Original Article

Association between Hypertension with and without Left Ventricular Hypertrophy and the Expression of a Panel of microRNAs in an Iranian Population

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Citation: Hajsadeghi S, Nekouian R, Mollababaei M, Motevalli H, Kalantar E, Iranpour A, et al. Association between Hypertension with and without Left Ventricular Hypertrophy and the Expression of a Panel of microRNAs in an Iranian Population. Res Heart Yield Transl Med 2025; 20(1): 21-28.

https://doi.org/10.18502/jthc.v20i1.19218

Highlights

- Left ventricular hypertrophy (LVH) is a critical independent predictor of cardiovascular mortality and is strongly associated with hypertension.
- Given the diagnostic potential of microRNAs (miRNAs) as biomarkers in hypertension, this study aimed to investigate their expression profiles.
- The findings underscore the significant involvement of miR-222 and miR-155 in hypertension and LVH pathogenesis.

Article info:

Received: 29 Dec. 2023
Revised: 20 Sep. 2024
Accepted: 6 Dec. 2024

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ABSTRACT

Introduction: Given the established role of microRNAs (miRNAs) in hypertension (HTN), this study aimed to analyze the expression of a defined miRNA panel in hypertensive individuals, both with and without left ventricular hypertrophy (LVH), compared with normotensive controls within an Iranian population.

Methods: We conducted a cross-sectional study with case-control sampling, comprising three study groups: controls, HTN, and HTN+LVH. Through an extensive literature review, we selected a panel of eight miRNAs (miR-1, miR-21, miR-29a, miR-29b, miR-133, miR-155, miR-221, and miR-222) for analysis using real-time PCR. Gene expression data were analyzed through a general linear model implemented in R programming.

Results: The study analyzed 100 total samples. We used miR-29a and miR-133 as endogenous controls for calculations. Analysis revealed no significant association between miR-29b or miR-221 expression and the study groups ($P>0.2$ for both). Additionally, miR-1 demonstrated downregulation in both hypertensive groups ($P<0.05$), although this effect lost significance after adjusting for potential confounders ($P=0.05–0.2$). The HTN+LVH group showed significant downregulation of miR-21 ($P<0.05$). The duration of HTN diagnosis correlated with the upregulated expression of both miR-155 and miR-222. The strongest association emerged for miR-222, with the study groups explaining 25.7% of its variation (the highest R^2 value among all models).

Conclusions: HTN without LVH might be associated with the downregulation of miR-1 and miR-155 and the upregulation of miR-222. HTN, along with LVH, might be associated with the downregulation of miR-1 and miR-21 and the upregulation of miR-222. Increasing years of experiencing HTN were correlated with the upregulation of miR-155 and miR-222. The largest effect size was for miR-222.

Keywords: Molecular Epidemiology; Genetic Epidemiology; Personalized Medicine

Introduction

Hypertension (HTN) represents a major global mortality risk and a significant contributor to heart disease, stroke, and kidney failure.

As blood pressure elevation often remains asymptomatic; its control is crucial for preventing these complications.[1] Among cardiovascular disorders associated with HTN, left ventricular hypertrophy (LVH), frequently detected via ECG in hypertensive patients,[2] stands out as an independent predictor of cardiac mortality strongly linked to elevated blood pressure.[3]

Genetic studies have established the heritable nature of LVH, with family and twin studies demonstrating its familial predisposition.[4,5] Subsequent research has explored genetic loci and molecular pathways underlying LVH development.[6] Nonetheless, a 2012 review emphasized that its primary genetic determinants remained largely unknown and underscored the need for further investigation.[7] Beyond DNA-level mechanisms, microRNAs (miRNAs) have emerged as key regulators in cardiovascular pathophysiology. These molecules exert documented effects on vascular endothelium, smooth muscle function, the renin-angiotensin-aldosterone system, and other mechanisms central to HTN pathogenesis.[8]

Considering the critical role of biomarkers in HTN management and the established influence of ethnic variability, conducting a biomarker investigation in the Iranian population became essential. Current gaps in knowledge highlight the need for population-specific biomarker profiles to advance personalized medicine approaches. Building on the demonstrated significance of miRNAs in cardiovascular pathophysiology and through comprehensive reviews of literature-reported expression patterns, we selected a panel of eight miRNAs for analysis: miR-1, miR-21, miR-29a, miR-29b, miR-133, miR-155, miR-221, and miR-222.

The present study aimed to examine the effects of HTN and LVH on expression changes within this miRNA panel. Given that LVH is an independent mortality risk factor in patients with HTN, identifying LVH-associated miRNAs could yield valuable diagnostic biomarkers for high-risk individuals. Such biomarkers could inform clinical strategies, including earlier treatment initiation or targeted use of anti-hypertrophic medications.

Methods

Study design

The current cross-sectional study was conducted in accordance with the STrengthening the Reporting of OBservational studies in Epidemiology – Molecular Epidemiology (STROBE-ME) statement, which is an extension of the STROBE statement.[9] The expression of miRNAs was considered a biomarker.

Biological samples

Venous blood was drawn from the subjects using a syringe under sterile conditions and the supervision of a physician. Approximately 3 to 5 mL of peripheral blood was collected from each patient in tubes containing anticoagulants. The plasma was then separated using a centrifuge and transferred to RNAse/DNAse-free tubes. The samples were stored at −70 °C until RNA extraction.

Following the protocol of the miRNA extraction kit (Norgen Biotek Corp., Canada), all miRNAs from the plasma were extracted. To ensure the absence of DNA contamination and phenol or thiocyanate, we measured the optical density (OD) at A260/A280 and A260/A230 using a spectrophotometer, with an acceptable absorbance lower than 1.8. Complementary DNA (cDNA) was synthesized using a cDNA synthesis kit (Norgen Biotek Corp., Canada) via the stem-loop method. Reverse transcriptase polymerase chain reaction (RT-PCR) was performed on the purified cDNA using the SYBR Green master mix (Norgen Biotek Corp., Canada).

Biomarker characteristics

The biomarkers analyzed in this study consisted of total serum miRNAs, with a specific focus on

miR-1, miR-21, miR-29a, miR-29b, miR-133, miR-155, miR-221, and miR-222.

Setting

Patient recruitment occurred from 2018 through 2022 following public announcements targeting hypertensive individuals. As the first investigation of its kind in Iran, this study was designed as a pilot investigation.

Participants

The sampling method employed was quota sampling. Cases were collected through announcements. The inclusion criteria included having HTN (patients receiving antihypertensive medication or those who, during two separate blood pressure measurements conducted correctly in the clinic, had a systolic blood pressure [SBP]≥140 mm Hg or diastolic blood pressure [DBP]≥90 mm Hg) and being between 40 and 60 years of age. The exclusion criteria included diabetes, obesity (body mass index [BMI]≥30), smoking, alcohol consumption, preexisting heart failure or other cardiac diseases, pregnancy, preexisting kidney disease (creatinine>1.5 mg/dL or proteinuria), hypo- or hyperthyroidism, obstructive sleep apnea, and the use of corticosteroids or oral contraceptives. In addition, a control group consisting of non-hypertensive individuals who did not meet the exclusion criteria was selected from the hospital's non-medical staff and included in the study.

After entering the study, participants were interviewed, and background information, including age, sex, race, and disease duration, was collected. All hypertensive patients underwent echocardiography to investigate LVH. Ultimately, the study groups were categorized as control, HTN, and HTN+LVH.

Variables

The outcome variables were the logarithmic expression levels of miRNAs. A logarithmic scale was employed to normalize the data and mitigate skewness. The main independent variable was the study groups, treated as a categorical variable, with the control group serving as the reference value. The demographic variables included age, sex, BMI, years since the diagnosis of HTN (variable name: HTN diagnosis), smoking status, SBP, DBP, and left ventricular ejection fraction (LVEF).

Data source/measurement

Gene expression was quantified using the -ΔCT method, calculated as CT (target) - CT (control). For control CT determination, we sorted all miRNA CT values by increasing variance and selected endogenous controls based on minimal variance across samples and variance inflation factor (VIF) > 2 (additional selection criterion).[10]

Bias

The most significant source of selection bias in this study was the use of non-probability sampling methodology.

Study size

As a pilot study, 100 individuals were included based on parameters from the study by Kontaraki et al.[11] Using four cyclic thresholds (CTs) as the standard deviation (SD) based on the researcher's archival gene expression data, and with a two-tailed significance level of 0.05, the a priori power calculations demonstrated statistical power of 0.236 for detecting mean differences of 1 CT (power=0.236), 2 CTs (power=0.697), 3 CTs (power=0.960), and 4 CTs (power=0.999).

Statistical Analysis

Descriptive statistics were reported as mean (SD) for normally distributed data and median (interquartile range) for non-normal distributions. Group comparisons of demographic variables employed Pearson chi-square tests, Fisher exact tests, or one-way analysis of variance (ANOVA) as appropriate. Missing CT values were imputed using the largest observed CT value when the missingness was unrelated to sample quality issues.

Gene expression analysis utilized a general linear model with ΔCT values (ΔΔCT) serving as β

coefficients, reported with their standard errors. Fold change (FC) was calculated as $2^{-\text{beta coefficient}} = 2^{-\Delta\Delta CT}$. All analyses were conducted using R version 4.0.0 (R Foundation for Statistical Computing), with statistical significance defined as a P-value<0.05.

Results

Participants

A total of 100 individuals were selected for this cross-sectional study. According to our case-control sampling, 20 cases were healthy controls from hospital personnel, while 80 cases of HTN were selected, composed of 60 cases without LVH and 20 cases with LVH. Eligible cases were directly selected, and there was no loss of individuals or biological samples.

Descriptive and demographic data

The mean age of the participants was 51.01±12.90 years: 34.75±7.08 years in the control group, 54.15±11.20 years in the HTN group, and 57.85±8.33 years in the HTN+LVH group (P<0.001). The cohort included 63 males and 37 females, with male representation of 60% (control), 61.7% (HTN), and 70% (HTN+LVH) (P=0.762). The mean BMI was 26.65±4.53 kg/m^2: 25.06±2.37 kg/m^2 in the control group, 27.82±5.32 kg/m^2 in the HTN group, and 24.75±1.59 kg/m^2 in the HTN+LVH group (P=0.006). The median and interquartile range of HTN diagnosis were 5 years and 2–9 years, respectively. Smoking status (19 total smokers) did not differ significantly between the groups (P=0.680). The mean SBP was 118.85±8.41 mm Hg in the control group, 142.38±13.48 mm Hg in the HTN group, and 145.2±5.32 mm Hg in the HTN+LVH group (P<0.001). The mean DBP was 74.35±9.10 mm Hg in the control group, 89.87±11.47 mm Hg in the HTN group, and 87.9±3.86 mm Hg in the HTN+LVH group (P<0.001). The median and interquartile range of LVEF were 55% years and 55–60% years, respectively.

Biomarker distributions

According to the VIF analysis, miR-29a and miR-133 had VIFs of 3.01 and 2.17, respectively. Further, the CT of miR-29a had the first rank of the least SD (3.047), and also the CT of miR-133 had the third rank of the least SD (3.391) among the miRNAs (range:3.243–5.343). Therefore, the mean value of miR-29a and miR-133 CTs was considered a calibrator for the calculation of ΔCTs.

Outcome data and main results

A general linear model was employed to analyze the effects of study groups on ΔCT values and the proportion of ΔCT variation explained by study groups.

The model specified study groups as a categorical factor with the control group as the reference. Potential confounders (age, sex, BMI, HTN duration, smoking status, and SBP) were incorporated into multivariable modeling using backward stepwise elimination (Wald test, retention threshold P>0.20). Both unadjusted and adjusted results are presented.

Accordingly, miR-1 demonstrated downregulation in both HTN and HTN+LVH groups (P<0.05), although this effect became nonsignificant after adjustment for age and smoking (P=0.05–0.20). Moreover, miR-21 showed significant downregulation specifically in the HTN+LVH group (FC=0.101; 95% CI, 0.016 to 0.625; P=0.015), with no confounding variables identified. No significant changes were observed for miR-29b (P>0.20). Additionally, miR-155 exhibited significant downregulation in the HTN group (P<0.05) in unadjusted analysis; nevertheless, multivariable modeling revealed that each additional year post-HT diagnosis was associated with 1.217-fold increased expression (P =0.015; adjusted for age and SBP). Furthermore, miR-221 showed no significant differential expression (P>0.20). According to our results, miR-222 displayed strong upregulation in both HTN (FC=64.40; 95% CI, 15.30 to 271.10) and HTN+LVH (FC=49.80; 95% CI, 8.57 to 289.26) groups (P<0.001), with adjusted analysis showing that each year post-HT diagnosis correlated with 1.124-fold increased expression (P = 0.006; adjusted for age and sex). All results are summarized in (Figure 1).

Other analyses

A correlation matrix was constructed for ΔCT values across all miRNAs, revealing the strongest positive correlation between miR-155 and miR-221 (r=0.67). The second strongest correlation occurred between miR-1 and miR-155 (r=0.49). These associations were observed independently of the calibrator miRNAs (miR-29a and miR-133) (Figure 1).

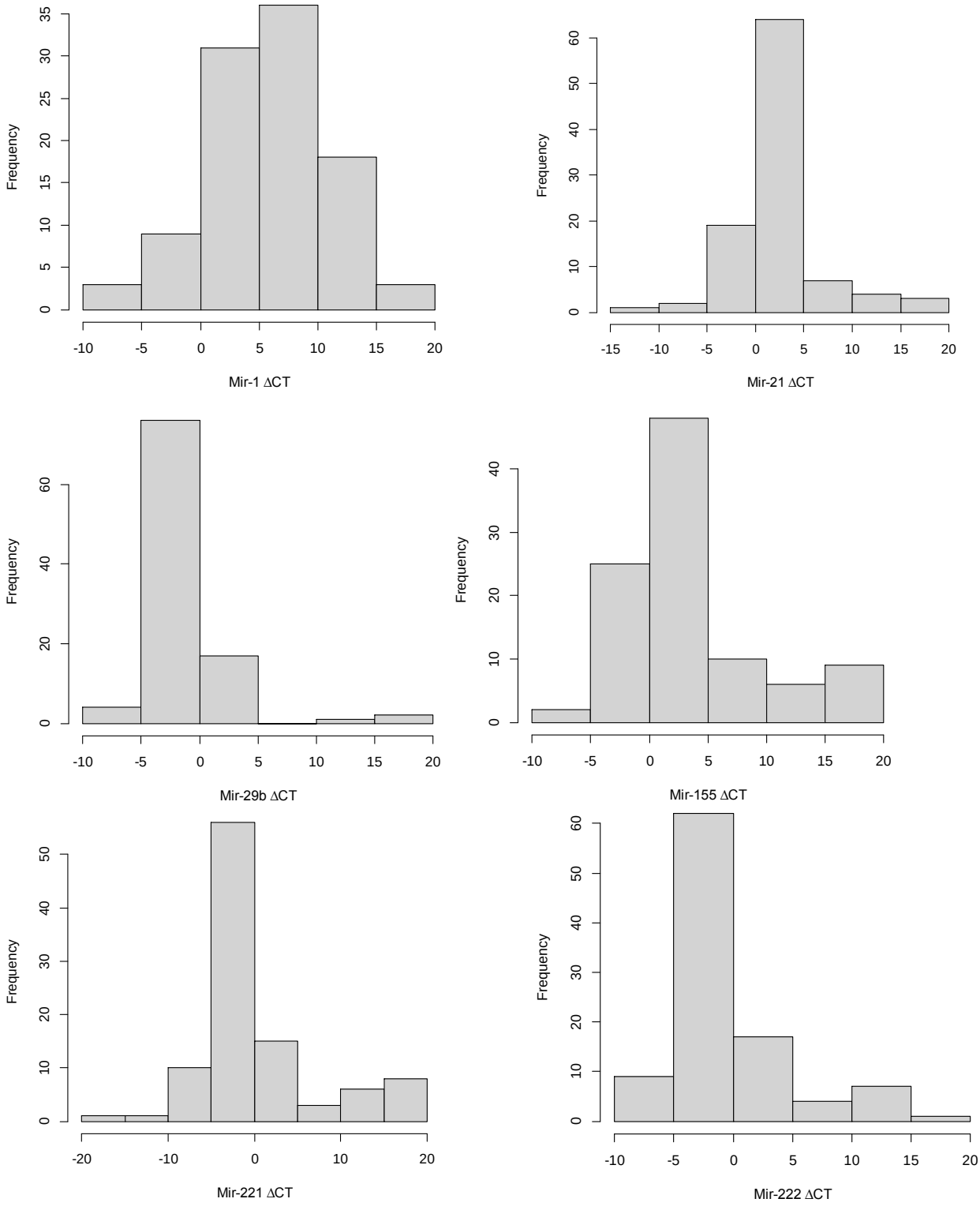

Figure 1. The image depicts the correlation matrix plot for the delta cycle threshold (ΔCT) values of the studied microRNAs

Discussion

Key results

The present gene expression study investigated the effects of HTN and LVH on miRNA expression patterns. Our analysis revealed that miR-29b and miR-221 showed no significant association with either study group. While miR-1 demonstrated downregulation in both hypertensive groups (HTN and HTN+LVH), this effect became nonsignificant after adjustment for potential confounders. We observed specific downregulation of miR-21 in the HTN+LVH group, with no confounding factors identified. A significant positive correlation was observed between the duration of HTN diagnosis and increased expression of both miR-155 and miR-222. The association was particularly strong for miR-222, with the study groups accounting for 25.7% of its expression variation (R^2=0.257), representing the highest explanatory power among all analyzed models.

Limitations

The cross-sectional design was the principal limitation, as the biomarkers did not have temporal precedence over the outcomes. Consequently, the interpretation should emphasize the role of HTN and LVH on the miRNAs, rather than the reverse. Non-probability sampling and the diverse demographic characteristics of the control group posed additional limitations. Still, multivariable modeling allowed us to adjust for potential confounding effects.

Several technical limitations should also be acknowledged. One was the lack of biomarker detection at the specified threshold in some miRNAs for certain individuals. Since this high CT was not attributable to sample quality issues, the missing data were replaced with the highest achieved CT. Another technical limitation of this study was the absence of a housekeeping gene, which has been a persistent challenge in miRNA studies. However, an endogenous control was established based on the observed variances.

A statistical limitation of this study was the small sample size, which resulted in diminished statistical power for small effect sizes and limited the generalizability of the findings.

Interpretation

Genetic biomarkers may be associated with the concurrent presence of LVH and HTN. Xin et al. [12] (2009) examined three genetic variants of the *NOS3* gene (endothelial nitric oxide synthase 3), namely, T786C (rs2070744), eNOS4a/b, and +G894T (rs1799983), in hypertensive individuals with and without LVH. Their findings indicated that homozygosity for the G894T variant (E298D) in *NOS3* was a risk factor for LVH in patients with HTN.

Wang et al.[13] (2010) investigated the relationship between three genetic variants of *GDF15* (growth differentiation factor 15) and LVH in patients with HTN. In multivariable analysis, the 3148G polymorphism was significantly associated with decreased LV end-systolic or diastolic diameter, as well as reduced LV mass and indexed mass.

In the current study, although some miRNAs exhibited significant changes in the HTN+LVH group compared with the control group, there was insufficient statistical power to differentiate between the HTN and HTN+LVH groups.

Shen et al.[14] (2014) investigated the role of the protein osteoprotegerin (OPG) and its encoding gene, *OPG*, in LVH development among hypertensive patients. Their findings revealed that serum OPG levels and the *OPG* 1181 G>C polymorphism were significantly and independently associated with the occurrence of LVH in this patient population.

In addition to genetic association studies, research has also focused on miRNAs.

Kontaraki et al.[11] (2015) examined the expression of several miRNAs in 102 hypertensive patients and 30 healthy individuals. Compared with the control group, patients with HTN exhibited lower expression levels of miR-26b and miR-133a, and higher expression levels of miR-499, miR-208b, miR-1, and miR-21. Furthermore, in hypertensive patients, miR-1 and miR-133a showed a significant negative correlation with LVH, whereas miR-208b, miR-26b, miR-499, and miR-21 demonstrated a significant positive correlation

with LVH. The present study yielded different results for miR-1 and miR-21. Chiming with our current investigation, the study by Kontaraki and colleagues did not observe the temporal precedence of miRNA expression relative to disease development.

Regarding the establishment of miRNA-based diagnostic or therapeutic targets, a 2010 review by Feidler et al. [15] indicated that normalizing miRNA expression through miRNA-targeting therapies could play a significant role in the future treatment of cardiovascular diseases. Be that as it may, the present study did not observe the temporal precedence of miRNA alterations relative to disease onset. Therefore, the research methodology employed in this study was consistent with the investigative focus highlighted in the aforementioned review.

Concerning miR-21, Düzgün et al.[16] (2023) demonstrated in a Turkish population that pulmonary arterial HTN was associated with the downregulation of circulatory miR-21. Conversely, Chang et al.[17] (2022), studying a Taiwanese population and a mouse model, found that miR-21 upregulation was associated with pressure-induced cardiac hypertrophy in aged hearts. These conflicting findings for miR-21 may be attributable to methodological differences, such as the choice of internal controls. For instance, the present study employed miR-29a and miR-133 as internal controls. Apropos of miR-221, its upregulation has been reported to be associated with pulmonary arterial HTN, an effect linked to its induction by hypoxia.[18]

Conclusion

In the present study of an Iranian population, using miR-29a and miR-133 as internal controls, HTN without LVH appeared to be associated with the downregulation of miR-1 and miR-155, and the upregulation of miR-222. Similarly, HTN coexisting with LVH seemed to be associated with the downregulation of miR-1 and miR-21, and the upregulation of miR-222. Nonetheless, after accounting for confounding factors, some of these associations did not retain statistical significance. A longer duration of HTN correlated with the upregulation of miR-155 and miR-222, with miR-222 exhibiting the largest effect size.

The current study lacked sufficient statistical power to definitively differentiate the specific impact of LVH on miRNA expression in hypertensive patients. Future case-control and cohort studies are recommended to establish the temporal relationship between miRNA expression and disease development. Furthermore, the influence of LVH warrants additional investigation.

Declarations:
Ethical Approval

We complied with the ethics of research involving human subjects as well as the Helsinki Declaration in the design and conduct of our research (Ethical code: IR.IUMS.REC 1396.32154). Informed consent was obtained from all participantas.

Funding

According to the authors, this article has no financial support.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

We extend our gratitude to the Rasool Akram Medical Complex for its assistance with human sample collection. The authors also wish to express their appreciation to Dr. Maryam Ghavidel Darestani for her valuable contributions and insightful comments regarding the genetic aspects of this study.

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