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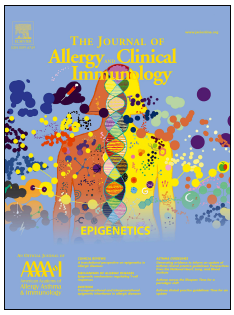

Flow mediated dilation shows impaired endothelial function in patients with mastocytosis

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Flow mediated dilation shows impaired endothelial function in patients with mastocytosis

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Abstract

Background: Mastocytosis is a rare disease characterized by clonal proliferation of mast cells in different organs. Clinical manifestations of mastocytosis are mostly due to the release of mediators from mast cells and, in many cases such as urticaria, flushing, angioedema and anaphylaxis, are expression of biological effects of mediators on endothelial cells. Chronic secretion of mediators in patients with mastocytosis may lead to alteration of endothelial function.

Objective: To investigate endothelial function in patients with mastocytosis by using a non-invasive technique of flow mediated dilation (FMD).

Methods: Twenty-five adult patients with indolent and advanced forms of mastocytosis and twenty healthy individuals used as control were enrolled in the study. Ultrasound assessment of FMD was performed by measuring changes in the diameter of brachial artery after 5 minutes of arterial occlusion. Changes in FMD were correlated with clinical parameters and serum tryptase level.

Results: Patients with mastocytosis had a lower FMD as compared to healthy controls ($p< 0.001$). Advanced and smoldering forms showed a lower FMD as compared to indolent forms ($p<0.001$). FMD inversely correlated with age and serum tryptase and directly with median arterial pressure and recurrent flushing episodes. No correlation was found between FMD and osteoporosis, recurrent anaphylaxis, presence of skin lesions and long term antihistamine treatment.

Conclusions: Endothelial dysfunction, demonstrated by FMD reduction, is detectable in patients with mastocytosis and is more severe in patients with high tryptase levels and advanced disease. Endothelial function appears to be negatively influenced by mast cells proliferation rather than by the severity of mediator-related symptoms.

Clinical implications: Endothelial function is impaired in patients with mastocytosis, particularly in those with high tryptase levels, and may contribute to an increased cardiovascular risk and to poor prognosis of advanced forms.

Capsule summary: Mastocytosis is associated with impaired flow-mediated dilation that is more evident in patients with advanced disease and elevated serum tryptase. Endothelial dysfunction seems to be related to mast cell burden rather than to mediator-related symptoms.

Key words: endothelial cells, endothelial function, flow mediated dilation, mast cells, mastocytosis, mediators, tryptase

List of abbreviations:

CysLTs: cysteinyl leukotrienes

EC: endothelial cells

ECG: electrocardiogram

FMD: flow-mediated dilation

ISM: indolent systemic mastocytosis

MAP: mean arterial pressure

MC: mast cells

NO: nitric oxide

PAF: platelet-activating factor

PGD_2: prostaglandin D_2

SM: systemic mastocytosis

SMC: vascular smooth muscle cells

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Introduction

Mastocytosis is a clinically heterogeneous disease characterized by clonal proliferation of mast cells (MC) in various tissues and organs including the skin, bone marrow, gastrointestinal tract, liver, spleen and lymphnodes[1]. Adult patients usually have the systemic form of the disease and show skin lesions and/or involvement of at least an extracutaneous organ, most frequently the bone marrow[2]. The current classification defines systemic mastocytosis as a complex disease with multiple variants ranging from indolent forms, with life expectancy similar to that of general population, to advanced forms associated with poor prognosis[3]. Clinical signs and symptoms of patients with mastocytosis are primarily due to the release of MC-derived pro-inflammatory and vasoactive mediators and to organ dysfunction due to progressive MC infiltration. While the latter are usually restricted to patients with the advanced forms of the disease, symptoms due to MC mediator release are seen in patients with all variants of mastocytosis and are independent from the rate of MC proliferation [4, 5]. Mediators released by MC include histamine, lipid mediators such as prostaglandin D_2 (PGD_2), cysteinyl leukotrienes (CysLTs) and platelet-activating factor (PAF), heparin, serotonin, tryptase and multiple cytokines and chemokines[6, 7]. Mediator-related symptoms are present in the majority of patients with mastocytosis and they are attributed to an uncontrolled, acute or chronic secretion of MC mediators. Symptoms due to MC mediators can be cutaneous (urticaria, pruritus, flushing and angioedema), gastrointestinal (abdominal pain, gastritis and diarrhea), musculoskeletal (bone pain and osteoporosis) and cardiovascular (hypotension, syncope or recurrent anaphylaxis)[8-10]. Many of these symptoms are due to the multiple effects of MC mediators on local (urticaria, angioedema, diarrhea) or systemic microcirculation (hypotension, anaphylaxis). It is well known that MC-derived mediators profoundly influence vascular tone and blood flow by inducing vasodilation and increasing vascular permeability. These effects are mainly due to the interaction of mediators with endothelial cells (EC) and vascular smooth muscle cells (SMC). The effects of mediators such as histamine, PGD_2, CysLTs and PAF on EC and SMC

functions have been fully elucidated over the last decades[11]. These mediators have pleotropic and redundant vasoactive proprieties including activation of EC and relaxation of SMC. Together these effects result in increased blood flow, reduced vascular resistance and increased endothelial leakage.

Despite plenty of evidence on the effects of MC mediators on vascular function, endothelial responses *in vivo* have not been investigated so far in patients with systemic mastocytosis. Flow-mediated dilation (FMD) is a widely used technique to study endothelial function in several cardiovascular diseases. It is a non-invasive method that measures flow-mediated changes in the brachial artery diameter induced by short-term ischemia[12]. Flow-mediated changes are caused by stress-induced generation of endothelial-derived vasoactive mediators. Since FMD is almost completely blocked by pre-treatment with nitric oxide synthase inhibitors, it has been proposed that this response is mainly due to endothelial release of nitric oxide (NO)[13]. MC secrete a variety of mediators that potentially interfere with generation of nitric oxide by EC and can, therefore, modulate endothelial response to ischemic stress. The aim of this study has been to investigate endothelial function in adult patients with SM by FMD.

Methods

Patient selection and data collection

In this cross-sectional study we included 25 patients with systemic mastocytosis (SM) referred to the Mastocytosis Reference Centre of the University of Salerno. Patients with SM were classified according to WHO criteria revised by Valent *et al.*[2]. Eighteen patients (72%) had indolent SM, two patients (8%) had smoldering SM, 4 patient (16%) had aggressive SM and one had SM associated with another hematological disorder (4%). KIT mutation analysis was performed in 8 (32%) patients and all of them carried the D816V mutation.

As control group, we enrolled 20 healthy subjects matched for age, sex, body mass index (BMI) and smoking status with the patient group. A previous history of cancer, myocardial infarction, venous thromboembolism, stroke, diabetes, arterial hypertension and heart failure were exclusion criteria for both patients and controls. Clinical history, anthropometric data and vital signs were collected immediately before FMD evaluation. Demographic and clinical characteristics of controls and patients are reported in **Table I**. Grading of mediator-related symptoms was determined according to Valent *et al.*[14].

A subset of patients analyzed in this study was on continuous antihistamine treatment defined as the daily intake of an oral antihistamine drug at recommended dosage for at least 3 months before FMD evaluation.

Besides antihistamines, additional anti-mediator drugs including H_2 blockers and antileukotrienes were given to 2 patients. At the time of FMD evaluation, no patient was on treatment with glucocorticoids, non steroidal antinflammatory drugs or supplements (vitamin C, vitamin E or N-acetylcysteine) that could influence FMD. Four patients with aggressive mastocytosis were treated with cytoreductive drugs (two with cladribine and two with midostaurin). However, FMD evaluation was done in all patients before the beginning of these treatments.

The study protocol was approved by the local Ethical Committee and was conducted in accordance with the principles embodied in the Declaration of Helsinki[15]. A written informed consent was obtained from all enrolled subjects.

Flow Mediated Dilation (FMD)

Ultrasound assessment of endothelial-dependent FMD was made according to the guidelines of the American College of Cardiology[16]. FMD was performed in a temperature-controlled room (22°C) with the subjects in a resting, supine state between 8 and 10 a.m. after at least an 8-hour fasting. Brachial artery diameter was imaged using a 7.5 MHz linear array transducer ultrasound system

(TOSHIBA, Minato, Tokyo) equipped with electronic calipers, vascular software for 2-dimensional imaging, color and spectral doppler, and internal ECG. The brachial artery was visualized 3 to 7 cm above the antecubital crease. To create a flow stimulus, a sphygmomanometer cuff was placed on the forearm and the cuff was inflated at least 50 mmHg above systolic pressure to occlude artery inflow for 5 minutes. At the end of the 5 minutes of ischemia, brachial artery diameter was measured above the cuff within 1 minute after releasing the occlusion. Measurement within 1 minute after ischemia allows an optimal assessment of flow-mediated dilation. All vasodilatation measurements were synchronized at beginning of R wave of ECG taken at the end of diastole. FMD was expressed as the change in post-stimulus diameter evaluated as the percent increase from the baseline diameter. To ensure consistency FMD was performed by the same operator in all patients.

Serum tryptase assay

Serum tryptase was measured from blood samples drawn by venipuncture after at least 8 h fasting using an immune enzymatic method (ImmunoCap, Thermofisher). In patients with recurrent anaphylaxis, serum tryptase level was measured at least 2 weeks after the acute episode.

Statistical analysis

Categorical variables were reported as counts (percentages) and continuous variables as means ± standard deviation or median and interquartile range, when appropriate. Categorical variables were compared using chi-square test. Normal distribution of parameters was assessed by Kolmogorove Smirnov test. The Student unpaired t test, Mann-Whitney U test and ANOVA test with Bonferroni post hoc analysis were used to compare continuous variables.

To explore correlations between FMD and clinical or laboratory parameters in patients with mastocytosis a univariate and multivariable linear regression analysis was performed. In all analyses, a p value <0.05 was considered statistically significant. Multivariable linear regression analysis was performed using variables with a $p<0.05$ at univariate analysis. Sample size calculation

was computed with respect to a two-tailed Student's t-test for independent groups, considering: 4% (δ) as difference for FMD between patients and controls, 3% as S.D., 0.05 (α) as type I error probability and 0.90 as power 1-β. The sample size was n = 26 patients, 13 patients for each group. All tests were two-tailed and analyses performed using computer software packages (IBM SPSS Statistics v22.0, Armonk, New York).

Results

The demographic and clinical characteristics of controls and patients are shown in **Table I**. There was no significant difference in age, sex, smoking status and clinical parameters relevant to FMD between the two groups. **Table II** shows the clinical characteristics of patients with mastocytosis. All patients underwent FMD evaluation as described in the Methods. Basal diameter of brachial artery at the beginning of the procedure was not significantly different between patients and controls (3.47 ± 0.67 mm *vs.* 3.43 ± 0.49 mm; p=0.854). Patients with mastocytosis had a significantly lower FMD as compared to controls (7.6 ± 3.9% *vs.* 13.0 ± 4.5%; p<0.001). FMD was significantly reduced in both patients with indolent and advanced form of the disease as compared to controls (**Figure 1**). Interestingly, however, the severity of the disease had a major impact on FMD since patients with advanced forms showed a significantly lower FMD as compared to patients with indolent forms (2.1 ± 1.1% *vs.* 8.4 ± 3.1 %; p<0.001). For this analysis patients with smoldering mastocytosis were included in advanced forms.

Serum tryptase is a surrogate marker of MC proliferation in mastocytosis and is a negative prognostic factor of the disease. We therefore investigated the relationship between FMD and tryptase levels in patients with mastocytosis. **Figure 2A** shows that there is a significant negative correlation between FMD and serum tryptase (p<0.001), indicating that severe endothelial dysfunction is detectable in patients with high tryptase levels. The inverse correlation between FMD

and tryptase remains highly significant in the subgroup of patients with indolent (**Figure 2B**) and advanced mastocytosis (**Figure 2C**).

Univariate analysis showed that, in addition to tryptase, FMD correlated negatively with age and recurrent flushing and positively with mean arterial pressure (MAP) (**Table III**). By contrast, sex, BMI and smoking status showed no correlation with FMD. Furthermore, no correlation was found between FMD and osteoporosis, recurrent anaphylaxis and presence of skin lesions. Multivariate regression analysis was further performed for variables that were significant at univariate analysis. This analysis confirmed that tryptase, age and flushing were strong independent variables associated with FMD reduction (**Table III**). Interestingly, **Figure 3A** shows that there is a highly significant negative correlation between FMD and age (p=0.002). The inverse correlation between FMD and age remains significant in the subgroup of patients with indolent (**Figure 3B**) but not in that of patients with advanced mastocytosis (**Figure 3C**).

Histamine is a powerful vasoactive mediator released from MC causing endothelium-dependent vasodilation through activation of H_1 receptors. To understand whether histamine was involved in causing FMD changes in patients with mastocytosis we compared FMD of patients on long term antihistamine treatment with that of untreated patients. FMD was not significantly different in patients with or without continuous antihistamine treatment, 8.0 ± 3.6 and 7.0 ± 4.5 % respectively; p = 0.565 (**Table III)**.

Finally, to explore whether there was any relationship between severity of mediator-related symptoms and FMD we compared endothelial function in patients with different symptom grading. Symptom grading was scored according to Valent *et al.*[14] with 0: no symptoms to 3: severe symptoms. There was no significant difference in FMD values between patients with grade 0 (FMD: 7.3 ± 3.3), grade 1 (FMD: 9.5 ± 4.2), grade 2 (FMD: 7.0 ± 4.8) and grade 3 (FMD: 7.4 ± 0.4); (p=0.717).

Discussion

Non-invasive assessment of endothelial function by FMD reveals reduced endothelial-mediated vasodilation in adult patients with mastocytosis. This study provides the first demonstration of the impairment of endothelial function in patients with SM in comparison to healthy controls. Impairment of FMD is greater in patients with advanced forms of mastocytosis as compared to those with indolent systemic mastocytosis (ISM).

FMD is a widely used technique to assess endothelial dysfunction defined as any form of functional and reversible alteration leading to an abnormal endothelial response to physiological stimuli. Endothelial function is then shifted toward a reduced vasodilation and the acquisition of a proinflammatory, proliferative and prothrombotic state[17]. It should be mentioned that the FMD technique does not fully discriminate between endothelium and SMC dysfunction. To analyze separately endothelial and SMC dysfunction, FMD evaluation should have been done after administration of nitroglycerin which causes an endothelium-independent relaxation. However, we decided to avoid this test since it can cause severe hypotension[16]. FMD is altered in atherosclerosis, diabetes, hypertension and dyslipidemia[18]. Several studies indicate that FMD provides independent prognostic information that exceeds the predictive value of traditional risk factors. This parameter appears to predict cardiovascular death and cardiovascular events such as myocardial infarction, stroke, heart failure and peripheral artery disease[19, 20].

Several mechanisms can be hypothesized for the reduced FMD in patients with mastocytosis. First, the reduced response may be related to persistent vasodilation induced by chronic release of MC-derived vasoactive mediators. It is tempting to speculate that continuous release of mediators from MC maintains a stable peripheral vasodilation that precludes the increase in blood flow during FMD. Mediators inducing vasodilation that are continuously secreted in patients with mastocytosis include histamine, PGD_2, CysLTs and PAF. Histamine induces vasodilation and increases vascular permeability through H_1 receptors that, in turn, activate Akt1 pathway leading to NO production[11].

In addition, it has been demonstrated that histamine may cause H_1 receptor desensitization[21] and, therefore, it can be hypothesized that, at the time of FMD, peripheral vessels may be unresponsive to the vasodilatory action of histamine. Urinary levels of histamine metabolites are consistently elevated in patients with mastocytosis and its levels are correlated with serum tryptase[22]. The latter observation is in line with our finding that FMD reduction correlates with serum tryptase. Our data show that a continuous antihistamine treatment does not influence FMD, thereby arguing against a role of histamine, by causing chronic H_1 receptor activation and/or desensitization, as the main cause of impaired FMD in patients with mastocytosis. It should be mentioned that the subgroup analysis in patients with or without continuous antihistamine treatment was performed with a limited number of cases and therefore further studies on a larger population sample are required to confirm our conclusions. Other vasoactive mediators secreted by MC, such as CysLTs, PGD_2 and PAF, may induce persistent vasodilation independently from histamine[23]. In addition, tryptase may be involved in endothelial dysfunction by inducing direct activation of protease-activated receptors 2 (PAR2)[24]. Activation of PAR2 on ECs induces leukocyte rolling and adhesion, NO impairment, increase in vascular permeability and over-expression of cyclooxygenase-2[25-27]. Together, these alterations may induce a procoagulant and proinflammatory state that could further contribute to generate endothelial dysfunction in mastocytosis.

A second explanation for reduced FMD in mastocytosis is an increased arterial stiffness related to MC infiltration of the arteriolar wall. Several studies have shown that in both healthy individuals and in many pathological conditions MC are located around blood vessels and that they can infiltrate all layers of the arterial wall[28]. Studies in animal models demonstrate that MC are abundant in close proximity of arterioles[29]. MC infiltration of precapillary arterioles might increase vessel stiffness and contribute to the reduced FMD detected in patients with mastocytosis. The observation that FMD is inversely related to serum tryptase, a reliable indicator of MC proliferation, supports this hypothesis.

Finally, a third mechanism by which FMD can be altered in mastocytosis is enhanced oxidative stress associated with MC proliferation causing an impairment in NO production by EC[30]. Recent evidence demonstrates high serum levels of two markers of oxidative stress (advanced glycation end products and advanced oxidation protein products) in patients with SM[31]. MC activation and proliferation are associated with enhanced ROS production mainly by NADPH-oxidase 2 (NOX2)[32, 33]. Furthermore, ROS play an important role in SM progression since they induce the expression of the anti-apoptotic protein DJ-1 in clonal MC[34]. ROS generation associated with MC proliferation could impair endothelial mediated dilation trough several mechanisms including i) inhibition of vasodilator prostacyclin production, ii) uncoupling endothelial nitric oxide synthase and iii) binding of NO with subsequent reduction of its bioavailability[35].

In patients with SM, univariate analysis shows that age, high serum tryptase and flushing are associated with a worse FMD whereas MAP is directly related to FMD. The correlation between FMD and age was previously demonstrated by several studies showing that aging is associated with a progressive reduction of vascular compliance[36]. Interestingly, the negative correlation between age and FMD is significant in the subgroup of patients with indolent mastocytosis, but not in patients with advanced disease. These data indicate that, in patients with severe forms of mastocytosis, the impact of the disease on endothelial function overcomes that of physiological aging of blood vessels and strongly point to factors associated with the disease as the primary cause of FMD alterations in mastocytosis. The presence of flushing is associated with a reduced FMD probably as an expression of altered vasomotor tone induced by PGD_2. It is interesting to note that a reduced FMD has been reported in patients with postmenopausal flushing[37].

An intriguing finding of our study is that FMD is reduced in patients with the lowest MAP. These data may apparently be in contrast with the well-known observation that reduced FMD is associated with hypertension. However, since hypertensive patients were excluded from our study, our finding

rather suggests that in patients with mastocytosis a low blood pressure, often detected in advanced forms, is associated with impaired vasodilation.

It is known that FMD impairment is associated with a high risk of major cardiovascular events[38]. The cardiovascular risk has not been fully evaluated in patients with mastocytosis. Two studies suggest that these patients may be at higher risk for certain cardiovascular events[39, 40]. More conclusive evidences of the cardiovascular risk in patients with mastocytosis are highly desirable and whether the reduction in FMD could be a predictive factor will be investigated in further long term studies.

In conclusion, our data show that patients with mastocytosis have endothelial dysfunction as indicated by a lower FMD as compared to healthy controls. FMD impairment in more evident in advanced forms of the disease and appears to be correlated with MC burden. Further studies are needed to understand the mechanisms underlying endothelial dysfunction in mastocytosis and to define its clinical and prognostic impact on the disease.

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Figure legends

Figure 1: FMD values in controls and patients with mastocytosis. FMD was measured as described in the methods in twenty healthy individuals (*controls*), 18 patients with indolent and 7 with advanced systemic mastocytosis. FMD is expressed as percent variation in the brachial artery diameter induced by 5-min ischemia. Lines indicate the mean value in each group.

Figure 2: Correlation between FMD and serum tryptase level in the whole population of patients with mastocytosis (A) and in patients with indolent (B) and advanced forms of the disease (C). FMD was measured as described in the methods and expressed as percent variation in the brachial artery diameter induced by 5-min ischemia. Serum tryptase was measured by immunoenzymatic method.

Figure 3: Correlation between FMD and age in the whole population of patients with mastocytosis (A) and in patients with indolent (B) and advanced forms of the disease (C).

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422 FMD was measured and expressed as percent variation in the brachial artery diameter induced by 5-

423 min ischemia.

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Table I. Demographic and clinical characteristics of controls and patients

	Controls (n = 20)	Mastocytosis (n = 25)	*p*
Age (years) range	43.8±14 24-67	45.3±12 24-65	0.213
Female sex, n (%)	11 (61)	16 (64)	0.578
Smokers, n (%)	4 (20)	5 (20)	0.712
Body Mass Index (kg/m^2)	24.4±3.7	24.8±3.1	0.581
Systolic Blood Pressure (mmHg)	122.7±10.6	118.4±15.7	0.466
Diastolic Blood Pressure (mmHg)	76.3±7.4	71.6±8.9	0.183
Mean Arterial Pressure (mmHg)	91.8±6.8	87.2±9.0	0.146
Heart Rate (bpm)	70.3±5.2	73±8.2	0.547
Hemoglobin (gr/dl)	13.1±1.7	12.9±1.6	0.689

Data are expressed as mean ± standard deviation. Values in brackets indicate percentage of patients

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Table II. Clinical characteristics of patients with mastocytosis.

All patients, n (%)	**25 (100)**
Patients with skin lesions (Urticaria pigmentosa), n (%)	17 (68)
<u>Mediator related symptoms</u>	
Osteoporosis, n (%)	16 (64)
Pruritus, n (%)	12 (48)
Flushing, n (%)	9 (36)
Diarrhea, n (%)	8 (32)
Urticaria, n (%)	7 (28)
Anaphylaxis, n (%)	6 (24)
Continuous antihistamine treatment, n (%)	10 (40)
Tryptase Median (µg/L) Interquartile range (µg/L)	 47.4 27.1-135.5

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Table III. Univariate and multivariate regression analysis of factors correlated with FMD in mastocytosis.

	Univariate analysis		Multivariate analysis	
	Beta	p	Beta	p
Age	-0.590	0.002	-0.419	0.004
Female sex	0.372	0.067	ns	ns
Body Mass Index	-0.260	0.210		
Mean Arterial Pressure	0.533	0.006	ns	ns
Smoking	-0.279	0.177		
Patients with skin lesions (Urticaria pigmentosa)	-0.216	0.322		
Osteoporosis	-0.058	0.781		
Pruritus	-0.069	0.744		
Flushing	-0.428	0.033	-0.329	0.013
Diarrhea	-0.111	0.597		
Urticaria	-0.045	0.830		
Anaphylaxis	-0.152	0.880		
Continuous antihistamine treatment	-0.121	0.565		
Tryptase	-0.738	< 0.001	-0.598	0.002

Multivariable linear regression analysis was performed using variables with a $p<0.05$ at univariate analysis. Beta is defined as correlation coefficient.

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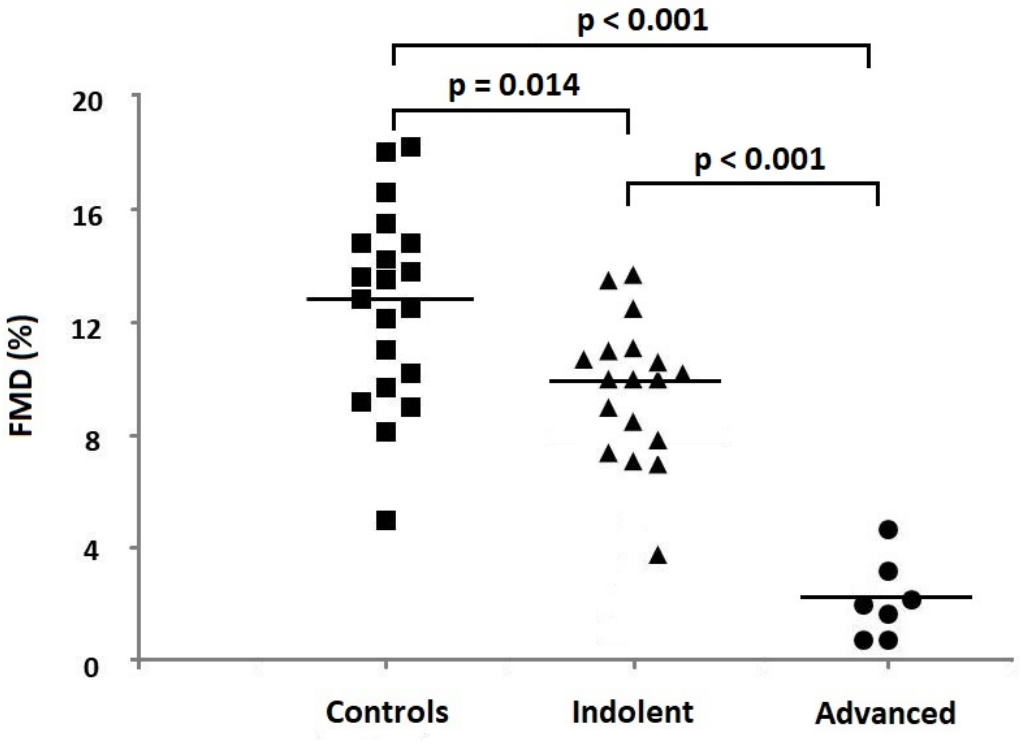
p < 0.001
p = 0.014
p < 0.001
20
16
12
8
4
0
FMD (%)
Controls
Indolent
Advanced

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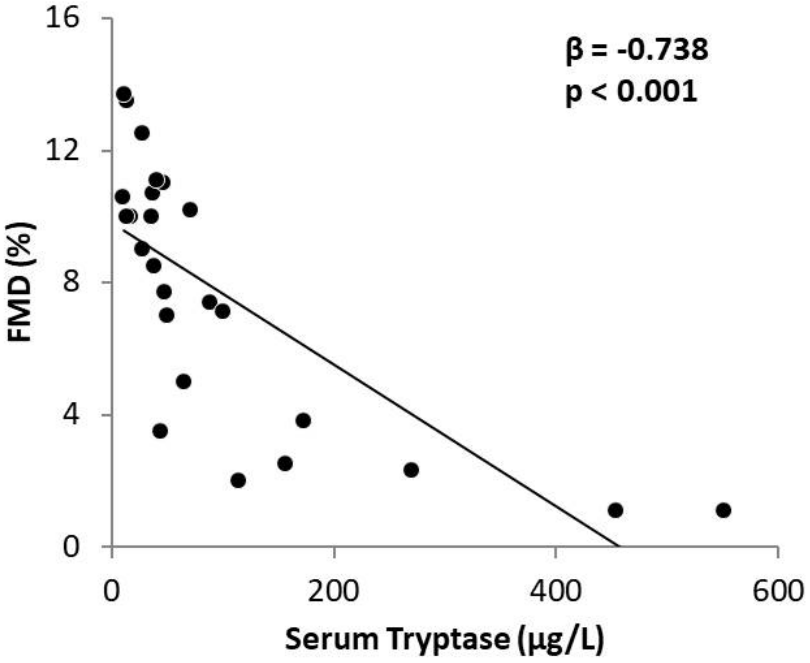

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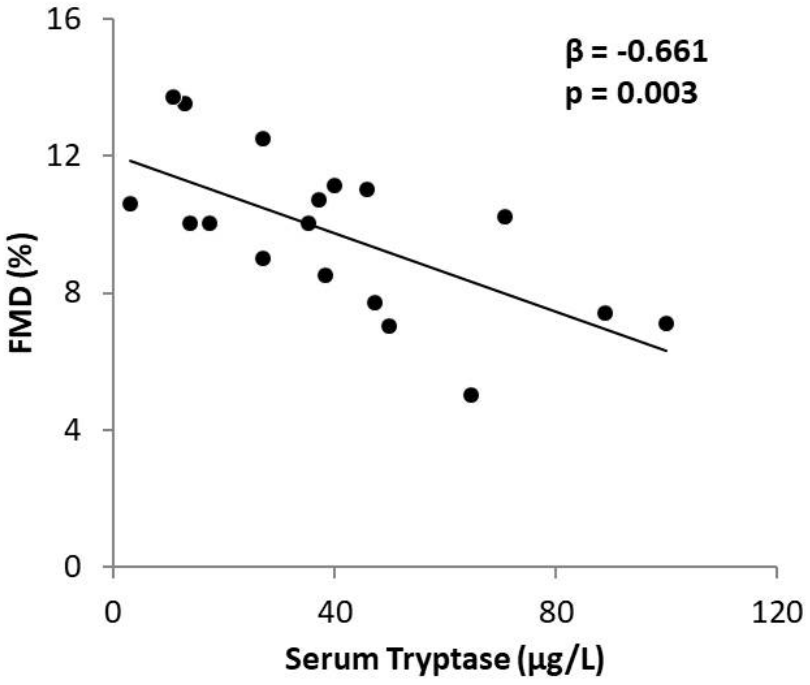

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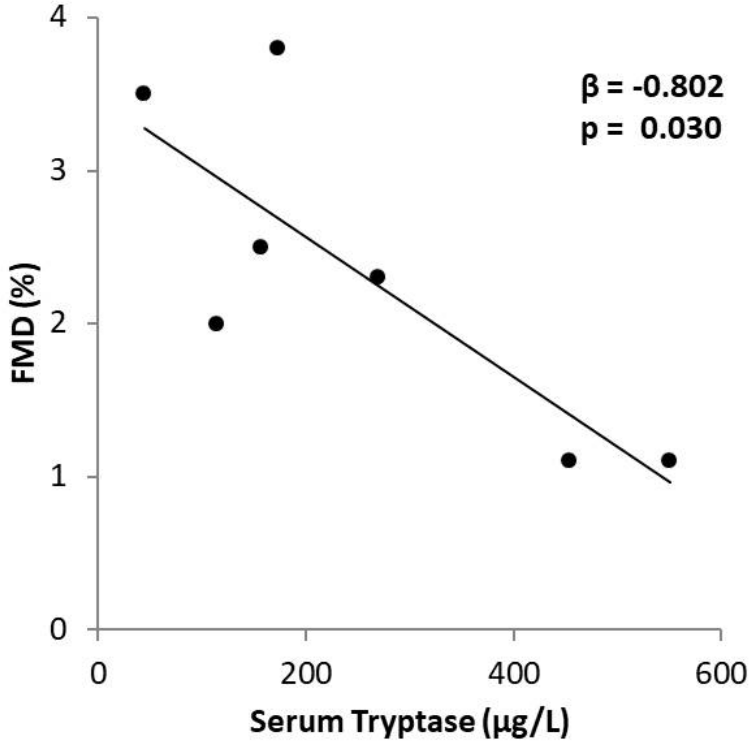

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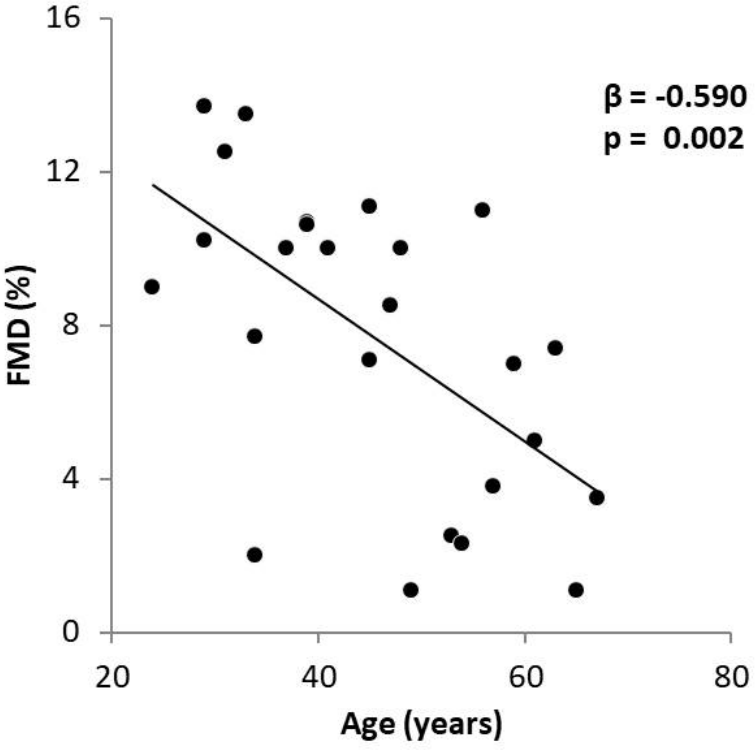

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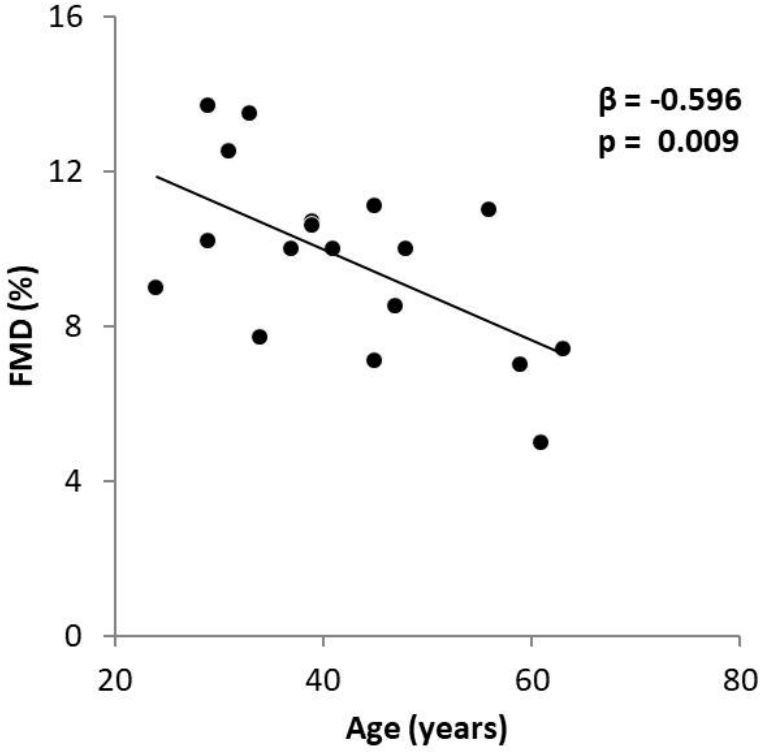

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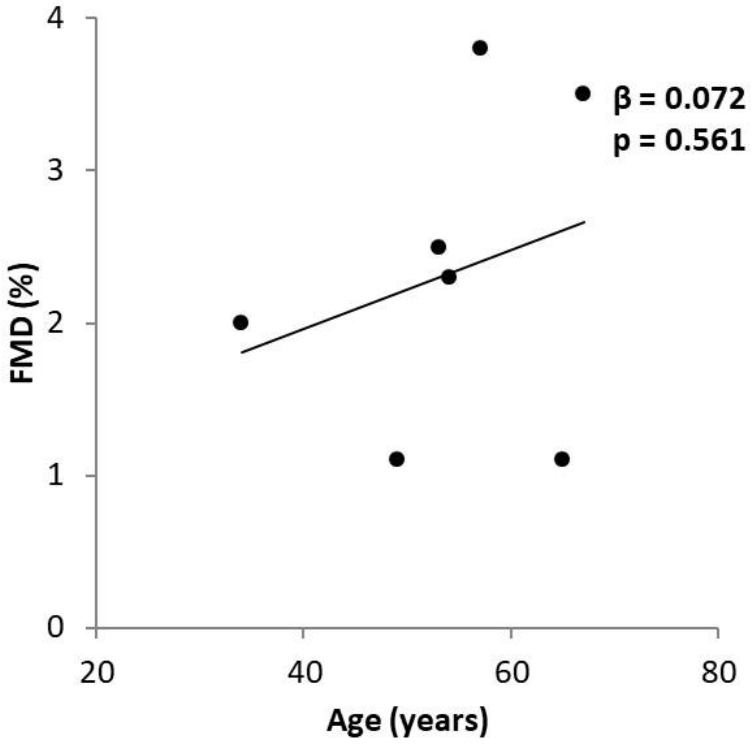

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