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Evaluation of Serum Advanced Glycation End Product Levels and Microvascular Complications in Children and Adolescents with Type 1 Diabetes Mellitus

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What is already known on this topic?

- *Serum advanced glycation end products (AGEs) levels are independent predictors of microvascular complications in diabetes mellitus (DM). There are a few studies in the literature on serum AGEs levels in type 1 diabetes mellitus (T1DM), but the association between serum AGEs levels and diabetic microvascular complications in children and adolescents has not been investigated.*

What this study adds on this topic?

- *This is the first study to investigate the relationship between serum AGEs levels and microvascular complications in children and adolescents with T1DM. Our study showed that serum AGEs levels were significantly associated with nephropathy, but not with retinopathy and neuropathy.*

ABSTRACT

Objective: Advanced glycation end products (AGEs) are irreversible macromolecules formed by nonenzymatic reactions due to chronic hyperglycemia. The aim of this study was to assess the relationship between AGEs and the microvascular complications of children and adolescents with type 1 diabetes mellitus (T1DM).

Materials and Methods: Twenty-six T1DM patients with microvascular complications and 58 complication-naive patients who were similar regarding age, sex, and pubertal status enrolled in the study. Anthropometric, biochemical, ophthalmologic, and neurologic variables were compared with serum AGEs levels by the fluorescence method.

Results: There was no significant difference observed between the patients with complications and those without complications in terms of serum levels of AGEs and other biochemical parameters. However, the duration of T1DM and urine microalbumin–creatinine ratio (uACR) were significantly higher in the complication-positive group ($P < .001$). Serum levels of AGEs were found to be similar when retinopathy, peripheral, and optic neuropathy were separately compared with the complication-naive group ($P > .05$). However, patients with nephropathy had significantly higher serum levels of AGEs than patients without complications ($P = .023$). In addition, there was a significant positive correlation between serum AGEs levels and uACR ($P = .042$) but not other parameters ($P > .05$).

Conclusion: This study is the first to evaluate the association between serum AGEs levels and microvascular complications in children and adolescents with T1DM. Our study highlights that serum AGEs levels are significantly correlated with nephropathy but not with retinopathy and neuropathy. Further long-term studies with a larger sample size are required to establish a better relationship between diabetic complications and AGEs.

Keywords: Advanced glycation end products, diabetic nephropathy, microvascular complications, neuropathy, retinopathy, type 1 diabetes

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease characterized by beta-cell damage and reduced insulin secretion, resulting in chronic hyperglycemia. Type 1 diabetes mellitus has a significant impact on patients' lives, as uncontrolled diabetes can lead to both microvascular and macrovascular complications, contributing to reduced life expectancy.[1] Optimal glycemic control is essential to reduce microvascular complications;

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however, fewer than 30% of patients achieve target hemoglobin A1c (HbA1c) levels.[2] As T1DM is a long-term disease, early detection of microvascular complications, especially in children and adolescents, is important to prevent further complications.

The chronic hyperglycemic state in diabetes mellitus leads to the formation of covalent adducts between glucose and proteins, DNA, and lipids through a nonenzymatic process known as the Maillard reaction. This process leads to the formation of advanced glycation end products (AGEs).[3] Advanced glycation end products are irreversible macromolecules and exert their biological activity through receptors of AGEs (RAGE).[4] Interaction between AGEs and RAGE disrupts oxidation–reduction reactions and triggers inflammatory and thrombogenic responses in endothelial cells. RAGE, highly implicated in proinflammatory responses and autoimmunity, contributes to cellular responses in diabetic vasculopathy, inflammation, and progression of the atherosclerotic process.[5,6] In addition, the AGE–RAGE axis can lead to increased production of reactive oxygen species (ROS) and oxidation of low-density lipoprotein (LDL), exacerbating plaque formation.[7]

The formation of AGEs progressively increases with aging, and they accumulate in human cartilage, the mesangium of kidneys, skin collagen, and lens and retina of the eye.[8] AGEs have an impact on the pathogenesis of diabetic complications, cataracts, atherosclerosis, and neurodegenerative diseases such as Alzheimer's disease.[3] AGEs interfere with normal protein functions through the disruption of molecular conformation, reduced enzymatic activity, and decreased receptor recognition.[9] The molecular mechanism involves protein and lipid denaturation, the accumulation of AGEs in tissue, the activation of receptor-mediated signal pathways in cells, an increase of oxidative and carbonyl stress, and the production of autoantibodies against serum AGEs.[10,11]

The half-life of AGEs varies depending on the specific compound and tissue, ranging from days to years. Elimination primarily occurs through renal excretion and metabolism by enzymes like glyoxalase and peptidases.[12] Animal studies have clearly shown that exposure to high levels of exogenous AGEs contributes to renal and vascular complications.[13]

Previous studies in the literature have investigated the association between AGE levels and microvascular complications in children and adolescents with T1DM. These studies have demonstrated that measuring AGEs using noninvasive techniques, such as skin autofluorescence (sAF), which evaluates AGEs in skin collagen, could be independent predictors of microvascular disease.[14-18] Nonetheless, there is inadequate research on the correlation between serum AGEs and diabetic complications in adults with type 2 diabetes mellitus (T2DM).[19-21] Although a few studies have analyzed the levels of serum AGEs in T1DM.[22,23] The relationship between serum AGE levels and diabetic microvascular complications has not yet been investigated in children and adolescents. In this study, we aimed to investigate the relationship between serum AGE levels, laboratory and anthropometric parameters, and microvascular complications (nephropathy, neuropathy, and retinopathy) in children and adolescents with T1DM.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of Dr. Behçet Uz Children's Research and Education Hospital in light of the Helsinki Declaration (600- 202l/13-09). Written informed consent was obtained from the patients who agreed to take part in the study.

Study Design and Participants

A total of 84 children and adolescents (aged 9-18 years) who had been under follow-up for more than 2 years with a diagnosis of T1DM in our pediatric endocrinology clinic between June 2021 and July 2022 were included in this study. The control group consisted of patients without any micro or macrovascular complications associated with T1DM, such as kidney disease, neuropathy, retinopathy, or cardiovascular disease. The groups were matched based on age, pubertal stage, and body mass index (BMI). Patients with a history of 2 episodes of ketoacidosis in the preceding 12 months, severe familial hypercholesterolemia, congenital heart and kidney diseases, autoimmune diseases including uncontrolled coeliac disease, Addison's disease, any congenital condition resulting in insulin-dependent diabetes, and/or eating disorders were excluded from the study. Adequate glycemic control in T1DM patients was defined as a glycated HbA1c concentration of 7.0% or below.[24]

Calculation of the Sample Size

The G*Power program version 3.1.9.2 for Windows was used for the calculation of sample size. According to the results of the power analysis, a sample size of 88 T1DM cases (27 T1DM patients with complications and 61 T1DM patients without complications) was needed for detecting a difference of serum AGEs (95% power, alpha error: 0.05). The power analysis of the study was calculated based on the mean ± standard deviation score (SDS) value of AGE levels measured by Banser et al[25] in children with diabetes.

Assessments

All study participants underwent the following evaluations: collection of demographic data, clinical data including age, body weight, body height, BMI, pubertal staging, blood pressure, age at diabetes onset, diabetes duration, insulin dosage, and laboratory tests including blood glucose, creatinine, HbA1c, lipid profiles (total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, non-HDL), uric acid, urinary albumin, glomerular filtration rate (GFR), and serum AGE levels measured using the fluorescence method.

Screening for microvascular complications was conducted in patients with T1DM within the first 5 years after the diagnosis of diabetes.[26] Screening for diabetic nephropathy, urine microalbumin levels were measured. A urinary albumin excretion (UAE) of 30-300 mg/g in 2 or more urine samples in a 24-hour period was considered as microalbuminuria, and an albumin level above 300 mg/g in the urine was defined as macroalbuminuria.[7] Estimated glomerular filtration rate (eGFR) was calculated using the Schwartz equation.

The diagnosis of retinopathy was based on direct and indirect ophthalmoscopy assessments performed by a retinal specialist after full mydriasis. Confirmation of suspected cases was done through fundus photography. The minimum requirement for

the diagnosis of diabetic retinopathy was the presence of at least 1 microaneurysm in any field in either eye.[27] For neuropathy evaluation, a neurological examination was conducted by a pediatric neurologist, and in suspected cases, electroneuromyography was performed. Neuropathy was diagnosed if the mean vibratory perception threshold (VPT) was ≥20 V. Optic neuropathies were assessed using visual evoked potential (VEP) testing, which evaluated visual function based on the latency of the first major positive component of the evoked response. All VEP transcripts were evaluated by the same pediatric neurologist using P100 latencies and amplitudes.[28] Abnormal VEP was defined as a delay or absence of the P100 wave.

Hypertension was diagnosed when the average systolic blood pressure (SBP) and/or diastolic blood pressure (DBP) were ≥95th percentile for gender, age, and height, as determined by blood pressure nomograms for Turkish children and adolescents.[29] Further evaluation using 24-hour Holter monitoring was performed to confirm hypertension.

Laboratory Measurements

Venous blood samples were collected from the participants after a 10- to 12-hour overnight fasting period. The blood was collected in plain tubes and then centrifuged at 1200 × g for 10 minutes. Serum samples were separated from the clots using plastic Pasteur pipettes and transferred to Eppendorf tubes. The serum samples for AGEs analysis were stored at −20℃ until further analysis. For the measurement of AGEs, 0.2 mL of serum sample was mixed with 0.05 mL of the AGE kit (Biovision Advanced Glycation End Products (AGEs) Assay Kit, California, USA). The fluorescence of AGEs (Ex/Em = 360/460 nm) was measured using a microplate fluorescence reader after differentiating between AGEs and unoxidized proteins. Oxidized bovine serum albumin (AGE-BSA) was used as a positive control. Reference ranges given by the manufacturer are 10000-100000 arbitrary units (AU)/mg.

Statistical Analysis

The data obtained from the study were analyzed using GraphPad Prism statistical software version 8.0.0 and the Statistical Package for the Social Sciences Statistics software, version 25.0 (IBM Corp., Armonk, NY, USA). The distribution of the data was evaluated with the Shapiro–Wilk test and the homogeneity of variance was evaluated with the Levene test. For numerical comparisons, the Student's *t*-test or Mann–Whitney *U*-tests were used to assess differences between the 2 groups according to the normal distribution of the measured parameters. In the comparison of more than 2 groups according to quantitative variables, the Kruskal–Wallis test was used, and the Dunn's test was used for post hoc analyses. Chi-square test was used to compare the categorical variables. Spearman's rho correlation was used to identify the associations between variables. A correlation coefficient below 0.20 was considered no correlation; correlation coefficients between 0.20-0.39 were considered weak; those between 0.40 and 0.69 were moderate correlation; those between 0.70 and 0.89 were strong correlation; and those between 0.90 and 1.0 were very strong correlation. While quantitative variables were expressed as mean ± standard deviation in the tables, categorical variables were shown as n (%). Variables were analyzed at the 95% confidence level, and a *P*-value of less than .05 was considered significant.

RESULTS

Demographic and Clinical Characteristics

A total of 26 T1DM patients (13 females and 13 males) with microvascular complications and 58 patients without complications (27 females) were included in the study. The mean age of the patients with complications and without complications was 14.9 ± 2.5 years and 14.1 ± 2.4 years, respectively ($P > .05$). The median duration of T1DM was significantly longer in patients with complications (7.4 ± 3.6 years) compared to patients without complications (5.6 ± 3.1 years) ($P = .037$). The mean age of patients with nephropathy was 14.4 ± 2.3 years, the mean duration of diabetes was 6.6 ± 3.8 years, and the mean HbA1c in the last 2 years was 8.2+1.7%.

Statistical Results

The mean HbA1c levels were 8.3 ± 1.3% in patients with complications and 8.5 ± 1.1% in patients without complications ($P > .05$). The frequencies of glycemic control were 15% in the patients with complications and 23% in the patients without complications. There were no significant differences between the 2 groups in terms of age, sex, age at diabetes onset, pubertal status, daily insulin dosage, HbA1c, mean HbA1c of the last 2 years, serum total cholesterol, LDL, HDL, non-HDL, triglycerides, uric acid, creatinine, and eGFR ($P > .05$). However, the duration of T1DM and urine microalbumin–creatinine ratio (uACR) were significantly higher in the group with complications ($P < .001$) (Table 1). There was no significant difference in serum AGE levels between patients with complications and patients without complications ($P > .05$). When comparing specific complications, patients with nephropathy had significantly higher serum levels of AGEs compared to patients without complications ($P = .023$) (Table 1). But there was no significant difference in age, diabetes duration, or HbA1c levels between patients with nephropathy and complication-positive patients without nephropathy (respectively, $P = .156$, $P = .123$, and $P = .317$) and complication-naïve patients (respectively, $P = .99$, $P = .86$, and $P = .27$). No significant difference in serum AGE levels was found between T1DM patients with good (42.8 ± 9.6 AU/mg × 103) and poor (44.3 ± 2.4 AU/mg × 10^3) metabolic control ($P = .804$).

Among all subjects, serum AGE levels showed a significant positive correlation with uACR ($P = .042$). However, serum AGE levels were not correlated with age, duration of diabetes, BMI SDS, the most recent HbA1c, mean HbA1c of the last 2 years, serum total protein, uric acid, total cholesterol, LDL, HDL, triglycerides, non-HDL, creatinine, and eGFR ($P > .05$) (Table 2). In post hoc power analysis, our study effect size *d* was 0.306 and power (1 – β err prob) was 0.675.

DISCUSSION

The present study showed that serum AGE levels in T1DM patients with and without microvascular complications were similar. Nevertheless, those suffering from nephropathy had significantly higher serum AGE levels compared to patients with other microvascular complications such as peripheral neuropathy, retinopathy, and optic neuropathy. Few studies have evaluated serum AGE levels in children and adolescents with T1DM. In a study, Berg et al[22] compared 68 T1DM cases with 25 healthy controls and found higher serum AGE

Table 1. Comparison of Clinical and Laboratory Characteristics in Patients With Complications and Without Complications

	Patients With Complication (n = 26)	Patients Without Complication (n = 58)	*P*	
Age (years)	14.9 ± 2.5	14.1 ± 2.4	.063[a]	
Sex (M/F)	15/13	28/27	.822[b]	
BMI SDS	−0.35 ± 1.2	0 ± 1.2	.264[a]	
Diabetes duration, years	7.4 ± 3.6	5.6 ± 3.1	**.037**[c]	
Age at diabetes onset, years	7.5 ± 3.2	9 ± 3.5	.065[a]	
Tanner puberty staging	4.1 ± 1.2	3.8 ± 1.3	.307[a]	
Insulin dosage, U/kg/day	1.07 ± 0.3	1.03 ± 0.2	.332[a]	
HbA1c, %	8.34 ± 1.3	8.47 ± 1.1	.566[a]	
Mean HbA1c, % (last 24 months)	8.55 ± 1.5	8.33 ± 1.6	.786[a]	
Total cholesterol mg/dL	169 ± 35	163 ± 33	.052[c]	
LDL mg/dL	87.1 ± 33	83.5 ± 27	.558[c]	
HDL mg/dL	64.2 ± 17	58 ± 12	.137[a]	
Triglycerides mg/dL	91 ± 43	111 ± 57	.066[c]	
Non-HDL mg/dL	87.1 ± 33	87.1 ± 33	.824[c]	
Uric acid mg/dL	3.05 ± 1.06	3.48 ± 1.12	.093[a]	
Creatinine mg/dL	0.7 ± 0.16	0.72 ± 0.11	.667[a]	
Urine microalbumin–creatinine mg/g	12.3 ± 8.1	8.7 ± 1.6	**<.001**[c]	
eGFR, mL/min/1.73 m^2	133 ± 33	126 ± 21	.831[c]	
Hypertension	5/28	3/56	.108[b]	
Dyslipidemia	3/28	5/56	.997[b]	
Serum AGEs (AU/mg×10^3)	49.6 ± 32.5	41.7 ± 16.4	.351[c]	
a) Nephropathy (n = 13)	64.8 ± 43.6	41.7 ± 16.4	**.032**[d]	**.023**[e]
b) Peripheral neuropathy (n = 3)	39.6 ± 13.6			.973[e]
c) Retinopathy (n = 3)	28.7 ± 18.8			.383[e]
d) Optic neuropathy (n = 14)	38.6 ± 10.6			.631[e]

BMI, body mass index; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SDS, standard deviation score.
[a]Student's *t*-test. [b]chi-Ssquare test. [c]Mann–Whitney *U*-test, [d]Kruskal–Wallis test. [e]Dunn's test.

levels, which were measured by the fluorometric immunoassay method in T1DM cases than in healthy controls. Kostolanska et al[23] included 81 children and adolescents diagnosed with T1DM, and they found higher serum AGE levels in T1DM patients compared to healthy controls. Furthermore, patients who had poor glycemic control had higher levels of AGEs. Although they

Table 2. Correlation Coefficients of Serum AGE Levels with Anthropometrics and Laboratory Parameters

	r	95% Confidence Interval	*P**
Age (years)	−0.001811	−−0.2214-0.2180	.987
BMI SDS	−0.06761	−0.2845-0.1558	.553
Diabetes duration, years	0.1232	−0.1084-0.3421	.295
Age at diabetes onset, years	−0.08684	−0.3008-0.1355	.443
HbA1c, % (the most recent)	0.06031	−0.1615-0.2764	.595
Mean HbA1c, % (last 24 months)	0.1154	−0.1071-0.3268	.308
Total protein mg/dL	0.06031	−0.1615-0.2764	.595
Uric acid mg/dL	−0.02517	−0.2656-0.2182	.841
Total cholesterol mg/dL	−0.1114	−0.3272-0.1154	.334
LDL mg/dL	0.002426	−0.2281-0.2327	.983
HDL mg/dL	−0.08697	−0.3050-0.1397	.452
Triglycerides mg/dL	−0.1668	−0.3767-0.05937	.147
Non-HDL mg/dL	−0.08189	−0.3004-0.1447	.478
Creatinine mg/dL	−0.1447	−0.3545-0.07893	.203
Urine microalbumin–creatinine mg/g	0.2413	0.008486-0.4493	**.042**
eGFR, mL/min/1.73 m^2	0.09336	−0.1365-0.3137	.425

BMI, body mass index; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SDS, standard deviation score.*P** Spearman's correlation analysis; Serum AGEs and urine microalbumin–creatinine mg/g levels as dependent variable

did not present the microvascular complications of the T1DM patients in their study, they suggested that serum AGE level measurement could be used to predict microvascular complications. Since our study did not include patients without diabetes, we could not make a direct comparison between the serum AGE levels of T1DM patients and healthy controls.

Most studies investigating the relationship between AGEs and microvascular complications in children and adolescents with diabetes have mainly used the sAF method, a noninvasive approach.[14-18] There are very few studies investigating serum AGE levels and diabetes microvascular complications in adults with T2DM.[19-21] In a study of 119 subjects with T2DM, Sampathkumar et al[20] demonstrated that serum AGE levels determined by the fluorescence method were higher in patients with diabetes than in those without diabetes. In addition, AGE levels were higher in patients with microvascular complications than in those without complications, with patients having both retinopathy and nephropathy exhibiting the highest levels. Similarly, Grossin et al[21] conducted a study with 30 T2DM patients and 29 healthy subjects and found that serum AGE levels measured by the competitive ELISA method were higher in diabetic patients than in the healthy control group, with the highest levels observed in diabetic patients with microvascular complications. Pia de la Maza et al[19] included 102 patients with T2DM in their study and suggested that cases without microvascular complications are associated with lower serum and urine AGE levels determined by the fluorescent AGEs by spect rophotofluorimetry method. In the current study, the serum AGE levels measured by the fluorescent method in cases of T1DM with and without complications were similar.

In the literature, sAF levels are linked with various complications of diabetes (retinopathy, neuropathy, and nephropathy) in adults.[30-32] Literatures showed that sAF levels were significantly higher in T1DM children and adolescents compared to healthy controls.[16,18] However, in the present study, there was no significant correlation between serum AGE levels and the presence of microvascular complications, such as neuropathy and retinopathy, in children and adolescents with T1DM. This may be due to the relatively small sample size and heterogeneity of the complications in our study cohort.

In adolescents with T1DM, diabetic nephropathy is the most commonly observed microvascular complication, and it manifests earliest.[33] Diabetic nephropathy may progress from microalbuminuria to end-stage renal disease, and this progression is enhanced by hyperglycemia and also parasympathetic dysfunction.[34] It has been demonstrated that the interaction between AGEs and RAGE, induces podocyte DNA damage and detachment. This interaction is partly mediated by the stimulation of the angiotensin II type 1 receptor and the accumulation of extracellular matrix proteins.[35,36] Orchard et al[16] showed that AGE levels by sAF were associated with renal damage beyond renal damage in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) cohort. Similarly, Tanaka et al[32] demonstrated that sAF had a positive correlation with chronic kidney diseases in adult cases. Forbes et al[18] showed that although sAF was significantly higher in diabetic patients compared to controls, circulating and urine AGEs were not independent factors in diabetic kidney disease of youth with T1DM, but higher eGFR and longer diabetes duration were detected. Consistent with the former view, we demonstrated that serum levels of AGEs were significantly higher in nephropathic patients among diabetic patients with microvascular complications and also correlated with UAE but not with eGFR. However, the cause-and-effect relationship between AGE levels and nephropathy is not clear.

Diabetic neuropathy is the most common and troublesome complication of diabetes mellitus and affects both autonomic and peripheral nerves.[35] It has been demonstrated that the precursor of AGEs, at physiological concentration, decreases the viability of rat Schwann cells, thus contributing to the pathogenesis and development of diabetic neuropathy, and it has also been shown that AGEs increase the excitability of neurons and facilitate the firing of nociceptive sensory neurons.[36,37] In the DCCT/EDIC study, Orchard et al[16] demonstrated the associations between polyneuropathy and HbA1c and sAF. A prospective study by Rajaobelina et al[38] confirmed the value of sAF in predicting the development of neuropathy in the adult T1DM cohort. In our study, we compared AGE levels between patients with subclinical peripheral and optic neuropathy, detected with electroneuromyography and VEP testing, respectively, and patients without any complications, and we found no significant difference. This may be due to the small number of patients in each subgroup, the relatively short disease duration, and also the relatively well-controlled disease activity in our cohort. There is an obvious need for larger studies with a longer follow-up period to clarify the relationship between AGE levels and T1DM patients with neuropathy.

Diabetic retinopathy is a serious microvascular complication of diabetes, and it remains an important cause of visual loss worldwide. Studies have shown that several AGE levels are higher in cataract and diabetic eye lenses than in normal human eye lenses, and apoptosis of retinal capillary pericytes and endothelial cells has been linked to AGEs.[39,40] In our study, we detected no significant differences in AGE levels between the retinopathy and naïve groups.

Our study has obvious limitations that need to be acknowledged. The most important one is that the rate of complications in childhood is much lower compared to adulthood. Therefore, the number of subgroups of patients with microvascular complications included in the study was quite low, and the complication-positive group was heterogeneous, which may adversely affect our results. Additionally, although there are various methods available for measuring AGEs, there is no widely accepted method for detecting AGE levels and applying them in our clinical practice. It is well known that circulating, urinary, and tissue AGE levels are closely related to dietary intake, but the effect of diet on serum AGEs levels was not investigated in the current study.[41,42] Finally, as this was a case–control study, we did not have follow-up data for the control groups to determine if and when they developed microvascular complications.

CONCLUSION

This is the first study to evaluate the relationship between serum AGEs levels and microvascular complications in children

and adolescents with T1DM. Our study showed that serum AGEs levels are significantly correlated with nephropathy but not with retinopathy and neuropathy. Further long-term studies with a larger number of participants are required to better establish the relationship between diabetic complications and AGEs.

Ethics Committee Approval: This study was approved by the Ethics Committee of Dr. Behçet Uz Children's Education and Research Hospital in light of the Helsinki Declaration (600-202l/13-09).

Informed Consent: Written informed consent was obtained from the patients who agreed to take part in the study.

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