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Association of BLK and BANK1 gene polymorphisms with systemic lupus erythematous in Egyptian patients

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Abstract

This study examined the genotype, allelic frequencies (polymorphisms) in a sample of Egyptian patients and examined the relationship between disease activity in systemic lupus erythematosus (SLE) and the B lymphoid tyrosine kinase (BLK) and B-cell scaffold protein with ankyrin repeats 1 (BANK1) gene. This case control study involved 70 SLE patients and 40 subjects matched for age and sex as a control group. Clinical data were gathered from each participant, including SLE-related clinical activity indicators. Utilizing the restriction fragment length polymorphism (RFLP)-polymerase chain reaction (PCR), the single nucleotide polymorphisms (SNPs) BLK rs13277113G/A and BANK1 rs10516487G/A were assessed. The most prevalent genotype among the study participants was BLK rs13277113; G/G (57.1%), and BANK rs10516487; GG/ genotype (74.3%). There were no substantial variations in the incidence of genotype and allelic polymorphism between patients and controls (p>0.05). In the studied SLE patients, however, there was no significant association between both alleles (BANK rs10516487 gene alleles, G/G and G/A & BLK rs13277113, G/A, G/G &A/A) and the SLE disease activity score. While there was a significant association between BLK rs13277113 genotype alleles and age. In conclusion, BLK rs13277113 G/A and BANK1 rs10516487 G/A alleles showed no difference between SLE Egyptian patients compared to controls with no link between both genes and SLE disease activity.

Keywords: Single nucleotide polymorphisms, BLK, BANK1, Systemic lupus erythematous, RFLP-PCR.

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Introduction

Systemic lupus erythematous (SLE) is an autoimmune illness characterized by heterogeneous systemic manifestations, formation of autoantibodies, involvement of environmental and complex genetic

components with no clear mendelian pattern of inheritance.[1]

The discovery of numerous genetic risk factors for SLE which are essential to the immune system's operation has increased our knowledge about disease's etiology. The recently discovered SLE-associated loci have shed light on the underlying genetic alterations.[2] Furthermore, we have been able to delve into the immune system pathways affected by these genes and pinpoint the symptoms triggered by these loci.[2]

SLE-associated variants affect signaling and regulatory pathways involved in many processes including apoptosis, DNA degradation and removal of immune complexes containing nuclear antigens. Additionally, SLE risk variants may have an impact on activation of pathways including type I interferon, Toll-like receptor activation, and nuclear factor kappa κB.[3] The majority of SLE susceptibility loci are common variations that have been verified across many ancestries, indicating shared pathways in disease causation.[3]

New susceptibility genes for SLE were identified including B Cell Scaffold Protein with Ankyrin Repeats 1 (BANK1). BANK1 encodes a scaffold protein which is unique for B cells development and activation. Its expression stimulates B cell receptor-induced calcium mobilization from intracellular calcium reserves.[4] BANK1 polymorphisms may be involved in dysregulation of the B cell receptor, which is a common feature found in SLE patients.[5]

B-cell receptor signaling, and development are also regulated by the Src family of nonreceptor tyrosine kinase, which is encoded by the B-lymphoid tyrosine kinase (BLK) gene. The disease related BLK haplotype is associated with significant low expression of BLK transcript in B lymphoblastoid cell lines, suggesting that lower production of BLK in human B cells confers risk for autoimmunity.[5]

It has been shown that BLK interacts with BANK1, an adaptor/scaffold protein that is mainly produced in B cells like other protein tyrosine kinases in the Src kinase family, as a result, these proteins have a significant impact on gene expression, protein function, and B-cell signaling and activation.[6] Although different BLK and BANK1 single-nucleotide variants have been examined in patients with SLE, the results are uncertain because a report showed an association with this disease,[7] while another report found no such associations.[8] However, the association of these gens and SLE has been scarcely evaluated in the Egyptian population. Thus, we aimed to investigate whether BANK1 and BLK polymorphisms could be replicated in SLE Egyptian patients and whether there is any relationship between these polymorphisms and disease activity.

Subjects and Methods

This was a case control study which involved 70 SLE patients fulfilling the SLE International Collaborating Clinics (SLICC) 2012 standards for SLE classification.[9] The participants were recruited from the outpatient rheumatology clinic of the Department of Internal Medicine at Al-Zahraa University Hospital during the period from December 2022 to May 2023. In addition, 40 subjects, matched for age and sex were included as a control group,

Detailed history and clinical examination were done for each patient, and the Systemic Lupus Erythematosus Disease Activity Index (SLEDI) score was used to determine the SLE disease activity, SLEDI > 6 points were defined as active disease.[10] Patients with other autoimmune diseases such as rheumatoid arthritis, Behcet's disease, myasthenia gravis, bronchial asthma, or Type I diabetes, chronic liver disease, malignancy, or those receiving biologic therapy were excluded from this study.

A venous blood sample (approximately 10 ml) was collected from each patient and control. Blood was aliquoted into ethylene diamine tetra-acetic acid (EDTA) tubes. A blood portion was used for assessment of erythrocyte sedimentation rate (ESR) mm/hr, using the Westergren method. Another blood portion was stored at -20°C for further DNA extraction. A final blood aliquot was used to separate sera. C-reactive protein (CRP) was determined by a turbidimetric assay kits (Lot Number 19420, Biosystems kit, Medical Expo, Spain), according to the manufacturer's instructions.

Laboratory measurements were performed for all participants. Serum urea and creatinine were determined by commercial kits (Biosystems S.A), Spain), according to the manufacturer's instructions. Serum albumin by commercial kits (Cat No. ALB103, Randox, United States), according to the manufacturer's instructions. Autoimmune markers (Anti-nuclear antibodies (ANA), the anti-double stranded DNA (anti-dsDNA) by commercial kits (Cat No. 2500 ANA HEp-2, Bio RAD, USA), according to the manufacturer's instructions. Complement (C3 and C4) by commercial kits (Thermo Fisher Scientific, USA), according to the manufacturer's instructions.

Assessment of BANK1 and BLK polymorphisms: DNA from the blood samples of patients and controls was extracted using commercial kits (Lot Number 00459333, Gene Jet whole blood Genomic DNA purification Mini Kit #K0781, Thermo Scientific Co. Ltd.'s, USA), according to the manufacturer's instructions. The concentration of the extracted DNA was determined by spectrophotometry using a photometer (Nano Drop spectrophotometer, Thermo Fisher, USA) and then stored at −70°C till used.

The TaqMan® single nucleotide polymorphisms (SNP) genotyping assay

To perform the PCR amplifications, we prepared a 5 µL reaction mixture using 2.5 µL of TaqMan Master Mix (Thermo Scientific), 0.0625 µL of a 40X probe, 2.435 µL of DNase-free water, and 50 ng of DNA, and 10 picomole of each primer. Following the manufacturer's protocol, two sets of primers (BLK and BANK1) were constructed using the Thermo Fisher's TaqMan® SNP genotyping assay, the primer sequences are reported in Table (1). Amplifications were carried out in a programmable thermal cycler (Biometra, Germany) using the following conditions: an initial denaturation step at 95°C, followed by 45 cycles, each of a denaturation at 95°C for 15 seconds, and annealing/extension at 60°C for 1 minute. Genotyping was performed with Step One™ and Step One Plus™ Real-Time PCR System Software version 2.3 (USA).

For Restriction Fragment Length Polymorphism (PCR-RFLP), PCR-RFLP, two restriction enzymes (Fast Digest BLK (rs13277113) and BANK1 (rs10516487), Thermo Scientific, USA) were used to digest the PCR products. Each enzyme was produced in two separate tubes. Tubes containing BLK and BANK1 restriction endonucleases were incubated at 37°C for 2 hours, with a negative control consisting of all reagents except the restriction enzyme. The reaction volume was 30 µL, consisting of 10 µL PCR products, 1 µL restriction enzyme, 5 µL buffer, and 14 µL sterile water. The digested products were then electrophoresed on a 2% agarose gel containing ethidium bromide and visualized using UV light.

Statistical Analysis

The Statistical Package of Social Science (SPSS) application for Windows (version 21) was utilized to analyze the data. The normality of the data was originally checked utilizing the Kolmogorov-Smirnov test. The terms used to describe qualitative data were number and percentage. When the projected cell count was less than 5, the Fischer exact test (FET) and the Monte Carlo test were utilized. The Chi-square test was utilized to explore relationships between categorical variables. Continuous variables with appropriately distributed data were given as mean± standard deviation (±SD), and non-normal data as median (min-max). The two study groups were compared utilizing the student t test for regular data and the Mann Whitney test for non-normal data. ANOVA (parametric test) and Kruskil-Wallis (non-parametric) tests were utilized to compare more than two groups. A p value <0.05 was considered significant.

Table 1. Primer sequence of BLK and BANK1.

Gene	Primer sequence	Cat.no.
BLK	AAGATTAAACACTTATCAGATCATT**[G/A]**TCTGCTTTTGGTTTTTCTAGTACCC	4351379
BANK1	AAAAGGGAAGCCATCCTGTTATATC**[G/A]**CTTGGAGAATTTCTCTTTTCGGCA	4351379

Results

The descriptive data for patients and control groups are summarized in Table 2. The median age of the SLE patients and controls were 35.40±7.09 and 31.95±6.16 years, respectively. The patients group included 63 (91%) female and 7 (9%) males, while the control group included 2 (5.0%) males and 38 (95.0%) females. The median duration of the disease was 6 years. Among the SLE patients, 64.1% had active disease, while 35.9% had inactive disease, with a mean SLEDI score of 6, ranging from 1 to 18.

The frequency of predominant clinical manifestations in the SLE patients were arthritis (82.9%), fever (68.6%), lupus nephritis (42.9%), and rash (37%) (Table 2).

Table 2. Description of demographic and clinical manifestations data of the studied groups.

	SLE patients No.=70	Control No.=40
Age (years, mean±SD)	35.40±7.09	31.95±6.16
Sex No. (%)		
Female	63 (91%)	38 (95.0%)
Male	7 (9%)	2 (5.0%)
Disease Duration (years)		
Median (Min-Max)	6 (1-16)	-
Disease activity No. (%)		
Active	45 (64.1%)	-
Inactive	25 (35.9%)	-
SLEDI		
Median (Min-Max)	6 (1-18)	-
Clinical manifestation No. (%)		
Lupus nephritis	29 (42.9%)	
Arthritis	57 (82.9%)	
Vasculitis	4 (5.7%)	
Cerebritis	2 (2.9%)	
Fever	47 (68.6%)	
Pleurisy	19 (28.6%)	
Leucopenia	10 (14.3%)	
Rash	26 (37.1%)	
Psychosis	4 (5.7%)	
Thrombocytopenia	10 (14.3%)	
Pericardial effusion	4 (5.7%)	
Alopecia	29 (42.9%)	
Seizures	6 (8.6%)	

The genotypic distribution of BANK rs10516487 and BLK rs13277113 SNPs in the SLE patients and control group were examined in this study. The most common genotype (codominant) BANK rs10516487 in the studied Egyptian SLE patients was G/G (74.3%) followed by G/A (25%). The dominant genotype for BLK rs13277113 in the SLE studied group was (G/G and G/A) (94.2%).

The genotypic and allelic frequencies of BLK rs13277113 were higher in SLE patients compared to the control group. The most frequent allele was G/G (57.1%) followed by G/A (37 %) and A/A alleles (5.7%) (Table 3). In

contrast, in the control group the allele frequencies were 55%, 45%, and 0% for G/G, G/A, and A/A, respectively. For BANK rs10516487, the G/G allele was less frequent in SLE patients (74.3%) compared to controls (80%), while the G/A allele was more frequent in SLE patients (25%) compared to controls (20%) as shown in Table 3.The most common genotype in the investigated patients were BLK rs13277113; G/G (57.1%) and BANK rs10516487 ; G/G (74.3%) (Figure 1 and 2).

Table 3. BANK rs10516487 and BLK rs13277113 genotype among systemic lupus erythematosus (SLE) and control groups.

	SLE group No.=70	Control group No.=40	*p* value
BANK rs10516487			
G/A	18 (25%)	8 (20%)	*NS
G/G	52 (74.3%)	32 (80%)	
BLK rs13277113			
G/A	26 (37.1%)	18 (45%)	**NS
G/G	40 (57.1%)	22 (55%)	
A/A	4 (5.7%)	0 (0%)	

*χ^2: Chi square test, **MC: Monte Carlo test. $p > 0.05$ is not significant (NS).

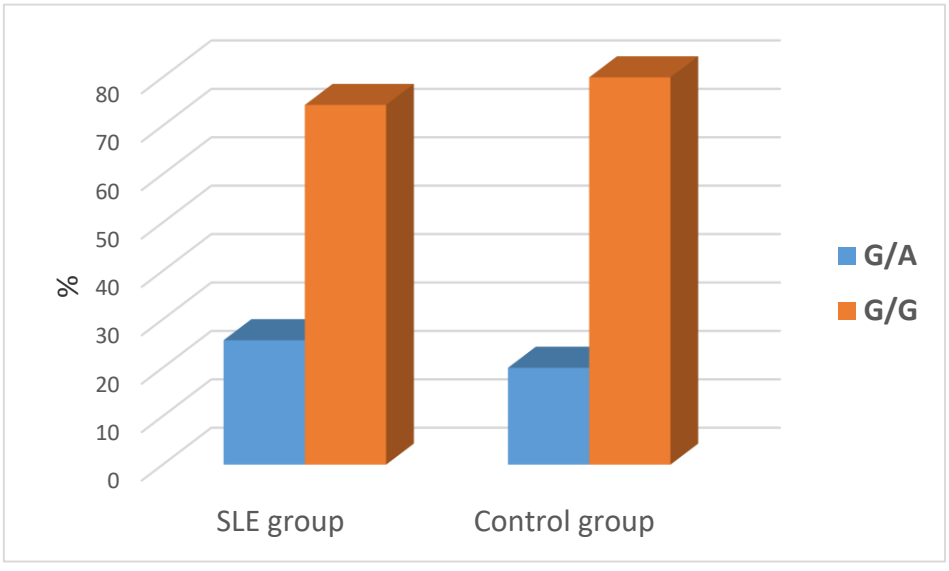

Figure 1. BANK rs10516487 genotype among systemic lupus erythematosus (SLE) and control groups.

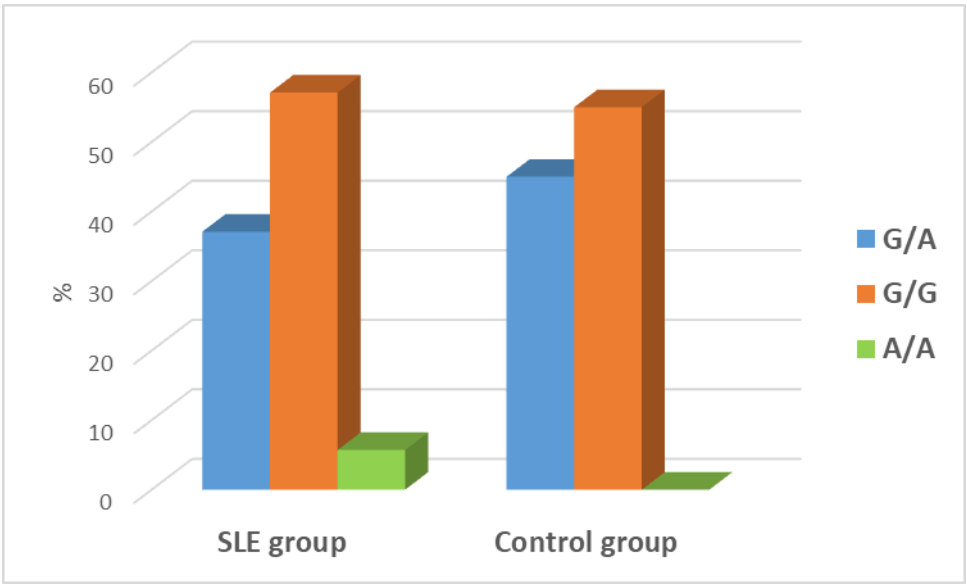

Figure 2. BLK rs13277113 genotype among systemic lupus erythematosus (SLE) and control groups.

Both BANK rs10516487 gene alleles, G/G and G/A which were found in the studied SLE patients showed no difference between both alleles and disease activity score (*p*=0.590), (Table 4 and Figure 1). The G/G allele was more frequent in both active and inactive groups (*p*= 0.911)**.** Many of the clinical findings in SLE patients' group including lupus nephritis, arthritis, fever, rash, and pleurisy were associated with G/G allele of BANK rs10516487, Table 4.

The A/A allele of BLK rs13277113 was more prevalent in younger SLE patients (*p*=0.005), while both G/A and G/G were detected in female patients (*p*= 0.624) and in patients with long disease duration (p=0.136). The G/A and G/G alleles were present in both active and inactive patients without significant difference in disease activity score (SLEDI) (*p*= 0.646). The G/G allele was more frequently detected than the other two alleles (G/A, and A/A) with the most presented clinical findings (Table 5).

Table 4. Association between BANK rs10516487 genotype and systemic lupus erythematosus (SLE) clinical findings.

	BANK rs10516487		*p* value
	G/A	G/G	
Age (years) Mean ± SD	31.55±6.69	36.73±6.85	^{t}NS
Sex Males Females	 4 (22.2 %) 14 (77.8%)	 2 (3.8%) 50 (96.2%)	^{FET}NS
Duration of disease	4 (2-15)	6 (1-16)	^{Z}NS
SELDI	5 (0.00-18)	6 (0.00-18)	^{Z}NS
Activity Active Inactive	 5 (55.6%) 4 (44.4%)	 15 (57.7%) 11 (42.3%)	χ^2NS

Table 4. Continued.

	BANK rs10516487		*p* value
	G/A	G/G	
Clinical manifestations			
Lupus nephritis	3 (33.3%)	12 (46.2%)	χ^2NS
Arthritis	7 (77.8%)	22 (84.6%)	^{FET}NS
Vasculitis	1 (11.1%)	1 (3.8%)	^{FET}NS
Cerebritis	0 (0 %)	1 (3.8%)	^{FET}NS
Fever	6 (66.7%)	18 (69.2%)	χ^2NS
Pleurisy	0 (0%)	10 (38.5%)	$\chi^2$0.028
Leucopenia	2 (22.2%)	3 (11.5%)	^{FET}**NS**
Rash	4 (44.4%)	9 (34.6%)	χ^2NS
Psychosis	1 (11.1%)	1 (3.8%)	^{FET}NS
Thrombocytopenia	1 (11.1%)	4 (15.4%)	^{FET}NS
Pericardial effusion	1 (11.1%)	1 (3.8%)	^{FET}NS
Alopecia	4 (44.4%)	11 (42.3%)	χ^2NS
Seizures	1 (11.1%)	2 (7.7%)	^{FET}NS

Frequency of BLK rs13277113 genotype alleles (G/A, G/G, A/A), $p > 0.05$ is not significant (NS).
χ2: Chi square test, Fisher's Exact Test (FET). Z: Z test.

Table 5. Association between BLK rs13277113 genotype and systemic lupus erythematosus (SLE) patient's characteristics.

	BLK rs13277113			*p* value
	G/A	G/G	A/A	
Age (years) Mean ± SD	32.15±6.20	38.40±6.21	26.50±6.36	^{F}0.005
Sex				
Male	2 (15.4%)	1 (5.0%)	0 (0%)	^{MC}NS
Female	11 (84.6%)	19 (95.0%)	2 (100%)	
Duration of disease	5 (2- 11)	7 (1- 16)	4.5 (4- 5)	^{KW}NS
SELDI	6 (0.0- 18)	5.5 (0.0- 18)	4 (0.0- 8)	^{KW}NS
Activity				
Active	7(53.8%)	12(60.0%)	1(50.0%)	^{MC}NS
Inactive	6(46.2%)	8(40.0%)	1(50.0%)	
Clinical manifestations				
Lupus nephritis	4(30.8%)	10(50.0%)	1 (50.0%)	^{MC}NS
Arthritis	11(84.6%)	16(80.0%)	2 (100 %)	^{MC}NS
Vasculitis	1(7.7%)	1(5.0%)	0 (0%)	^{MC}NS
Cerebritis	0(0 %)	1(5.0%)	0 (0 %)	^{MC}NS
Fever	10(76.9%)	13(65.0%)	1 (50.0%)	^{MC}NS
Pleurisy	3(23.1%)	6(30.0%)	1 (50.0%)	^{MC}NS
Leucopenia	2(15.4%)	3(15.0%)	0(0 %)	^{MC}NS
Rash	2(15.4%)	10(50.0%)	1(50.0%)	^{MC}NS
Psychosis	0 (0%)	2 (10.0%)	0 (0%)	^{MC}NS
Thrombocytopenia	4(30.8%)	1(5.0%)	0 (0 %)	^{MC}NS
Pericardial effusion	1(7.7%)	1(5.0%)	0 (0%)	^{MC}NS
Alopecia	6(46.2%)	8(40.0%)	1 (50.0%)	^{MC}NS
Seizures	1(7.7%)	2(10.0%)	0 (0%)	^{MC}NS

$p > 0.05$ is not significant (NS). MC: Monte Carlo test. KW:-Kruskil-Wallis test, F: Fischer exact test.

Discussion

In the present study we investigated whether BANK1 and BLK polymorphisms could be replicated in SLE Egyptian patients and to determine any relationship between these polymorphisms and disease activity. In our study, single nucleotide polymorphisms (SNPs) in BANK1 and BLK were shown to be associated with SLE in Egyptian patients, this association between these variant genes and SLE was observed in other population studies as well.[11,12] In the present study, the predominant genotype (codominant) for BANK rs10516487 was G/G (74.3%) followed by G/A (25%). These findings were consistent with a previous research study.[13] However, we did not find a relation between the GG haplotype containing the two main alleles and susceptibility to SLE. Nevertheless, we found that the AA haplotype containing the BANK1 rs10516487A decreases the risk for SLE. We found a link between SLE susceptibility and the GA haplotype bearing the rs10516487G major allele. It has been hypothesized that rs10516487 could potentially alter the affinity of BANK1 for inositol 1,4,5-trisphosphate receptor, altering B cell signaling and thus increasing the susceptibility to SLE.[14]

Both alleles of the BANK1 rs10516487 gene, G/G and G/A, showed no significant differences with respect to age and gender within the SLE patient group (p=0.058, p=0.090 respectively). In addition, there was no difference detected between both alleles and the disease activity score in our study, this may indicate that these two variants may confer risk for SLE per se with no impact on disease activity or severity. The G/G allele was more frequent in both active and inactive SLE patients than G/A allele. The current study found a relation between BLK and BANK1 SNPs with many clinical characteristics of SLE. Hence, there might be a link between the BLK rs2736340T/C-rs13277113A/G and BANK1 rs10516487G/A-rs3733197G/A polymorphisms and disease severity.[5] Upregulated mRNA expression of BANK1 was detected in renal biopsies from lupus nephritis patients, suggesting a role for B cells in SLE pathogenesis and its related clinical manifestations.[15]

The BLK rs13277113A/G genotypic and allelic frequencies were associated with susceptibility to SLE disease in a study from China, specifically involving patients from Hong Kong, and Thailand.[16] However, our study as well as a study involved a Mexican cohort did not validate this link.[17] Such variation in the results may be related to the difference in sample size and ethnicities, some of the disease susceptibility loci operate in some ethnic groups, but not all.[16]

In the present study, the most frequent allele in SLE patients was G/G (57.1%) followed by G/A (37 %) and A/A alleles (5.7%), compared to control group (55% and 45% and 0 %, respectively). In contrast to our results, a study by Pamuk, et al., 2017, showed that the relative expression of the BLK gene was considerably lower in the SLE group (0.52 times, 95% CI: 0.19-0.85) compared to controls. When compared to controls, SLE patients were more likely to have the heterozygous genotypic pattern (GA) for the rs13277113 polymorphism (48.8 vs. 31.4%, for control, p= 0.035).[18] The discrepancy between those earlier results and ours may be explained by different geographical regions.

One of the strengths of this study is the presence of clinical data of the patients to correlate with the BLK and BANK1 variants. However, the limitations are the small sample size, and it was a single-center study performed with no external funding. In conclusion, based on our study findings, there is insufficient evidence to establish a link between the BLK rs13277113 and BANK1 rs10516487 variations and SLE.

Author Contributions

HAAA, RAM, AMT; Conceptualization. AMT, OAE, HAAA, NS; Methodology. AAA; Software. AAA; Validation. NS; Investigation. SE, OAE; Data Collection. HAAA, RAM, AAA; Writing. HAAA. RAM; Original Draft Preparation. RAM. and HAAA; Writing, Reviewing & Editing. HAAA; Supervision. SME; Project Administration. All authors; Funding Acquisition.

Declaration of Conflicting Interests

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Ethical approval

The protocol of the study was reviewed and approved by the Research Ethics Committee, Faculty of Medicine for Girls, Al-Azhar University, (approval dated 8 September 2021, study ID: 979).).

Informed consent

Each patient and control subject provided a written informed consent before being included in the study.

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