Impaired endothelial function irrespective of systemic inflammation or atherosclerosis in mastoscytosis

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Abstract

Background: Knowledge on endothelial dysfunction and its relation to atherosclerosis in mastocytosis is limited. Aim: To investigate the endothelial function in mastocytosis by flow mediated dilatation (FMD) and biomarkers related to vascular endothelia, the presence of subclinical atherosclerosis by carotid intima media thickness (CIMT). Method: Forty-nine patients with mastocytosis and 25 healthy controls (HCs) were included. FMD and CIMT during transthoracic echocardiography, biomarkers including endocan, endothelin-1 (ET-1), vascular endothelial growth factor (VEGF) were measured in sera of participants. Tumor necrosis factor-alpha (TNF-α), interleukine-6 (IL-6) and high sensitive c-reactive protein (hsCRP) were determined as inflammatory biomarkers. Result: The mean FMD% was lower in the patients than HCs (11.26±5.85% vs 17.84±5.27% p<0.001) and was the lowest in the advSM and SSM group among the patients (p=0.03). The median value of VEGF was significantly higher in patients than HCs. [73.30 pg/mL; min-max (32.46-295.29) pg/mL vs (46.64 pg/mL; min-max 11.09-99.86 pg/mL; p:0.001] and it was the highest in the advSM and SSM group (p:0.01). FMD was inversely correlated with endocan (r:-.390, p:0.006), ET-1 (r:-.363, p:0.01) and VEGF (r:-.402, p:0.004) but there were no correlations between FMD and TNF-α, IL-6, and hsCRP. No differences in CIMT values between patients and HCs and no correlation between CIMT and the biomarkers were observed. Conclusion: Endothelial dysfunction in mastocytosis becomes evident with decreased FMD and elevated serum VEGF, in the absence of atherosclerosis or systemic inflammation and is related to disease severity. Keywords: CIMT, Endocan, Endothelial function, Endothelin-1, FMD, VEGF

Introduction

Mastocytosis is a rare heterogeneous disease characterized by abnormal proliferation and accumulation of mast cells (MCs) in different organs. According to the World Health Organization (WHO) 2016 classification, mastocytosis is classified as cutaneous mastocytosis, systemic mastocytosis and mast cell sarcoma [(1-3)].

MCs are frequently found in perivascular area and lymphatic tissue, release various mediators [(4)] during inflammation which can cause vasodilatation by relaxing smooth muscle cells in the vessels and activate the endothelial cells (ECs) leading to a breakdown in tight junctions[(4)]. Sustained activation of ECs can cause endothelial dysfunction by increasing the vasoconstrictor endothelial derived factors and reducing the vasodilator ones[(5, 6)] which have been reported in various systemic diseases [(7, 8)].

Flow mediated dilation (FMD) as a gold standard noninvasive technique measuring endothelial function by quantifying NO-dependent arterial vasodilatation [(9)] is mostly used for clinical research in cardiovascular

diseases. It measures arterial flow mediated changes as percentage dilatation of the brachial artery after short-term occlusion [9, 10]. Furthermore, endocan, Vascular Endothelial Growth Factor (VEGF), endothelins (ETs) are some circulating biomarkers used to evaluate endothelial function [8]. Endocan (also known as endothelial cell specific molecule-1) plays a role in endothelial cytoskeleton rearrangement and inflammation and is secreted from ECs [11]. It is a modulator of VEGF signaling whereas its expression is further induced by VEGF, revealing a bidirectional interaction in between which plays a critical role in inflammation [7]. VEGF , an important growth factor in promoting angiogenesis during embryogenesis [12] , can increase during inflammation due to uncontrolled endothelial activation[13]. ET-1, as a circulating aminopeptide in human mainly produced by ECs, plays an important role in atherosclerosis which can further cause vascular dysfunction [14-16]. Tumor necrosis factor alpha (TNF-α) and Interleukin-6 (IL-6) are inflammatory biomarkers that can stimulate the release of ET-1. Furthermore, TNF-α plays a role in atherosclerosis development [15] and IL-6 can be a potential mediator of MCs activation and accumulation[17]. High sensitive C-reactive protein (hsCRP) as a marker of systemic inflammation has been shown to be associated with increased risk of vascular pathologies [18].

Beside allergic inflammation, MCs can play an important role in atherosclerotic lesions in human [19]. Degranulation of proinflammatory cytokines, histamine and neutral proteases, such as tryptase from mast cells upon activation can lead to atherosclerotic plaque progression, increasing plaque destabilization and risk of intraplaque hemorrhage [20]. The carotid intima media thickness (CIMT) is measured between intraluminal and the medial-adventitial interfaces of the carotid artery by B-mode carotid ultrasound to detect the presence and progression of atherosclerosis. Increased CIMT is a predictor of future vascular atherosclerotic events[21, 22].

Although the effects of MCs on vascular structure and progression of atherosclerosis are known, there are limited knowledge about endothelial function in mastocytosis [5]. Endothelial impairment in mastocytosis has not been evaluated with biomarkers and its possible relation with subclinical atherosclerosis has not been studied before. In this study our aim was to investigate the endothelial function in mastocytosis patients by FMD and related biomarkers and to assess the presence of subclinical atherosclerosis by CIMT.

Material and Method

Inclusion of study participants

Forty-nine adult mastocytosis patients who were diagnosed according to recent diagnosis and classification criteria[1] and were followed at outpatient clinics of adult immunology and allergic diseases and adult hematology divisions of our faculty were included in the study . Accordingly, patients were grouped as follows; Group-1: "Cutaneous Mastocytosis (CM)", Group-2: "Indolent Systemic Mastocytosis (ISM)" and Group-3: "Advanced Systemic Mastocytosis (AdvSM) and Smoldering Systemic Mastocytosis (SSM)". Aggressive SM (ASM) and systemic mastocytosis (SM) with an associated hematologic neoplasm (SM-AHN) were considered within the advSM group[1]. Age, sex, body mass index and smoking habits matched 25 healthy control (HCs) were also enrolled. Individuals with a history of diabetes, hypertension, cancer, hyperlipidemia, auto-inflammatory diseases, venous thromboembolism, atherosclerotic diseases that can contribute to the development of endothelial dysfunction and/or atherosclerosis were excluded. None of the study participants were under ascorbic acid, corticosteroids, non-steroidal anti-inflammatory drugs or estrogen therapy which can affect FMD results.

This study was approved by the local institution's ethics committee (Approval Number:1293) in accordance with the Declaration of Helsinki and written approval was received from all study participants .

Measurement of FMD and CIMT

FMD and CIMT were performed by a single assessor with transthoracic echocardiography iE33xMATRIX ultrasound system (Philips Medical Systems) according to the recommendations in the guidelines[23] . FMD was evaluated using the method described in the previous reports [24, 25]. FMD % was calculated with the formula below.

FMD (%) = (brachial artery maximum diameter - brachial artery diameter at rest) × 100 / brachial artery diameter at rest [24]

CIMT was defined as the distance between lumen-intima and the media-adventitia of the carotid arterial wall on the ultrasound. Carotid artery measurements were applied in supine position according to the recommendations. The mean CIMT was calculated from six measurements obtained in two scans [26].

Clinical data collection and measurement of serum biomarkers

Demographic and all clinical features including medications were collected from medical records. Measurements of heart rate, systolic and diastolic blood pressures at least 20 minutes of relaxing and body mass index were initially performed in all study participants. Serum baseline tryptase levels, full blood count, serum glucose level, triglyceride, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, erythrocyte sedimentation rate (ESR) and high sensitive c-reactive protein (hsCRP) were measured in all study participants after at least 8 hours of fasting. In patients with recurrent anaphylaxis episodes, serum tryptase levels were measured at least 2 weeks after the last attack. Ten ml peripheral venous blood samples were collected for the measurement of endocan, ET-1, IL-6, VEGF, TNF-α levels in both groups and centrifuged at 4000 rpm for 10 minutes and then stored at-80C^0 until the day of measurement. Serum endocan, ET-1, VEGF, TNF-α and IL-6 levels were measured by the enzyme-linked immunosorbent assay (ELISA) (IL-6 ELISA Kit and TNF- α ELISA Kit: Diaclone SAS, Besancon, France; Endocan ELISA Kit, ET-1 ELISA Kit and VEGF ELISA Kit: Abbkine Scientific, Wuhan, China) and serum tryptase by ELISA Immunoassay (EIA) (fluoroenzyme immunoassay, Phadia, Uppsala, Sweden). Serum biomarkers, FMD and CIMT measurements in the patient group were evaluated before the start of management in order not to cause any interference of certain drugs on study outcome.

Statistical Analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS Inc.) v22.0 and GraphPad Prism Software 8 was used for graphics. Demographic and clinical features were assessed by descriptive analysis and shown as percentages and mean ± standard deviation or median according to distribution of the data. Continuous variables were compared by independent t test or Mann Whitney-U test between two groups and Kruskal Wallis or One Way Anova test with Bonferroni correction between multiple groups depending on the distribution of the data. The categorical variables were compared with X^2 test. Correlation analysis were performed by Pearson' or Spearman's correlation tests according to the distribution of the data. Cut off values of FMD and biological markers were determined by receiver operator characteristic curve (ROC) analysis. Binominal Exact Test was used to compare Area Under Curve (AUC) of biological markers and FMD. In all analysis, p value less then <0.05 was considered statistically significant.

Results

Clinical and demographic features of the study participants

The mean age of patients with mastocytosis and HCs were 42.80±10.47 and 41.03±9.03 years, respectively ($p>0.05$). There was no significant difference between the patients and the HCs regarding age, sex and clinical features which can possibly affect the presence of vascular diseases (Table 1).

According to WHO mastocytosis classification criteria, 8 (16.3%), 34 (69.4%), 6 (12.2%) and 1 (2.1%) patients had CM, ISM, advSM and SSM, respectively. Aggressive SM (ASM) (n:4, 8.2%) and systemic mastocytosis (SM) with an associated hematologic neoplasm (SM-AHN) (n:2, 4.1%) were considered within the advSM group. Polycythemia vera (n:1) and essential thrombocytosis (n:1) were accompanying SM-AHN. The median duration time of mastocytosis was 52 (min-max:11-264) months. Twenty-two (44.9 %) patients had history of anaphylaxis and the most common trigger of anaphylaxis was venom allergy (n=9, 18.4%). Idiopathic anaphylaxis, drug induced anaphylaxis and food induced anaphylaxis were seen in 6 (12.2%), 4 (8.2%) and 2 (4.1%) patients, respectively. Forty-two patients (85.7%) had c-Kit D816V mutation. In 37 (75.5%) patients (8 CM, 29 ISM patients), skin involvement was observed and the most common skin manifestation was urticaria pigmentosa (n=36, %73.5). Osteoporosis and osteosclerosis were observed in

11 (22.4%) and 4 (8.2%) patients, respectively. Median serum baseline tryptase level 34.36 (min-max:3.5-1648) kU/L and 3.00 (min-max:1.0-6.0) kU/L in patients with mastocytosis and HCs, respectively ($p<0.001$) However, median total IgE level were 26 (min-max: 2.3-480) μg/L and 17.60 (min-max:5-169) μg/L in patients with mastocytosis and HCs, respectively ($p>0.05$).

FMD values in study participants

The baseline brachial artery diameters were 4.02±0.66 and 4.07±0.68 mm in patients and HCs, respectively ($p>0.005$). The mean FMD % value was significantly lower in the patients than HCs (11.26±5.85% vs 17.84±5.27% $p<0.001$). The Anova analysis showed that the mean FMD% values were significantly different ($p<0.001$) among four groups as (17.84±5.27%) in HCs, (12.25±5.17%) in group-1, (11.94±6.19%) in group-2 and (6.85±2.26%) in group-3 patients. According to post-hoc analysis, the differences between HCs and group-2, and HCs and group-3 were significant ($p=0.001$, $p<0.001$, respectively) whereas FMD% values were not statistically different between group-1 and HCs. Patients in the group-3 revealed lower FMD values than group-1 and group-2 ($p=0.03$) (Figure-1).

Results of biomarkers

Among endocan, VEGF, ET-1, TNF-α, IL-6, hsCRP and ESR, the median value of VEGF was significantly higher in the patients than HCs ($p=0.001$) while there were no significant differences between groups regarding other biomarkers (Table 2).

The Kruskal Wallis test revealed that the median (min-max) VEGF levels were different between four groups including HCs [46.64 (11.09-99.86) pg/mL], group-1 [47.56 (40.47-225.53) pg/mL], group-2 [74.01 (32.46-295.29) pg/mL] and group-3 [104.17 (49.25-248.46) pg/mL] (p:0.002) (Table:3). Post-hoc analysis showed that both group-2 and group-3 revealed higher VEGF levels than HCs ($p=0.01$, for each) and group-3 had the highest values.

Correlation analysis of FMD and other parameters

In the correlation analysis of FMD with biomarkers, there was a significant negative correlation between FMD and VEGF ($p=0.004$, r=-0,402), ET-1 ($p=0.01$, r=-0,363), endocan ($p=0.006$, r=-0,390), systolic ($p=0.009$, r=-0.368) and diastolic ($p=0.003$, r=-0.419) blood pressure in the patients (Supplement Figure-1, Table-4). FMD values did not differ according to gender, smoking habits, family history of coronary artery diseases, history of anaphylaxis and other clinical features including c-kit positivity, serum baseline tryptase levels and the presence of hepatosplenomegaly, skin or bone lesions ($p>0.05$).

CIMT results and correlation analysis with other parameters

The mean CIMT values were 0.56±0.14mm and 0.56±0.15 mm in patients and HCs, respectively ($p>0.05$). The mean CIMT was not different among mastocytosis subgroups ($p>0.05$). There was no correlation between CIMT with endocan, ET-1, VEGF, TNF-α, IL-6,hsCRP, tryptase levels and FMD. As expected, a significant positive correlation was observed between CIMT values and age in the patients ($p=0.03$, r=0.306) (Table-4).

Predictive values of FMD and VEGF for endothelial dysfunction in mastocytosis

In the ROC curve analysis, less than 14.5% was determined as the optimal cut-off value for FMD in mastocytosis patients with the sensitivity, specificity, positive and negative predictive values of 75.5%, 76%, 84.4% and 62%, respectively (AUC:0.796, 95% CI:0.696 to 0.897, $p<0.001$). In 37 (75.5%) patients, FMD was less than 14.5%. Also, odds ratio (OR) was detected as 8.883 (%95 CI: 2.954-26.717). Higher than 62.7 pg/mL was selected as the optimal cut-off value for VEGF in mastocytosis patients with 65.5% and 64% sensitivity and specificity rates, respectively. Positive and negative predictive values were 78.05% and 48.48%, respectively and OR was 3.346 (%95 CI:1.223-9.155).

The area under curve (AUC) in ROC analysis for FMD% was determined as 79.7% and standard error was determined 5.13%. For VEGF, AUC was determined 72.9% and with 6.16 % standard error. Comparing these two AUC values, there was no statistically significant difference in the level of predicting the patients

with mastocytosis who had endothelial dysfunction. The cut-off values of FMD and VEGF, AUC, specificity and sensitivity values were shown in Figure-2.

Discussion

This novel study demonstrates that mastoscytosis patients possess endothelial impairment in the absence of systemic inflammation and subclinical atherosclerosis which is excessive in advanced forms and is related to serum VEGF biomarker. It also determines predictive values of FMD and VEGF for endothelial dysfunction in mastocytosis patients for the first time.

It is well known that MCs are found in perivascular area and lymphatic endothelium [(4)] and increased MC count and its mediators can increase oxidative stress by effecting the vessel endothelium [(27)]. In accordance with this knowledge, the reason of impaired FMD in patients with mastocytosis in our study can be related to increased MC accumulation in the vessel walls and decreased stress response of the brachial artery is due to this accumulation. In addition, when patients were allocated into mastocytosis subgroups, FMD was low in all subgroups while the lowest value was detected in advSM and SSM group. Since MC accumulation in advSM and SSM is expected to be more than other subgroups[(1, 2, 28)], our this finding supports the hypothesis that increased MCs and related mediators may cause impaired FMD in patients with mastocytosis. Interestingly, FMD was found lower in CM patients than HCs although it was not statistically significant. Since CM is limited to the skin, the largest organ of our body, and it probably hosts increased number of MCs in skin vessels, lower FMD in CM patients can be expected than HCs [(29)]. Similar to our study, endothelial dysfunction in mastocytosis was reported in a recent study of limited number of systemic mastocytosis patients. This study showed a negative correlation between serum baseline tryptase levels and FMD [(5)] which is not found in our study probably due to the heterogeneity and the large number of the patients involved. Similarly, in our study FMD inversely correlated with systolic and diastolic blood pressure in accordance with this knowledge[(24, 25)].

It has been shown that MCs may affect the improvement and rupture of an atheroma plaque [(30)] However, in our study endothelial dysfunction observed in the patient group was not related to subclinical atherosclerosis determined with CIMT measurement [(21)]. Since age, smoking habits, fasting glucose level and lipid profile were similar in patients with mastocytosis and HCs in our study, the risk factors for atherosclerosis were considered as similar in both groups. In a previous study, Unal et al indicated that higher IgE levels in various allergic diseases can be related to the development of atherosclerosis but failed to show a correlation between total IgE and CIMT levels [(31)]. Similarly, in our study we did not observe such a correlation. Consequently, our study revealed that impairment of FMD in mastocytosis can occur in the absence of atherosclerosis and follow-up of these patients may be important considering the increased risk of developing cardiovascular diseases.

VEGF is mainly secreted by ECs but also secreted by macrophages, platelets, activated T-cells, leukocytes and tumor cells[(32)]. It leads to decreased vessel tonicity by increasing calcium/calmodulin, endothelial nitric oxide synthase (eNOS) activity and prostacyclin in ECs[(33)] and its serum level can increase in inflammation due to uncontrolled endothelial activation [(13)]. Two studies showed that VEGF can be secreted by atypical MCs seen in mastocytosis when compared to non-neoplastic MCs [(34, 35)]. In our study the median VEGF level was significantly higher in patients than HCs , most prominently in advSM patients. Since MC accumulation in advSM and SSM is expected to be more than ISM and CM [(1, 2, 28)], we may speculate that the increase in VEGF observed in the patients can be directly related to the atypical MC counts in the patients. Moreover, the inverse correlation between the two parameters which are probably related to MC counts, FMD and VEGF, found in our study strengthens our hypothesis that increased accumulation of MCs can cause endothelial dysfunction in mastocytosis.

Studies showed that endocan can be used as a marker for endothelial dysfunction in diabetes, hereditary angioedema (HAE), sleep apnea syndrome and cardiovascular diseases[(11, 36-39)]Demirtürk et al. found that endocan can be a marker for endothelial dysfunction in attack free periods in C1 inhibitor deficient HAE (C1 INH HAE) patients [(11)]. Others reported that higher serum endocan levels might reflect endothelial dysfunc-

tion in primary hypertension irrespective of blood pressure results[38]. In our study, endocan was evaluated for the first time in mastocytosis. Although there was no significant difference in endocan levels between the patients and HCs, there was a negative correlation between FMD and endocan levels. Since endocan induces VEGF to bind its receptor and also a prominent decrease in the expressions of the cytoskeleton-associated proteins occludin and ZO-1, which are important elements of tight junctions in vascular endothelium[40], we believe that further studies are needed to investigate endocan in relation to VEGF in mastocytosis. Similar to endocan we could not find any significant relation with ET-1 and mastocytosis although it has been shown in endothelial dysfunction in other diaseases [41]. Since there was a negative correlation with FMD and ET-1 and a positive correlation between ET-1 and endocan levels in our study, larger studies can further elucidate possible relations in between.

Although previous data showed that inflammatory biomarkers TNF-α and IL-6 can stimulate the release of ET-1, however TNF-α plays a role in the development of atherosclerosis [15], IL-6 can increase in severe mastocytosis [42], and hsCRP as an independent risk factor of cardiovascular disease can increase in inflammation and atherosclerosis [39], there were no differences in any of these inflammatory biomarkers between the patients and the HCs in our study. Although ESR was not different between the patients and HCs, it was significantly increased in ISM, advSM and SSM patients when compared to CM patients, probably due the deepened anemia observed in these patients. All these findings remind us that endothelial dysfunction occurs in the absence of systemic inflammation in mastocytosis.

According to our ROC analysis, we propose that when FMD % level is $< 14.5\%$ and VEGF is >62 pg/mL, endothelial dysfunction may be evident in mastocytosis patients and further cardiovascular monitoring may be necessary. Since the AUC values of VEGF and FMD are not statistically different, either of the test can be performed to predict according to its availability.

Although our study is a comprehensive study evaluating endothelial dysfunction in mastocytosis, the heterogenous distribution of patients in mastocytosis subgroups may negatively affect statistical significance. As a limitation, patients in the advSM and SSM group (group-3) were relatively low when compared to other groups however we believe that this distribution reflects the presentation of the disease in real life.

In conclusion, our study shows that endothelial function is impaired in mastocytosis patients which can be easily recognized by FMD and VEGF analysis early before cardiovascular events are evident in the course of the disease. Further multicenter studies in different populations with a high number of patients including molecular analysis are needed to confirm our findings.

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Table 1: Clinical and demographic features of patients with mastocytosis and HCs

Features	Patients with mastocytosis (n=49)	Healthy Controls (n=25)	p
Age (years, mean±SD)	42.80±10.47	41.52±9.51	NS
Gender Female (n,%) Male (n,%)	25(62.5%) 24(70.6%)	15(37.5%) 10(29.4%)	NS
Body mass index (kg/m^2, mean±SD)	25.75±3.93	25.29±4.50	NS
Smoking habits (n,%)	32(69.6%)	14(30.4%)	NS
Alcohol consumption (n,%)	24 (70.5%)	10(29.5%)	NS
Family history for cardiovascular diseases (n,%)	17(58.6%)	12 (41.4%)	NS
Systolic blood pressure (mm/Hg, mean±SD)	118.08±11.85	118.00±8.29	NS
Diastolic blood pressure (mm/Hg, mean±SD)	72.08±9.34	72.08±5.46	NS
Heart rate (/minutes, mean±SD)	80.63±9.03	82.04±6.27	NS
Hemoglobin (gr/dL, mean±SD)	13.69±1.75	14.06±1.47	NS
Fasting glucose level (mg/dL, mean±SD)	92.67±15.41	89.12±6.59	NS
LDL cholesterol level (mg/dL, mean±SD)	103.73±31.26	114.88±16.52	NS
HDL cholesterol level (mg/dL, mean±SD)	48.11±15.00	53.09±11.17	NS
Tyrgliseride level (mg/dL, mean±SD)	136.73±88.65	105.27±30.34	NS

Abbreviations : HDL: High Density Lipoprotein, LDL:Low Density Lipoprotein

Table-2: Comparison of the median values of inflammatory markers between patients with mastocytosis and healthy controls

Biological Markers	Patients With Mastocytosis (n:49)	Healthy Controls (n:25)	p
Endocan [median (min-max), pg/mL]	30.99 (18.89-634.01)	30.64 (12.94-90.23)	NS
ET-1 [median (min-max), pg/mL]	26.19 (13.79-295.38)	35.95 (10.26-85.17)	NS
VEGF [median (min-max), pg/mL]	73.30 (32.46-295.29)	46.64 (11.09-99.86)	0.001
TNF-α [median (min-max),pg/mL]	8.69 (2.51-15.18)	8.65 (3.33-11.58)	NS
IL-6 [median (min-max), pg/mL]	5.30 (3.29-29.10)	4.48 (3.34-9.86)	NS
hsCRP [median (min-max),mg/dL]	2.30 (0.02-18.05)	1.40 (0.17-6.74)	NS

Biological Markers	Patients With Mastocytosis (n:49)	Healthy Controls (n:25)	p
ESR [median (min-max), mm/hr]	8.30 (1-39)	5 (2-22)	NS

Abbreviations : ET-1: Endothelin-1, VEGF: Vascular Endothelial Growth Factor, TNF-α: Tumor Necrosis Factor-Alpha, IL-6:Interleukine-6, hsCRP: High Sensitive C-Reactive Protein, ESR:Eritrocyte Sedimentation Rate

Table-3: Comparison of the median values of inflammatory markers between each mastocytosis subgroups and healthy controls

Biological Markers	Group-1 (n:8)	Group-2 (n:34)	Group-3 (n:7)	HCs (n:25)	p
Endocan [median (min-max), pg/mL]	27.89 (22.83-634.01)	31.60 (23.00-469.99)	31.68 (18.89-231.68)	30.64 (12.94-90.23)	NS
ET-1 [median (min-max), pg/mL]	27.13 (18.62-295.38)	26.71 (13.79-221.03)	23.96 (21.55-229.79)	35.95 (10.26-85.17)	NS
VEGF [median (min-max), pg/mL]	47.56 (40.47-225.53)	74.01 (32.46-295.29)	104.17* (49.25-248.46)	46,64* (11.09-99.86)	0.002
TNF-α [median (min-max), pg/mL]	9.13 (7.68-11.22)	8.81 (2.51-15.18)	7.35 (3.47-13.87)	8.65 (3.33-11.58)	NS
IL-6 [median (min-max), pg/mL]	4.11 (3.43-5.66)	5.58 (3.29-29.10)	5.30 (3.55-9.00)	4.48 (3.34-9.86)	NS
hsCRP [median (min-max), mg/dL]	2.47 (0.18-18.5)	1.54 (0.02-13.00)	5.90 (2.75-11.90)	1.40 (0.17-6.74)	NS
ESR [median (min-max), mm/hr]	4 (1-20)	9 (2-39)	8.30 (1-34)	5 (2-22)	0.019

Abbreviations : HCs: Healthy Controls ET-1: Endothelin-1, VEGF: Vascular Endothelial Growth Factor, TNF-α: Tumor Necrosis Factor-Alpha, IL-6:Interleukine-6, hsCRP: High Sensitive C-Reactive Protein, ESR:Eritrocyte Sedimentation Rate

* Post-hoc analysis showed that both group-2 and group-3 revealed higher VEGF levels than HCs (p=0.01, for each)

Table-4: Correlation of biomarkers, age, body mass index, demographic features, biochemical markers with FMD and CIMT in patients with mastocytosis

	FMD		CIMT	
	r	p	r	p

		FMD		**CIMT**	
Age	-	-	NS	0.306	0.033
BMI	-	-	NS	-	NS
Systolic blood pressure	-0.368	-0.368	**0.009**	-	NS
Diastolic blood pressure	-0.419	-0.419	0.003	-	NS
Heart rate	-	-	NS	-	NS
Fasting glucose level	-	-	NS	-	NS
LDL cholesterol level	-	-	NS	-	NS
HDL cholesterol level	-	-	NS	-	NS
Trigliseride level	-	-	NS	-	NS
Total IgE level	-	-	NS	-	NS
Hemoglobin	-	-	NS	-	NS
Endocan	-0.390	-0.390	0.006	-	NS
ET-1	-0.363	-0.363	0.01	-	NS
VEGF	-0.402	-0.402	0.004	-	NS
TNF-α	-	-	NS	-	NS
IL-6	-	-	NS	-	NS
hsCRP	-	-	NS	-	NS
ESR	-	-	NS	-	NS
Serum basal tryptase level	-	-	NS	-	NS
FMD	-	-	-	-	NS
CIMT	-	-	NS	-	-

Abbreviations : FMD: Flow-mediated Dilatation, CIMT: Carotid Intima Media Thickness, BMI: Body Mass Index, HDL: High Density Lipoprotein, LDL:Low Density Lipoprotein, IgE: Immunoglobulin E, ET-1: Endothelin-1, VEGF: Vascular Endothelial Growth Factor, TNF-α: Tumor Necrosis Factor-Alpha, IL-6:Interleukine-6, hsCRP: High Sensitive C-Reactive Protein, ESR:Eritrocyte Sedimentation Rate

Figure Legends

Figure-1: Mean FMD% values in patients with mastocytosis and HCs. In A; FMD were compared between HCs and all patients with mastocytosis. In B; FMD were compared between each mastocytosis subgroups. Group-1: Cutaneous Mastocytosis, Group-2: Indolent systemic mastocytosis, Group-3: Advanced systemic mastocytosis and Smoldering systemic mastocytosis

Figure-2: In A: Cut-off, sensitivity%, specificity% and AUC of FMD. In B: Cut-off, sensitivity%, specificity% and AUC of VEGF.

Supplement Figure-1: Correlation analysis between FMD with serum biomarkers. In A; Correlation between FMD with VEGF was shown. In B; Correlation between FMD with endocan was shown. In C; Correlation between FMD with ET-1 was shown.