out of 10 (median 3.9). Using a Food Frequency Questionnaire, 24/50 (48%) participants reported use of dietary supplements in the previous year. A third (18/50; 36%) had used self prescribed, non-iron containing dietary supplements, ingesting between 1 and 3 different supplements each day. Eight (16%) used fish oils. Despite having more severe epistaxis ($p = 0.012$), the 12 iron supplement users had higher serum iron concentrations, and were able to maintain their red blood cell indices. In contrast, there was no evident benefit for the participants using non iron supplements. Furthermore, platelet counts and serum fibrinogen tended to be lower in fish oil/supplement users, and one fish oil user demonstrated reduced in vitro platelet aggregation. In conclusion, in this small study, a third of HHT patients used non-iron dietary supplements, and one in six ingested fish oils, unaware of their known anti-platelet activity. The scale of use, and potential of these "natural health supplements" to exacerbate nosebleeds has not been appreciated previously in HHT.**

Keywords: Epistaxis, Diet, Iron, epistaxis severity score (ESS), fish oils

1. Introduction

Patients with hereditary haemorrhagic telangiectasia (HHT) provide an intriguing real-life model to evaluate potential precipitants of haemorrhage. Due to a causative mutation most commonly in an *ENG*, *ACVRL1* or *SMAD4* gene, HHT leads to the development of vascular abnormalities, particularly visceral arteriovenous malformations, and smaller nasal and gastrointestinal telangiectasia (*1,2*). Patients with HHT can report nosebleeds (epistaxis) in real time, and the magnitude of their nasal haemorrhagic losses are such that iron deficiency anaemia commonly results, permitting objective evaluations (*3*).

Understanding nosebleed precipitants is also important in the overall management of people with HHT. Despite a battery of potential interventional,

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surgical, and medical approaches (*4*), HHT patients are commonly iron deficient and/or anaemic because replacing iron lost through recurrent haemorrhage demands very high iron intakes (*3*). In a recently surveyed international group, 273 of 1,288 (21.2%) had received iron infusions and 396 (30.8%) had received blood transfusions, 105 (8.1%) on at least 10 occasions (*5*). Additionally, arterial rate bleeds can cause acute haemodynamic disturbances (*3,4,6,7*); epistaxis severity is a major predictor of reduced quality of life (*8-11*); and epistaxis is the primary outcome measure in nine of ten clinical trials of new therapeutic agents recruiting in HHT (*12*).

Understanding what provokes HHT nosebleeds at particular times offers insights into haemorrhagic precipitants, and potential strategies to limit healthcare demands, improve quality of life, and optimise clinical trial design. HHT nosebleeds are highly variable, and generally difficult to predict. However, in recent studies by our group, HHT patients reported that nosebleeds could be precipitated by certain food groups (*13,14*). Spontaneously volunteered precipitant foods included red wine, spices, chocolate, coffee, berries, oily fish, and other food items that contain high levels of naturally occurring salicylates and other anti-platelet agents (*13*). We hypothesised that for some people, suggesting dietary changes may also detrimentally impact on their quality of life, and therefore examined nosebleed relationships with dietary supplements that may be more readily modified.

2. Materials and Methods

2.1. *Ethics*

The study was given a favourable Ethics opinion by the London Wandsworth Research Ethics Committee (11/H0803/8), and all participants gave written informed consent.

2.2. *Survey methodology*

Based on previous experience from pilot study numbers, an unselected group of 50 patients with HHT attending our tertiary care clinic were recruited into a blood sample and questionnaire-based study during two study periods to coincide with recruiter availability, April-September 2011, and March-May 2013. Inclusion criteria were a definite diagnosis of HHT using the Curaçao Criteria (*15*) and not residing in the same household as another study participant. Within this period, ~180 eligible patients were approached by letter or in person. All recruited participants recorded nosebleed severity by the validated Epistaxis Severity Score (ESS) (*16*). This rates nosebleed severity on a scale of 0-10, with the minimal important difference recently identified as 0.71 (*17*). Dietary supplement intake was assessed in an unbiased manner on the final page of the validated European Prospective Investigation into Cancer and Nutrition (EPIC) Food Frequency Questionnaire (*18*). Participants were asked "Have you taken any vitamins, mineral, fish oils, fibre or other food supplements during the past year?" and then asked to detail the brand, strength, amount and frequency (6 frequency options were provided).

2.3. *Haematological evaluations*

Blinded to epistaxis severity scores and supplement use, full blood count, prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen concentrations, and biochemical analyses were measured as part of routine clinical care. Blood samples were obtained by a professional phlebotomist in the early to mid-afternoon, and centrifuged to obtain platelet-rich plasma (PRP) and platelet poor plasma (PPP). Blinded to supplement use and other blood results, PRP was used to determine platelet aggregation to freshly-prepared adenosine diphosphate (ADP), by a Helena Aggregometer.

2.4. *Statistical analysis*

Statistical analyses were performed using GraphPad Prism 6 (GraphPad Software, San Diego, CA). Participants were categorised by supplement use as described in the text. Where there were three categories (*e.g*. no supplement use, iron supplement, and non iron supplement use; or no supplement use, fish oil and non fish oil supplement use) *p* values were calculated using Kruskal Wallis. Where there were two categories (*e.g*. supplements use, or no use of supplements), *p* values were calculated using Mann Whitney.

3. Results and Discussion

The 50 study participants ranged in age from 18 to 72 (median 53) years with an even gender distribution. Haemoglobin ranged from 5.9 to 17.8 (median 14.1) g/dL, with higher values in participants with hypoxaemia (low blood oxygen levels) induced by pulmonary arteriovenous malformations (*19*) that affect at least 50% of people with HHT (*20*).

Of the 50 participants, 24 (48%) had used dietary supplements in the previous year. Twelve of the HHT-affected study participants used iron supplements which would be fully appropriate given their high iron requirements, but 18/50 (36%) also used between 1-3 non-iron containing supplements (Figure 1). There were no evident gender differences, but compared to the 26 study participants using no supplements (median age 53, IQR 40, 63 years), there were trends for iron supplement users to be younger (median age 47, IQR 39.5, 62.3 years) and non-iron supplement users to be older (median age 58.5, IQR 49.5, 66 years).

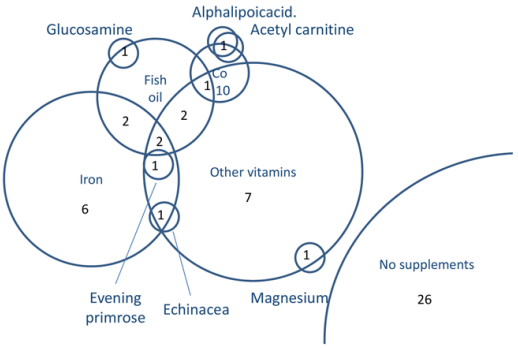

Figure 1. Dietary supplement use by 50 HHT patients. Venn diagram indicating overlapping use of dietary supplements, with circles to scale according to number of participants ingesting the item in the previous year.

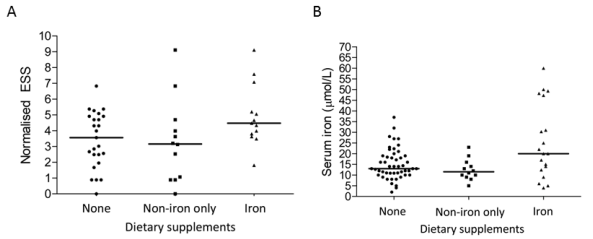

Figure 2. Nosebleeds and serum iron, categorised by dietary supplement use. (A) Epistaxis severity score (ESS): The ESS ranges from zero (no nosebleeds) to 10. As outlined in Figure 1, the non iron supplements comprised vitamins (N = 15), fish oil (N = 8), glucosamine, coenzyme Q10, alpha lipoic acid, echinacea, magnesium, and evening primrose. Median values indicated. 3 group comparison, p = 0.075 by Kruskal Wallis. **(B)** Serum iron in the 50 study participants. Note that the institutional normal range is 7-27 µmol/L. Median values indicated. Overall Kruskal Wallis p-value = 0.033. Data from all 50 study participants.

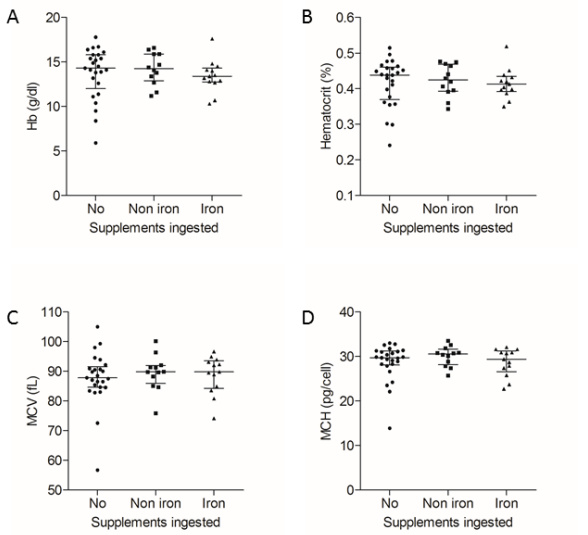

Figure 3. Red blood cell indices, categorised by dietary supplement use. (A) Haemoglobin (Hb, Kruskal Wallis p = 0.41); **(B)** Haematocrit (Kruskal Wallis p = 0.62); **(C)** Mean corpuscular volume (MCV, Kruskal Wallis p = 0.69); **(D)** Mean corpuscular haemoglobin concentration (MCH, Kruskal Wallis p = 0.66). Median and interquartile range indicated. Data from all 50 study participants.

The cohort demonstrated the full spectrum of nosebleed severity with epistaxis severity scores ranging from 0 (none) to 9.1 out of 10 (median 3.9; IQR 2.5, 5.0). The highest ESS of 9.1 was observed in two supplement users- one used fish oil and vitamins, and one used iron (Figure 2A). Overall, HHT patients with more nosebleeds tended to use iron supplements: the median ESS in the 38 non-iron users combined was 3.2 (IQR 1.8, 4.8) compared to 4.7 (IQR 4.0-5.2) in the 12 iron supplement users (p = 0.012 by Mann Whitney).

The difference in nosebleeds between the iron and non-iron using HHT cohorts substantially exceeded the minimal important difference for the epistaxis severity score (*17*), and corresponded to the difference between one nosebleed per week, typically lasting 1 to 5 minutes, and an average of one a day, typically lasting 6-15 minutes (*16*). For a male, the haemorrhage-adjusted iron requirement (HAIR) (*3*) would increase from 9.1 to 35 mg/day, compared to the UK recommended nutrient intake (RNI) of 8.7 mg of iron per day (*21*).

However, the HHT patients using iron supplements had higher serum iron concentrations than the other study participants (Figure 2B), and despite their more frequent nosebleeds, were able to maintain their red blood cell indices (Figure 3). There was no discernable trend in mean corpuscular volume (MCV) or mean corpuscular haemoglobin (MCH), indices that are generally unaffected by the secondary erythrocytosis observed in hypoxaemic patients with PAVMs (*19*). There were only possible trends towards lower haemoglobin and haematocrit values (Figure 3).

As noted in Figure 1, 18/50 (36%) study participants used non-iron containing supplements. These were most commonly vitamins (N = 15) or fish oil (N = 8), and included glucosamine, coenzyme Q10, alpha lipoic acid, echinacea, magnesium, and evening primrose. The one individual using echinacea had stopped taking it, because it seemed to make their nosebleeds worse.

Fish oil supplements are known to have anti-platelet activity (*22-25*). In the cohort as a whole, epistaxis severity scores did not differ between users of fish oils and any other group (data not shown). However, subtle differences emerged examining indices related to platelets in blood from fish oil users compared to non users of fish oils. Platelet counts were lower in supplement users (p = 0.037, Figure 4A), while serum fibrinogen, which can act as a circulating protein for platelet aggregation (Figure 4B), also tended to be lower in fish oil supplement users (p = 0.07). For a subgroup of 11 patients, 'selected' based purely on dates of attendance at clinic when the study was in progress, platelet aggregation studies were undertaken and provided provocative, preliminary findings: Within this very small sample, although there was no apparent difference in platelet aggregation to ADP between iron/

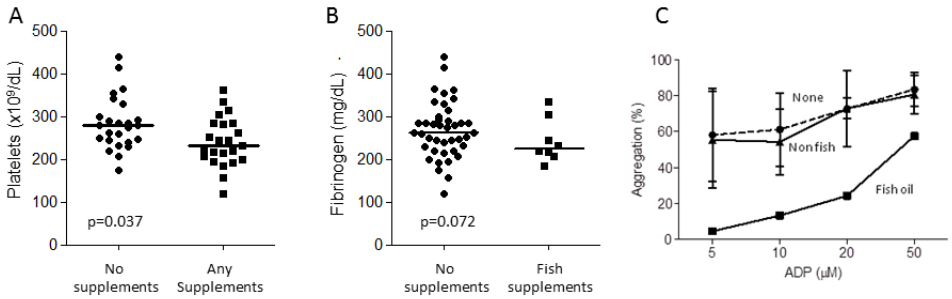

Figure 4. Coagulation and platelet aggregation. (A) Platelet counts and **(B)** Fibrinogen concentrations, in the 50 study participants, categorised by supplement use, median values indicated. There was no apparent difference in routine coagulation screens (prothrombin time, activated partial thromboplastin time, data not shown). **(C)** Platelet aggregation to stated concentrations of adenosine diphosphate (ADP) in the subgroup of 11 participants undergoing platelet aggregation studies, categorised by ingestion of no supplements ('None', $N = 7$), supplements other than fish oil ('non fish', $N = 3$), and fish oil ($N = 1$). Means and standard deviations illustrated.

other vitamins, and non supplement users (Figure 4C), the one individual using fish oil demonstrated markedly reduced platelet aggregation to ADP (Figure 4C).

The strengths of the study were the use of validated questionnaires to capture epistaxis severity and dietary supplement intake, and the evaluation of a patient population able to recognise and report their haemorrhagic losses in a quantifiable manner. It is clearly a small study that should be repeated in larger HHT cohorts, and the observational nature means it is difficult to infer causality, but we believe it does allow helpful conclusions to be drawn.

First, it was surprisingly common for people with HHT to use dietary supplements. The non-iron supplements were generally instituted by the patients themselves. The supplements were not recorded on communications to us, and we suspect, were not recognised within standard clinical care pathways.

Second, the supplements which were self-prescribed included fish oils with recognised anti-platelet activity. The study participants appeared to demonstrate subtle differences in platelet activity, although differences in epistaxis scores did not emerge in the small cohorts. High proportions of HHT patients avoid therapeutic antiplatelet and anticoagulant agents, often on medical advice (*26*). It is not known whether any potential anti-platelet activity for example, from Omega-3 compounds (*22-25*), would be sufficient to exacerbate HHT nosebleeds in the subgroup of individuals who report that their HHT nosebleeds are exacerbated by aspirin and/or clopidogrel (*26*). We are however, unaware of any medical recommendation for their use in haemorrhagic disorders, and in the clinic, find patients to be surprised and concerned that these "healthy supplements" may act as natural blood thinners.

Third, within this small observational sample, despite having worse nose bleeds, iron supplement users had higher serum iron than non iron users, and were able to maintain their baseline haemoglobin and red cell indices. This would support data from other sources suggesting oral iron absorption is appropriate in people with HHT (*i.e.* enhanced in iron-deficiency (*3*)). It may be of concern however, that for five iron users, serum iron concentrations were at least twice the upper limit of normal (Figure 2B), in keeping with recent data from a healthy volunteers study (*5*). One in 20 HHT iron users report nosebleeds are worse after iron treatments (*5,27*), and recent data suggest one plausible biological explanation through activation of endothelial DNA damage response pathways by 10 μM iron (*28*), an order of magnitude lower than examined in recent iron toxicity studies (*29,30*).

In conclusion, this study highlights how frequently people with HHT self-medicate with dietary supplements that may influence nosebleed (epistaxis) severity and platelet function. The scale of use, and potential of "natural health supplements" to exacerbate nosebleeds has not been appreciated previously in HHT. We suggest management of people with HHT and other haemorrhagic disorders should include a discussion of dietary supplements, and particularly the fish oil supplements that have recognised antiplatelet activity (*22-25*). For individuals with troublesome haemorrhage or iron deficiency, a trial of cessation of non-iron containing supplements may be indicated unless there has been clear benefit from their use.

Acknowledgements

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Case Report

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The retrieval of atrial septal defect closure device embolized into aortic arch

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Summary **Percutaneous atrial septal defect (ASD) closure has become an increasingly simplified procedure over the past decade. The device embolization is seen rarely but it can be fatal. Although percutaneous retrieval is feasible, surgical removal might be preferred when the endothelialization status of the device is unknown. We report a comlication of such closure in a 43-year-old woman: embolization of the ASD occluder device into aortic arch 12 months after implantation. We removed the device surgically and closed the ASD.**

Keywords: Atrial septal defect, septal closure device, migration, embolism, aortic arch

1. Introduction

Ostium secundum atrial septal defect (ASD) is one of the most common congenital heart defects in adults. As an alternative to surgery with a high rate of safe and success, the closure of ASD by percutaneous intervention has been the standard treatment approach. Device embolization is a complication which is rarely encountered and may be potentially resulted in death during or after the process, or in the late period. It is seen in about 0.3-0.6% of the cases (*1,2*).

2. Case Report

43-year-old female patient was admitted to our clinic with the complaint of chest pain and cyanosis of the lips occurring by the exercise. It was detected in the anamnesis that the patient underwent the percutaneous ASD closure operation approximately 12 months ago in another hospital. It was also found that the patient didn't go for routine checks. Chest x-ray revealed that the Amplatzer septal occluder was located into the aortic arch (Figure 1A). In the transthoracic echocardiography,the ASD was observed, but it was also detected that the device embolized into aortic arch (Figure 1B). In order to be able to identify the location of the device more clearly, she was examined by fluoroscopy and thorax computed tomography. It was confirmed by fluoroscopy and thorax computed tomography that the device was in aortic arch (Figure 1C and Figure 1D). As it was not known when the device embolized, it was decided to retrieve the device by a surgical operation considering the possibility of endothelialization into aortic arch and the thrombosis of the device.

Although the patient was stabile hemodynamically, she was taken to the operating room under emergency conditions. Axillary artery cannulation after median sternotomy, and unicaval two stage venous cannulation from right atrial appendage were performed. The patient was cooled to 24 degrees by entering the pump. The right atriotomy was made after potassium blood cardioplegia arrest. 2 × 3 cm size secundum ASD was detected. It was repaired primarily with 4/0 polypropylene suture.Then cross clamp was put to the innominate artery at 24 degrees hypothermia,and aortic arch was opened. Meanwhile, antegrade cerebral perfusion was continued with 500 mL of flow. It was observed that the embolized device was attached to the side wall of aortic arch, and it was almost completely endothelizedand largely buried in the aortic wall (Figure 2A and Figure 2B). After carefully excised, the defect

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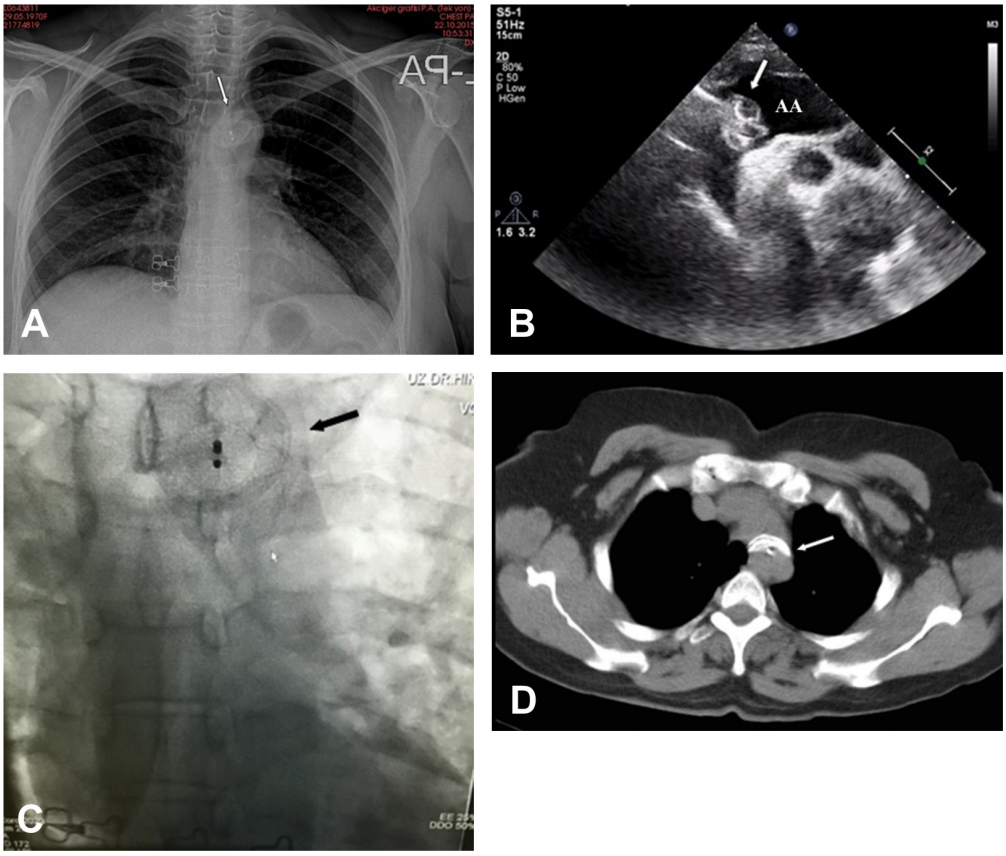

Figure 1. The images on admission. (A), Chest x-ray showing the device migration into the aortic arch. **(B)**, Transthoracic echocardiography showing atrial septal defect occluder device embolized into aortic arch. **(C)**, Fluoroscopy showing atrial septal defect occluder device embolized into aortic arch. **(D)**, Thorax computed tomography showing atrial septal defect occluder device embolized into aortic arch.

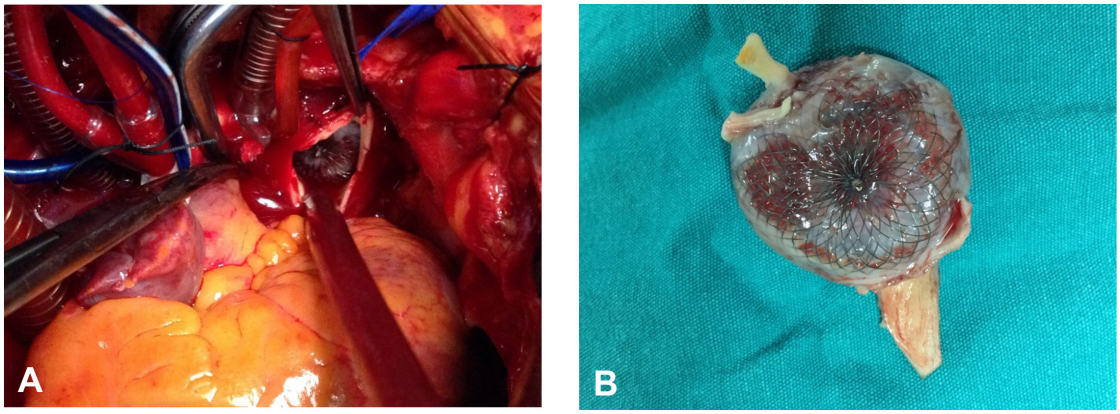

Figure 2. Intraoperative and macroscopic images. (A), Removal of the device with an incision of the aortic arch. **(B)**, Macroscopic image of the removed device.

on the aortic wall and the regions with endothelial damage were repaired from inside with the sutures reinforced with Teflon. Aortotomy was primarily repaired.After filling and air extraction process, the clamp in the innominate artery was retrieved and switched to perfusion with normal flow. While heating the patient, the right atriotomy was closed. The perfusion was completed smoothly.The postoperative course was uneventful and the patient was discharged with full recovery on the 7th day.

3. Discussion

The device embolization is the most frequently seen among the complications requiring surgical intervention in the percutaneous ASD closure. This complication is seen at a rate of 0.5% (*3*), but has a great importance because it can be fatal. Although generally, the device embolization occurs within the first few days after the percutaneous ASD closure, it may also be seen many years after the intervention (*4*).

The closure of ASD by a percutaneous intervention may fail for several reasons. These reasons may include a very large defect, and the lack of strength of rims to carry the device, extremely mobile interatrial septum, rupture occurrence in septum and the technical problems related to the insufficient experience of the intervention team (*5*). The failure of the insertion of the device and the acute embolization occurring at the same time is usually associated with the malposition of the device and the use of device with non-suitable size for defects. It is considered that the subacute embolization occurring within the first few days after the implantation is largely related to the erosion occurrence in aortic rim or the septum weakness (*3*). The device mobility after the implantation and the aortic rim narrower than 5 mm also increase the risk of early and late embolization (*6*).

The surgical or percutaneous interventional methods may be used in the retrieval of the embolized devices (*5*). Pala *et al*. reported the case of interatrial septal closure device embolized into aortic arch. During the process, the device embolized into aortic arch was successfully retrieved percutaneously using bioptome (*7*). In our case, the embolization time of the device was not known. Therefore,because of the high risk of thrombosis and endothelization of the device, surgical treatment was implemented and successful results were achieved. The surgical approach makes possible to both retrieve the device and to close the defect in the cases ASD is not suitable for percutaneous closure intervention. Also, there are those who argue the only surgical method is to be preferred in the retrieval of embolized device as it enables to detect the damage in the device embolisms in pulmonary artery and other cardiac cavities, and the damage that may occur during the embolization in intracardiac structures (*8*).

We think that the reason of device embolization may be the shortness and flexibility of inferoposterior rim in the defect observed during the surgery. Also, the endothelialization status of the retrieved device showed that embolization may have occurred months earlier. Because of the high risk of thrombosis and endothelialization of the device into aortic arch due to the fact that the time of embolization of the device was not known, the percutaneous intervention was not performed.

4. Conclusion

Device embolization is a rare but potentially fatal complication of transcatheter ASD closure. Although percutaneous retrieval is feasible, surgical removal might be preferred when the endothelialization status of the device is unknown. This case underlines the importance of proper patient selection and routine follow-up after the procedure.

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Case Report

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Cleidocranial dysplasia: A report of two cases with brief review

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Summary **Cleidocranial dysplasia (CCD) is a genetic disorder primarily causing dysplasia of bones and teeth with autosomal dominant inheritance pattern. Affected individuals presented with several skeletal and dental abnormalities mainly hypoplasia of clavicles, open fontanelles, short stature, retention of primary teeth, supernumerary teeth, delayed eruption of permanent teeth, multiple impacted permanent teeth *etc*. The present series of two cases illustrates the clinical and radiological features of pediatric patients with cleidocranial dysplasia. The early diagnosis of the condition helps in proper orientation of the treatment thereby offering better quality of life to such patients.**

Keywords: Marie and Sainton's disease, *CBF-1* gene, supernumerary teeth

1. Introduction

Cleidocranial dysplasia (CCD) is a well-known, congenial, developmental disorder that primarily affects bones undergoing intramembranous ossification *i.e*. calvarial bones and clavicles and teeth (*1*). This rare disease can occur spontaneously or by an autosomal dominant inheritance pattern, with no predilection of genre or ethnic group. Hypoplasia or agenesis of clavicles with a narrow thorax, which allows approximation of the shoulders in front of the chest and delayed ossification of the skull are the manifestations. Boys and girls have an equal chance of getting affected. The condition is of clinical significance to the dental practitioners due to the involvement of facial bones, altered eruption patterns, presence of retained primary teeth, delayed eruption of permanent teeth and multiple supernumerary teeth *etc*. (*2*)

The aim of this article is to describe the clinical features, radiological features and associated dental abnormalities of two cases of pediatric patients with CCD.

2. Case Report

Case report I A 14 year old male child patient reported to the department of pedodontics and preventive dentistry with the chief complaint of pain in the right and left upper posterior back teeth region and delayed eruption of teeth in the lower front teeth region. Detailed medical history revealed him to be a known case of CCD. No other members of his family suffered from this present medical condition.

General physical examination demonstrated short stature, thin and lean build, slurred speech and drooping shoulders that can be brought easily forward in the midline if asked. Macrocephaly, frontal, parietal and occipital bossing was seen that gives a skull a large globular shape (Arnold head). Depressed nasal bone, hypertelorism, mid-face hypoplasia and mandibular prognathism were also noticed (Figure 1). Intraoral examination showed high arched and narrow palate, presence of multiple retained primary teeth and delayed eruption of permanent teeth. Supernumerary teeth were seen in the maxillary midline and bilateral mandibular premolar region. Radiological investigations included orthopantomogram (OPG) that revealed several unerupted permanent teeth and supernumerary teeth in both maxilla and mandible. Gonial angles on both sides of mandible were missing and maxillary sinuses were underdeveloped. Narrow thorax with oblique ribs and hypoplastic clavicles were seen on chest radiograph. Lateral cephalogram and PA view of skull revealed open fontanelles, depressed skull, multiple wormian bones and prognathic mandible (Figure 2A, 2B, and 2C; Figure 3A, 3B, and 3C).

Case report II A 10 year old child patient reported to the department of pedodontics and preventive dentistry

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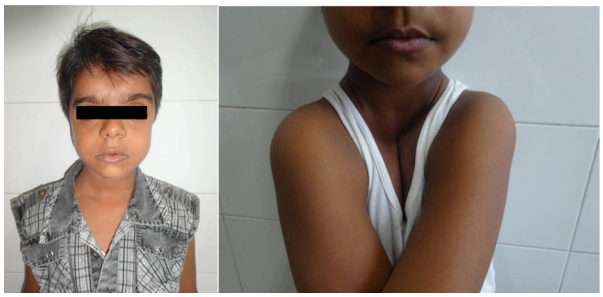

Figure 1. Frontal view of the patient with approximation of shoulders in midline.

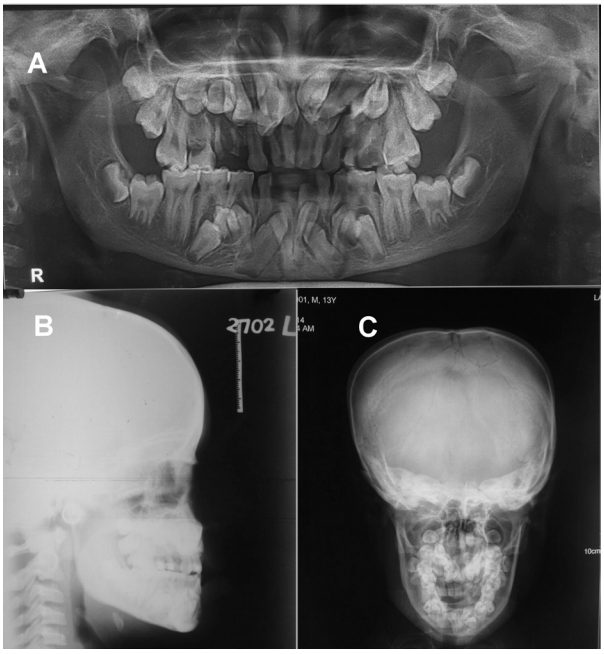

Figure 2. Radiographs showing dental abnormalities (A), OPG shows supernumerary teeth, multiple impacted teeth and over retained primary teeth. **(B)**, Lateral view of skull shows broad sutures and wormian bones. **(C)**, Anteroposteriar view of skull showing sunken sutures and depressed calvaria.

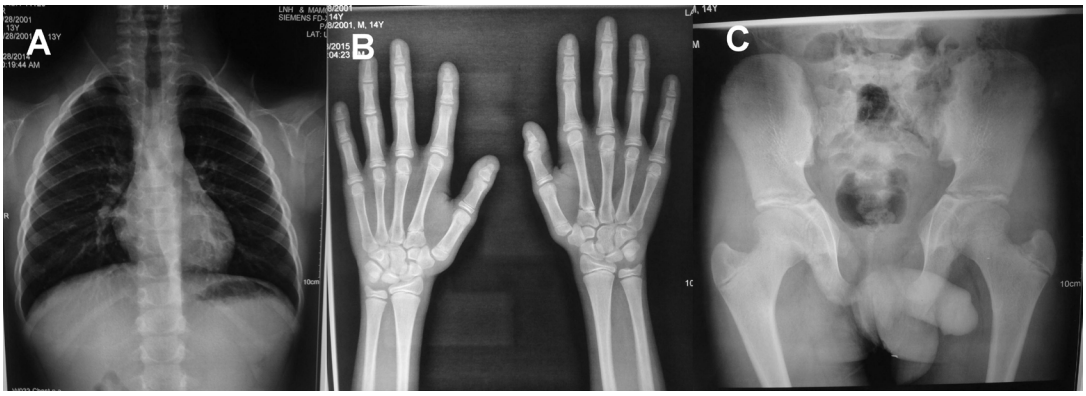

Figure 3. Radiographs showing skeletal abnormalities (A), Chest radiograph showing hypoplastic clavicles and narrow thoracic cage. **(B)**, Hand wrist x-ray showing metacarpel pseudoepiphysis and **(C)** x-ray pelvis shows wide pubic symphysis ,broad femoral heads and short femoral necks.

with a chief complaint of delay eruption of teeth in the upper front teeth region. Detailed medical history was obtained through parents that described him a follow up case of celiac disease with primary hypoparathyroidism with CCD and global developmental delay.

Short height, apparently well built, narrow thoracic cage and shrugged shoulders which were easily apposable were the features seen on general examination. Extraoral findings were hypertelorism, brachycephaly, depressed nasal bridge, prominent forehead and maxillary hypoplasia (Figure 4). Intraoral findings were multiple retained permanent teeth, delayed exfoliation of primary teeth. Radiological investigations such as OPG, lateral cephalogram, chest radiograph, P-A view of skull and pelvis were carried out to confirm the diagnosis. Multiple unerupted permanent teeth, supernumerary teeth in the region of 11 and 45 were seen on OPG. Cranial abnormatilies such as calvarial thickening, open fontanelles, wormion bones, sunken sagittal sutures were also evident (Figure 5A, 5B, and 5C). Barrel shaped thorax with hypoplastic clavicles were also noticed on chest x-ray Metacarpal pseudoepiphysis, absence of carpel bones and wide pubic symphysis with short femoral neck were also seen on hand wrist and pelvic x-ray respectively (Figure 6A, 6B, and 6C).

3. Discussion

CCD is also known as Marie and Sainton's disease, Scheuthauer-Marie-Sainton syndrome, Mutational dysostosis. It was first described by Pierre Marie and Paul Sainton in 1898 (*3*). They coined the term

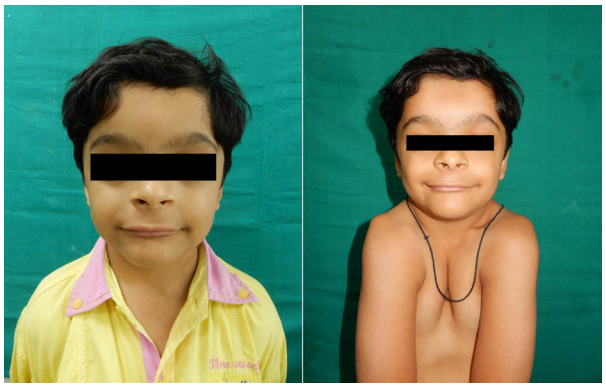

Figure 4. Characteristic feature of CCD patient: Adduction of shoulders.

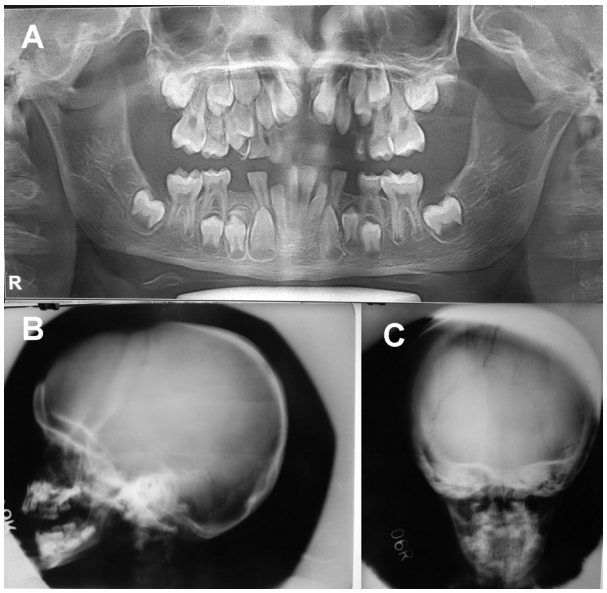

Figure 5. Radiographs showing dental abnormalities (A), Retained primary teeth and supernumerary teeth seen on OPG. **(B)** and **(C)** Cranial abnormalities such as open fontanelles, sunken sutures, wormian bones seen on lateral view and anteroposterior view of skull.

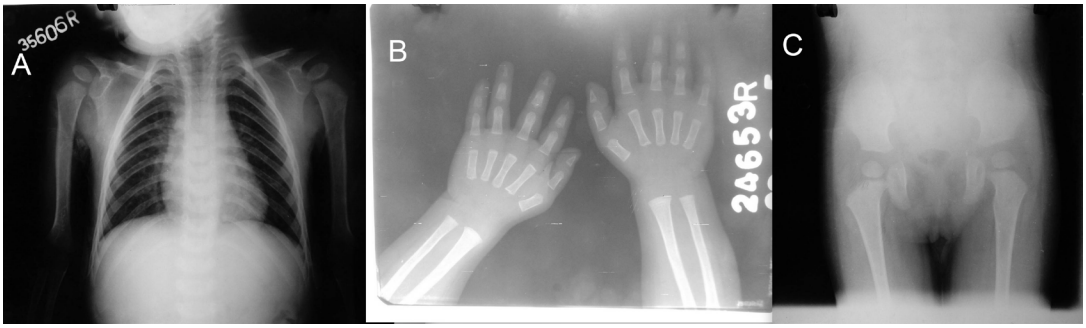

Figure 6. Radiographs showing skeletal abnormalities (A), Chest radiograph shows hypoplastic clavicles bilaterally. **(B)**, Absence of carpel bones seen on hand wrist x-ray. **(C)**, Pelvic abnormalities seen on radiograph.

'Cleidocranial Dysostosis' (*4*). This condition is known to be associated with several chromosomal abnormalities such as rearrangement of long arm of chromosome 8 and 6 or mutation in the core-binding factor alpha-1 (*CBFA-1*) gene, located on chromosome 6p21, which encodes a protein necessary for the correct functioning of osteoblast cells (*5*). *CBFA-1* also called as *RUNX2*-Runt related transcription factor 2 gene, is expressed specifically in chondrocyte and osteoblast progenitors, as well as in mature osteoblasts. It regulates the expression of several important osteoblast proteins including osterix (another transcription factor needed for osteoblast maturation), osteopontin, bone sialoprotein, type I collagen, osteocalcin and receptor activator of NF kβ ligand (*5*).

Patients with CCD have short height and have frontal, parietal and occipital bossing leading to bulging calvarias. There is a partial or complete absence (in about 10% of cases) of the clavicle permitting an abnormal mobility of shoulders, open fontanelles, wormian bones, a wide pubic symphysis, short middle phalanges of the fifth fingers, and various vertebral and dental abnormalities. Dental manifestations are underdeveloped maxilla, relative mandibular prognathism, retained primary dentition, multiple impacted permanent dentition, delayed eruption of permanent teeth, multiple supernumerary teeth, crown and root abnormalities, crypt formation around impacted teeth, and a high palate (*6-8*). Various scientific views has been postulated regarding etiology of non-eruption of permanent teeth, such as lack of cellular cementum (*9*), defectiveness in post cementum formation (*10*), presence of thick connective tissue between oral epithelium and dental follicle (*11*), delayed tooth formation and maturation (*12*).

Dental management of cleidocranial dysplasia is largely dependent on the chronological and dental age of the patients. Timely diagnosis with appropriate treatment plan is essential for attaining successful results (*13,14*). A multidisciplinary approach for the management of these patients utilizing a pedodontist, an orthodontist, an oral surgeon and prosthodontist is

recommended. Various treatment options available are: removal of the impacted permanent, supernumerary and primary teeth, combined with fabrication of over-dentures; surgical removal of the primary and supernumerary teeth, combined with orthodontic traction of the impacted permanent teeth; removal of the supernumerary teeth immediately after completion of mineralization of their crowns, combined with removal of the overlying bone of the permanent teeth to facilitate their eruption (*13,15,16*). However, Pusey and Durie also suggested removal of only erupted teeth and use of a removable prosthesis to minimize alveolar bone loss (*17*).

The present case report highlights the need for awareness among pedodontists about the prosthodontic rehabilitation of pediatric patients with CCD syndrome as it not only causes physical discomfort but also leads to psychological problems. Therefore, along with achieving a well functioning dentition and an esthetically satisfying facial appearance, proper motivation and psychological support for the patients and their parents are also important.

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Case Report

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A case of split notochord syndrome: Presenting with respiratory failure in the neonatal period

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Summary **Split notochord syndrome (SNS) is a very rare congenital anomaly. This report describes a male newborn with a neuroenteric cyst in the posterior mediastinum and multiple vertebrae anomalies presenting with respiratory failure and pulmonary hypertension. This report also discusses the embryological development and the etiologic theories of SNS.**

Keywords: Split notochord syndrome, newborn, respiratory failure

1. Introduction

Split notochord syndrome (SNS) is a very rare congenital malformation that is associated with anomalies of the vertebrae, central nervous system, and gastrointestinal tract (*1,2*). In this syndrome, anomalies arise from a connection between the endoderm and dorsal ectoderm (*3,4*). The variety of malformations and clinical symptoms depends on when abnormal splitting of the notochord occurs and the size and location of that split (*5*). The current report describes a newborn with SNS presenting with respiratory symptoms, and this report also discusses SNS and its different presentations.

2. Case Report

A 12-day-old boy, weighing 2,920 g, was referred to the Neonatal Intensive Care Unit (NICU) of Goztepe Medical Park Hospital, Bahcesehir University School of Medicine with respiratory failure.

The baby was born full-term *via* a Caesarean section as the first child of a 24-year-old mother. The mother had no history of radiation exposure, drug ingestion, alcohol use, or smoking during pregnancy, no family history of congenital anomalies, and there was no consanguinity.

At birth, the infant was admitted to the NICU for dyspnea and tachypnea. A few hours later, intubation was performed and an intratracheal surfactant was administered. The infant was administered antibiotics for 10 days because of elevated CRP levels. The initial diagnosis was congenital pneumonia. However, the infant could not be weaned from mechanical ventilation and a computed tomography scan of the chest revealed a cyst in the right lung. The infant was transferred to this facility to determine the etiology of and the treatment for the cyst in the right lung.

Upon physical examination, the infant had severe respiratory failure and decreased breath sounds in the right lung but other findings were normal. An anteroposterior (AP) chest radiograph revealed a large, well-defined mass in the right hemithorax, displacing the mediastinum to the right, and cervico-thoracic vertebral anomalies (Figure 1). Ultrasonography of the right pleura revealed pleural effusion, and magnetic resonance imaging of the chest and abdomen revealed a homogeneous, unilocular hyperintense cystic mass in the right prevertebral region without extension to the spinal canal (Figure 2). Echocardiography findings were consistent with pulmonary hypertension. Blood tests revealed elevated levels of C-reactive protein (71.17 mg/L) and an elevated white blood cell count (28,650 cells per cubic mm). Blood, endotracheal, and urine cultures were negative. The antibiotics vancomycin and

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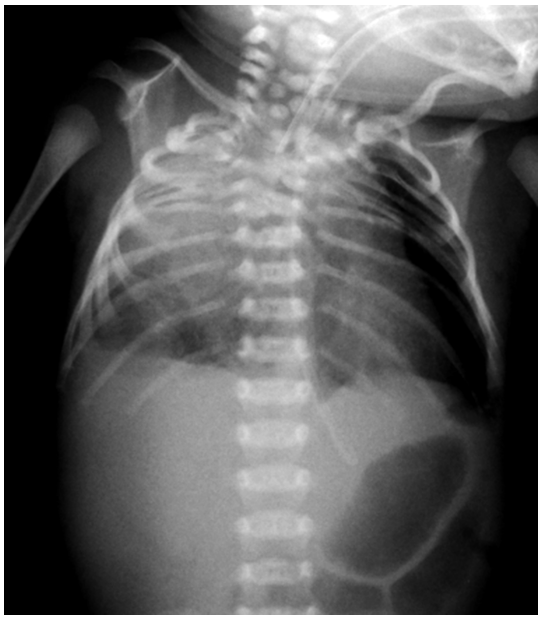

Figure 1. AP chest radiograph showing a large, well-defined mass in the right hemithorax, displacing the mediastinum to the right, and cervico-thoracic vertebral anomalies.

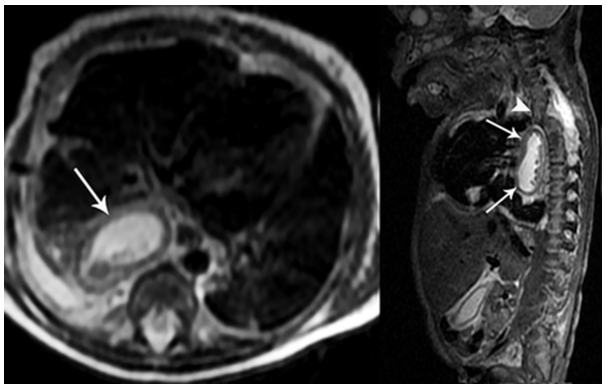

Figure 2. Axial (right) and sagittal (left) T2-weighted magnetic resonance images showing a homogeneous unilocular high signal cystic mass in the right prevertebral region without extension to the spinal canal.

meropenem were initially administered and fluconazole was added. Sildenafil was administered to treat pulmonary hypertension. A chest tube was placed to drain the pleural effusion. The patient was intubated and on mechanical ventilation in the synchronized intermittent mandatory ventilation (SIMV) mode. Since blood gases revealed respiratory acidosis and hypercarbia, the mode of the mechanical ventilator was switched from SIMV to High frequency oscillatory ventilation (HFOV). Ventilation in HFOV mode was continued for 8 days. On the 9th day of hospitalization, mechanical ventilation was changed to SIMV mode, and the infant was sent to Pediatric Surgery for surgery to remove the cyst in his right lung. A right thoracotomy was performed. The mass was in the posterior mediastinum and it was completely removed. Histopathology revealed well-differentiated simple columnar epithelium with glandular organization and smooth muscle (type B neuroenteric cyst, Wilkins and Odom system for histopathological classification of neuroenteric cysts) (Figure 3). After surgery, the infant remained on mechanical ventilation for 18 days. On the 10th day postoperatively, enteral feeding was started and gradually continued. The infant was discharged from the hospital at 53 days of age.

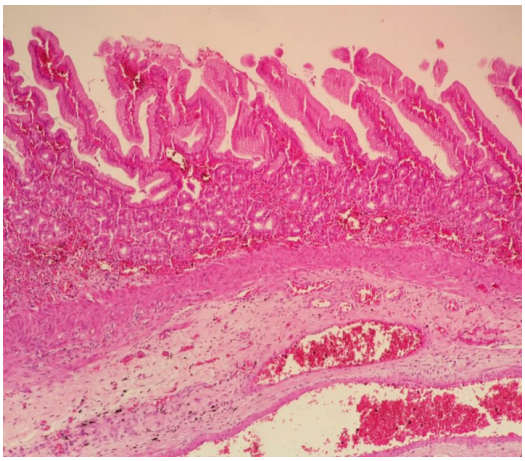

Figure 3. Resected specimen. Well-differentiated simple columnar epithelium with glandular organization and smooth muscle (type B neuroenteric cyst, Wilkins and Odom system for histopathological classification of neuroenteric cysts).

3. Discussion

SNS was first described by Rembe in 1887 (*6*). The congenital anomalies in SNS include anomalies of the vertebrae (anterior and posterior spina bifida and butterfly vertebrae), the central nervous system (diastematomyelia, diplomyelia, and myelomeningocele), and the gastrointestinal tract (fistulas, dermal sinus tract, diverticula, and enteric cysts) (*7,8*). The syndrome manifests as a cleft in the dorsal midline of the body through which intestinal segments are exteriorized, myelomeningocele, and occasionally as a teratoma. Hydrocephalus and diastematomyelia/diplomyelia are also associated with the syndrome (*9*). Congenital anomalies associated with SNS can be detected antenatally (*4*). The current case involved a newborn with an esophageal duplication cyst in the posterior mediastinum.

During the embryological development of the human embryo, the human embryo consists of ectoderm, mesoderm, and endoderm layers in the third week of gestation. The notochordal process appears as a tube in the mesodermal layer by day 20. The ventral wall of the notochordal process then begins to fuse with the endoderm and thus forms the notochordal plate. In a short amount of time, an open neuroenteric canal is formed between the yolk sac cavity and the amniotic cavity. The final remnants of this canal are located at the tip of the os coccygis. The notochordal process then forms the notochord. The paraxial mesoderm forms somitomeres and somites, and somites differentiate into

sclerotomes. These give rise to the vertebral bodies, vertebral arches, and part of the back of the skull. In the fourth week of the development, the neural plate transforms into the neural tube. Vertebral anomalies result because of inadequate closure of the neural tube (*10*). SNS is a failure of the notochord to split from the foregut, resulting in a fistula or cyst. Attachment of a cyst to the notochord prevents the fusion of vertebral bodies. This results in vertebral column anomalies such as scoliosis, hemivertebrae, and spina bifida. As the embryo grows, these cysts move caudally and the intrathoracic viscera descend, and vertebral anomalies are often found in the lower cervical spine. The current patient had anomalous fusion of the upper cervical vertebrae. The clinical findings of a duplication cyst depend on the origin, size, and nature of the cyst. The most common form is midgut duplication (*11,12*).

Bremer and Sanders have described 2 theories regarding the etiology of SNS. Bremer suggested that a dorsal intestinal fistula may result from the complete retention or only partial obliteration of the primitive neurenteric canal. A widely accepted view is that presented by Sanders, who suggested that a split or localized duplication of the notochord may cause this anomaly and that the primitive gut or endoderm herniates through the opening and adheres to the dorsal ectoderm (*2*).

Although most reported cases of SNS involve the cervical and thoracic region, signs of the syndrome can be found at any level of the spine. Schurink *et al*. described a 5-year-old boy with a mass between the shoulder blades, and they noted that the tumor affected the sixth and seventh thoracic vertebrae *(13*). Although the mass was successfully removed, the thoracic vertebrae had partially collapsed. The current case involved a neuroenteric cyst in the posterior mediastinum and multiple cervical and thoracic vertebral anomalies. A significant symptom in the current case is severe respiratory failure. Plain chest x-rays revealed vertebral and rib anomalies. During follow-up, the infant was unsuccessfully extubated several times, so lung tomography was performed, followed by MR imaging of the chest and abdomen.

A neuroenteric cyst does not present with any pathognomonic signs, so the infant's chest x-rays showed no specific features of a cyst. Magnetic resonance imaging of the chest and abdomen revealed a cyst in the mediastinum that communicated with the esophagus. After excision of the cyst, histologic examination of specimens revealed gastric mucosa, gastric submucosa, and pancreatic tissue. Pathology proved that the cyst was a neuroenteric cyst.

In conclusion, SNS is very rarely seen, so the management of SNS must be tailored to the different anomalies present in each case. In the present case, excision of a cyst resulted in improvement of respiratory symptoms. Early diagnosis and treatment of SNS improves the prognosis for patients.

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Case Report

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Infantile systemic hyalinosis: Report of two severe cases from Saudi Arabia and review of the literature

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Summary **Infantile systemic hyalinosis (ISH) (OMIM 228600) is a rare fatal autosomal recessive disorder characterized by extensive deposition of hyaline material in many tissues. Consanguinity has been recorded in many cases. Herein we present two new Saudi cases with review of the literature. Our first proband was a 9 month-old male who was the first baby for parents descended from a closed consanguineous pedigree. The second proband was a 13 month-old male who was the first baby for consanguineous parents (3rd C). Both cases presented with bilateral painful limited limb movement with joints contractures, low birth weight (< P5), severe generalized stiff skin, hyper-pigmented skin over bony prominences, fleshy perianal masses and gingival hypertrophy. The first child died at 18th month as a result of recurrent chest infections. The second proband showed a severe progressive course of joint contractures, and died at 19th month because of failure to thrive and recurrent infections. Although the clinical features of ISH are characteristic, the disease is under/miss diagnosed. The role of consanguinity needed to be highlighted to the community. Careful clinical examination and molecular diagnosis will be helpful for genetic counseling, prenatal diagnosis and early treatment.**

Keywords: Hyalinosis, AL Madinah, consanguinity

1. Introduction

Infantile systemic hyalinosis (ISH) (OMIM 228600) is a very rare fatal autosomal recessive disorder of connective tissue belonging to the heterogeneous group of genetic fibromatosis (*1*). Both males and females are equally affected. The disease presented usually at birth or within the first few months of life with progressive painful joint contractures, skin hyperpigmentation over bony prominences, and papules on the face, scalp, and neck (*2*).

Other notable characteristic features are gingival hypertrophy and thickened skin. However, the children with ISH are found to be intellectually normal. Affected children suffer from osteopenia which results in increased susceptibility to bone fractures. Intractable diarrhea as a result of protein loss enteropathy and/or recurrent infections are commonly reported in children suffering from ISH. Because of multisystem failure resulting from complicacy, many patients die in infancy (*3,4*).

The disease was first called Molluscum Fibrosum by Murray in 1873 (*5*) then Juvenile Hyaline Fibromatosis (JHF) and ISH. (Drescher *et al*., 1967; Landing and Nadorra, 1986) (*2,6*). Due to similarity of the clinical features of both JHF and ISH, the Hyaline Fibromatosis (HF) term was used by some authors. ISH could be differentiated from JHF by its severe phenotype. About 150 cases have been reported in the literature without notable ethnic or geographic predisposition (*7*).

Histologically ISH is characterized by deposition of hyalinized fibrous material (glassy translucent substance of glycolprotein) in many tissues like skin, skeletal muscle, cardiac muscle, gastrointestinal tract, lymph nodes, spleen, thyroid, and adrenal glands (*8*).

Capillary morphogenesis gene 2 - capillary morphogenesis protein 2 (*CMG2*)/Anthrax Toxin Receptor 2 (*ANTXR2*) - mapped to chromosome 4q21.21, was identified as the gene responsible for these two rare autosomal recessive human genetic disorders, ISH, (MIM

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236490) and JHF, (MIM 228600) (*7,9*).

Type I membrane protein, which is universally expressed in all human tissues, with exception of the brain, is encoded by the *CMG2* gene (*10*). Scobie *et al*., 2003 reported *CMG2* as the subsequent receptor for the anthrax toxin, hence its certified name of *ANTXR2*. Almost 34 different mutations have been investigated, predominantly in exons and spread from exon 1 to exon 15. Although most reported mutations have been identified only once, there is a mutational hotspot at position (1074-1077) in exon 13 where insertion or deletion of one or two bases has been described (*7,10*).

Non-cancerous tissue proliferation and nodules are the most outstanding external features found for all patients and are the hallmark of the disease. These nodules are not seen at birth but may develop in the first month of life. Since those nodules develop over areas of mechanical stress, upregulation of microtrauma repair mechanisms might be the suggested pathogenesis (*11*). The nodules were found to be rather cellular at the onset, while older ones contained mainly extra cellular matrix (*12,13*).

Currently the diagnosis of ISH is based on clinical data but molecular genetic testing is available on a research basis only. Treatment of ISH is palliative as physical therapy and nutritional support improve the quality of life of the patients (*14*).

In this report we present two new Saudi cases from Almadinah Almonwarh province referred to us for genetic evaluation during our work in Taibah University from September 2013 to September 2014. These two cases were of interest because of closed consanguineous pedigree, severe course of the disease, huge popular-nodules at the perianal region, generalized joint contractures, failure to thrive and death before 2 years of age. We aimed to present a fine characterization of the phenotype of ISH in order to facilitate early diagnosis of cases, which are very important for genetic counseling and early treatment.

2. Case 1

A 9-month old male child who was the first baby of an apparently healthy young couple descended from a closed consanguineous pedigree (Figure 1) was referred for a medical genetics consultation. The family history was negative for birth defects and genetic disorders except for primary infertility. He was a full term baby born by vaginal delivery after induction. Pregnancy was complicated with threatened abortion during the first trimester that was treated with hormonal therapy. Reduced and weak intrauterine fetal movement was noticed by his mother. Prenatal ultrasound examination at 36 weeks of gestation showed small sized baby with normal amount of amniotic fluid.

At birth, his weight was 1,300g (less than 5th percentile). His cry was delayed with limited painful limb movement bilaterally. Within the first three months of life, parents noticed progressive limb contractures with limitation of joint movements of the four limbs and pain on minimal handling together with obvious perianal masses.

Genetic examination was done at 9th month of age, his height was difficult to be measured due to severe joint contractures, weight was 5 kg (less than 5th Percentile), and head circumference was between 10th and 25th percentile. Weak cry and irritability was noticed with high sensitivity and excessive crying on passive movement of his limbs. Failure to thrive and delayed motor milestones like neck support and sitting were diagnosed.

Craniofacial examination revealed minimal coarse faces like broad forehead, down slanting palpebral

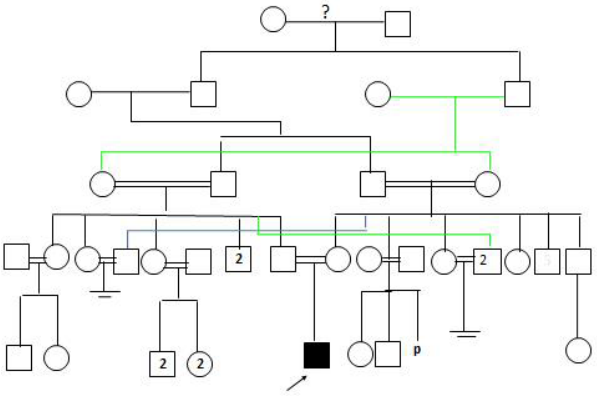

Figure 1. Pedigree of the studied family of the first case: three generations were studied from both parents. Close consanguinity and primary infertility on both paternal and maternal side were noted. P: current pregnancy at the time of the study.

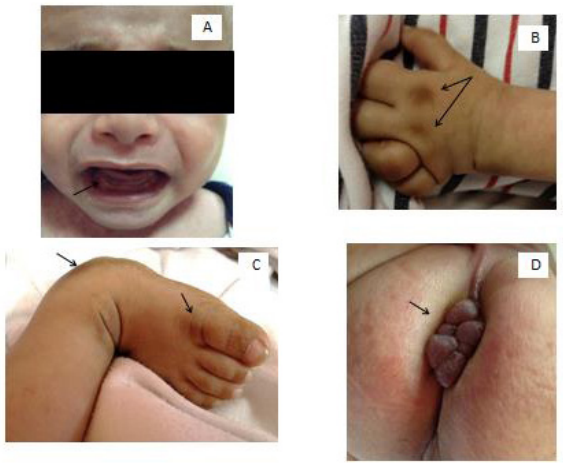

Figure 2. Clinical features of case 1. (A) Gingival hypertrophy with delayed teeth eruption. **(B)** Skin overlying metacarpophalangeal and proximal phalangeal joints was shiny, hyperpigmented and thickened. Contractures of metacarpophalangeal and interphalangeal joints. **(C)** Both feet showed marked contractures of metacarpophalangeal joints, dorsiflexion, skin hyperpigmentation. Heel skin is thickened and hyperpigmented. **(D)** Giant papular perianal nodules.

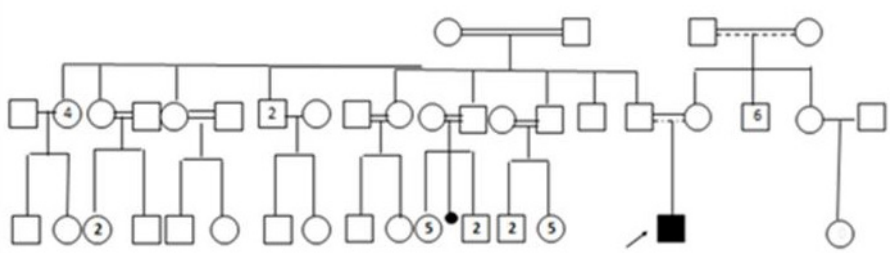

Figure 3. Pedigree of the studied family of the second case. The parents were third cousin relatives and close consanguinity was manifested on the paternal side.

fissures, depressed nasal bridge, low set ears and gingival hypertrophy. By cutaneous examination, his skin was thick and shiny showing hyperpigmentation over bony prominences like malleoli and knuckles of upper and lower limbs but there were no focal nodules and/or papules. Limited joint movement with tenderness and pain with no redness or hotness was seen. Flexion deformity was present at elbows, wrists, knees, ankles and small joints of both hands (Figure 2).

Chest, abdomen and genitalia were clinically free. By inspection, fleshy perianal nodules were seen. Hematological and biochemical investigations were normal. Lysosomal storage diseases were excluded by MS/LS analysis and chromosomal malformation syndrome was absent as shown by normal male karyotype (46, XY).

Radiological skeletal survey demonstrated generalized osteopenia with bilateral periosteal reaction of radii, ulnae, tibia and fibulae. MRI brain showed slight prominence of ventricular system and cortical sulci. Abdominal ultrasound and computerized tomography (CT) screening organs were normal. Ophthalmological examination was normal.

The course of the disease was severe and progressive, and the child had recurrent episodes of pneumonia. At 18th month he suffered from pneumonia with no response to treatment, deterioration of the general condition occurred and the child passed away.

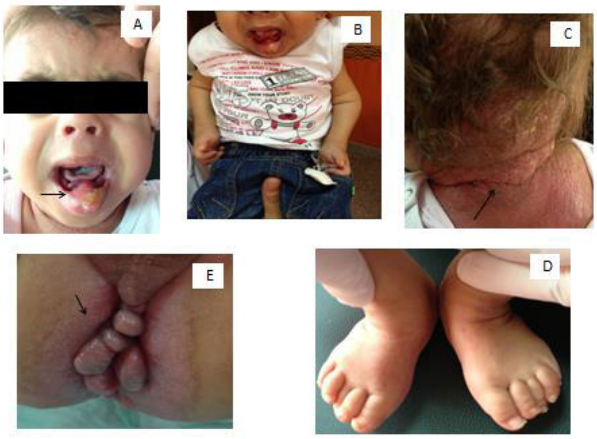

Figure 4. Clinical features of case 2. (A) Marked gingival hypertrophy with normal pink firm gingival tissue which almost covered erupting teeth. The lower lip is everted due to prominent gingival mass. **(B)** Contractures and flexion deformity at both elbow joints. **(C)** Severe eczematous papules and nodules in the auricular area and neck. **(D)** dorsiflexion of foot with hyperpigmented skin patches over heels. **(E)** Large papular perianal nodules and severe eczema in perianal area.

3. Case 2

A full term male baby who was the first child of an healthy consanguineous couple, a mother aged 23 years and the father was 28 years (3rd Cousins) (Figure 3). The family history was negative for similar conditions or any genetic disorders. Pregnancy course was normal except for reduced fetal movement. Prenatal follow up ultrasound showed normal amniotic fluid. Labor was induced at 40th month of gestation by caesarian section due to fetal bradycardia.

Birth weight was 3,000 g with a weak cry, the baby was stable and discharged from the hospital. Few days later, the mother noticed painful limited limb movement had started on the right upper limb then limited mobility became bilateral and involved the four limbs with severe pain on handling. The child was admitted to the hospital for clinical evaluation. Laboratory investigations were normal and CT brain was unremarkable. Perianal masses were observed by the parents at 9th month of age and oral mass appeared at the first year.

Genetic examination was done at 13th month of age, his height was 62 cm with joint contractures, his weight was less than 5th percentile (7.5 kg), head circumference was 47 cm. his motor milestones were delayed.

Craniofacial examination revealed broad forehead, down slanting palpebral fissures, depressed nasal bridge, low set ears and gingival hypertrophy with a marked central swelling extended deep in the bottom of the tongue. On cutaneous examination, skin was of normal texture with normal hair distribution. Hyperpigmentation over bony prominences like malleoli and knuckles was seen, with no focal nodules and/or papules. Severe eczema around the neck and in napkin area was present. Limited joint movement with pain and tenderness in all extremities was seen, and there were no redness or hotness and flexion deformity present at elbows, wrists, knees, ankles and small joints of both hands (Figure 4). Chest, abdomen and genitalia were clinically free. By

inspection, large fleshy perianal nodules were seen.

Radiological skeletal survey demonstrated generalized osteopenia with bilateral periosteal reaction. CT brain was normal with wide extra-axial CSF places. Abdomenal ultrasound was normal with no organomegally and ophthalmological examination was normal. The case had normal enzymatic studies for various lysosomal storage diseases. Chromosomal analysis of this case showed normal male karyotype (46, XY).

The general condition was deteriorating and the child died at 19th month of age because of failure to thrive and recurrent chest infections.

4. Discussion

ISH and JHF shares many clinical resemblances, both are caused by mutations in the *CMG2/ANTXR2* gene. Both syndromes are characterized by gingival hypertrophy, bilateral generalized joint contractures, and subcutaneous and perianal fleshy nodules. A progressive severe course, an earlier onset and death in early childhood characterize ISH. They share characteristic histological similarities (*15*).

Since ISH is rare it is difficult to diagnose, and it should be differentially diagnosed very carefully from other diseases that present with chronic pain, subcutaneous nodules, multiple malformations and joint contractures.

Chronic pain and joint contractures in infancy have very limited differential diagnosis. The most important one is lysosomal storage diseases (LSD). ISH may have some resemblance to LSD. The two studied patients herein had normal enzymatic studies for various LSD including, Farber disease (Farber lipogranulomatosis) [OMIM 228000] which presents with skin nodules over joints, painful movement and diminished cognitive function. On the contrary ISH child is cognitively normal. Also in ISH the abnormal hyaline material is deposited extracellularly in the dermis, while in LSD it is accumulated intracellularly. Hematoxylin and eosin staining of skin biopsy from perianal papular-nodules, in both cases, showed normal epidermis with deposits of hyaline material throughout the papillary dermis.

The abnormal physical findings seen in ISH might suggest conventional cytogenetic analysis to rule out multiple malformations. In our studied cases, normal karyotypes have been documented. Multiple large sized perianal nodules/papules were found in both studied cases. Other authors reported perianal nodules/papules but our cases had marked large sized nodules (*16,17*). The perianal nodule, although it is highly characteristic of ISH, might be misdiagnosed as condylomata which herein has been ruled out by a negative test for human papilloma virus (*18*).

Marked gingival hyperplasia, results in difficulties in suction, mastication and leads to malnutrition, and has been reported earlier as in our cases (*19*).

Almost all characteristic clinical features of ISH, such as gingival hyperplasia, limited painful joint contractures, diffuse, thickened skin, hyperpigmented plaques on bony prominences, perianal fleshy nodules, frequent severe infections, and fatal outcome, were detected in our probands.

Malnutrition due to feeding problems, perioral stiffness, difficult mastication, and protein-losing enteropathy due to thickening and hyaline infiltration of the intestinal walls were noted in both cases. These finding were the main causes of growth retardation and failure to thrive.

Laboratory investigations showed low levels of serum IgG, protein, albumin and hemoglobin in both cases. In the literature some cases have similar laboratory abnormalities, and others have not (*11,19*).

Both cases suffer from recurrent respiratory problems, which might be related to hyaline deposits in the lungs. The same findings have been previously reported in the literature (*20*).

Although ISH is considered a rare disease, a review of 19 cases were reported from a referral center in Saudi Arabia in 2005. While ISH was reported globally and in all ethnic groups, many cases reported from Arabian countries are due to a high rate of consanguinity (*21-24*). In Saudi Arabia there are high consanguinity rates (25-60% of all marriages are consanguineous), and particularly the practice of first cousin marriages. In some areas (like Al Madina) the society is still tribal. Tribal groups and families descending from restricted ancestors may accumulate genetic and congenital diseases (*25*). Further detailed genetic studies might help to elucidate this issue.

It has been reported that ISH has a very poor prognosis since it results in death within the first two years of life (*2,25,26*). Both studied cases died by the age of two years due to recurrent respiratory infections.

Up to date there is no known specific treatment for ISH, only nutritional support and physiotherapy could improve the child's quality of life.

5. Conclusion

Clear guidelines are needed for early, correct diagnosis of the disease. Clinical data and detailed analysis of genetic *CMG2/ANTXR2* mutations will lead to better understanding of the disease pathogenesis, which may in turn help to reduce the high morbidity and mortality associated with ISH. These data provide the basis for diagnostic testing and genetic counseling for families of affected cases.

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Arabia., is acknowledged for referral of case number one for genetic counseling and diagnosis.

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Case Report

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Fibro-epithelial polyps in children: A report of two cases with a literature review

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Summary **A fibro-epithelial polyp is the most common epithelial benign tumor of the oral cavity. Such a polyp is of mesodermal origin and it is a pink, red, or white knob-like painless growth that is sessile or pedunculated. A fibro-epithelial polyp commonly occurs on buccal mucosa, the tongue, or the gingiva. A fibro-epithelial polyp is an inflammatory hyperplastic lesion in response to chronic irritation due to calculus, sharp tooth edges, irregular denture borders, or overhanging restorations. Such a polyp rarely occurs before the fourth decade of life and its prevalence is not sex-specific. The current paper presents two cases where an intraoral fibro-epithelial polyp was successfully managed in children. Conservative surgical excision was performed in both cases. A follow-up at 3 months revealed uneventful healing of the site without reoccurrence of the lesion.**

Keywords: Fibro-epithelial polyp, traumatic fibroma, benign tumor

1. Introduction

The oral cavity is a dynamic region that is constantly exposed to various external and internal stimuli, resulting in a myriad of diseases, from developmental to reactive and neoplastic (*1*). Fibroma of the oral mucosa is the most common benign neoplasm of the oral cavity, and such a fibroma originates from fibrous connective tissues (*2*). A fibroma of the oral mucosa is most commonly seen in older adults but can occur at any age, with a prevalence of 1-2%. A fibroma is an inflammatory hyperplastic lesion of the connective tissue. This local response to tissue insult results in an increase in the size of an organ or tissue due to hyperplasia of the constituent cells. In the oral cavity, a fibroma usually occurs due to chronic irritation from sources such as lip/cheek biting, irregular denture borders, overhanging restorations, calculus, sharp tooth edges, or other oral prostheses (*3*). Fibrous hyperplasia is the healed end product of an inflammatory hyperplastic lesion (*4*). Daley et al. suggested the term "focal fibrous hyperplasia," which implies a reactive tissue response, as preferable to the term "fibroma" (*5*). Fibroma of the oral mucosa is also known as irritation fibroma (IF), traumatic fibroma, a fibrous nodule, or a fibro-epithelial polyp (*6*). Usually, a fibroma of the oral mucosa clinically presents as painless swelling that is sessile or occasionally pedunculated; the affected site can be firm and resilient or soft with a spongy consistency (*7*). Cooke referred to any pedunculated lesion of the mucosal surface as a "polyp" (fibro-epithelial polyp) (*8*) and any pedunculated or sessile lesion in the gingiva as "epulides." Fibro-epithelial polyps most commonly occur in the buccal mucosa along the line of occlusion while epulides commonly occur in the maxillary anterior region (*9*).

The present report describes the rare case of a fibro-epithelial polyp on the buccal mucosa in one child and on the maxillary anterior gingiva in another.

2. Case Report

2.1. *Case 1*

A 12-year-old boy who had developed a growth in the left buccal region of the mouth four months earlier was seen by Pediatrics and Preventive Dentistry. The patient's history revealed a habit of chronic cheek biting during mastication since one year of age. The lesion

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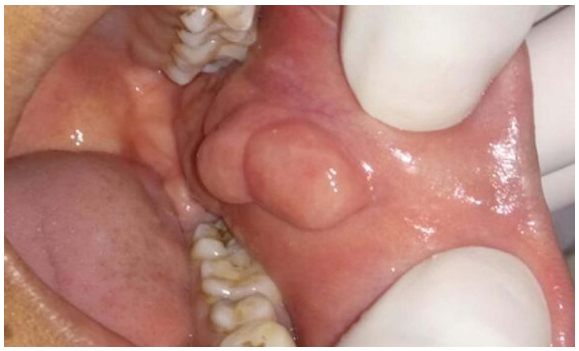

Figure 1. Pre-operative intraoral photograph of the lesion in Patient 1.

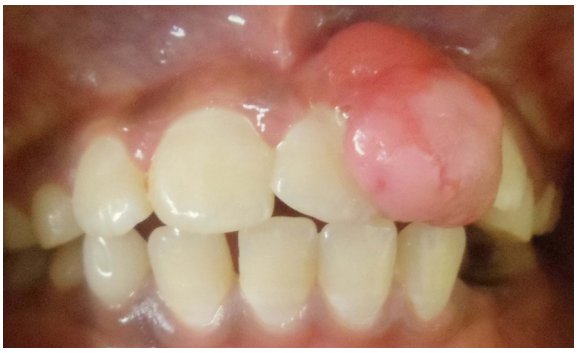

Figure 2. Pre-operative intraoral photograph of the lesion in Patient 2.

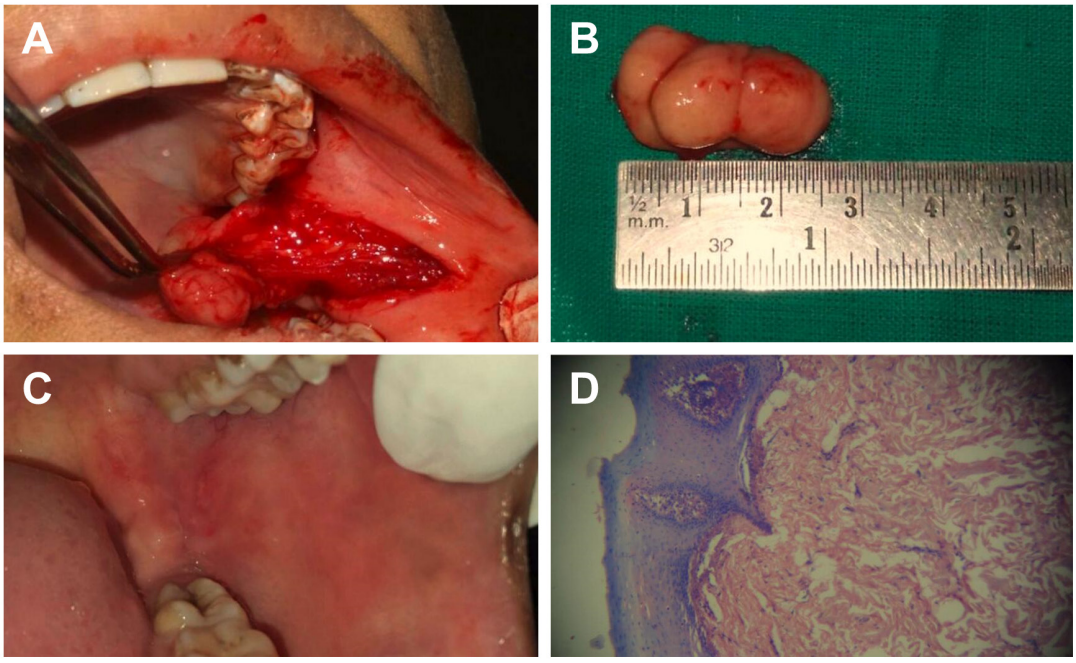

Figure 3. Clinical and histological photographs of the lesion from Patient 1. (A), Intraoperative photograph depicting fibrous attachment of the lesion to the tissue below. **(B)**, Excised mass. **(C)**, Post-operative intraoral photograph during follow-up. **(D)**, Histopathological appearance of the lesion.

started as a small nodule and grew, but no change in size was noted over the last four months. Upon oral examination, smooth, well-defined swelling that was lobulated and sessile was noted. The color of the swelling resembled normal mucosa. The swelling was located on the left buccal mucosa along the line of occlusion and the swelling was up to 5 cm in diameter. On palpation, the growth had a firm consistency and it was attached to the surface below. No other signs and symptoms of any syndromes were detected. The patient was clinically diagnosed with a fibro-epithelial polyp on the left buccal mucosa (Figure 1).

2.2. *Case 2*

An 11-year-old girl who developed swelling in relation to an upper front tooth one month earlier was seen by Pediatrics and Preventive Dentistry. An oral examination revealed a solitary, oval-shaped, painless, well-defined, and pedunculated growth between the left permanent maxillary central incisor and the left permanent maxillary lateral incisor. The growth had a firm consistency and was smooth and shiny in appearance. The growth was slightly red than normal mucosa and measured about 1.5 × 2 cm in diameter. The patient was clinically diagnosed with a fibro-epithelial polyp on the left maxillary gingiva. An intraoral periapical radiograph was taken to rule out any associated bone changes (Figure 2).

3. Treatment

In both cases, initial phase I treatment was planned. This included scaling & root planing and emphasizing oral hygiene. An excisional biopsy was performed under local anesthesia. The wound was sutured in Patient 1 (Figure 3) while a periodontal dressing was applied in Patient 2 (Figure 4). On gross examination, the excised

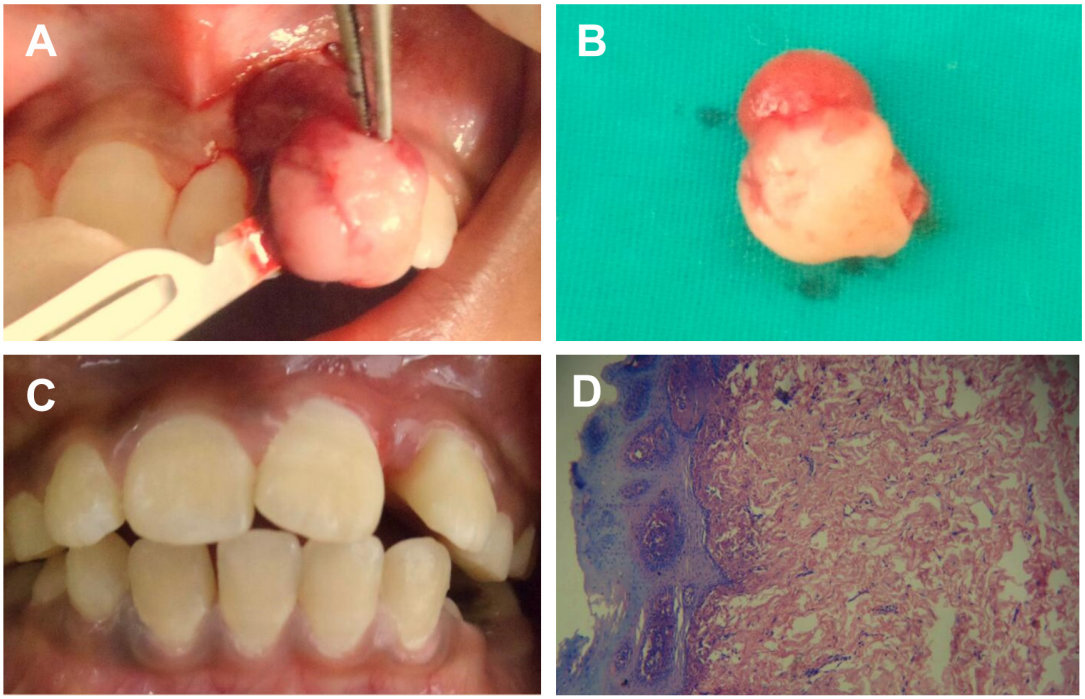

Figure 4. Clinical and histological photographs of the lesion from Patient 2. (A), Intraoperative photograph depicting fibrous attachment of the lesion to the tissue below. **(B)**, Excised mass. **(C)**, Post-operative intraoral photograph during follow-up **(D)**, Histopathological appearance of the lesion.

mass appeared to be fibrous in nature. In both cases, histopathology revealed atrophic squamous epithelium that was parakeratinized and stratified and that had dense connective tissue stroma below. The connective tissue had dense bundles of collagen fibers, fibroblasts, and fibrocytes along with chronic inflammatory cells. Therefore, histopathological findings confirmed the diagnosis of a fibro-epithelial polyp in both cases.

4. Discussion

A fibro-epithelial polyp or fibrous hyperplasia is the most common benign soft tissue tumor seen in the oral cavity (*10*). A fibroma occurs as a result of a chronic repair process that includes granulation tissue and scar formation resulting in a submucosal fibrous mass (*11*). Axell reported that fibromas have a prevalence of 3.25% among the adult Swedish population (*12*). Fibromas rarely occur before the fourth decade of life and its prevalence is not sex-specific (*12*). In the current cases, both patients were younger than 20 years, a finding that contrasts with the affected age group described by the literature. The clinical features of a fibro-epithelial polyp are not exclusive and the lesion must be differentiated from a peripheral ossifying fibroma and a peripheral giant cell granuloma. A peripheral ossifying fibroma appears exclusively on the gingiva, and it may be firmer because of calcified material in the stroma (*13*), thus distinguishing it from a fibro-epithelial polyp. A pyogenic granuloma and a peripheral giant cell granuloma generally appear to be more vascular and may bleed when palpated (*13*). A fibro-epithelial polyp is diagnosed based on the location of soft tissue swelling. If swelling is located on the tongue, the possibility of a neurofibroma, neurilemmoma, or granular cell tumor must be considered. Swelling on the lower lip or buccal mucosa may be a mucocele, lipoma, or salivary gland tumor. Another important distinguishing feature is that a traumatic fibroma exhibits two different patterns of collagen arrangement, a radiating pattern and a circular pattern, depending on the amount of irritation and the site of the lesion. An irritation fibroma with a radiating pattern is associated with sites that are immobile in nature (*e.g.* the hard palate) and with more severe trauma while an irritation fibroma with a circular pattern is associated with sites that are flexible in nature (*e.g.* the cheeks) and with less severe trauma, but a true fibroma exhibits neither of those patterns (*14*). A fibro-epithelial polyp does not pose a risk of malignancy (*7*). Recurrence rates are low (*15*) and recurrence is mostly caused by repetitive trauma at site of the lesion. In addition to surgery, other treatment modalities are electrocautery, an Nd:YAG laser, a flashlamp-pumped pulsed dye laser, cryosurgery, intralesional injection of ethanol or corticosteroids, and sodium tetradecyl sulfate sclerotherapy. However, the crucial step is to examine the tissue histopathologically in order to distinguish a fibro-epithelial polyp from a malignant tumor since those polyps mimic the clinical features of a true fibroma.

5. Conclusion

Diagnosis of an inflammatory hyperplastic lesion is quite difficult for clinicians since all lesions exhibit

overlapping clinical features. Their presence may hinder/obstruct the insertion of an oral prosthesis, cause difficulty with mastication or speech, or even cause bleeding and ulceration following a secondary infection. Therefore, the key to preventing the recurrence of that lesion is its surgical excision *in toto* along with elimination of the source of irritation that led to the lesion.

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Case Report

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Tip of nose tuberculosis: A rare presentation of extra pulmonary tuberculosis

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Summary **Tuberculosis is notorious that it affects various sites of the human body and presents in different ways. One of the uncommon or rather rare presentation of extra pulmonary tuberculosis is nasal tuberculosis. The nose apart from its physiological functions also contributes to facial aesthetics and gives a defined appearance and its deformity imparts cosmetic disfigurement and unsightly appearance. Both primary and secondary forms of nasal tuberculosis are rare but should be considered in the differential diagnosis of ulcerative or crusting lesions of the nose. Here we report such a case of nasal tuberculosis, which presented as an ulcerative and crusting lesion over the tip of the nose in a female child. The patient was given antituberculous chemotherapy after establishing the diagnosis and responded well to treatment.**

Keywords: Nasal, tuberculosis, granuloma

1. Introduction

Though tuberculosis has this unwanted honor of being a major global health problem, the involvement of nose, nasopharynx and para nasal sinus by tuberculosis is rare (*1*). Trauma and atrophic changes may facilitate introducing bacilli into the nasal lining in the otherwise resistant nasal mucosa (*2*). Giovanni Morgagni, Professor of Anatomy of Italian origin described nasal tuberculosis in 1761 for the first time while reporting an autopsy of a patient with ulceration of nose, palate and nasopharynx who had concomitant pulmonary tuberculosis (*3*). Herzog described 20 cases of primary nasal tuberculosis among 80 cases of nasal tuberculosis in the 18th century (*3*) and Butt described 35 cases of nasal tuberculosis in a review of the 20th century medical literature (*4*). In this paper, we report a case of nasal tuberculosis manifesting as an ulcerative lesion on tip of nose and we review the relevant literature.

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2. Case report

A 10 year old female child presented to our department with complaints of a non healing ulcerative and crusted lesion over tip of the nose for almost three months (Figure 1). The patient also had complaints of pus discharge from the lesion and nasal stuffiness. There were no significant constitutional symptoms. She was given a Mantoux test, other routine investigations (Table 1) and was sent to department of otorhinolaryngology for examination of nasal cavity and for obtaining a histopathological specimen from the lesion. Her X-Ray paranasal sinus didn't reveal any significant abnormality. Chest X ray was grossly normal and sputum for acid fast bacilli was negative on two occasions. Meanwhile she was kept on antibiotics and was asked to report back with histopathology report.

Patient's histopathology from the nasal cavity tissue was suggestive of tuberculosis, with microscopy showing well formed granulomas comprised of epitheloid cells with areas of fibroblastic proliferation entrapping fat as well as fair number of langhans giant cells and evidence of necrosis was also observed (Figure 2). Tissue sections were negative for 10% Potassium Hydroxide (KOH) mount for fungus and fungal culture. The patient's Mantoux test had a induration of 15 mm. She was given anti tuberculous chemotherapy

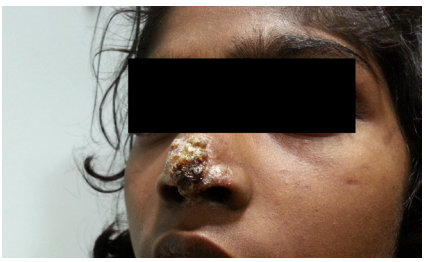

Figure 1. Picture of patient before treatment showing ulcerative & crusted lesion over Tip of nose.

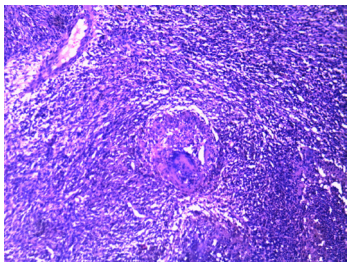

Figure 2. Hematoxylin & Eosin stained slide showing a well formed epitheloid granuloma with necrosis surrounded by inflammatory cells.

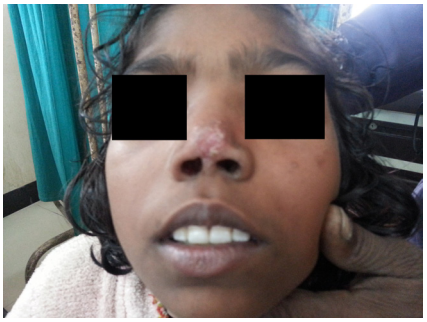

Figure 3. Picture of patient after treatment showing resolution of ulceration & crusting.

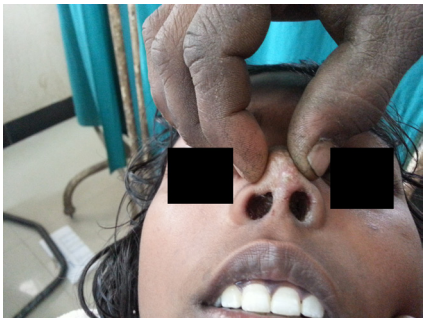

Figure 4. Another picture of patient after treatment showing roomy nasal cavity.

Table 1. Pre treatment evaluation of the patient

Items	Results
Hemogram and Blood sugar	Hb-11.6 g/dl, TLC-9,000/cu/mm, DLC:N-63, L-33,M-02, E-02, RandomBloodSugar-84 mg%
Kidney Function Tests (KFT)	Blood Urea-23.5 mg/dl Serum Creatinine-0.8 mg/dl
Liver Function Tests (LFT)	Serum Bilirubin (Total-0.5 mg/dl, direct-0.3 mg/dl, indirect-0.2 mg/dl) SGOT-46 IU/L, SGPT-76 IU/L, SALP-312 IU/L
Chest X ray	Normal
X ray Para-nasal sinus	No significant Abnormality
Sputum For Acid Fast Bacilli	Negative on two occasions

comprising of Rifampicin, Isoniazid, Ethambutol and Pyrazinamide according to her body weight and was directed for follow up at two months. Patient responded to the treatment and the lesion considerably improved with resolution of the nasal blockade and pus discharge (Figure 3 and Figure 4).

3. Discussion

Extra pulmonary tuberculosis has been on the rise during the past few decades constituting around 15 to 20 percent of tuberculosis cases in immunocompetent subjects and more than 50 percent of cases in human immunodeficiency virus (HIV) positive and other immunocompromised states. The most common sites affected by it are lymph nodes followed by pleura although other sites including bones, genito urinary system, abdomen, and meninges are also involved in a fair number of cases.

Extra pulmonary tuberculosis manifesting on unusual sites is a diagnostic challenge for the treating physician and nasal tuberculosis is one of them because the signs and symptoms are nonspecific (*5*). Nasal tuberculosis is usually secondary either to pulmonary tuberculosis or to lupus vulgaris of facial skin (*6*). Primary disease is rare. Ciliary movement, the bactericidal effect of nasal secretions, filtering by nasal vibrissae and the inherent resistance offered by nasal mucosa can explain the lower occurrence of nasal tuberculosis (*5*). Differential diagnosis can include various infectious as well as non-infectious causes which are shown (*2*) (Table 2).

Most cases of nasal tuberculosis are unilateral and lesions can be proliferative, infiltrative or ulcerative

Table 2. Differential diagnosis of Granulomatous lesions of nose

Items	Diagnosis
Infectious	• Bacterial: Rhinoscleroma, Leprosy, Syphilis, Yaws • Fungal: Mucormycosis, Aspergillois, Blastomycosis, Histoplasmosis • Parasitic: Leishmaniasis, Rhinosporidiasis
Non infectious	• Wegener's Granulomatosis, Sarcoidosis
Malignant	• Midline Granuloma
Miscellaneous	• Trauma, Cocaine abuse

(*4*). Pulmonary and extra pulmonary tuberculosis at adjacent sites have been reported in cases of nasal tuberculosis though rarely constituting roughly 2-6% of extrapulmonary cases (*7,8*).

Sometimes the role of computed tomography (CT) and magnetic resonance imaging (MRI) come into play in evaluating the various kinds of nasal cavity masses as they may differentiate the contents, and they may localize the lesion by different contrast enhancement patterns. In fully ossified lesions like in rhinolitis, osteomas and fibrous dysplasias diffuse hypointensity on MRI and sclerosis on CT hints towards benign pathology. Cysts, secretions and mucoceles appear as smoothly bordered non enhanced lesions. Masses with different enhancements with smooth borders hint towards nerve tissue or minor salivary gland tumors. Uniform contrast enhancement can be observed in hemangioma or angiofibroma. Destruction, invasion and necrosis may be observed with malignant neoplasms, granulomatous reactions, metastasis and fungal infections (*9*). A definitive diagnosis is established by isolating tuberculous bacilli from tissue specimens obtained for biopsy or from surgery. Management of this condition in the past was more or less symptomatic before the introduction of anti-tubercular chemotherapy including irrigations with alkaline antiseptics, use of hydrogen peroxide, lactic acid, trichloroacetic acid, use of galvanocautery, use of astringents and pyrogallic ointments *etc*. The treatment of nasal tuberculosis is the same as for the other extrapulmonary sites, it can be accompanied by surgical intervention with cosmetic correction depending on the case and extent of involvement.

The following three types of sinonasal tuberculosis have been described in the literature *i*) mucosal involvement leading to formation of polyps with minimal pus discharge; *ii*) bony involvement and fistula formation with abundant discharge of acid-fast bacilli; and *iii*) hyperplastic changes with formation of tuberculoma. In the case of sinus involvement the most commonly affected is the maxillary sinus (*10*).

Few important differential diagnoses can be considered while dealing with a case of nasal granuloma like in Rhinosporidiasis histological examination of the lesion shows characteristic appearance of sporangia, oval or round in shape filled with spores which may be seen bursting through its chitin walls (*11*). Rhinoscleroma is another differential diagnosis with demonstration of characteristic Mikulicz cells in its histology (*12*). In cases of aspergillosis of nose the histological examination of tissue demonstrates infiltrating aspergillus hyphae (*13*). Histology of sarcoidosis demonstrates non caseating granulomas. Midline granuloma is a lethal malignant condition characterized by angiocentric and angiodestructive growth patterns with extensive mucosal ulceration and lymphomatous infiltrate (*14,15*).

Many times the diagnosis is delayed and it may lead to further involvement of the adjacent structures and cosmetic disfigurement but fortunately in our patient there was a superficial ulcerative and crusting lesion over the tip of her nose which probably prompted her parents to seek early consultation and so based on clinical suspicion followed by histopathological confirmation with demonstration of epitheloid granulomas, necrosis and other supportive features suggestive of tuberculosis the patient was started on antitubercular chemotherapy. The patient responded well to the treatment with resolution of her lesion and improvement in general well being and didn't require any sort of cosmetic surgery.

4. Conclusion

Extra pulmonary tuberculosis has a wide spectrum of presentation and may manifest with constitutional symptoms, which sometimes are the only diagnostic clue. Sometimes it is not possible to do a bacteriological and histopathological confirmation of the diagnosis and treatment is given based on clinical suspicion. Just like pulmonary tuberculosis, the patients of extra pulmonary tuberculosis respond well to antitubercular chemotherapy in most cases, however some cases may proceed to complications or may be left with remnants of the primary disease.

Our patient was a case of tuberculosis of tip of the nose, which is a rare presentation of extra pulmonary tuberculosis and was successfully treated with standard antitubercular chemotherapy. Early diagnosis and treatment are the cornerstone of management and can prevent complications.

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Commentary

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New perspective on molecular markers as promising therapeutic targets in germ cell tumors

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Summary **Testicular germ cell tumors (TGCTs) are the most frequent solid malignant tumors in men 20-40 years of age and the most frequent cause of death from solid tumors in this age group. TGCTs comprise two major histologic groups: seminomas and non-seminomas germ cell tumors (NSGCTs). NSGCTs can be further divided into embryonal carcinoma, Teratoma, yolk sac tumor, and choriocarcinoma. Seminomas and NSGCTs present significant differences in clinical features, therapy, and prognosis, and both show characteristics of the Primordial Germ Cells (PGCs). Many discovered biomarkers including HMGA1, GPR30, Aurora-B, estrogen receptor β, and others have given further advantages to discriminate between histological subgroups and could represent useful therapeutic targets.**

Keywords: Testicular germ cells tumors, seminomas, Aurora B, GPR30, PATZ1; HMGA

Testicular germ cell tumors (TGCTs) are histologically classified as seminomas and non-seminomas according to the international classification of oncological diseases. Both these tumors display an invasive phenotype and are believed to be derived from a common ancestor, intratubular germ cell neoplasia unclassified (IGCNU) and gonadoblastoma, where the generation and expansion of tumor cells are limited to within the seminiferous tubules (*1-4*). Non-seminomas, including embryonal carcinoma and teratoma, contain stem cells as well as cells that have differentiated toward somatic lineages to various degrees, thus giving rise to a morphologically pleiotropic appearance (*5-9*). In contrast, seminomas have a rather uniform appearance, at least at the histological level. Due to this apparently homogenous cell composition, seminomas are particularly suitable for investigations of tumor-associated alterations in gene expression. In addition, the cells that constitute seminomas resemble the primordial germ cells and/or the cells in the IGCNU. Thus, the gene expression profile in seminomas is interesting not only with regard to understanding their oncogenesis, but it also may be useful for research into primordial germ cells (PGCs) (*9*).

A substantial increase in cure rates of the medical treatment of advanced testicular cancer has raised from approximately 25% in the mid-1970s to nearly 90% today. This is the highest cure rate in solid tumor. Improved survival is primarily due to effective chemotherapy (*10*). A great advance in chemotherapy for TGCT was the introduction of cisplatin in association with vinblastine, and bleomycin. The response rate increased to more than 80% with the use of this regimen in combination with surgery (*10*).

The International Germ Cell Cancer Collaborative Group (IGCCCG) was formed and a universal classification scheme was developed. In this stratification system, patients are separated into good-, intermediate-, and poor prognostic groups according to predicted outcome to cisplatin-combination chemotherapy, based on histology, but also on primary site, sites of metastasis, and serum tumor marker elevation (*11,12)*.

Although most patients with testicular cancer will be cured and can expect decades of additional life, thousands of men around the world will still die from testicular cancer every year, and many challenges remain. Cytotoxic chemotherapy remains the mainstay of therapy for advanced disease. A deeper understanding of the molecular mechanisms underlying the development of TGCTs may provide new tools to specifically target neoplastic cells and could contribute to overcome acquired and intrinsic chemotherapy resistance.

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Both clinical and epidemiological evidence strongly suggest that genetic and environmental factors play an important role in the genesis and development of TGCTs. Several genes are implicated in the pathogenesis of TGCTs, but the involvement of other genetic factors remains unknown (*2,3,12,13*). Susceptibility genes and environmental factors may deregulate normal differentiation processes of PGCs. In fact, TGCT have an invasive phenotype and are believed to be derived from a common ancestor, IGCNU, where the generation and expansion of tumor cells are limited to within the seminiferous tubules (*12,13*). A number of environmental factors have been investigated to explain the possible links. Some evidence suggests association of increased TGCTs risk and maternal smoking during pregnancy, adult height, body mass index, diet rich in cheese, and others (*14*). However, the biological mechanisms remain to be elucidated. The incidence of seminomas has been increasing over the last decades. Diagnosis is usually based on identification of histological subgroups. In last years, many potential therapeutic targets has been discovered, including SOX2, SOX17, HMGA1, and HMGA 2, Aurora B, PATZ1, GPR30 and others (*15-29*). Promising molecules capable to selectively target neoplastic cells, that are, serine-threonine kinases, TKs, HMGAs, GPR30 antagonist, proangiogenic factors inhibitors, and micro-RNAs already under clinical evaluation will open a new scenario for TGCTs treatment.

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IRDR
Intractable & Rare Diseases Research

Guide for Authors

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Original Articles should be well-documented, novel, and significant to the field as a whole. An Original Article should be arranged into the following sections: Title page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgments, and References. Original articles should not exceed 5,000 words in length (excluding references) and should be limited to a maximum of 50 references. Articles may contain a maximum of 10 figures and/or tables.

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