EVALUATION OF MACROVASCULAR INVOLVEMENT IN SYSTEMIC SCLEROSIS

MD RHEUMATOLOGY

NAME: PANDI PERUMAL s

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CERTIFICATE

This is to certify that this dissertation entitled **"Evaluation of macrovascular involvement in systemic sclerosis"** presented here is the original work done by Dr.S.Pandiperumal Postgraduate in the Department of Rheumatology, Madras Medical College and Govt. General Hospital, Chennai-3 in partial fulfillment of the University rules and regulations for the award of D.M.Branch VII- Rheumatology, under my guidance and supervision during the academic period from 2008-2011.

Dr.V.KANAGASABAI, MD.,
Dean,
Madras Medical College,
Chennai-600 003.

DR.S.RUKMANGATHARAJAN,
MD., DM., FMMC.,
Professor and Head,
Dept. of Rheumatology,
Madras Medical College,
Rajiv Gandhi Govt. General Hospital,
Chennai-600 003.

DECLARATION

I solemnly declare that dissertation titled **"EVALUATION OF MACROVASCULAR INVOLVEMENT IN SYSTEMIC SCLEROSIS"** is done by me at the Department of Rheumatology, Madras Medical College& Rajiv Gandhi Government General Hospital, Chennai, during January 2009-March 2011 under the guidance and supervision of Prof.Dr.S.Rukmangatharajan M.D,D.M., FMMC.

The dissertation is submitted to The Tamil Nadu Dr.M.G.R. Medical University towards the partial fulfillment of requirements for the award of D.M., degree in Rheumatology.

Dr.S.PANDIPERUMAL
DM Post graduate Student,
Department of Rheumatology,
Madras Medical College &
Rajiv Gandhi Govt. General Hospital,
Chennai.

Place: Chennai
Date:25.5.2011

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INTRODUCTION

Systemic sclerosis (SSc) is associated with chronic inflammation, fibrosis and obliterative vasculopathy within the skin and visceral organs. SSc is an autoimmune disorder characterized by widespread vasculopathy. The microvasculature is primarily affected. However, large-vessel disease also occurs in SSc [1–6]. Accelerated atherosclerosis has been described in some patients with SSc [7]. This occurred despite the absence of classical vascular risk factors. Macrovascular disease was observed in 58% of patients with limited cutaneous SSc (lcSSc) [2, 4].Intermittent claudication, cardiovascular disease (CVD) and cerebrovascular disease were detected in 22%, 15% and 6.5% of SSc patients, respectively [7].

Vascular abnormalities have long been recognized as being central to the pathogenesis of SSc, and SSc has been suggested to be primarily a vascular disease [8,9]. Both structural and functional changes occur, which interrelate [10]. Structural changes affect digital microcirculation (well demonstrated with nailfold capillaroscopy) and the digital arteries, in which the most characteristic histologic lesion is marked intimal hyperplasia/fibrosis [11]. Although involvement of peripheral arteries proximal to the digital arteries was previously believed to be unusual, in the past 15 years considerable interest has

been shown in the hypothesis that patients who have SSc have an increased prevalence of large vessel disease [12-14]

Endothelial dysfunction is thought to be an important factor in the pathogenesis of atherosclerosis. In healthy subjects, endothelium is more than a physical barrier and has several functions, like: (a) continuous regulation of vascular tone, (b) leucocytes adhesion, (c) maintenance of the balance between thrombotic and anticoagulant properties of the blood [15]. When these functions of the endothelium are affected, endothelial dysfunction appears. Endothelial cell activation is an initiating step in atherogenesis [16].

Endothelial function can be assessed noninvasively by means of several methods, based on the inability of dysfunctional endothelium to cause vasodilation of the vessels by its inability to release endothelium-derived vasodilatory mediators.

Endothelium independent vasodilation can be studied by using glyceryl nitrate, causing vasodilation by direct action on the smooth muscle [17,18]. Measurement of flow-mediated dilatation (FMD, endothelium-dependent) and endothelium-independent vasodilatation via high ultrasound techniques allowed the detection of endothelial dysfunction in children and adults with risk factors for atherosclerosis [17]. A relationship among endothelial dysfunction, IMT (Intima-Medial Thickness) and cardiovascular risk factors has been established[19].

Angiography demonstrated rigidity of various arteries [6]. Increased intimal-medial thickness of the common carotid artery (ccIMT) has been described in 64% of SSc patients [3, 6, 20] Early endothelial dysfunction has been described in rheumatoid arthritis (RA) and lupus (reviewed in [3, 4]). However, there are very few studies assessing flow-mediated (FMD) or nitrate-mediated vasodilatation (NMD) in SSc. FMD measured on the brachial artery at rest and during reactive hyperaemia indicates endothelium-dependent vasodilatation, while NMD assessed after administration of sublingual nitroglycerin, is an indicator of endothelium-independent vasodilatation [8, 21].

High-frequency ultrasonographic imaging of the brachial artery to assess endothelium-dependent flow-mediated vasodilatation (FMD) was developed in the 1990's .The technique provokes the release of nitric oxide, resulting in vasodilatation that can be quantitated as an index of vasomotor function. The noninvasive nature of the technique allows repeated measurements over time to study the effectiveness of various interventions that may affect vascular health[22].

Very high interest exists in determining the clinical utility of brachial artery FMD. Investigators have hypothesized that endothelial function may serve as an integrating index of risk factor burden and genetic susceptibility, and that endothelial dysfunction will prove to be a preclinical marker of cardiovascular disease [23]. Several studies

suggest that the presence of endothelial dysfunction in the coronary circulation is an independent predictor of cardiovascular disease events [24,25]. The technique is particularly well suited for study of the earliest stages of atherosclerosis in children and young adults, thus providing maximal opportunity for prevention. Recently, endothelial dysfunction has also been found in patients with systemic vasculitis and has been reversed by administration of immunosuppressive therapy [26]. As endothelial dysfunction may represent an early stage in atherogenesis, it is important to understand the mechanisms of its development in a condition such as SSc. It is also important to determine whether it is associated with other Coronary Heart Disease (CHD) risk factors or early atheroma.

REVIEW OF LITERATURE

Systemic sclerosis is a generalized disorder of connective tissue [27] characterized clinically by thickening and fibrosis of skin and by distinctive form of involvement of internal organs notably the heart, lungs, kidney and G.I.T [28]. Morbidity and mortality are substantial and are directly related to the extent and severity of visceral involvement [27]. Scleroderma or systemic sclerosis is characterized by fibrosis and microvascular occlusion. Similar to other autoimmune rheumatic diseases, including Systemic Lupus Erythematosis (SLE) and Rheumatoid Arthritis (RA), macrovascular disease can occur in SSc, although its frequency is unknown.

Three major types of abnormalities have been described in the pathogenesis of the disease. First, there is an impairment of the immune system resulting in chronic inflammation, abnormal T cell activation, abundant production of proinflammatory cytokines such as interleukin 4, B cell dysfunction and the production of characteristic autoantibodies including anticentromere antibodies in limited cutaneous SSc (lcSSc) and antitopoisomerase 1 and anti-RNA polymerase I/III antibodies in diffuse cutaneous SSc (dcSSc) [29,30]Second, there is functional impairment of the microvasculature including the vascular endothelium, contributing to tissue hypoxia [31,32]. Finally, there is a structural vasculopathy caused by extracellular matrix deposition into the vessel

wall. At the cellular and molecular level, this phenomenon has been associated with disproportionate fibroblast activity, as well as increased serum levels of hyaluronan, matrix metalloproteinases (MMP) and tissue inhibitors of metalloproteinases (TIMP) [33,34]. Some features of the disease resemble the processes taking place in an atherosclerotic plaque [35].

The etiology of SSc remains elusive despite significant advances in the understanding of the pathogenetic mechanisms of the disease since its initial description in the medical literature almost 250 years ago. SSc can be conceptualized as tripartite disease in which dysfunction of the immune system, endothelium and fibroblasts give rise to a heterogenous phenotype that is marked by prominent fibrosis.

Autoimmunity is manifested by the elaboration of circulating disease specific autoantibodies [36]. Fibroblast dysfunction is manifested as fibrosis of skin and internal organs as the result of increased synthesis and deposition of extracellular matrix (ECM) proteins [37,38]. Raynaud's phenomenon, capillary dropout, enthothelial injury/ apoptosis and abnormalities in vascular tone are manifestations of endothelial dysfunction.

Genetic factors may play a role in the dysregulation of these components by affecting host susceptibility to disease or modifying clinical presentation and organ damage. Available data are consistent

with the paradigm that the phenotype identified as SSc is the end result of a complex interaction of genetic factors and unknown environmental influences.

The pathologic hallmark of systemic sclerosis is an excessive accumulation of ECM in the dermis, which leads to taut skin. In the early stages of SSc, an infiltration of mononuclear inflammatory cells, predominantly T cells [39], surrounding the dermal blood vessels is concentrated at the border between the reticular dermis and subcutaneous fat. There is intimal proliferation with luminal narrowing at the arterial and arteriolar levels. Capillary loss can be seen in vivo in the skin by widefield nailfold capillaroscopy.

Pathogenic factors involved in SSc-associated vascular damage include increased low density lipoprotein (LDL), homocysteine and C reactive protein (CRP) production [3, 31, 32].

Neudecker et al have recently described the association of 5,10-methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism with homocysteine, vitamin B12 production and macrovascular abnormalities in SSc [33]. They also detected increased serum adhesion molecule levels in SSc patients with macrovascular disease [34].

Atherosclerosis is considered to be a chronic inflammatory disorder. Several autoimmune rheumatic diseases are characterized by premature and accelerated atherosclerosis in which both classical and

non-classical risk factors contribute to atherogenesis. In SSc patients admitted to the hospital because of acute myocardial infarction, the odds ratio of having normal coronary arteries was 33.89 compared with the patients admitted from the general population, suggesting microvascular and not macrovascular disease in these patients [40]

Also, cerebrovascular involvement in SSc has rarely been documented, although the opposite has been stated [41]. Only one retrospective cohort study is available, showing no increased prevalence of cerebrovascular disease in 31 female SSc patients compared with matched controls (prevalence 26 vs 19%, RR 1.3 with 95% CI of 0.5, 3.3) [42].

An increased prevalence of peripheral vascular disease in SSc patients compared with healthy controls (21.7 vs 4.6%) has been observed by Veale et al. [43], using a questionnaire for intermittent claudication, and by Youssef et al. [42], using data available from angiography, Doppler ultrasound or physical examination (prevalence 58 vs 10%, RR 6.0 with 95% CI of 2.0, 18). When angiographic findings of the lower and upper limb in SSc patients were related to cardiovascular risk factors, an association was observed between these risk factors and proximal peripheral artery disease, but not distal peripheral artery disease [44]. Distal peripheral artery disease is present in the digits of many SSc patients, showing a high frequency of digital

stenosis and occlusions in the digital arteries of patients. Lesions were most frequently found in the 2nd to 5th palmar digital artery, the ulnar artery and the superficial palmar arch [45-48]. As a consequence, digital ischaemia, ulceration or amputation is a well-known, but feared manifestation in SSc [49-51].

An increased prevalence of distal peripheral artery disease in the digits has been found. The prevalence of coronary artery disease and cerebrovascular disease is not increased, although studies using intima–media thickness of the carotid artery as a marker of early atherosclerosis showed discrepant results. Besides traditional risk factors, as present in the general population, non-traditional risk factors are present in SSc as well, such as increased lipoprotein (a), oxidized LDL, inflammation, vasospasm and endothelial dysfunction. Moreover, markers of vascular damage in atherosclerosis, like antibodies to oxidized LDL, and increased levels of soluble vascular adhesion molecules, have been described in association with vascular damage in SSc. Nevertheless, generalized premature atherosclerosis has not been detected in SSc. Therefore, further research is necessary to assess the prevalence of clinically manifest or subclinical early atherosclerosis in SSc.

In ulnar artery biopsies, intimal thickening and transmural lymphocytic infiltrates were detected without the presence of atherosclerotic plaques [6]. Microvascular abnormalities and Raynaud’s

Phenomenon (RP) are well-known as major sites of pathology [52-55], but less attention has been paid to macrovascular abnormalities. The prevalence of vascular abnormalities in SSc has been considered to be inversely proportional to the size of blood vessels studied [56]. Macrovascular disease was considered extremely rare.

There have been several reports that macrovascular disease is increased in SSc. Taken together, these studies provide evidence that there is increased macrovascular pathology, with severalfold greater risk of symptomatic disease and possibly also increased asymptomatic disease. Important questions remain unanswered though, especially whether it relates directly to SSc or whether it reflects other genetic or environmental characteristics of the scleroderma population.Factors related to the vasospastic or fibrotic pathologies of SSc might be involved in the development of co-existing macrovascular disease.

Large vessel involvement has important implications for organ-based complications, such as renal disease, peripheral ischemia and bowel involvement. Noninvasive studies have shown blood flow abnormalities in the large vessels in the cerebral and renal circulation in patients with SSc [57] and symptomatic and asymptomatic macrovascular disease are increased [58,59]. A more recent study of macrovascular involvement showed a predilection for the ulnar artery [60]. Postmortem studies have reported that cerebrovascular disease, especially with

vascular calcification, may be disproportionately severe in patients with lcSSc compared with macrovascular disease at other sites [59a].

Although a mild vasculitis may sometimes be present, the vascular pathology of SSc is not necessarily inflammatory and is best characterized as a vasculopathy. Arterial occlusion in SSc is common, particularly in the ulnar arteries in patients with the lcSSc and in association with anticentromere antibodies [70].

Studies investigating the prevalence of lower limb large vessel disease have shown conflicting results. Veale and colleagues [61] reported that 10 of 46 patients (21.7%) who had SSc who participated in a questionnaire - based study experienced claudication, compared with 4.5% of the population of a neighboring region in Scotland. The same investigators then reported ankle-brachial pressure indices (ABPI) of less than 0.9 in 9 of 53 patients who had SSc (17%) but in none of 43 controls, with 21 (40%) patients who had SSc having an ABPI of less than 1 compared with 4 (10%) controls [62]. However a later study of 119 patients who had SSc reported that only 12% had an ABPI of less than 1.0, but this study didn't include a control group [63]. A recent study found no differences in ABPIs between 53 patients who had SSc and 43 controls, despite higher intima-media thickness (an index of atherosclerotic carotid artery disease) in the SSc group [64].

If macrovascular disease is increased in SSc, it may be from the SSc disease process or because of an increased prevalence of atheromatous disease as reported in other rheumatic diseases. Despite whether the prevalence of large vessel peripheral arterial disease is increased in SSc, atheromatous disease is common , especially in older patients, and the coexistence of proximal and small vessel disease in SSc is potentially limb threatening. It is important not to miss, for example, a subclavian or superficial femoral artery stenosis that may be amenable to angioplasty or surgery.

Upper and lower limb arteriographic studies in patients who have SSc, mostly with critical digital ischemia, have shown a combination of proximal and digital arterial disease, although in small numbers of patients. Hasegawa and colleagues [65] described arteriographic findings in 8 patients who had SSc and severe digital ischemia; 7 had digital artery occlusions, but 4 also had arterial occlusions proximal to the digits and in 3, the ulnar artery was involved .These authors reviewed the earlier literature on arteriographic findings in SSc [65]. Three previous groups of investigators reported ulnar artery occlusion on angiography in patients who had SSc: in 11 of 31 patients [66], 10 of 24 has (12 patients) [67] and 9 of 29 patients [68]. In contrast, radial artery involvement was only rarely reported. Ulnar artery disease was also reported in a retrospective study by Stafford and colleagues [69], who found that 10 of 19 patients who had SSc who had ulnar arterial

wall abnormalities on Doppler ultra sound, and by Tailor and colleagues [70], who reported ulnar artery involvement in all 15 patients (9 had bilateral involvement) who underwent arteriography based on severe digital ischemia / ulceration and a positive Allen's test (all 15 patients also had digital artery disease). Why the ulnar artery should be involved more than the radial artery is unknown?

Avascular necrosis (AVN) is caused by death of the bone, likely caused by interrupted circulation to the marrow and trabeculae [71]. It rarely can occur in scleroderma, probably secondary to both macro and microvacsular abnormalities that are part of the underlying disease process [72]. Initial case reports of bilateral hip[73] and bilateral ankle [73] ischemic necrosis in SSc were notable in the disease of significant corticosteroid therapy, arthropathy, vasculitis or anticardiolipin (Acl) antibodies.Fewer reports describe osteonecrosis of the wrist in SSc. One report of AVN of the carpal scaphoid was in a patient treated with 25 gm of prednisone over 8 yrs [74]. Another subject who suffered bilateral lunate osteonecrosis had severe Raynaud's Phenomenon and vasculitis [75]. In the author's centre, 3 patients, two of whom had no corticosteroid exposure, developed osteonecrosis of the lunate [76]. It is important to recognize AVN early, because revascularization of the involved bone may be prevent bone death.

LIPOPROTEIN PROFILE

Decreased HDL levels have been detected in patients with limited cutaneous SSc (lcSSc) compared with healthy controls [77]. No studies are available on LDL levels in SSc. However, patients with SSc showed increased susceptibility to oxidation of LDL, a process in which oxidized LDL (OxLDL) is formed [78]. OxLDL is a proatherogenic lipoprotein, which, amongst others, promotes foam cell formation, vascular oxygen radical formation, tissue remodelling, endothelial dysfunction and even vasospasm [79, 80]. Another cardiovascular pathogenic factor is lipoprotein(a) [Lp (a)] [81]. Its exact mechanism is unknown, but Lp (a) counterbalances the pro- and anti-coagulant, pro- and anti-inflammatory and vasorelaxing and vasoconstricting properties of the endothelium, in which raised concentrations are linked with atherosclerosis and thrombosis [82, 83]. In patients with SSc, increased concentrations of Lp (a) without further differences in lipid profile in comparison with healthy controls have been found, both in lcSSc and dcSSc [84, 85].

INFLAMMATION

Inflammation is a hallmark of systemic autoimmune diseases. Also in SSc, inflammation is present when the disease is active. Disease activity in SSc can be assessed by using the preliminary European Scleroderma Study Group (EScSG) activity indices (a score ranging from 0 to 10). A score of >3 denotes active disease [86, 87]. One of the

items of this scale is CRP, found to be a strong marker of cardiovascular risk in asymptomatic subjects and subjects with a history of a cardiovascular event [88-93]. Elevated acute-phase reactants have been found especially in dcSSc (41.8%) compared with lcSSc (24.6%), as have been described in the large EULAR Scleroderma Trials and Research (EUSTAR) group database [94]. Therefore, inflammation might play a role in vascular abnormalities in SSc. No data are available concerning acute-phase reactants and atherosclerosis in SSc.

Large vessel involvement has been suggested in SSc since altered elastic properties of the carotid artery, increased stiffness of the aorta and decreased arterial distensibility have all been reported in SSc patients [95, 96-101].

The capacity of blood vessels to respond to physical and chemical stimuli in the lumen confers the ability to self-regulate tone and to adjust blood flow and distribution in response to changes in the local environment. Many blood vessels respond to an increase in flow or more precisely shear stress, by dilating. This phenomenon is designated FMD. A principal mediator of FMD is endothelium-derived NO. The precise mechanisms for the acute detection of shear forces and subsequent signal transduction to modulate vasomotor tone are not fully understood. The endothelial cell membrane contains specialized ion channels, such as calcium-activated potassium channels, that open in response to shear

stress[102,103and104].The effect of potassium channel opening is to hyperpolarize the endothelial cell, increasing the driving force for calcium entry (there are no votage-gated calcium channels in endothelial cells). Calcium activates an enzyme, endothelial nitric oxide synthase (eNOS) and the subsequent generation of NO appears to account for FMD [105,106]. Indeed, endothelial denudation or treatment with a nitric oxide synthase (NOS) inhibitor abolishes FMD in a variety of arterial vessels. However, it was recently shown that blood vessels from mice genetically engineered to lack the eNOS enzyme (eNOS knockout mice) still respond to shear stress by dilating. In the eNOS knockout mice, FMD seems to be mediated by endothelium-derived prostanoids, as it is blocked by indomethacin [107]. Thus, there is some redundancy in the system, and more than one endothelial mediator is capable of acting as the signal between endothelium and smooth muscle. It is unknown whether other mediators, such as the putative endothelium-derived hyperpolarizing factor, can cause FMD if both NO and prostanoids are deficient.

Several mechanisms may underlie the increase in NO in response to changes in shear stress. Very acute changes may be mediated by the increase in intracellular calcium that occurs when ion channels open (see the previous text). Over slightly longer time periods (minutes), shear-stress-induced phosphorylation of eNOS via a serine/threonine protein kinase, Akt/PKB, increases eNOS activity, even at low calcium

concentrations, and this may be important to allow continued output of NO [108,109]. In addition, other posttranslational modifications of the enzyme (myristilation or palmitoylation) or interaction with caveolin can affect intracellular localization of the enzyme and thereby alter its function. Over longer time periods (many minutes or hours), eNOS gene transcription is activated, and this can result in continued increases in NO generation if shear stress is maintained at high levels.

ENDOTHELIAL DYSFUNCTION

Endothelial cell activation is an initiating step in atherogenesis [110]. Endothelial function can be assessed noninvasively by means of several methods, based on the inability of dysfunctional endothelium to cause vasodilation of the vessels by its inability to release endothelium-derived vasodilatory mediators.

To assess endothelial function noninvasively, brachial arteries are scanned with high resolution ultrasound imaging, under baseline conditions (at rest) and during hyperemia induced by inflation and deflation of a sphygmomanometer cuff mostly around the forearm distal to the site to be scanned with ultrasound. The induced shear stress caused by the increased blood flow following transient ischemia induces nitric oxide (NO) release, which in turn causes local arterial vasodilatation. Endothelial function, defined as flow mediated dilatation (FMD), is estimated as the percentage increase in vessel diameter from

baseline conditions to maximum vessel diameter during hyperemia. Impaired endothelial function of the brachial artery assessed in this manner has been reported in asymptomatic children and adults with elevated cardiovascular risk factors such as smoking [111], hypercholesterolemia[112], hypertension [113], diabetes mellitus [114], and hyperhomocysteinaemia [115]. Although the results of these studies are likely to be internally valid, comparison of the FMD values across studies is troublesome.FMD values vary considerably across populations, ranging from – 1.9 to 19.2%.

Endothelium independent vasodilation can be studied by using glyceryl nitrate, causing vasodilation by direct action on the smooth muscle [116,117]. Measurement of flow-mediated dilatation (FMD, endothelium-dependent) and endothelium-independent vasodilatation via high ultrasound techniques allowed the detection of endothelial dysfunction in children and adults with risk factors for atherosclerosis [116]. A relationship among endothelial dysfunction, IMT and cardiovascular risk factors has been established[118].

Interestingly, an association between impairments of FMD and increased IMT was found in some [119,120] but not all studies [121]. Several groups have studied subclinical, early atherosclerosis in SSc. Using IMT of the carotid artery as a marker of early atherosclerosis discrepant results were reported. No differences in IMT or intraluminal

diameter of the common carotid artery (CCA) between SSc patients and controls were noted by some authors [95,122-124], while others found significantly increased IMT values or increased prevalence of carotid artery disease in SSc patients (Table 1) [125-129]. IMT values of the femoral artery in SSc patients and healthy controls were comparable [95,130].

Treatment with immunosuppressive agents, especially corticosteroids, influences the atherogenic process. Corticosteroids are considered to have atherogenic properties, [131] like azathioprine, [132] whereas for hydroxychloroquine [131] and methotrexate [133] a protective effect against atherosclerosis has been described.

Recently, endothelial dysfunction has also been found in patients with systemic vasculitis and has been reversed by administration of immunosuppressive therapy [26].

An important goal is to extend these preliminary studies and to determine whether any of the therapies currently used for Raynaud's and scleroderma might influence development of macrovascular disease.

GUIDELINES FOR THE ULTRASOUND ASSESSMENT OF ENDOTHELIAL-DEPENDENT FLOW-MEDIATED VASODILATION OF THE BRACHIAL ARTERY. A REPORT OF THE INTERNATIONAL BRACHIAL ARTERY REACTIVITY TASK FORCE MARY C. CORRETTI MD, FACC ET AL

SUBJECT PREPARATION

Numerous factors affect flow-mediated vascular reactivity, including temperature, food, drugs and sympathetic stimuli, among others. Therefore, subjects should fast for at least 8 to 12 h before the study, and they should be studied in a quiet, temperature-controlled room. All vasoactive medications should be withheld for at least four half-lives, if possible. In addition, subjects should not exercise, should not ingest substances that might affect FMD such as caffeine, high-fat foods and vitamin C or use tobacco for at least 4 to 6 h before the study.

EQUIPMENT

Ultrasound systems must be equipped with vascular software for two-dimensional (2D) imaging, color and spectral Doppler and a high-frequency vascular transducer. A linear array transducer with a minimum frequency of 7 MHz, attached to a high-quality mainframe ultrasound system, is used to acquire images with sufficient resolution for subsequent analysis. Image resolution is enhanced with broadband (multiple-frequency: 7 to 12 MHz) linear array transducers.

IMAGE ACQUISITION

The subject is positioned supine with the arm in a comfortable position for imaging the brachial artery. The brachial artery is imaged above the antecubital fossa in the longitudinal plane. A segment with clear anterior and posterior intimal interfaces between the lumen and vessel wall is selected for continuous 2D grayscale imaging.

ENDOTHELIUM-DEPENDENT FMD

To create a flow stimulus in the brachial artery, a sphygmomanometric (blood pressure) cuff is first placed either above the antecubital fossa or on the forearm. A baseline rest image is acquired, and blood flow is estimated by time-averaging the pulsed Doppler velocity signal obtained from a midartery sample volume. Thereafter, arterial occlusion is created by cuff inflation to suprasystolic pressure. Typically, the cuff is inflated to at least 50 mm Hg above systolic pressure to occlude arterial inflow for a standardized length of time. This causes ischemia and consequent dilation of downstream resistance vessels via autoregulatory mechanisms. Subsequent cuff deflation induces a brief high-flow state through the brachial artery (reactive hyperemia) to accommodate the dilated resistance vessels. The resulting increase in shear stress causes the brachial artery to dilate. The image of the artery is recorded continuously from 30 s before to 2 min after cuff deflation.

Studies have variably used either upper arm or forearm cuff occlusion, and there is no consensus as to which technique provides more accurate or precise information. When the cuff is placed on the upper part of the arm, reactive hyperemia typically elicits a greater percent change in diameter compared with that produced by the placement of the cuff on the forearm [134-136]. This may be due to a greater flow stimulus resulting from recruitment of more resistance vessels or possibly to direct effects of ischemia on the brachial artery. However, upper-arm occlusion is technically more challenging for accurate data acquisition as the image is distorted by collapse of the brachial artery and shift in soft tissue. The change in brachial artery diameter after cuff release increases as the duration of cuff inflation increases from 30 s to 5 min. The change in diameter is similar after 5 and 10 min of occlusion; therefore, the more easily tolerated 5 min occlusion is typically used. Also, FMD may be studied in the radial, axillary and superficial femoral arteries. Notable caveats are that arteries smaller than 2.5 mm in diameter are difficult to measure, and vasodilation is generally less difficult to perceive in vessels larger than 5.0 mm in diameter [137-139].

ENDOTHELIUM-INDEPENDENT VASODILATION WITH NITROGLYCERIN

At least 10 min of rest is needed after reactive hyperemia (i.e., FMD) before another image is acquired to reflect the reestablished

baseline conditions. In most studies to date, an exogenous NO donor, such as a single high dose (0.4 mg) of nitroglycerin (NTG) spray or sublingual tablet has been given to determine the maximum obtainable vasodilator response, and to serve as a measure of endothelium-independent vasodilation reflecting vascular smooth muscle function. Peak vasodilation occurs 3 to 4 min after NTG administration; images should be continuously recorded during this time, and NTG should not be administered to individuals with clinically significant bradycardia or hypotension. Determining the vasodilator responses to increasing doses of NTG, rather than a single dose, may further elucidate changes in smooth muscle function or arterial compliance that might be playing a role in any observed changes in FMD.

ANATOMIC LANDMARKS

The diameter of the brachial artery should be measured from longitudinal images in which the lumen-intima interface is visualized on the near (anterior) and far (posterior) walls. These boundaries are best visualized when the angle of insonation is perpendicular. Thus, clear visualization of both the near and far wall lumen-intima boundaries indicates that the imaging plane is bisecting the vessel in the longitudinal direction, and diameters measured from these images likely reflect the true diameter. Once the image for analysis is chosen, the boundaries for diameter measurements (the lumen-intima or the media-adventitia interfaces) are identified manually with electronic calipers or

automatically using edge-detection software. The variability of the diameter measurement is greatest when it is determined from a point-to-point measurement of a single frame, and least when there is an average derived from multiple diameter measurements determined along a segment of the vessel.

The diameter measurement along a longitudinal segment of vessel is dependent upon the alignment of the image. Skew occurs when the artery is not completely bisected by the plane of the ultrasound beam. Some edge-detection programs can account for skew from transducer angulation.

TIMING OF FMD

Flow-mediated vasodilation is an endothelium-dependent process that reflects the relaxation of a conduit artery when exposed to increased shear stress. Increased flow, and thereby increased shear stress, through the brachial artery occurs during postocclusive reactive hyperemia. Several studies have suggested that the maximal increase in diameter occurs approximately 60 s after release of the occlusive cuff, or 45 to 60 s after peak reactive hyperemic blood flow [142,139]. The increase in diameter at this time is prevented by the NOS inhibitor NG-monomethyl-arginine, indicating that it is an endothelium-dependent process mediated by No [143,144]. Other measures of vasodilator

response include time to maximum response [145], duration of the vasodilator response [146] and the area under the dilation curve.

CHARACTERIZING FMD

Flow-mediated vasodilation is typically expressed as the change in post-stimulus diameter as a percentage of the baseline diameter.

EVALUATING PRECISION OF THE TECHNIQUE

Intraobserver and interobserver variability in image acquisition and analysis should be established and periodically reassessed for each condition, including baseline, reactive hyperemia and NTG administration. Image variability is best judged by having two sonographers independently scan the same series of subjects at different times. The highest reproducibility is likely to be shown over a short interval, during which the individual vasodilator response is unlikely to have changed owing to environmental or other influences. This can be accomplished by taking two measurements on the same day within a 10- to 15-min interval, or on separate days in otherwise identical circumstances. Longitudinal studies in which interventions over weeks to months are tested require that reproducibility measurements be performed at longer intervals. The image analysis and measurement of the vasodilator response from repeated studies should be performed by an individual who is blinded as to sequence. Measurement variability is assessed, typically, by a designated core laboratory for multicenter

trials, prior to site certification and periodically thereafter to analyze for temporal drifts.

Assessment of FMD of the brachial artery in clinical trials has increased because of its seeming ease of use, efficiency and noninvasive nature. Owing to the biological and technical variability of the measurement, several caveats should be considered when planning a clinical trial where FMD is the end point of interest. These include study design, sample size and uniform technique.

STUDY DESIGN

Recent studies have reported on the effect of pharmacologic or physiologic interventions on FMD of the brachial artery. These include both acute [149-151] and longer-term intervention trials [152-154]. Both parallel-group and crossover designs have been successfully employed.

SAMPLE SIZE

Typically, significant improvement in FMD can be seen with 20 to 30 patients in a crossover design study and 40 to 60 patients in a parallel-group design study. In studies of this size, the minimal statistically significant improvement that can be detected with intervention is an absolute change in FMD of 1.5% to 2%. The sample size depends greatly on the variance of repeated

measurement in the control group in a particular vascular laboratory.

With intervention trials, an important parameter to report is the time-dependent reproducibility of FMD. For example, in the placebo group, the pretreatment and postintervention FMD measures are usually reported, and often are very similar. However, if the mean difference between the two measurements for each patient is quite high, it indicates that the variance of the technique might limit interpretation of the study results. An acceptable reproducibility is a mean difference of 2% to 3% in FMD over time (on a baseline vasodilation of about 10%) [137]. This value has not been readily available in published trials.

METHODOLOGY

As discussed above, several techniques have been employed to measure FMD. Laboratories should select the method that gives them the most reproducible results, and for multicenter studies, the same scanning protocol should be employed at all sites.For studies employing repeated measurements following intervention, FMD might change as a result of the intervention. However, FMD could also be affected by a change in the hyperemic stimulus. Therefore, the flow stimulus should be consistent. Otherwise, any change in FMD of the conduit artery may be related to changes in

flow (indirectly mediated by changes in the microcirculation) rather than improvement of endothelial function of the conduit vessel per se.

Ultrasound assessment of brachial artery FMD has yielded important information about vascular function in health and disease, yet several new approaches and technological advances have emerged. Most prior studies examined FMD at a single time point, typically 1 min after cuff release. This practice evolved from the observations that the maximal dilator response occurs at approximately 1 min in healthy subjects and that the necessity for manual acquisition and measurement placed a practical limit on the number of image frames that could be analyzed.

AIM

1) To evaluate macrovascular involvement in SSc with high resolution ultra sonogram (HR-USG) by using the measures of

   a. Flow-mediated dilatation (FMD), nitroglycerine mediated dilatation of the brachial artery and

   b. Carotid intima-media thickness (ccIMT) .

2) To find out any correlation between FMD, NMD, ccIMT and SSc disease activity and clinical characteristics.

MATERIALS AND METHODS

SUBJECTS:

Patients attending outpatient department and inpatients of Department of Rheumatology, Rajiv Gandhi Government General Hospital, Chennai were recruited during the period from October 2008 to March 2011. 32 eligible cases older than 16 years of age were enrolled. They fulfilled ACR criteria for SSc and were willing to undergo measurement of FMD, NMD and ccIMT.

Controls were the healthy relatives of the cases who were age and sex matched.

All subjects gave written informed consent to take part in this study, which was approved by the ethical committee.

INCLUSION CRITERIA

ACR Systemic Sclerosis: Preliminary Classification Criteria

- ☑ Major criteria or two minor criteria for diagnosis
- ☑ Major criterion: Proximal Scleroderma
- ☑ Minor Criteria
  - o Sclerodactyly
  - o Digital pitting scars or loss of substance from finger pad
  - o Bibasilar pulmonary fibrosis

(Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980;23:581-590)

EXCLUSION CRITERIA

1. Overlap syndrome
2. Mixed Connective Tissue Disease (MCTD)
3. Unclassified Connective Tissue Disease (UCTD)
4. Malignancy
5. Systemic hypertension
6. Diabetes mellitus
7. Smoking
8. Alcoholism

CLINICAL AND LAB ASSESSMENT

Detailed history, physical examination and evaluation were done in all cases (Photo-A). Laboratory evaluations included complete blood count, ESR (Westergren's method), liver function tests, renal function tests, blood sugar and fasting lipid profile. Immunlological assays were CRP (Latex agglutination method), ANA (ELISA / HEP2) and ENA profile.

Other investigations included ECG, X-ray Chest PA, USG abdomen, Echo-cardiogram and HRCT lungs. Barium swallow and meal studies and vascular imaging were also done.

The extent and severity of skin tightening was recorded with Modified Rodnan Skin Scoring System (MRSSS). To assess disease activity the preliminary European Scleroderma Study Group (EscSG)

activity indices (a score ranging from 0-10) was used. A score higher than 3 denotes active disease. (Appendix-I) Also, the revised preliminary SSc severity scale (Medsger's severity scale-Appendix-II), a measure of activity, damage and severity, was used. This scale is a 9-organ disease severity scale in which for each organ system a score of 0 to 4 is applied, with 0 being normal and 4 denoting end stage organ involvement.

ASSESSMENT OF ENDOTHELIAL FUNCTION

Endothelial function was assessed with high-resolution B-mode Doppler (ESAOTE S.P, A-Italia, EIZO NANAO Corporation, Canada with a 7-12 MHz linear array transducer) (Photo-B) examination of the brachial artery using the protocol described as in the guidelines cited previously. Radiologist converse with the techniques of vascular imaging assessed flow-mediated dilation (FMD) in response to reactive hyperemia. All the subjects were studied between 8 and 11am after a 12-hour overnight fast. The brachial artery was scanned 10 cm above the antecubital fossa (Photo-C). Distance measured was from anterior to posterior M lines (media-adventitia interface), and every measurement was taken by sonologist blinded to cases and controls. Then, ischemia was induced by inflating the pneumatic cuff of sphygmomanometer to a pressure 50 mmHg above systolic one, in order to obliterate the brachial artery. After 5 minutes, the cuff was deflated and the diameter was measured after 60 seconds postdeflation (Df) (Photo-D).

FMD is calculated as follows:

$$100\% \times \left(\frac{\text{Post Deflation diameter- resting diameter}}{\text{Resting Diameter}} \right)$$

ASSESSMENT OF ENDOTHELIUM-INDEPENDENT VASODILATION WITH NITROGLYCERIN

After 20 minutes of taking FMD measurement, 0.4 mg of nitroglycerine (NTG) is sprayed sublingually. After 5 minutes of sublingual NTG, the diameter of the brachial artery is taken as mentioned above (Photo-E).

NMD is calculated as follows

$$100\% \times \left(\frac{\text{Post NTG diameter – Resting Diameter}}{\text{Resting Diameter}} \right)$$

CAROTID ARTERY INTIMA-MEDIA THICKNESS AND PLAQUE

Cases and control subjects also had the intima-media thickness (IMT) of their carotid artery measured using high-resolution B-mode Doppler (ESAOTE S.P, A-Italia, EIZO NANAO Corporation, Canada with a 7-12 MHz – linear array transducer). The common carotid artery was scanned longitudinally and the IMT measurement was taken in the proximal part of the common carotid artery, 1cm proximal to the carotid bulb as the maximum distance between the intima- lumen and adventitia-media interfaces. IMT was determined as the average of 6 measurements, 3 each from the left and right common carotid arteries. (Photo-F)

To assess reproducibility of our technique, we looked at the reliability of reading scans on 2 separate occasions by a single blinded observer. For this 10 scans from cases or control subjects were chosen at random and baseline diameter, FMD, NMD and ccIMT were measured.

STATISTICAL ANALYSIS:

The statistical analysis was performed using the SPSS (version 11.5).

Results are presented as mean SD, except for frequencies, which are expressed as percentages.

Unpaired student's t- test has been used for comparing the FMD, NMD, ccIMT and other features of the study and control group.

P (less than) 0.05 was considered statistically significant.

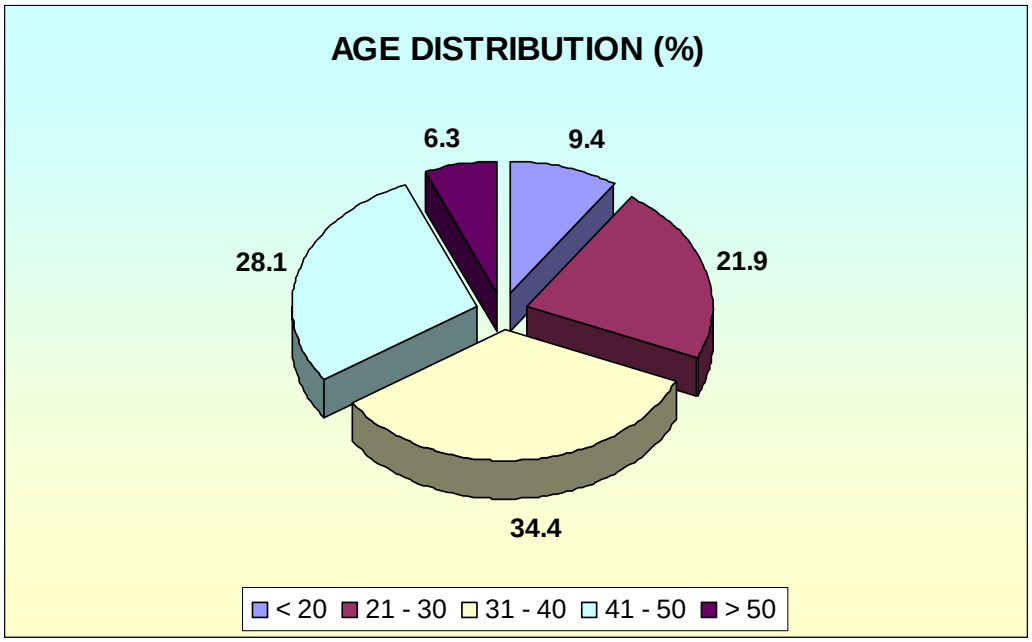
Pearson's test has been used for studying the correlation between FMD and variables.

RESULTS

Total number of cases were 32. The mean age was 36 years. The range was from 19-60 years. Majority of them were females – 24(75%). Female: Male ratio is 3:1.

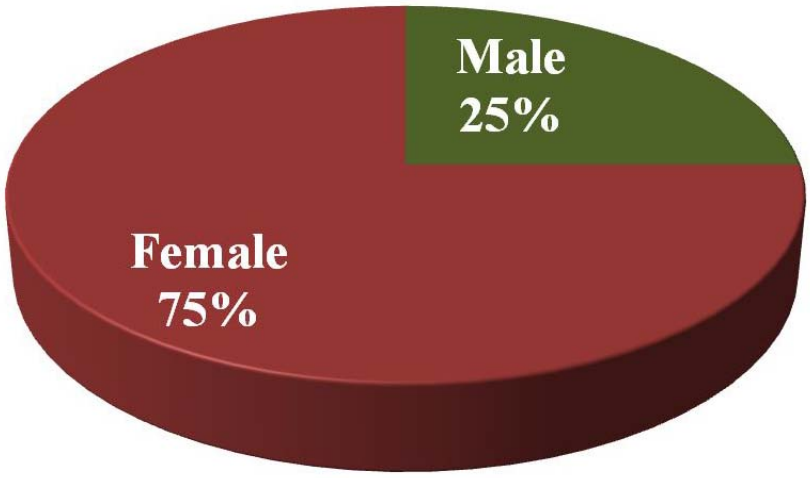
AGE DISTRIBUTION

Age (Years)	*Frequency (n)*	*Percent (%)*
< 20	3	9.4
21 - 30	7	21.9
31 - 40	11	34.4
41 - 50	9	28.1
> 50	2	6.3
Total	32	100



SEX DISTRIBUTION

Sex	*Frequency (n)*	*Percent (%)*
Male	8	25
Female	24	75
Total	32	100



CLINICAL CHARACTERISTICS OF CASES:

Limited cutaneous disease was observed in 26 (81%). Diffuse cutaneous disease was observed in 6 (19%). Raynaud's Phenomenon was observed in 21 (91%). Digital pitting scar was observed in 17 (53%). Finger contractures were noted in 11 (34%). Poly Arthralgia was noted in 9 (28%). Digital tip ulcerations was noted in 6 (19%).

Pulmonary involvement was noted in 23 (72%). ILD was seen in 23 (72%), ILD with traction bronchiectasis was seen in 2 (6%), ILD

with emphysematous changes was seen in 2 (6%) and ILD with pulmonary hypertension was seen in 2 (6%).

Cardiac involvement was noted in 6 (19%). ECG changes (ST segment/ T wave changes, ventricular premature beats and intra-ventricular conduction disturbances) was seen in 4 (12%.5), decreased LVEF by Echo was seen in 4 (12.5%).

Gastro Intestinal involvement was noted in 14 (44%), Esophageal dysmotility was seen in 14 (44%), Small bowel dysmotility was seen in 2 (6%).

Carpal tunnel syndrome was observed in 3 (9%). Sensory neuropathy was noted in 3 (9%), Vasculitic Neuropathy was seen in 1(3%).

Osteonecrosis of both tibiae and calcaneum was noted in 1 (3%). Non nephrotic macro-albuminuria was observed in 1 (3%).

The duration of disease varied from 6 months to 17 years . Mean duration of disease was 3.96 years.

28 cases (87.5%) were ANA positive. 4 cases (12.5%) were ANA negative.

15 cases were on pulse cyclophosphamide, 5 were on azathioprine and 2 each were on azathioprine and methotrexate.

CORRELATIONS BETWEEN FMD, NMD, IMT AND OTHER PARAMETERS IN CASES

Parameters		***FMD***	***NMD***	***ccIMT***
FMD	r- Value	-	0.370	-0.085
	P - Value	-	0.037*	0.642
NMD	r- Value	0.370	-	-0.024
	P - Value	0.037*	-	0.896
ccIMT	r- Value	-0.085	-0.024	-
	P - Value	0.642	0.896	-
Age	r- Value	0.105	-0.109	0.601
	P - Value	0.566	0.552	0.000*
Sex	r- Value	-0.008	0.223	-0.235
	P - Value	0.966	0.220	0.195
Disease Subtypes	r- Value	-0.031	0.093	-0.279
	P - Value	0.866	0.614	0.122
Disease Duration	r- Value	-0.287	-0.391	0.152
	P - Value	0.111	0.027*	0.406
ESR	r- Value	-0.191	-2.100	0.214
	P - Value	0.296	0.248	0.241
CRP	r- Value	-0.179	-0.072	-0.061
	P - Value	0.327	0.694	0.739
Active / Inactive disease (EUSTAR Score)	r- Value	0.137	-0.042	-0.186
	P - Value	0.455	0.819	0.309
Medsger-General	r- Value	0.114	0.121	-0.071
	P - Value	0.533	0.509	0.700

Parameters		***FMD***	***NMD***	***ccIMT***
Medsger-Peripheral Vascular	r- Value	-0.160	-0.264	0.067
	P - Value	0.381	0.144	0.715
Medsger-Skin	r- Value	-0.385	-0.440	0.251
	P - Value	0.029*	0.012*	0.165
Medsger-Joint / tendon	r- Value	-0.452	-0.227	0.288
	P - Value	0.009*	0.211	0.110
Medsger-GIT	r- Value	0.328	0.091	-0.350
	P - Value	0.067	0.621	0.050
Medsger-Lung	r- Value	0.052	0.325	0.020
	P - Value	0.778	0.070	0.915

The size of any correlation generally evaluates as follows:

Value of r	***Qualitative Description of the Strength***
-1	Perfect negative
(-1, -0.75)	Strong negative
(-0.75, -0.25)	Moderate negative
(-0.5, -0.25)	Weak negative
(-0.25, 0.25)	No linear association
(0.25, 0.5)	Weak positive
(0.5, 0.75)	Moderate positive
(0.75, 1)	Strong positive
1	Perfect positive

There was significant weak positive correlation between FMD and NMD. Significant moderate positive correlation was observed between age and ccIMT. Significant weak negative correlation was noted between disease duration and NMD. Significant weak negative correlation was seen between Medsger skin and FMD. Significant weak negative correlation between Medsger joint tendon and FMD non-significant weak negative correlation between Medsger G.I.T and ccIMT. Non-significant weak negative correlation between Medsger Lung and NMD.

CONTROLS

There was no significant difference between patients and controls in BMI, BP and serum lipid profile. There was significant difference in FMD and ccIMT between cases and controls. FMD was significantly decreased in cases compared to controls. The mean FMD in cases was 8.13% compared to 13.07% in controls. ccIMT was significantly increased in cases compared to controls . The mean cc IMT in cases was 0.67mm compared to 0.57 mm in controls. There was no significant difference in baseline diameter and NMD between cases and controls. There was significant moderate positive correlation between age and ccIMT.

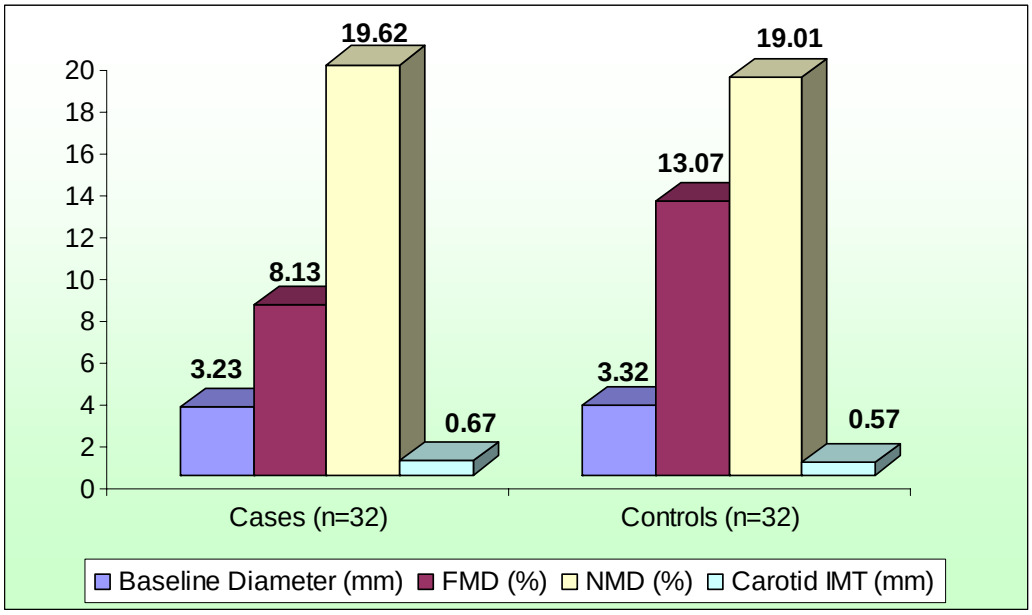
CORRELATIONS BETWEEN CASES AND CONTROLS

Parameters	***Cases (n=32)***	***Controls(n=32)***	***P - Value***
Male / Female	8/32	8/32	1.000
Mean age	36.34±10.28	36.63±10.05	0.912
BMI (kg/m2)	20.53±1.88	20.53±2.125	1.000
Mean duration of symptoms	3.96	-	-
Systolic BP (mmHg)	120.75±11.69	122.06±11.68	0.655
Diastolic BP (mmHg)	82.50±5.54	81±6.12	0.308
Total Cholesterol (mg/dl)	167.72±23.62	175.03±19.74	0.184
Triglycerides (mg/dl)	119.16±23.29	114.34±9.89	0.336
HDL (mg/dl)	44.06±4.23	42.41±2.49	0.061
LDL (mg/dl)	99.78±22.01	109.44±19.16	0.066

DIFFERENCES BETWEEN CASES AND CONTROLS

Parameters	***Cases (n=32)***	***Controls (n=32)***	***P - Value***
Baseline Diameter (mm)	3.23±0.26	3.32±0.22	0.158
FMD (%)	8.13±2.08	13.07±2.45	0.000
NMD (%)	19.62±3.91	19.01±3.46	0.511
Carotid IMT (mm)	0.67±0.19	0.57±0.10	0.011

Parameters	*Cases (n=32)*	*Controls (n=32)*
Baseline Diameter (mm)	3.23	3.32
FMD (%)	8.13	13.07
NMD (%)	19.62	19.01
Carotid IMT (mm)	0.67	0.57



There was no significant difference between the 2 values of baseline diameter, FMD,NMD and ccIMT between cases and controls (to assess reproducibility of our technique)

For baseline diameter, the p value was 0.429.

For FMD, the p value was 0.585.

For NMD, the p value was 0.367.

For ccIMT, the P value was 0.809.

Correlations between FMD, NMD and IMT with Age (Control Groups)

		FMD	**NMD**	**ccIMT**	**AGE**
FMD	r-Value	1	-.167	-.107	-.170
	P- Value	-	.362	.559	.353
NMD	r-Value	-.167	1	-.044	.041
	P- Value	.362	-	.813	.825
ccIMT	r-Value	-.107	-.044	1	.565*
	P- Value	.559	.813	-	.001
AGE	r-Value	-.170	.041	.565*	1
	P- Value	.353	.825	.001	-

DISCUSSION

Similar to our study, **G. Szucs et al** [157] observed in their 29 SSc patients, the FMD was significantly lower (4.82 ± 3.76%) in comparison with the controls (8.86 ± 3.56%) (P <0.001). No difference was found in NMD between patients (19.13 ± 17.68%) and controls (13.13 ± 10.40%) (P >0.1). As against our study in which there is significant difference, in their study the difference was not significant (P=0.067) between increased ccIMT in SSc patients (0.67 ± 0.26mm) and healthy subjects (0.57 ± 0.09).Like our study, a significant positive correlation between ccIMT and age in SSc (r = 0.470, P = 0.013) was detected, as well as in healthy controls (r = 0.61, P = 0.003), but no correlation was found between FMD and age ,in their study. Unlike our study, in which NMD displayed significant inverse correlation with the disease duration, in their study, ccIMT but not FMD and NMD, displayed significant correlation with disease duration (r = 0.472, P=0.011), NMD displayed significant inverse correlation with the age in SSc patients (r = −0.492, P = 0.012), but not in controls. In contrast to our study, in which there is significant positive correlation between FMD and NMD, they did not find any correlation between FMD, NMD, ccIMT and SSc subtype.

In concurrence with our study, **Bartoli.F et al** [158a and b] in their study of 35 SSc patients and 20 healthy controls, observed FMD

was significantly impaired (3.41%± 4.56% versus 7.66%± 4.24%; $P < 0.037$) and IMT was significantly elevated compared with healthy controls (0.93 ± 0.29 mm versus 0.77 ± 0.13 mm; $P < 0.005$). FMD was not significantly different in SSc with increased IMT compared with those with normal IMT). No correlation was found between risk factors for atherosclerosis and the impairment of FMD or IMT in SSc patients.

In contrast to our study in which NMD is preserved, **Rossi et al** [159] observed flow-mediated vasodilation (FMD) and NMD were significantly decreased in patients with SSc (10.3 ± 8.6 versus 26.6 ± 7.4%, $P<0.001$; 24.2 ± 8.4 versus 33.3 ± 10.1%, $P<0.001$, respectively), in their study of 14 SSc patients and 14 healthy controls matched for age and for cardiovascular risk factors.

After the first reports suggesting an increased prevalence of macrovascular involvement in SSc, several studies have been performed in the last decade using IMT of the carotid artery as a marker of early atherosclerosis.

Lekakis et al. [160] reported impaired FMD and NMD in 12 patients with either SSc or RP. Unfortunately, authors did not separate SSc from RP. **Andersen et al.** [24] found similar FMD and NMD in 24 SSc patients compared with controls. However, their patient population also included mixed connective tissue disease (MCTD) patients.

Lekakis et al, [160] **Kaloudi et al,** [161] and **Bartoli et al** [158aandb] found strongly increased IMT values in the CCA in SSc patients compared with controls.

Unlike our study, **Hettema et al** [162] in their study of 49 SSc patients, reported that the mean IMT (median, interquartile ranges; 0.69 mm (0.62-0.79)) was not significantly increased compared to healthy controls (0.68 mm (0.56-0.75, p=0.067). Also, after correction for the confounders age, HDL-cholesterol and LDL-cholesterol (p=0.328), no difference in IMT was present between SSc patients and healthy controls. In patients no correlations were found between maximum IMT and disease related variables.

Cheng et al [163and164] found no differences in IMT values in SSc patients compared with controls.

IN OUR STUDY:

The endothelium independent vasodilatation assessed by NMD in SSc patients is still preserved giving an opportunity of nitroglycerine therapy.

There is weak negative correlation between NMD and disease duration. This may be explained by the impairment of the vascular media late in the disease course by the pathogenic mechanisms.

There is no correlation between ccIMT and disease duration. This may be probably due to long term use of immunosuppressives in many patients affecting the atherogenic mechanisms favourably.

Thus, our middle-aged SSc patients represent a population, where there is sub-clinical cardiovascular disease indicated by surrogate measures (decreased FMD and increased ccIMT) of sub-clinical atherosclerosis. At the same time, the preserved NMD gives us a possibility, to introduce nitroglycerin treatment or nitric-oxide donor agents in order to decrease cardiovascular risk.

FMD using vascular ultrasonography on brachial artery represents a useful, noninvasive method for the assessment of endothelial dysfunction. Reactive hyperemia produces a shear stress stimulus that induces the normal endothelium to release nitric oxide (NO), which acts as a vasodilator. Impaired enthothelial function is associated with a reduced release of No and a lower vasodilation [165]. The effect of disease states and/ or interventions on the blood flow response to cuff occlusion (reactive hyperemia) is under explored. Current technology limits the utility of spectral Doppler to reproducibily assess changes in flow, which might provide useful information about endothelial function of the microvasculature.

Ongoing studies in several large populations, including the Framingham Heart Study and the Cardiovascular Health Study , shall

determine whether endothelial dysfunction in the brachial artery will identify patients at risk for developing coronary artery disease, cerebral vascular disease and/ or peripheral vascular disease. The technique is particularly well suited for study of the earliest stages of atherosclerosis in children and young adults, thus providing maximal opportunity for prevention.

Numerous studies have demonstrated that brachial artery reactivity improves with risk factor modification and treatment with drugs known to reduce cardiovascular risk. It remains unknown whether an improvement in endothelial function directly translates into improved outcome. In the future, however, practitioners may use brachial artery FMD to assess response to drug therapy and to individualize patient risk factor modification programs. Further studies are needed to determine whether the methodology is sufficiently reproducible and whether biological variability is sufficiently low to make assessment of FMD a clinically useful measure of cardiovascular risk on an individual or group basis. To that end, the methodology will need to mature, with formal opportunities for training, certification and continuing medical education, as currently exist for other cardiovascular testing modalities.

FUTURE DIRECTIONS

Commercially available technology now makes it possible to acquire multiple images of the brachial artery automatically using the

ECG signal as a trigger and to measure arterial diameter automatically using computer-based edge-detection techniques. This approach allows investigators to examine the entire time course of brachial dilation in response to reactive hyperemia, the true peak response, the time to peak and the overall duration of FMD as discussed in the previous text. The time course and extent of brachial expansion within a single cardiac cycle, possibly reflecting vessel compliance, can be examined. In the carotid artery, compliance has been shown to correlate with cardiovascular risk [166]. About 70% of the dilation observed 1 min after cuff release is attributable to NO synthesis.

Further studies are needed to evaluate other vasoactive mechanisms and to determine whether various disease states influence the kinetics and/or extent of FMD. Careful examination of the vasodilator response to NTG provides another potential avenue for investigation. Although most studies have detected little effect of disease states on this response, there is evidence that cardiovascular risk factors might impair the vasodilator response to NTG, especially when a dose-response curve is measured. These findings are consistent with experimental studies demonstrating that inactivation of NO by reactive oxygen species is an important mechanism of vascular dysfunction.

Further information about the causes of vascular dysfunction and the response to interventions may be gained by examining the response to a submaximal dose of NTG or a series of NTG doses.

The effect of disease states and/or interventions on the blood flow response to cuff occlusion (reactive hyperemia) is under explored. Current technology limits the utility of spectral Doppler to reproducibly assess changes in flow, which might provide useful information about endothelial function of the microvasculature.

CONCLUSION

1) Macrovascular involvement is also seen in SSc patients even in the absence of traditional cardiovascular risk factors.

2) There is an impairment of endothelium dependent vasodilatation indicated by low FMD (8.13%) in SSc patients.

3) The endothelium independent vasodilatation assessed by NMD is still preserved providing an opportunity of nitroglycerine therapy for favourably modifying the course of macrovascular pathology.

4) Carotid atherosclerosis as measured by ccIMT is higher in SSc patients (0.67mm) than in controls (0.57mm).

5) ccIMT has significant correlation (moderate positive) with age, in controls and in SSc patients.

6) There is no moderate or strong correlation between FMD, NMD, ccIMT and SSc disease activity and clinical characteristics.

7) FMD using vascular ultrasonography on brachial artery represents a simple, inexpensive, non-invasive and reproducible tool for the assessment of endothelial function.

8) Future prospects of FMD technique include:

a. Early screening module for evaluation of endothelial dysfunction and to start on drugs with pleomorphic effects favourably modifying the clinical events like statins, ACE inhibitors and aspirin.

b. Serial screening method to ascertain improvement of macrovascular and endothelial function while on treatment with immunosuppressive agents and vasodilators.

9) The assessment of macrovascular involvement in SSc may have important diagnostic, prognostic and therapeutic implications in the management of systemic sclerosis.

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ABBREVIATIONS

ACR	:	American College of Rheumatology
ANA	:	Anti Nuclear Antibody
AVN	:	Avascular Necrosis
BMI	:	Body Mass Index
ccIMT	:	Common Carotid Intima Medial Thickness
CHD	:	Coronary Heart Disease
CRP	:	C-Reactive Protein
CVD	:	Cardiovascular Disease
DcSSc	:	Diffuse Cutaneous Systemic Sclerosis
ECG	:	Electro Cardiogram
ECHO	:	Echo Cardiogram
ECM	:	Extra Cellular Matrix
ELISA	:	Enzyme Linked Immuno Sorbent Assay
ENA	:	Extractable Nuclear Antigen
ESR	:	Erythrocyte Sedimentation Rate
EUSTAR	:	Eular Scleroderma Trials and Research
FMD	:	Flow Mediated Dilatation
GIT	:	Gastrointestinal Tract
HDL	:	High Density Lipoprotein
HRCT	:	High Resolution Computerized Axial Tomography
ILD	:	Interstitial Lung Disease
LcSSc	:	Limited Cutaneous Systemic Sclerosis
LDL	:	Low Density Lipoprotein

MCTD	:	Mixed Connective Tissue Disease
MRSSS	:	Modified Rodnan Skin Scoring System
NMD	:	Nitroglycerin Mediated Dilatation
NO	:	Nitric Oxide
NTG	:	Nitroglycerin
SSc	:	Systemic Sclerosis
UCTD	:	Undifferentiated Connective Tissue Disease
USG	:	Ultrasonogram

APPENDIX- I

EUSTAR Systemic Sclerosis Activity Score

Parameter	SCORE*	Assessment
Modified Rodnan skin score > 14	1	Assessment of skin thickness on a scale from 0 (normal thickness) to 3+ (severe thickness) at 17 anatomic areas (values from 0 – 51).
Scleredema	0.5	Increase in soft tissue mass (particularly at the fingers) due to dermal skin folds.
Skin	2	The patient states "worsened" in response to the physician's question "Did your skin manifestations change during the last month ?"
Digital necrosis	0.5	Active digital ulcers ranging from small infarcts of the digital tips to digital gangrene
Vascular	0.5	The patient states "worsened" in response to the physician's question "Did your blood flow manifestation change during the last month ? "
Arthritis **	0.5	Symmetric swelling and tenderness of the peripheral joints
DLCO***	0.5	DLCO less than 80% of the predicted values evaluated by the single breath method
Heart/Lung	2	The patient states "worsened" in response to the physician's question "Did your cardiopulmonary manifestations change during the last month ?"
ESR > 30	1.5	Westergreen method
Hypocomplementemia	1	Either low C3 or low C4 by whatever method (nephelometry, double radial immuno-diffusion)

APPENDIX- II

Table 2 Medsger Disease Severity Scale (22,47)

Organ system	0 (normal)	1 (mild)	2 (moderate)	3 (severe)	4 (end stage)
General	Wt loss <5%; PCV 37%+; Hb 12.3+ g/dl	Wt loss 5–10%; PCV 33–37%; Hb 11.0–12.2 g/dl	Wt loss 10–15%; PCV 29–33%; Hb 9.7–10.9 g/dl	Wt loss 15–20%; PCV 25–29%; Hb 8.3–9.6 g/dl	Wt loss 20+%; PCV 25%; Hb <8.3 g/dl%
Peripheral vascular	No Raynaud's; Raynaud's not requiring vasoldialtors	Raynaud's requiring vasodilators	Digital pitting scars	Digital tip ulcerations	Digital gangrene
Skin	TSS 0	TSS 1–14	TSS 15–29	TSS 30–39	TSS 40+
Joint/tendon	FTP 0–0.9 cm	FTP 1.0–1.9 cm	FTP 2.0–3.9 cm	FTP 4.0–4.9 cm	FTP 5.0+ cm
Muscle	Normal proximal muscle strength	Proximal weakness, mild	Proximal weakness, moderate	Proximal weakness, severe	Ambulation aids required
Gastrointestinal tract	Normal esophagram; normal small bowel series	Distal esophageal hypoperistalsis; small bowel series abnormal	Antibiotics required for bacterial overgrowth	Malabsorption syndrome; episodes of pseudo-obstruction	Hyperalimentation required
Lung	DLCO 80+%; FVC 80+%; No fibrosis on radiograph; sPAP <35 mmHg	DLCO 70–79%; FVC 70–79%; Basilar rales; fibrosis on radiograph; sPAP 35–49 mmHg	DLCO 50–69%; FVC 50–69%; sPAP 50–64 mmHg	DLCO <50%; FVC <50%; sPAP 65+ mmHg	Oxygen required
Heart	EKG normal; LVEF 50+%	EKG conduction defect; LVEF 45–49%	EKG arrhythmia; LVEF 40–44%	EKG arrhythmia requiring Rx; LVEF 30–40%	CHF; LVEF <30%
Kidney	No history of SRC with serum creatinine <1.3 mg/dl	History of SRC with serum creatinine <1.5 mg/dl	History of SRC with serum creatinine 1.5–2.4 mg/dl	History of SRC with serum creatinine 2.5–5.0 mg/dl	History of SRC with serum creatinine >5.0 mg/dl or dialysis required

Wt, weight; PCV, packed cell volume (hematocrit); Hb, hemoglobin; TSS, total skin score; FTP, fingertip to palm distance in flexion; DLCO, diffusing capacity for carbon monoxide, % predicted; FVC, forced vital capacity, % predicted; sPAP, estimated pulmonary artery pressure by Doppler echo; EKG, electrocardiogram; LVEF, left ventricular ejection fraction; Rx, treatment; CHF, congestive heart failure; SRC, scleroderma renal crisis.

<u>சுய ஒப்புதல் படிவம்</u>

ஆய்வு செய்யப்படும் தலைப்பு

ஸ்கிலிரோடெர்மா- இரத்தக் குழாய் நோய் பற்றிய ஆய்வு

ஆராய்ச்சி நிலையம் : மூட்டு, தசை, இணைப்புத்திசு மருத்துவத்துறை
சென்னை மருத்துவக்கல்லூரி,
சென்னை - 3.

பங்கு பெறுபவரின் பெயர் :

பங்குபெறுபவரின் எண் :

பங்கு பெறுவர் இதனை (✓) குறிக்கவும்.

மேலே குறிப்பிட்டுள்ள மருத்துவ ஆய்வின் விவரங்கள் எனக்கு விளக்கப்பட்டது. என்னுடைய சந்தேகங்களை கேட்கவும், அதற்கான தகுந்த விளக்கங்களை பெறவும் வாய்ப்பளிக்கப்பட்டது. ☐

நான் இவ்வாய்வில் தன்னிச்சையாகதான் பங்கேற்கிறேன். எந்த காரணத்தினாலோ எந்த கட்டத்திலும் எந்த சட்ட சிக்கலுக்கும் உட்படாமல் நான் இவ்வாய்வில் இருந்து விலகி கொள்ளலாம் என்றும் அறிந்து கொண்டேன். ☐

இந்த ஆய்வு சம்பந்தகமாகவோ, இதை சார்ந்த மேலும் ஆய்வு மேற்கொள்ளும் போதும் இந்த ஆய்வில் பங்குபெறும் மருத்துவர் என்னுடைய மருத்துவ அறிக்கைகளை பார்ப்பதற்கு என் அனுமதி தேவையில்லை என அறிந்து கொள்கிறேன். நான் ஆய்வில் இருந்து விலகிக் கொண்டாலும் இது பொருந்தும் என அறிகிறேன். ☐

இந்த ஆய்வின் மூலம் கிடைக்கும் தகவல்களையும், பரிசோதனை முடிவுகளையும் மற்றும் சிகிச்சை தொடர்பான தகவல்களையும் மருத்துவர் மேற்கொள்ளும் ஆய்வில் பயன்படுத்திக் கொள்ளவும் அதை பிரசுரிக்கவும் என் முழு மனதுடன் சம்மதிக்கிறேன். ☐

இந்த ஆய்வில் பங்கு கொள்ள ஒப்புக்கொள்கிறேன். எனக்கு கொடுக்கப்பட்ட அறிவுரைகளின்படி நடந்து கொள்வதுடன் இந்த ஆய்வை மேற்கொள்ளும் மருத்துவ அணிக்கு உண்மையுடன் இருப்பேன் என்றும் உறுதியளிக்கிறேன். என் உடல் நலம் பாதிக்கப்பட்டஏாலோ அல்லது எதிர்பாராத வழக்கத்திற்கு மாறான நோய்க்குறி தென்பட்டாலோ உடனே அதை மருத்துவ அணியிடம் தெரிவிப்பேன் என உறுதி அளிக்கிறேன். ☐

இந்த ஆய்வில் எனக்கு இரத்தம், சிறுநீர், எக்ஸ்ரே, ஸ்கேன் பரிசோதனை செய்தஉகொள்ள நான் முழு மனதுடன் சம்மதிக்கிறேன்.

பங்கேற்பவரின் கையொப்பம் ..... இடம் ..... தேதி

கட்டைவிரல் ரேகை

பங்கேற்பவரின் பெயர் மற்றும் விலாசம் .....

ஆய்வாளரின் கையொப்பம் ..... இடம் ..... தேதி

ஆய்வாளரின் பெயர் .....

CONSENT FORM

Study Title
EVALUATION OF MACROVASCULAR INVOLVEMENT IN SYSTEMIC SCLEROSIS

Study Centre : Department of Rheumatology,
Madras Medical College, Chennai – 600 003

Patient's Name :

Patient's Age :

Identification Number :

Patient may check (✓) these boxes

I confirm that I have understood the purpose of procedure for the above study. I have the opportunity to ask the question and all my questions and doubts have been answered to my complete satisfaction. ☐

I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving any reason, without my legal rights being affected. ☐

I understand that sponsor of the clinical study, others working on the sponsor's behalf, the ethics committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the study. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from this study. ☐

I agree to take part in the above study and to comply with the instructions given during the study and to faithfully co-operate with the study team, and to immediately inform the study staff if I suffer from any deterioration in my health or well being or any unexpected or unusual symptoms. ☐

I hereby consent to participate in this study on 'Evaluation of macrovascular involvement in systemic sclerosis'. ☐

I hereby give permission to undergo complete clinical examination and diagnostic tests including hematological, biochemical, radiological and urine examination. ☐

Signature / Thumb Impression _____ Place _____
Date _____

Patient's Name and Address: _____

Signature of the Investigator : _____ Place _____
Date _____

Study Investigator's Name : _____

INSTITUTIONAL ETHICAL COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI -3

Telephone No: 04425305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. Pandiperumal
PG in DM Rheumatology
Madras Medical College, Chennai -3

Dear Dr. Pandiperumal

The Institutional Ethical Committee of Madras Medical College reviewed and discussed your application for approval of the project / proposal / clinical trail entitled " **Evaluation of macrovascular involvement in systemic sclerosis**" No 09072010

The following members of Ethical committee were present in the meeting held on 21.07.2010 conducted at Madras Medical College,

1.	Prof. S.K. Rajan, MD	-- Chairperson
2.	Prof. J. Mohanasundaram, MD,Ph.D,DNB Dean, Madras Medical College, Chennai -3	-- Deputy Chairman
3.	Prof. A. Sundaram, MD Vice Principal , MMC, Chennai -3	-- Member Secretary
4.	Prof. R. Sathianathan, MD Director, Institute of Psychiatry	-- Member
5.	Prof R. Nandhini, MD Director, Institute of Pharmacology, MMC, Ch-3	-- Member
6.	Prof. Pregna B. Dolia , MD Director, Institute of Biochemistry, MMC, Ch-3	-- Member
7.	Prof. C. Rajendran , MD	-- Member
	Director, Institute of Internal Medicine, MMC, Ch-3	-- Member
8.	Prof. Geetha Subramanian, MD,DM Professor & Head , Dept. Of Cardiology	-- Member
9.	Prof. V. Shruti Kamal, MS Professor of Surgery, MMC, Ch-3	-- Member
10.	Prof. Md. Ali, MD, DM	-- Member
	Professor & Head ,,Dept. of MGE, MMC, Ch-3	-- Member

We approve the trail to be conducted in its presented form.

Sd/. Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, any SAE occurring in the course of the study, any changes in the protocol and patient information / informed consent and asks to be provided a copy of the final report

Member Secretary, Ethics Committee.

Photo - A

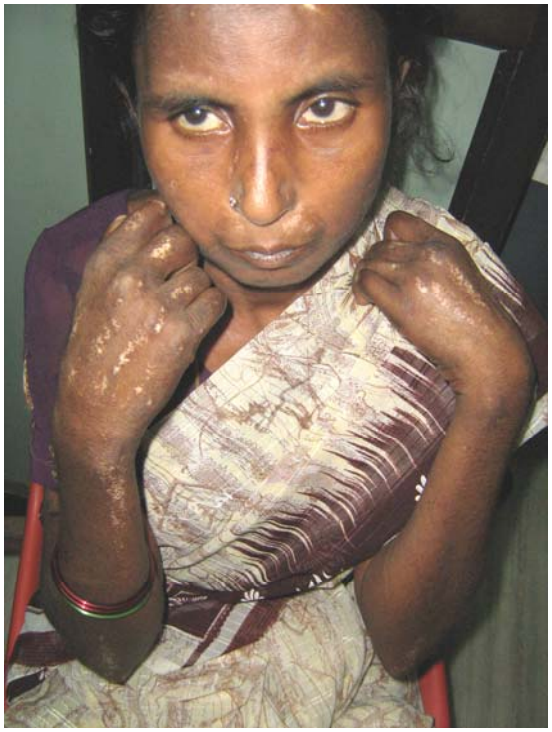


Photo - B

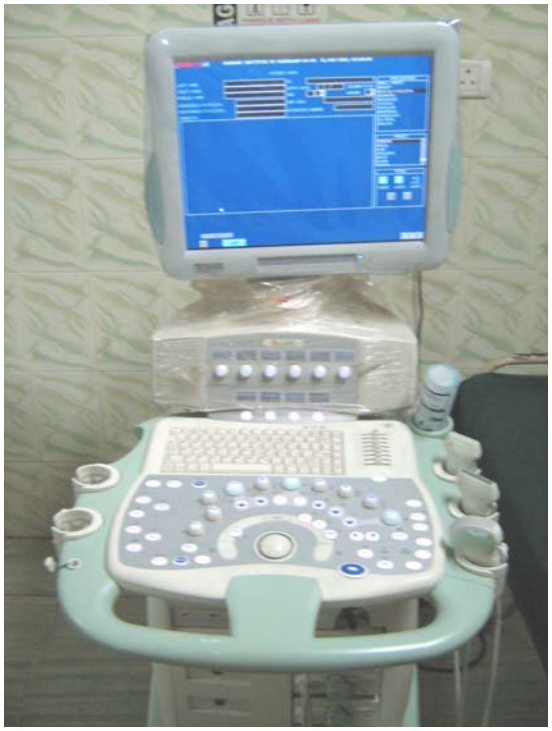
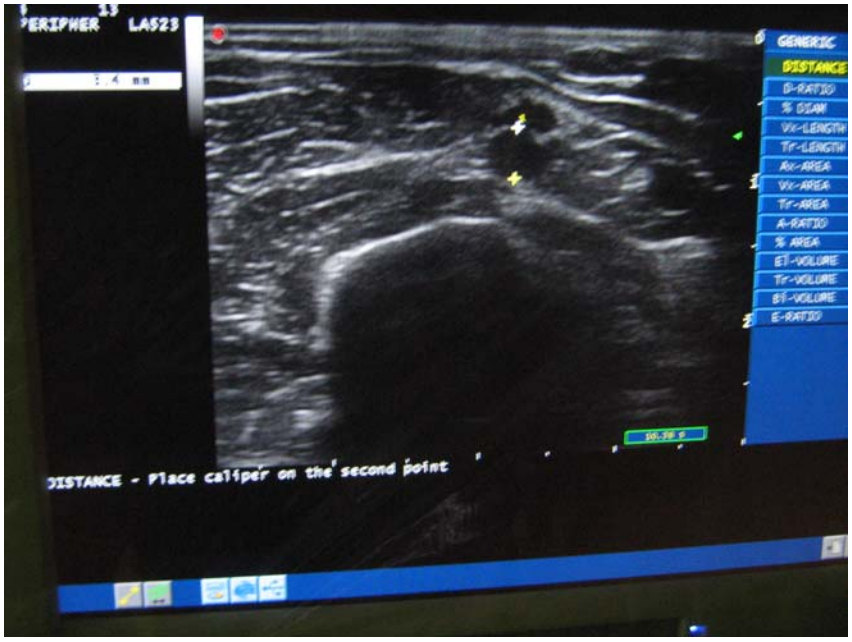


Photo - C

Photo – D

Photo-E



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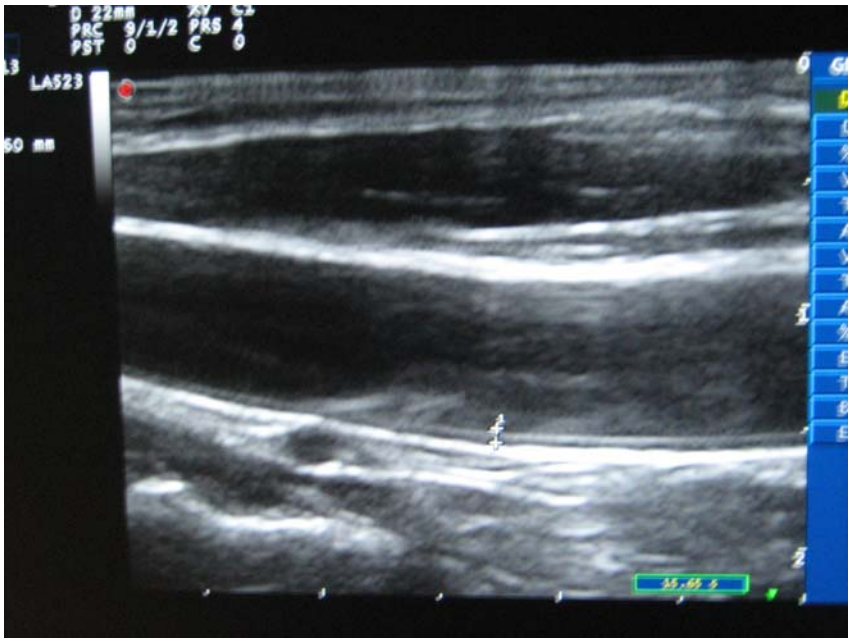

S. NO	RCC NO	AGE	SEX	duration Subtype		FMD%	NMD%	ccIMT (mm)	T. cholest	TGL	HDL	LDL	ESR	CRP	M. Gener	M.Per. vas	M. Skin	M.Jt. Tend	M. Muscle	M.G.I.T	M. Lung	M. Heart	M. Kidney	BMI	syst.BP	diast.BP	B.dm (mm)
1	51715	33	F	2Y	Lc	6.25	18.75	0.55	161	124	43	93	38	6	2	3	2	2	0	1	4	0	0	21	120	84	3.2
2	47973	28	F	5Y	Lc	8.33	19.44	0.54	140	115	40	77	34	12	4	2	1	0	1	1	0	1	0	22	124	80	3.6
3	53694	19	F	2Y	Lc	8.82	14.7	0.42	146	110	40	84	45	0	3	2	1	0	0	3	0	1	0	19	130	84	3.4
4	49214	36	M	8Y	Lc	9.67	22.58	0.85	169	114	46	100	15	0	2	2	1	0	0	1	1	0	0	18	120	86	3.1
5	52063	30	F	1Y6M	Lc	6.25	18.75	0.65	176	92	44	114	40	6	3	0	1	0	0	0	2	0	0	20	130	84	3.2
6	52169	59	F	1Y6M	Lc	11.11	22.22	0.7	170	160	42	96	10	0	3	3	1	0	0	1	1	0	0	19	132	78	3.6
7	52278	25	M	1Y6M	Lc	5.71	17.14	0.65	140	110	48	70	12	0	1	3	2	0	0	1	0	0	0	22	128	76	3.5
8	53218	39	F	4Y	Lc	9.37	15.62	0.65	187	127	56	106	10	6	2	2	1	0	1	0	3	0	0	17	130	84	3.2
9	46208	28	F	5Y	Lc	6.25	18.75	0.6	170	113	42	105	28	6	3	2	1	0	0	0	0	1	0	23	134	86	3.2
10	42652	30	F	10Y	Lc	9.09	30	0.68	172	113	42	107	30	0	2	1	1	0	0	1	2	0	0	22	130	86	3
11	53065	38	F	6M	Lc	9.67	19.35	0.66	118	124	40	53	45	0	3	1	1	0	1	0	1	0	0	18	120	88	3.1
12	40172	43	F	17Y	Lc	5.7	14.28	0.9	180	130	42	112	114	12	3	2	2	4	0	0	0	0	1	19	122	80	3.5
13	53298	20	F	1Y	Lc	8.82	17.64	0.6	187	70	42	131	10	0	1	2	1	0	0	1	0	0	0	20	130	84	3.4
14	53164	34	M	5Y	Lc	12.5	21.87	0.37	180	122	41	115	20	0	2	2	1	0	0	1	1	1	0	20	106	90	3.2
15	46486	35	F	5Y	Dc	6.66	20	0.7	137	108	42	73	35	0	1	3	2	0	1	0	1	0	0	19	130	84	3
16	53502	45	F	3Y	Lc	6.66	16.66	0.77	181	143	50	102	50	0	2	2	1	0	0	1	1	0	0	21	126	90	3
17	53258	42	F	6M	Lc	9.09	24.24	1.15	197	123	53	119	40	6	1	0	2	2	0	0	1	0	0	16	100	82	3.3
18	43105	27	F	3Y	Lc	3.44	24.13	0.6	185	114	44	118	40	12	3	0	1	0	1	0	2	0	0	22	134	80	2.9
19	53195	19	F	1Y	Lc	12	28	0.41	180	130	40	114	68	12	2	0	1	0	0	1	0	0	0	23	140	90	2.5
20	53230	25	F	4Y	Dc	6.25	18.75	0.5	180	128	42	112	16	0	2	2	2	1	0	0	1	0	0	19	120	82	3.2
21	49206	48	M	3Y6M	Lc	9.37	21.87	0.9	176	140	42	106	30	0	3	2	1	0	0	0	0	0	0	18	126	82	3.2
22	50394	46	M	3Y	Dc	8.57	14.28	0.9	173	222	46	83	55	12	1	2	3	0	0	0	1	0	0	24	130	86	3.5
23	51976	60	F	1Y6M	Lc	10.3	20.68	0.83	191	113	49	119	40	6	3	0	1	0	1	1	0	1	0	18	120	84	2.9
24	36349	50	F	10Y	Lc	6.25	21.87	0.6	174	126	45	104	65	12	2	1	1	0	0	1	1	0	0	20	130	86	3.2
25	51884	38	F	3Y	Lc	8.1	18.91	0.5	160	106	40	99	40	0	3	2	1	0	0	0	0	2	0	19	90	60	3.7
26	51286	43	M	6Y	Dc	5.88	17.64	1.2	226	110	52	152	130	0	2	3	2	1	0	0	0	0	0	17	100	74	3.4
27	52909	40	F	5Y6M	Lc	9.67	16.12	0.67	146	100	46	80	80	0	3	1	2	0	1	1	1	0	0	17	120	88	3.1
28	53306	32	F	8M	Lc	9.37	25	0.7	188	102	42	126	55	12	2	0	1	0	1	0	4	1	0	20	110	78	3.2
29	53137	31	F	4Y	Lc	9.09	18.18	0.51	177	124	44	108	120	12	2	2	1	0	0	0	1	1	0	21	120	86	3.3
30	53510	36	F	2Y	Lc	7.14	21.42	0.55	118	108	46	50	28	0	1	0	2	0	0	1	2	0	0	19	130	80	2.8
31	53762	42	M	1Y	Dc	5.4	13.51	0.73	146	127	41	80	71	12	1	0	2	1	0	0	0	0	1	19	100	70	3.7
32	53854	42	M	5Y	Dc	9.37	15.62	0.45	136	65	38	85	30	12	1	0	2	0	0	1	0	0	0	20	110	80	3.2

S. NO	RCC NO	AGE	SEX	duration Subtype		FMD%	NMD%	ccIMT (mm)	T. cholest	TGL	HDL	LDL	ESR	CRP	M. Gener	M.Per. vas	M. Skin	M.Jt. Tend	M. Muscle	M.G.I.T	M. Lung	M. Heart	M. Kidney	BMI	syst.BP	diast.BP	B.dm (mm)
1	51715	33	F	2Y	Lc	6.25	18.75	0.55	161	124	43	93	38	6	2	3	2	2	0	1	4	0	0	21	120	84	3.2
2	47973	28	F	5Y	Lc	8.33	19.44	0.54	140	115	40	77	34	12	4	2	1	0	1	1	0	1	0	22	124	80	3.6
3	53694	19	F	2Y	Lc	8.82	14.7	0.42	146	110	40	84	45	0	3	2	1	0	0	3	0	1	0	19	130	84	3.4
4	49214	36	M	8Y	Lc	9.67	22.58	0.85	169	114	46	100	15	0	2	2	1	0	0	1	1	0	0	18	120	86	3.1
5	52063	30	F	1Y6M	Lc	6.25	18.75	0.65	176	92	44	114	40	6	3	0	1	0	0	0	2	0	0	20	130	84	3.2
6	52169	59	F	1Y6M	Lc	11.11	22.22	0.7	170	160	42	96	10	0	3	3	1	0	0	1	1	0	0	19	132	78	3.6
7	52278	25	M	1Y6M	Lc	5.71	17.14	0.65	140	110	48	70	12	0	1	3	2	0	0	1	0	0	0	22	128	76	3.5
8	53218	39	F	4Y	Lc	9.37	15.62	0.65	187	127	56	106	10	6	2	2	1	0	1	0	3	0	0	17	130	84	3.2
9	46208	28	F	5Y	Lc	6.25	18.75	0.6	170	113	42	105	28	6	3	2	1	0	0	0	0	1	0	23	134	86	3.2
10	42652	30	F	10Y	Lc	9.09	30	0.68	172	113	42	107	30	0	2	1	1	0	0	1	2	0	0	22	130	86	3
11	53065	38	F	6M	Lc	9.67	19.35	0.66	118	124	40	53	45	0	3	1	1	0	1	0	1	0	0	18	120	88	3.1
12	40172	43	F	17Y	Lc	5.7	14.28	0.9	180	130	42	112	114	12	3	2	2	4	0	0	0	0	1	19	122	80	3.5
13	53298	20	F	1Y	Lc	8.82	17.64	0.6	187	70	42	131	10	0	1	2	1	0	0	1	0	0	0	20	130	84	3.4
14	53164	34	M	5Y	Lc	12.5	21.87	0.37	180	122	41	115	20	0	2	2	1	0	0	1	1	1	0	20	106	90	3.2
15	46486	35	F	5Y	Dc	6.66	20	0.7	137	108	42	73	35	0	1	3	2	0	1	0	1	0	0	19	130	84	3
16	53502	45	F	3Y	Lc	6.66	16.66	0.77	181	143	50	102	50	0	2	2	1	0	0	1	1	0	0	21	126	90	3
17	53258	42	F	6M	Lc	9.09	24.24	1.15	197	123	53	119	40	6	1	0	2	2	0	0	1	0	0	16	100	82	3.3
18	43105	27	F	3Y	Lc	3.44	24.13	0.6	185	114	44	118	40	12	3	0	1	0	1	0	2	0	0	22	134	80	2.9
19	53195	19	F	1Y	Lc	12	28	0.41	180	130	40	114	68	12	2	0	1	0	0	1	0	0	0	23	140	90	2.5
20	53230	25	F	4Y	Dc	6.25	18.75	0.5	180	128	42	112	16	0	2	2	2	1	0	0	1	0	0	19	120	82	3.2
21	49206	48	M	3Y6M	Lc	9.37	21.87	0.9	176	140	42	106	30	0	3	2	1	0	0	0	0	0	0	18	126	82	3.2
22	50394	46	M	3Y	Dc	8.57	14.28	0.9	173	222	46	83	55	12	1	2	3	0	0	0	1	0	0	24	130	86	3.5
23	51976	60	F	1Y6M	Lc	10.3	20.68	0.83	191	113	49	119	40	6	3	0	1	0	1	1	0	1	0	18	120	84	2.9
24	36349	50	F	10Y	Lc	6.25	21.87	0.6	174	126	45	104	65	12	2	1	1	0	0	1	1	0	0	20	130	86	3.2
25	51884	38	F	3Y	Lc	8.1	18.91	0.5	160	106	40	99	40	0	3	2	1	0	0	0	0	2	0	19	90	60	3.7
26	51286	43	M	6Y	Dc	5.88	17.64	1.2	226	110	52	152	130	0	2	3	2	1	0	0	0	0	0	17	100	74	3.4
27	52909	40	F	5Y6M	Lc	9.67	16.12	0.67	146	100	46	80	80	0	3	1	2	0	1	1	1	0	0	17	120	88	3.1
28	53306	32	F	8M	Lc	9.37	25	0.7	188	102	42	126	55	12	2	0	1	0	1	0	4	1	0	20	110	78	3.2
29	53137	31	F	4Y	Lc	9.09	18.18	0.51	177	124	44	108	120	12	2	2	1	0	0	0	1	1	0	21	120	86	3.3
30	53510	36	F	2Y	Lc	7.14	21.42	0.55	118	108	46	50	28	0	1	0	2	0	0	1	2	0	0	19	130	80	2.8
31	53762	42	M	1Y	Dc	5.4	13.51	0.73	146	127	41	80	71	12	1	0	2	1	0	0	0	0	1	19	100	70	3.7
32	53854	42	M	5Y	Dc	9.37	15.62	0.45	136	65	38	85	30	12	1	0	2	0	0	1	0	0	0	20	110	80	3.2