



DOPPLER ULTRASONOGRAPHIC ASSESSMENT OF MAJOR SALIVARY GLANDS IN TYPE 2 DIABETES MELLITUS PATIENTS IN CORRELATION WITH SALIVARY QUANTITY AND SERUM MAGNESIUM LEVELS – A CROSS SECTIONAL STUDY

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ABSTRACT

Aims: Diabetes mellitus is a multifactorial genetic disorder and studies have found that it affects the salivary gland function and micronutrients. The aim of the study was to assess the Doppler Ultrasonographic changes of major salivary glands in patients with Type 2 Diabetes Mellitus and to correlate it with the quantity of salivary secretion and Serum Magnesium levels.

Materials: The study was conducted on 90 study patients with Type II Diabetes Mellitus who were categorized into 3 groups of 30 each namely Group A (well controlled), Group B (moderately controlled) and Group C (poorly controlled) based on the HbA1c levels and 30 normal individuals as controls. Doppler ultrasonography was used to assess the horizontal and vertical dimensions of major salivary gland and the vascularity of the glands. The ultrasonographic measurements of parotid and submandibular gland were tabulated. Serum magnesium levels and salivary quantity were calculated and compared with ultrasonographic changes.

Results: The mean horizontal and vertical dimensions of parotid glands were $4.1 \pm 0.3 * 4.2 \pm 0.3$ cm (Group A), $4.4 \pm 0.4 * 4 \pm 0.5$ cm (Group B), $4.7 \pm 0.5 * 4 \pm 0.5$ cm (Group C), in Group A,B,C respectively and $3.1 \pm 0.3 * 2.9 \pm 0.3$ cm in controls. The range of mean value of submandibular gland was $2.5 \pm 0.3 * 2.6 \pm 0.3$ cm (Group A), $2.9 \pm 0.3 * 2.9 \pm 0.3$ cm (Group B), $3 \pm 0.1 * 2.9 \pm 0.2$ cm (Group C), in Group A,B,C respectively while $2.7 \pm 0.1 * 2.5 \pm 0.1$ cm in controls which was highly significant with p value of < 0.01 . The mean values of salivary flow in Group A,B,C were 0.37 ± 0.15 , $0.320.37 \pm 0.15$, $0.10.37 \pm 0.12$ and that of controls were $0.380.37 \pm 0.15$ ml/min. Magnesium levels were 1.83 ± 0.3 mg/dl, 1.25 ± 0.32 mg/dl, 1.13 ± 0.37 mg/dl in group A,B,C respectively and that of controls were 1.83 ± 0.3 mg/dl.

Conclusion: To conclude, increased HbA1c levels were associated with increased ultrasonographic measurements and decreased levels of serum magnesium and salivary flow.

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INTRODUCTION

Diabetes mellitus being an unrevealed mystery and a window for various diseases, is a growing health problem worldwide. Diabetes mellitus is a multifactorial disorder characterized by increased insulin secretion. It is an epidemic disorder associated with a multitude of life style changes. India is announced as a capital for diabetes by WHO and it is predicted that by 2025, the maximum prevalence of diabetes would be in India.[1] Diabetes mellitus being a multifactorial disorder affects various systems in the body. Salivary glands are also affected due to diabetes mellitus. Salivary glands are important glands of digestive system and serves functions which influence oral health in general. Studies have found that Diabetes mellitus affect the salivary gland function and composition potentiating it as an indicator of disease

process.[2] Oral cavity is supposed to be the mirror of systemic diseases and saliva is the key feature which is involved in various functions from digestive system till immune system. Hence saliva is used as the diagnostic marker in systemic diseases. Recent research studies are focused mainly on the involvement of saliva in other diseases. [3] Diabetes is a disorder which affects metabolism of the body. There are various micro and macronutrients involved in this metabolism. Magnesium is a second predominant micronutrient in the metabolism. It is an intracellular component and a regulator in cellular process. Its function is related to synthesis of tissue constituents, growth and thermogenesis, in the metabolism of glucose. It acts as a cofactor in the glucose transporting mechanism of the cell and also plays an important role in glucose metabolism. [4] Diabetes is life threatening when it is associated with chronic complications like retinopathy,

abnormal platelet function, cardiovascular disease and hypertension. Low serum magnesium level is involved in micro and macro vascular complications of diabetes.[5] Imaging technologies are wide spread in analyzing early stages of a disease. Studies are done to analyze and diagnose diabetes at early stages through imaging technologies. Ultrasonography is a non invasive method of imaging the soft tissue pathologies. It is being widely used in diagnosing due to the feature that it is non invasive and does not produce any damage to the tissues. [6] With these factors the aim of the study was to assess the Doppler Ultrasonographic changes of major salivary glands in patients with Type 2 Diabetes Mellitus and to correlate the changes with the salivary quantity and serum magnesium levels.

METHODS

Sample Selection

The study group comprised of 120 patients of both sexes reporting to the Department of oral medicine and radiology, Indira Gandhi Institute of Dental sciences and Department of General Medicine, Mahathma Gandhi Medical College & Research Institute, Puducherry. The patients were selected from age group 35 – 55 years. The study was conducted with 90 Type II Diabetes Mellitus patients who were grouped into Group A (well controlled), Group B (moderately controlled) and Group C (poorly controlled) based on the HbA1c levels (Group A – 6.5-7.5%, Group B – 7.5-8.5%, Group C - >8.5%) and the control group which consisted of 30 patients. This observational cross sectional study was approved by Institutional ethical committee. Written consent was obtained from the participants.

Ultrasonographic Assessment of Major Salivary Gland

Doppler ultrasonographic assessment of major salivary gland was performed in the Department of Radiology, Mahathma Gandhi Medical College & Research Institute, Puducherry using 12MHZ linear probe to determine echogenicity and 8 to 9 MHZ to measure the gland size. Both bilateral Parotid and submandibular glands were scanned in 2 planes, parallel and perpendicular to submandibular plane.

Salivary Collection

Saliva was collected by non stimulatory method. Patients were asked to bend the head downwards and allowed to collect the saliva in floor of the mouth. The whole saliva was obtained by standardized spitting technique for 2 minutes. The amount of saliva collected was measured.

Blood Samples

Blood samples were collected and serum Magnesium level was estimated using calorimetric method. 2ml of venous blood sample was collected by veni-puncture from the antecubital vein under aseptic precautions. 1ml of blood was dispensed in test tube containing EDTA for HbA1c analysis. 1ml was dispensed in test tube without EDTA for serum magnesium analysis.

Statistical Analysis

All statistical analyses were performed using SPSS software (version 16.0. 2007, SPSS Inc., Chicago, IL, USA). The mean values of the parotid and submandibular gland were compared with groups and healthy subjects using Student 't' test. The association between serum Magnesium level, salivary

quantity and ultrasonographic changes was carried out using ANNOVA test.

RESULTS

The study showed 23 cases under 35 – 40 age group (19.16 %), 56 cases under 41-45 age group (46.6%), 16 cases under age group 46 – 50 (13.3%), 22 cases under age group 51 – 55 (18.3%). Of the 120 patients, 57 were males (47.5 %) and 63 were females (53.5%). In group A 17(56.7 %) were females and 13 (43.3 %) were males. In group B 13(43.3 %) were females and 17(56.7 %) were males. In group C 16(53.3%) were females and 14(46.7 %) were males. Majority of age group were from 41 – 45 years and females being the most predominant gender.

Results of Serum Magnesium Levels

There was a decrease in the values of serum Magnesium levels in Group B and C (1.25 ± 0.32 , 1.13 ± 0.37) but similar values with Group A and Controls (1.83 ± 0.3). The groups were compared with ANNOVA test which showed statistically significant value with $F(3,101) = 27.914$, $p < 0.05$. (where F is the f statistical value). It was observed that serum Magnesium levels were similar in Control group and Group A whereas it was decreased in group B & C compared with Control group. (TABLE 1)

Table 1 Serum Magnesium Levels

	Group A	Group B	Group C	Controls
Mean	1.83 mg/dl	1.25 mg/dl	1.13 mg/dl	1.83 mg/dl
SD	0.3	0.3	0.3	0.3

Results Of Ultrasonographic Changes

The range of mean value of parotid gland was (Horizontal * vertical dimensions) $4.1 \pm 0.3 * 4.2 \pm 0.3$ cms (Group A), $4.4 \pm 0.4 * 4 \pm 0.5$ cms (Group B), $4.7 \pm 0.5 * 4 \pm 0.5$ cms (Group C), while $3.1 \pm 0.3 * 2.9 \pm 0.3$ cms were Controls in the horizontal and vertical dimensions respectively. The range of mean value of submandibular gland was (Horizontal * vertical dimensions) $2.5 \pm 0.3 * 2.6 \pm 0.3$ cms (Group A), $2.9 \pm 0.3 * 2.9 \pm 0.3$ cms (Group B), $3 \pm 0.1 * 2.9 \pm 0.2$ cms (Group C), while $2.7 \pm 0.1 * 2.5 \pm 0.1$ cms in Controls in the horizontal and vertical dimensions respectively. The groups were compared with ANNOVA test which showed statistically significant value with $F(3,101) = 82.977$, $p < 0.05$. (Table2). It was observed from the study that ultrasonographic changes of parotid glands and submandibular glands were increased between groups as the HbA1c levels are increased. It was statistically significant and the chance of variation between the groups were more between Group A,B,C and controls. It was also observed that the Doppler ultrasonographic studies showed a normal vascular flow in Group A,B,C and controls.

Table 2 Ultrasonographic changes

	Parotid gland		Submandibular	
	RV	RH	RV	RH
Group A	4.1 cms	4.2 cms	2.5 cms	2.6 cms
Group B	4.4 cms	4 cms	2.9 cms	2.9 cms
Group C	4.7 cms	4 cms	3 cms	2.9 cms
Controls	3.1 cms	2.9 cms	2.7 cms	2.5 cms

Results Of Salivary Quantity

Table 3 shows decrease in the values of salivary quantity in Group C (0.2 ml/min respectively) compared with Group A, B and normal subjects (0.35, 0.37, 0.38ml/min) which had equal

values. It was observed that the compared groups were different significantly with $F(3,101) = 9.43, p < 0.05$.

Table 3 Salivary Quantity

	Group A	Group B	Group C	Controls
Mean	0.35 ml/min	0.37 ml/min	0.2 ml/min	0.38 ml/min
SD	0.15	0.16	0.12	0.15

Correlation Between Ultrasonographic Measurements And Serum Magnesium

Association between serum Magnesium levels was correlated using ANNOVA test. It was observed that, there was a highly significant decrease in the value of serum Magnesium levels as salivary gland measurements increase. $p < 0.05$ (Table 4)

Table 4

	Parotid gland		Submandibular		Serum Magnesium	
	RV	RH	RV	RH	SM	p value
Group A	4.1cms	4.2cms	2.5cms	2.6cms	1.83mg/dl	0.1
Group B	4.4cms	4cms	2.9cms	2.9cms	1.25 mg/dl	0.06
Group C	4.7cms	4cms	3cms	2.9cms	1.13mg/dl	0.002
Controls	3.1cms	2.9cms	2.7cms	2.5cms	1.83mg/dl	0.001

Correlation Between Ultrasonographic Measurements And Salivary Quantity

Association between serum Magnesium levels was correlated using ANNOVA test. It was observed that, there was a highly significant decrease in the value of salivary quantity levels as salivary gland measurements increase with $p < 0.05$. (Table 5)

Table 5

	Parotid gland		Submandibular		Salivary flow	
	RV	RH	RV	RH	SF	p value
Group A	4.1cms	4.2cms	2.5cms	2.6cms	0.35 ml/min	0.1
Group B	4.4cms	4cms	2.9cms	2.9cms	0.37 ml/min	0.88
Group C	4.7cms	4cms	3cms	2.9cms	0.2 ml/min	0.002
Controls	3.1cms	2.9cms	2.7cms	2.5cms	0.38 ml/min	0.001

Correlation of Ultrasonographic Changes With Serum Magnesium And Salivary Flow

Table 6 shows correlation of ultrasonographic changes with serum Magnesium and salivary flow between groups using Pearson's correlation coefficient. It was observed that parotid gland ultrasonographic measurements showed positive pearsons coefficient values with that of serum magnesium and salivary quantity. This showed that there was a positive correlation between the serum magnesium and salivary flow with the ultrasonographic changes of parotid gland which was statistically significant with p value less than 0.05.

The submandibular gland ultrasonographic measurements showed negative pearsons correlation coefficient values with the serum magnesium and salivary quantity. This showed that there was a negative correlation between the serum magnesium and salivary flow with the ultrasonographic changes of submandibular gland which was statistically not significant with p value greater than 0.05. It was observed that reliability on the correlation between salivary flow and serum magnesium with ultrasonographic changes of parotid glands are more compared with the submandibular gland. In parotid gland correlation Group C (poorly controlled) has highly significant positive correlation (p value of 0.02) compared with that of Group A and B. This showed reliability on the correlation factors are more in poorly controlled groups with $p = 0.02$.

Table 6 - Group A

	Serum magnesium level		Salivary flow	
	Pearson's co efficient	P value	Pearson's co efficient	P value
PRV	0.341	0.06	0.344	0.06
PRH	0.342	0.06	0.333	0.07
PLV	0.257	0.01	0.396	0.005
PLH	0.328	0.07	0.347	0.06
SRV	-0.915	0.91	-0.756	0.84
SRH	-0.845	0.86	-0.643	0.21
SLV	-0.832	0.52	-0.932	0.11
SLH	-0.764	0.32	-0.784	0.32

Group B

	Serum magnesium level		Salivary flow	
	Pearson's co efficient	P value	Pearson's co efficient	P value
PVR	0.205	0.02	0.347	0.06
PRH	0.073	0.0703	0.338	0.015
PLV	0.107	0.572	0.320	0.085
PLH	0.090	0.06	0.230	0.02
SRV	-1.309	0.20	-1.428	0.86
SRH	-1.429	0.16	-0.225	0.88
SLV	-0.225	0.82	-1.034	0.68
SLH	-0.304	0.31	0.711	0.62

Group C

	Serum magnesium level		Salivary flow	
	Pearson's co efficient	P value	Pearson's co efficient	P value
PVR	0.223	0.02	0.045	0.08
PRH	0.105	0.05	0.253	0.01
PLV	0.180	0.03	0.071	0.07
PLH	0.152	0.04	0.149	0.04
SRV	-0.45	0.76	-0.190	0.26
SRH	-0.292	0.63	-1.195	0.14
SLV	-0.043	0.79	-1.137	0.26
SLH	-0.153	0.57	-1.515	0.62

Controls

	Serum magnesium level		Salivary flow	
	Pearson's co efficient	P value	Pearson's co efficient	P value
PVR	0.012	0.09	0.018	0.09
PRH	0.029	0.06	0.095	0.06
PLV	0.141	0.045	0.075	0.06
PLH	0.046	0.08	0.068	0.07
SRV	-0.366	0.63	-2.168	0.63
SRH	-0.413	0.16	-0.256	0.76
SLV	-0.236	0.17	-1.759	0.90
SLH	-0.497	0.26	0.473	0.15

PRV – Parotid Right vertical PRH – Parotid Right Horizontal SM – Serum Magnesium SRV – Submandibular Right vertical SRH – Submandibular Right Horizontal SF – Salivary Flow

DISCUSSION

Results from the present study showed average age of study group was 44.7 ± 10.7 years. The controls had a mean age of 42.3 ± 5.28 years. Gupta *et al* (2011) conducted a study on the prevalence of Diabetes Mellitus among Puducherry comprising a study group of 616 samples in which majority of the patients were within age group of 35 – 49 and > 50 (34.25% and 34.25%). Our study group were in accordance with this study.[7] Anu Gaikwad *et al* (2012) in a study on prevalence of Diabetes Mellitus showed a result of out of 117 samples majority of them were under age group of 30-45. This in correlation with our study which enumerates that there is a major evolutionary change in various factors including dietary and sedentary lifestyle change among the younger age groups.[8] In the present study we had female predilection of gender with 44 males (48%) and 46 (61%) females who belonged to three categorized groups. Chris E. Ekpenyong *et*

al (2012) in a study to determine the age and gender predilection of Type 2 Diabetes Mellitus concluded that out of 3500 samples 1986 were females which showed female predilection. [9] This was in co ordinance with our study.

Serum Magnesium

Magnesium plays an important regulatory role in the stimulus secretion coupling events in a number of epithelial secretory cells including the exocrine pancreas, lacrimal and the parietal cells. [10] Magnesium is a cofactor in glucose transporting mechanism of cell membrane. It is involved at multiple levels in insulin secretion, binding and activity [11]. In our study serum Magnesium levels were similar in Control group and Group A whereas it was decreased in group B & C. Iryna N. Andrusishina (2010) investigated 39 persons among which 27 were diabetic and 12 with controls to evaluate the serum Magnesium and salivary Magnesium levels with calcium levels. The result showed that the levels of salivary Magnesium and serum Magnesium were nearly equal. It was decreased in the experimental groups. In controls serum Magnesium were $1.02 \pm 0.2 \text{ mg/dl}$ (75%) and that of cases were $0.74 \pm 0.2 \text{ mg/dl}$ (67.5%). In controls salivary Magnesium levels were $1.20 \pm 0.2 \text{ mg/dl}$ (75%) and that of cases were $0.60 \pm 0.2 \text{ mg/dl}$ [12]. This proves to the state that serum and salivary Magnesium decreases with increased glucose levels.

Asha and Hierensanghani (2013) studied the serum Magnesium and HbA1c levels in diabetic patients. A total of 75 subjects were included in the study, 50 patients were divided into two groups: Group I (25 diabetic patients without complications) and Group II (25 patients with micro or macro vascular complications). The serum Magnesium level were measured as $1.94 \pm 0.26 \text{ mg/dl}$, $1.32 \pm 0.28 \text{ mg/dl}$ and $2.47 \pm 0.24 \text{ mg/dl}$ in Group I, Group II and Group III, respectively. This showed a decrease in serum Magnesium level with increase in HbA1c level. In the study Magnesium levels were correlated with triglyceride levels, the average serum triglyceride levels were 152.08 ± 8.93 , 177.44 ± 13.47 and $119.96 \pm 10.19 \text{ mg/dl}$ in Group I, Group II and Group III, respectively. These values showed that higher incidence of increase in serum cholesterol as HbA1c levels increase. With this reference it can be correlated to the pathogenesis of sialosis in salivary gland which is due to fat infiltration in acinar cells. [13]

Salivary Flow

In the present study unstimulated saliva was measured for both experimental and control groups which was observed that there is a significant decrease in the salivary flow in Group B and C as HbA1c level increases. Harijanti *et al* (2007) conducted a study to determine the prevalence of xerostomia and its relation with level of blood glucose in Type 2 DM. It was concluded that 38 patients (76%), were affected with xerostomia and most of the patients (32 patients=84%) were under poorly controlled group. This reference was in correlation with our study. [14]

Ultrasonographic Assessment of Major Salivary Gland

Morphological changes of salivary gland associated with Diabetes Mellitus are still being under research. It was observed from the study that ultrasonographic changes of parotid glands and submandibular glands were increased between groups as the HbA1c levels are increased. It was statistically significant and the chance of variation between the groups were more between Group A, B, C and controls. It was also observed that

the dopplerultrasonographic studies showed a normal vascular flow in Group A, B, C and controls. Ahlam A. Fattah *et al* (2010) to determine the ultrasonographic measurements of parotid gland in poorly controlled Type 2 Diabetes Mellitus female patients. The study sample comprised of 35 female patients with Type 2 Diabetes Mellitus, and 35 healthy females. The results showed $48.8 \pm 2.3 * 39.3 \pm 5.7 \text{ mm}$ in horizontal and transverse dimension of parotid gland. The measurements of submandibular glands were $21.9 \pm 1.1 * 10 \pm 3.7 \text{ mm}$ in horizontal and transverse dimension. Control groups measured $42.8 \pm 2.3 * 39.3 \pm 5.7 \text{ mm}$, $6.4 \pm 0.8 * 17.7 \pm 1.1 \text{ mm}$ in parotid and submandibular gland respectively [15]. These results were in correlation with our present study which showed increase in value of diabetic groups compared with control. Divi Cornec *et al* (2013) conducted a study to determine the accuracy of salivary gland ultrasonography (SGUS) for diagnosing primary Sjogren's syndrome (SS) and to suggest modifications of the American-European Consensus Group (AECG) classification criteria. The study comprised of 158 patients, 78 had diagnosis of primary SS according to the experts, including 61 patients (78.2%) who met the AECG criteria. Doppler waveform analysis and gland size was measured in the study which showed no differences between patients with primary SS and those without primary SS in terms of the baseline or stimulated transverse facial artery resistive index. [16] This study was in correlation with our study contributing to the Doppler waveform assessment.

Correlating Features

It was observed in our study that the serum Magnesium and salivary flow were highly significant in correlation with ultrasonographic changes in parotid glands of group C. The submandibular gland had no significant values compared with parotid gland values with serum Magnesium and salivary flow. Sandrine *et al* (2007) conducted a study to diagnose primary Sjogren's syndrome (pSS) and to evaluate treatment responses with ultrasonographic changes. The study sample consisted of 16 patients fulfilling the new American-European consensus group criteria for pSS and 9 controls. Compared to controls, the untreated patients had significant abnormalities in salivary gland structure ($p < 0.001$) and parotid size ($2.05 \pm 0.33 \text{ cm}$ versus $1.70 \pm 0.28 \text{ cm}$; $p = 0.001$). It was stated in the study that there were no difference in submandibular gland size between the controls and patients. With this reference in our study, the submandibular gland size was not significant compared with the serum Magnesium and salivary flow. This is due to the probability that these glands are more difficult to delineate than the parotids as mentioned by Sandrine *et al* (2007) [17]. It was observed that serum Magnesium and salivary flow had highly significant correlation values with Ultrasonographic changes in Group C compared with Group A & B. Asha and Hierensanghani (2013) studied the serum Magnesium and HbA1c levels in diabetic patients. The serum Magnesium levels were measured as $1.94 \pm 0.26 \text{ mg/dl}$, $1.32 \pm 0.28 \text{ mg/dl}$ and $2.47 \pm 0.24 \text{ mg/dl}$ in Group I, Group II and Group III, respectively. This showed a decrease in serum Magnesium level with increase in HbA1c level. In the study Magnesium levels were correlated with triglyceride levels, the average serum triglyceride levels were 152.08 ± 8.93 , 177.44 ± 13.47 and $119.96 \pm 10.19 \text{ mg/dl}$ in Group I, Group II and Group III, respectively [13]. This study contribute to fact on predominant increase is seen in Group C, which is due to the increase in serum cholesterol levels leading to infiltration of fat in the acinar cells. Since there is no significant correlation

in Group A and B ultrasonographic changes were also insignificant. Mata *et al* (2003) conducted a study to investigate specifically the effect of perturbation of extracellular Magnesium (Mg) on both basal and secretagogue-evoked amylase secretion and total protein output and intracellular free calcium concentrations (Ca) in the rat submandibular and parotid glands. It was concluded that in rat acinar cells there was decreased levels of Mg in Type 2 Diabetes Mellitus patients which interfere with the Ca exit from the extracellular side. It was stated that hypomagnesemia led to a diminished intracellular Mg concentration which led to impairment of function of Mg dependent enzymes regulating salivary secretion. This reference could be added to the fact that hypomagnesium leads to alteration in the salivary flow [18]. In our study Magnesium has significant decrease in the poorly controlled patients which could be added as a support for decrease in salivary flow.

Progress of Diabetes mellitus is interlinked with various associated factors and their related signs in human body. Poor glycemic control leads to increased salivary gland size which is due to adipose infiltration in parenchyma. As stated by Asha and Hierensanghani (2013) sialosis is associated with the biochemical alteration in saliva in Type 2 diabetes mellitus were magnesium levels were decreased in our study (13). Diabetes mellitus is associated with decreased magnesium levels this is because of altered reabsorption of magnesium in Distal convoluted tubules (19). Decrease in serum magnesium levels in turn affects the secretory function of salivary glands which is related to decrease in salivary flow.

CONCLUSION

To conclude, the present study was successful in determining the ultrasonographic changes, salivary flow and serum magnesium levels in type 2 diabetes mellitus patients. Our research revealed that individuals with higher HbA1c levels are associated with decreased levels of serum magnesium and salivary flow. Doppler ultrasonography is an essential way to assess the morphological, volumetric as well as vascular flow of the glands. Evaluation of ultrasonographic changes of salivary gland along with serum magnesium levels will serve as auxiliary tool in identifying patients with uncontrolled diabetes and could be a valuable additional diagnostic clue for an underlying micro and macrovascular disease leading to cardiac failure. Further research in developing indices for vascularity could be a valuable aid in diagnosing microvascular disease.

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