Receptor of advanced glycation end product (RAGE) polymorphism and oxidative status in Hashimoto’s thyroiditis in Egyptian female patients: case control study

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Abstract

Background: Hashimoto’s thyroiditis is the most widespread autoimmune illness targeting a specific organ. “Redox homeostasis” is achieved when the production of Reactive Oxygen Species and their elimination are in balance. Advanced glycation end products (AGEs) are formed when glucose and/or α-oxaloaldehydes react non-enzymatically with the amino groups of lipids, proteins, and DNA. Nowadays, many studies are concerned with AGEs, the polymorphisms of their receptors, and their association with increased risk of HT. However, few studies investigated the role of receptors of advanced glycation end product (RAGE) SNP in Egyptian females.

Objective: The goals of this investigation were to ascertain whether oxidative stress plasma malondialdehyde (MDA) and total antioxidant capacity (TAC) were associated with HT, in addition, to assess the association of RAGE polymorphisms (−374 T > A and the −429 T > C and Gly82Ser) with HT.

Subject and methods.

Our case–control study has 80 patients enrolled who have newly been diagnosed with HT and 80 age and sex-matched healthy female controls. Each participant underwent a thorough medical history, physical examination, and laboratory investigations, which included Genotyping of RAGE Gly82Ser, −374 T > A and −429 T > C using polymerase chain reaction-restriction fragment length polymorphisms (PCR–RFLP).

Results: Chi-square revealed a significant association regarding the distribution of RAGE (−374 T < C) genotypes TT and CC between patients and control (P = 0.04). Non-significant associations regarding the distribution of Gly82Ser genotypes Gly/Gly, Gly/Ser, Ser/Ser were found between patients and control (P = 0.5), and non-significant associations related to −429 T > C gene polymorphism were revealed. In addition, patients with HT had higher MDA and lower TCA compared with controls.

Conclusion: The elevated MDA and decreased TAC as an antioxidant may be one of several risk factors associated with the prevalence of HT in individuals with the −429 T > C RAGE mutation polymorphism that is associated with an increased risk of HT in Egyptian females.

Keywords: Hashimoto’s thyroiditis, Advanced glycation end product (AGE), Reactive Oxygen Species (ROS), Receptor of Advanced glycation End product (RAGE)

Introduction

Hashimoto’s Thyroiditis (HT) is the most frequently occurring autoimmune disease that targets a specific organ [1], which causes dysfunction of the thyroid in
variable grades [2]. It is a multifactorial disorder that is characterized by lymphocyte infiltration of the thyroid gland, elevation of serum anti-thyroid antibodies in the form of thyroglobulin autoantibodies (Tg), thyroid peroxidase (TPO), and the thyroid-stimulating hormone receptor (TSH-R), which are all markers of autoimmune thyroid diseases (AITD) [3]. Besides, there is a sign of goitrous or atrophic gland and the gradual destruction of thyroid cells by apoptosis, which determines the outcome [4]. Several years later, the patients who are initially euthyroid may have Hypothyroidism [5].

Reactive Oxygen Species (ROS) and the free radicals developed by the metabolism of the normal cell are well recognized for both beneficial and harmful effects on cells. The presence of low ROS levels is mandatory for various biochemical reactions within the cells. However, excessive ROS produces injury to the cell by its reaction with lipids, proteins, DNA, and inhibition of the normal functions [6, 7]. Normally, there are either enzymatic or non-enzymatic defense systems, called antioxidants, for the prevention of damage. Thus, "redox homeostasis" occurs when ROS generation and elimination are in balance [8]. Meanwhile, oxidative stress occurs by the imbalance between prooxidants and antioxidants, which causes damage to macromolecules, disruption of ions in redox signaling, and alterations of proteins [9]. Many studies found an association between increased oxidative stress and HT [10]. Though, the exact link between them is still debated [11].

Advanced Glycation End products (AGEs) are formed when glucose and/or α-oxaloaldehydes react non-enzymatically with the amino groups of lipids, proteins, and DNA. Those changes cause modification of the structure and function of proteins, and intramolecular and intermolecular cross-link formation. In the vascular endothelium, the binding between the receptor of the advanced glycation end product (RAGE) by AGEs leads to endothelial cells and pericytes changing that are characteristic of many inflammatory diseases such as Hashimoto thyroiditis [12].

Recently, many studies have been concerned with the RAGE roles, the polymorphisms of the correlated receptor, and its association with many autoimmune diseases. RAGE is one of the cell surface receptors which belongs to the immunoglobulin superfamily [13], and it is found in high concentrations in different cells and tissues [14]. The RAGE gene is located on chromosome 6p21.3 in the MHC locus III region and it spans a 1–7-kb 50 flanking area and 11 exons [15].

Various RAGE polymorphisms have been linked to the occurrence of cardio-metabolic syndrome besides vascular complications [16]. Furthermore, the most extensively investigated RAGE polymorphisms, −429 T>C and −374 T>A, are located in the gene's promoter region. Additionally, within the exons, a frequent variant (Gly82Serine) and three unusual modifications have been identified (Thr18Pro, Gly329Ala, and Ala389Gln). Some studies reported that Gly82Ser polymorphism in the RAGE gene is linked with HT. However, other studies did not support these findings. A meta-analysis study was done by Jun Wang, who found no significant relationships between −429 T/C polymorphism and risk of myocardial infarction [16].

Numerous studies have explored the possible relation of RAGE polymorphisms with different diseases. A meta-analysis study was performed by Wenjie Xia et al. to investigate the relation between 82G/S, −374 T/A, and −429 T/C polymorphisms and the occurrence of cancer. This meta-analysis showed that 82G/S polymorphism is related to a marked rise in cancer incidence, while −374 T/A polymorphism is related to a decreased occurrence of cancers [17]. The study of Martens et al. reported that −429 T>C polymorphism is more frequent in systemic lupus erythematosus (SLE) [18]. Nonetheless, Tiszlavicz et al. stated that 374 AA of RAGE gene polymorphism is a protective factor for multiple sclerosis [19, 20]. One study was concerned with RAGE polymorphism and thyroid autoimmunity (HT). To the best of our knowledge, no other studies were concerned with RAGE polymorphism and HT, especially in Egyptian females.

The goal of this study was to define the viable role of oxidative stress levels (plasma MDA and TAC) and RAGE receptor polymorphisms (−374 T>A, −429 T>C, and Gly82Ser) in Egyptian females with HT.

Subject and methods

Study population

Our case–control study was conducted on 80 female patients with recently discovered Hashimoto’s disease attending to internal medicine clinic and Endocrinology Clinic of Zagazig University hospital. Hashimoto's thyroiditis diagnosis is based on anti-thyroid peroxidase antibodies (anti-TPO-AB) >60U/L and antithyroglobulin antibodies (anti-Tg-AB) >180 IU/ML in addition to typical thyroid hypoechogenicity on high-resolution sonography [5]. The control group involved 80 healthy female adults of similar age and sex. All patients were euthyroid, non-pregnant, non-breastfeeding, cardiac, hepatic and renal and other autoimmune diseases were excluded.

Ethical consideration

Consents were obtained from both patients and controls. Moreover, the current study was authorized by the Zagazig University Hospital’s Ethical Committee.
Methods
The study included 2 ml of whole blood and 3 ml plasma samples of 80 euthyroid Egyptian patients who suffered from newly diagnosed HT (Centrifugation of venous blood was done at 1500 rpm for 10 min, and separation and storage of plasma at −80 °C until analysis). Each patient underwent a comprehensive history taking and clinical assessment. The weight and height of patients and controls were measured and the Body Mass Index (BMI) was calculated. PCR–RFLP was used to detect the RAGE (−374 T > A, −429 T > C, and Gly82Ser) polymorphisms in patients and controls.

Biochemical analysis

- Analyses of serum thyroid hormones thyroxine (T4) total, triiodothyronine(T3) total, free triiodothyronine(T3), free thyroxine (T4), and thyroid stimulated hormone(TSH) total values

Serum total T4, total T3, free T4, free T3, and TSH levels were determined by ELISA. Total T4 and total T3 ELISA kits were purchased from BioVendor (RCD025R).
- Antibodies against thyroglobulin (anti-Tg-AB)

Anti-Tg-AB was detected in serum using ELISA kits purchased from Aeskue (Hamburg, Germany).
- Assessment of oxidative status by evaluation of the TAC and plasma MDA

Serum TAC was calorimetrically measured according to Koracevic et al. [21]. The MDA level was measured by the spectrophotometer via the reaction between MDA and Thiobarbituric acid with the pink pigment production. According to Buege and Aust [22], kits were purchased from (Nanjing Jiancheng Bio-engineering Institute, Nanjing, China).

Steps of testing RAGE (−374 T > A, −429 T > C, and Gly82Ser) polymorphisms using PCR–RFLP

Genomic DNA was isolated from whole blood using the commercially available G-spin TM Total DNA Extraction Kit (iNtRON Biotechnology, Seongnam, Korea). The purity and concentration of DNA were determined using a spectrophotometer set at 260 and 280 nm. Then DNA was stored at −20 °C until the following usage. The following primers were used for RAGE (−374 T > A and −429 T > C) polymorphisms forward primer 5′GGGGGCAGTTCTCTCTCTC-3′ and reverse primer 5′-TCAGAGCCCCCGATCCTATTT-3′. Moreover, for Gly82Ser, we used forward primer 5′-CACTGTCTTAGGC CCTGCTTC-3′ and reverse primer 5′-GGAATTCTTT ACGGTAGACACGG-G3′ (BiosourceEurope SA, Belgium, Netherlands, Germany) according to Maria Giannakou et al., 0.2017[20]. Amplification was carried out using the polymerase chain reaction (PCR) in a 25 µL volume containing 25 pmol of each primer. Cycling conditions were initial denaturation of 94 °C for 4 min followed by 40 cycles of 94 °C for 30 s, 58 °C for 30 s, and the final extension occurred at 72 °C for 30 s. Final extension of 72 °C for 10 min using DNA thermal cycler 480 PERKIN ELMER (Norwalk. CT 06,856, USA, Serial No.P16462). Restriction analysis was done overnight at 37 °C on the PCR amplified mix using AluI for the −429 T > C and MfeI for the −374 T > A polymorphisms and AluI (Arthrobacter luteus) (LifeTechnologies) was used for 16 h at 37 °C for Gly82Ser. Separation of the restriction products by 3% agarose electrophoresis (Maxicell, EC 360 M-E-C apparatus Cooperation. St Petersburg Florida USA) and visualization in the ultraviolet (UV) light after staining with ethidium bromide.

Digestion with AluI revealed fragments 344 bp for the −429 T allele (wild type) and 215, 129 bp for the −429C allele. Digestion with MfeI revealed fragments 256, 88 bp for the wildtype allele −374 T and 344 bp for the mutated allele -374A [23]. The polymorphism is discovered to be homozygous for both the CC/TC and AA/TA genotypes (429 CC/TC, 374 AA/TA). Regarding Gly82Ser, a wild type for Gly82 allele (82Gly/Gly) 122, 67, 49 bp bands denote homozygosity for the Ser82 allele (82Ser/82Ser). Bands of 189, 122, 67, 49 bp represent a heterozygote state for the Gly82 and Ser82 alleles (82Gly/Ser).

Statistics analysis
Mean ± standard deviation was used to express all demographic and clinical parameters. To compare quantitative data, Student’s t-test was utilized. The Hardy & Weinberg equilibrium was verified using the Chi-square test. The strength of genetic risk was determined using an odds ratio with a 95% confidence interval between the case and control groups. To compare genotype groups, the unpaired Student’s t-test was used. Statistical significance was well-defined as a two-tailed p-value of (p < 0.05).

Results
The RAGE gene polymorphism was genotyped in 80 female patients with HT and 80 healthy control females. Standard clinical and demographic attributes of HT patients are summarized in Table 1. Age, BMI, blood glucose level, FT3, and FT4 didn’t significantly differ between patients with HT and the control group. There is a statistically measurable difference in TSH levels between HT and the control group (p <0.001). As
Table 1 Demographic data of studied groups

| Parameter          | Cases group N = 80 | Control group N = 80 | P value |
|--------------------|--------------------|----------------------|---------|
| Age (years)        | 35.39 ± 11.81      | 33.56 ± 11.27        | 0.48 (NS) |
| BMI                | 27.24 ± 7.06       | 26.34 ± 7.79         | 0.58 (NS) |
| Systolic BP (mm Hg)| 122.0 ± 11.4       | 118.5 ± 8.7          | 0.03*(S) |
| Diastolic BP (mm Hg)| 77.0 ± 9.20       | 74.1 ± 6.20          | 0.01*(S) |
| Cholesterol (mg/dL)| 192.5 ± 31.9       | 181.6 ± 31.2         | 0.03*(S) |
| Triglyceride (mg/dL)| 120.4 ± 68.0     | 103.3 ± 28.9         | 0.04*(S) |
| HDL-C (mg/dL)      | 56.4 ± 14.4        | 60.0 ± 11.8          | 0.000***(S) |
| LDL-C (mg/dL)      | 107.8 ± 28.2       | 101.5 ± 26.6         | 0.14(NS) |
| ESR (mm/h)         | 14.0 ± 15.4        | 7.86 ± 5.14          | P < 0.000***(S) |

BMI body mass index, BP blood pressure, HDL-C high density lipoprotein-cholesterol, LDL-C low density lipoprotein-cholesterol, ESR erythrocyte sedimentation rate. Significance (S), Non-significance (NS)

All data was expressed as Mean ± standard deviation

P value represent Student’s t-test, Significant differences were found: *p < 0.05; **p < 0.01; ***p < 0.001

Table 2 Clinical characteristics of glucose, thyroid hormone, thyroid antibodies, and oxidative stress parameters in control and Hashimoto’s thyroiditis

| Parameter           | Cases group N = 80 | Control group N = 80 | P value |
|---------------------|--------------------|----------------------|---------|
| Glucose (mg/dl)     | 96.1 ± 12.1        | 96.5 ± 29.3          | 0.93(NS) |
| FT3 (pg/ml)         | 3.37 ± 1.12        | 3.44 ± 0.33          | 0.7 (NS) |
| FT4 (pg/ml)         | 10.33 ± 1.66       | 11.11 ± 2.03         | 0.063 (NS) |
| TSH                 | 17.76 ± 11.03      | 1.2 ± 1.14           | <0.001*(S) |
| anti-Tg-AB (IU/L)   | 380.93 ± 173.02    | 2.7 ± 1.4            | <0.001*(S) |
| MDA (nmol/ml)       | 9.1 ± 1.22         | 1.33 ± 0.06          | <0.001*(S) |
| TAC (nmol/L)        | 1.2 ± 0.21         | 2.6 ± 0.9            | <0.001*(S) |

FT3 (free triiodothyronine), FT4 (free thyroxine), TSH (thyroid-stimulating hormone, Tg (anti-thyroglobulin antibody), MDA (malondialdehyde), TAC total antioxidant capacity

The data is represented as mean ± SD

(NS)non-significant, (S)* Significant values

expected, anti-Tg-AB (IU/L) was considerably increased in HT compared to control (p < 0.001). Additionally, although TAC levels were lower in HT patients than in controls, MDA levels were higher in HT patients than in controls, as shown in Table 2.

The genotype frequencies for RAGE − 429 T > C were consistent with the HWE in controls (P = 0.96) and patients (P = 0.89) respectively. In the patient group, the frequencies of TT, TC, and CC genotypes were 82.5, 12.5, and 5%, respectively. While in the control group, the frequencies were 92.5, 7.5, and 0%, respectively. Chi-square revealed a significant difference regarding the distribution of RAGE (− 429 T > C) genotypes TT and CC between patients and control (X2 = 7.49, P = 0.04). In terms of risks of developing HT, a higher frequency of the CC genotype was significantly related to an increased risk of developing HT (OR = 5.5 (0.259–120.8), 95% confidence interval CI, and P = 0.04). The increased C allele frequencies were significantly correlated with increased risk HT (OR = 3.25 (0.84–12.94) (Table 3).

The genotype frequencies for RAGE − 374 T > A were consistent with the HWE in patients (P = 0.89) and controls (P = 0.96), respectively. The frequencies of the TT, TA, and AA genotypes in the patient group were 52.5, 25, and 22.5%, respectively, and were 27.5, 47.5, and 25%, respectively, in the control group. Chi-square revealed a non-significant difference regarding the distribution of RAGE (− 374 T > C) genotypes TT and AA between patients and control (P = 0.2). In terms of risks of developing HT, the increased AA genotype frequency was non-significantly correlated with an increased risk of HT (OR = 0.47 (0.147–1.5)). The increased A allele frequencies were non-significantly correlated with increased risk HT (OR = 0.57 (0.3–1.06).

The genotype frequencies in Hashimoto’s patients for Gly82Ser (Gly/Gly, Gly/Ser, Ser/Ser) genotypes were 90%, 5%, and 5%, respectively, while in the control group, the frequencies were 95, 5, and 0%, respectively. Chi-square revealed a non-significant difference regarding the distribution of Gly82Ser genotypes Gly/Gly, Gly/Ser, Ser/Ser between patients and control (P = 0.5). In terms of risks of developing HT, the increased Ser/Ser genotype frequency was non-significantly correlated with an increased risk of HT (OR = 0.5 (0.009–4.2)) (Table 4).

Discussion

The main purpose of the current work was to correlate oxidative stress plasma malondialdehyde (MDA) and total antioxidant capacity (TAC) with HT, in addition,
to assess the association of RAGE polymorphisms (−374 T > A and the −429 T > C and Gly82Ser) with HT.

Oxidative stress is a condition that results from either an excess of free radicals production or a deficit of antioxidant defense systems. The pathogenesis of a range of conditions, especially autoimmune diseases, is affected by oxidative damage to molecules [23]. It was suggested that increased oxidative stress is associated with HT. Therefore, in the current investigation, plasma MDA is studied in newly diagnosed HT Egyptian females as a biomarker of oxidative stress and tissue injury. Additionally, TAC measurement is considered the most reliable indicator of antioxidant defense, as plasma TAC evaluation is more useful than individual antioxidant measurement [24]. MDA was significantly higher while TAC was significantly lower in the HT group compared with the control, indicating increased oxidative stress in recently diagnosed HT females. Our results agreed with previous researchers Gerenova J, Erdamar H, and Lassoued et al.[24, 25, 27], who reported the same results; their argument was reinforced by the possibility that HT is a precancerous lesion, as the incidence of carcinoma is increased in HT patients. In addition, HT was found to have proliferating nodules and cytological changes close to papillary thyroid carcinoma [25–27]. Moreover, several studies have discovered significantly higher malondialdehyde, nitrite, and myeloperoxidase levels in patients with overt hypothyroidism caused by HT. Additionally, other researchers discovered that HT patients have much lower glutathione (GSH) levels than healthy controls [28]. To the best of our knowledge, there are few studies evaluating the changes in oxidative balance in a large series of euthyroid HT patients. The majority of current research has focused on oxidative stress in people with Hyperthyroidism who also have thyroid dysfunction as both hypothyroidism and hyperthyroidism have been linked to an increase in oxidative stress [29–31]. From that finding, supplementation of antioxidants has been suggested as a therapeutic agent in Graves’ hyperthyroidism and orbitopathy [32]. However, their therapeutic role in HT is debated [34,35,36 and 37]. Supported by our results, we highly recommend testing the supplementation of

Table 3  Distribution of studied groups according to RAGE (−429 T > C and −374 T > A) and Gly82/Ser: shows a significant difference between HT cases and control regarding distribution of single nucleotide polymorphism (SNP) of −427 T>C gene and shows nonsignificant differences regarding -347>T>A and Gly 82 ser.

| Parameter | Cases group N = 80 | Control group N = 80 | P value | OR |
|-----------|--------------------|----------------------|---------|----|
| RAGE − 429 T > C | TT 66(82.5%) | 74(92.5%) | 0.04* (S) | 1 |
| | TC 10(12.5%) | 6(7.5%) | 1.86 (0.414–8.428) | |
| | CC 4(5%) | 0 (0%) | 5.5 (0.259–120.8) | |
| RAGE − 374 T > A | TT 42 (52.5%) | 22 (27.5%) | 0.2 (NS) | 1 |
| | TA 20(25%) | 38(47.5%) | 0.27 (0.095–0.793) | |
| | AA 18(22.5%) | 20 (25%) | 0.47 (0.147–1.5) | |
| Gly82 /Ser82 RAGE | Gly82/Gly 72(90%) | 76(95%) | 0.5 (NS) | 1 |
| | Gly82/Ser 4(5%) | 4(5%) | 1.05 (0.14–7.8) | |
| | Ser82/Ser 4(5%) | 0 (0%) | 0.52 (0.009–4.2) | |

RAGE Receptor of advanced glycation, OR ODDS RATIO
S*: significant, NS: Non significant

Table 4  Allele frequency for case and control OR ODDS RATIO The increased A allele frequencies was non significantly associated with increased risk HT (OR = 0.57 (0.3–1.06)

| Allele RAGE -429 T > C | Cases group N = 80 | Control group N = 80 | OR (95% CI) |
|------------------------|--------------------|----------------------|-------------|
| T 71(88%) | 77(96%) | 1 |
| C 9(12%) | 3(4%) | 3.25 (0.84–12.94) |
| RAGE −374 T > A | Cases group N = 80 | Control group N = 80 | OR (95% CI) |
| T 52(65%) | 41(51%) | 1 |
| A 28(35%) | 39(49%) | 0.57 (0.3–1.06) |
| Gly82/Ser82 | Cases group N = 80 | Control group N = 80 | OR (95% CI) |
| Gly82 allele 74/80(92.5%) | 78/80(97.5%) | 1 |
| Ser82 allele 6/80(7.5%) | 2/80(2.5) | 0.31 (0.06–1.6) |
antioxidants as therapeutic agents in Egyptian HT euthyroid females.

Recently, we have made considerable advances in our understanding of the genetic risk factors for human autoimmune illnesses. Genetic variations, such as single nucleotide polymorphisms may contribute to the development of HT. Additionally, genome-wide association (GWAS) studies are becoming increasingly prevalent and extensive to create more precise diagnostic and therapeutic solutions for a variety of human illnesses [37].

Our current findings demonstrate a significant correlation between the RAGE system and HT. Egyptian patients only regarding the distribution of the −429 T > C polymorphism, not the −374 T > A polymorphism. Our results did not support a role for the RAGE−374 T > A gene polymorphism in the formation and intensity of HT. However, the RAGE−429 T > C polymorphism was significantly more prevalent in HT Egyptian female groups than in controls. These findings are in agreement with some studies conducted on patients with SLE [38]. Relationships between RAGE polymorphisms and HT may suggest that the RAGE system’s −429 T > C polymorphism is associated with increased inflammation associated with autoimmune thyroid disease. In another study, a meta-analysis conducted by Jun Wang, showed no significant relation between −429 T/C polymorphism and risk of myocardial infarction [39].

Our results reported that the Gly82Ser polymorphism was not associated with HT’s susceptibility. Secondly, we found no significant differences regarding genotyping frequencies or allele frequencies of Gly82Ser amongst HT patients and control. Thus, we could suggest that the Gly82Ser polymorphism is not associated with HT development. The effect of the Gly82Ser polymorphism on receptor function at a supposed N-linked glycosylation site and the AGEs binding site in the same immunoglobulin variable domain are explained by these findings.

To the best of our knowledge, the current study is the first to investigate the association between Gly82Ser polymorphism and HT. Our results are consistent with Yousri M Hussein et al. 2017, who reported that Gly82Ser polymorphism in the RAGE gene is not associated with type 2 diabetes susceptibility or diabetic retinopathy (DR) development in type 2 diabetic subjects. In addition, Gao J X found no association between Gly82Ser polymorphism of the RAGE gene and type 2 diabetes in Chinese patients [40]. However, our work has some limitations because only three polymorphisms were genotyped and linkage disequilibrium analysis was not possible. In terms of genetic variability research, the sample size is quite tiny.

Conclusions

Increased MDA and decreased TAC as an antioxidant may be among the risk factors for HT in individuals carrying the −429 T > C RAGE polymorphism. Additionally, further research is necessary to establish the validity of the existing findings. We recommend that elevated serum MDA and decreased TAC levels in individuals with HT can be a guide for the usage of antioxidant treatment for thyroid function improvement.

Abbreviations

HT: Hashimoto’s thyroiditis; AGE: Advanced glycation end product; RAGE: Receptor of Advanced glycation end product; MDA: Malondialdehyde; TAC: Total antioxidant capacity; RFPLP: Restriction fragment length polymorphism; TPO: Thyroid peroxidase; Tg: Thyroglobulin; AITD: Autoimmune thyroid diseases; ROS: Reactive Oxygen Species; TSH-R: Thyroid-stimulating hormone receptor; PCR: Polymerase chain reaction.

Acknowledgements

Not applicable

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by NMM, and AA. The first draft of the manuscript was written by NMM and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

No funding was delivered from any institution.

Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

An informed consent was taken from patients and control. The study was approved by the Ethical Committee of Zagazig University Hospital.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent to publish

In the present study, all the testing procedures were performed using non-invasive techniques and adhering to the conditions of the ethical approval committee of the institute. Agreement with written knowledgeable consent was gained from the participant.

Competing interests

The authors have no conflict of interests to report.

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Received: 17 November 2021 Accepted: 22 May 2022

Published online: 31 May 2022
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