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Original Research Article

Association Between Interleukin 9 Gene Polymorphism and Serum Interleukin 9 Levels in Type 2 Diabetes Mellitus Patients in a Tertiary Care Hospital of Chhattisgarh

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Abstract:

Background: Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder often complicated by systemic inflammation. Interleukin 9 (IL-9), a cytokine with immune-modulatory functions, has recently been implicated in T2DM pathogenesis through its influence on inflammatory pathways.

Objective: This study aimed to investigate the association between IL-9 gene polymorphisms and serum IL-9 concentrations in patients with T2DM to elucidate potential genetic and inflammatory contributions to disease severity.

Methods: A cross-sectional observational study involving 160 clinically diagnosed T2DM patients at Pt. JNM Medical College, Raipur, was conducted. Genomic DNA was extracted from peripheral blood using the Qiagen QIAamp DNA Blood Mini Kit. Polymerase chain reaction (PCR) was performed with custom-designed primers to genotype IL-9 polymorphisms. Serum IL-9 levels were quantified by ELISA, and fasting blood glucose was measured by the glucose oxidase-peroxidase method. Data were analyzed statistically to assess correlations.

Results: The most prevalent genotype was AA (45%), followed by AG (42.5%) and GG (12.5%). Serum IL-9 and fasting glucose levels were significantly elevated in GG genotype carriers compared to AA and AG groups ($p < 0.001$).

Conclusion: The AA genotype predominates in this population, but the GG genotype is associated with increased IL-9 expression and worse glycemic control, suggesting IL-9 polymorphisms influence inflammatory and metabolic dysregulation in T2DM.

Keywords: Type 2 diabetes mellitus, Interleukin 9, Gene polymorphism, PCR, ELISA.

Introduction

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder characterized by persistent hyperglycemia due to insulin resistance and pancreatic β-cell dysfunction [1]. Chronic low-grade inflammation is recognized as a key contributor to the onset and progression of T2DM. Cytokines secreted by immune cells mediate this inflammatory response, impacting insulin signaling and β-cell survival. Among these cytokines, Interleukin 9 (IL-9) has gained attention for its multifaceted role in immune regulation, inflammation, and tissue homeostasis [2,3].

IL-9 is mainly produced by T helper 9 (Th9) cells and exerts effects on various immune cells, including mast cells, T cells, and macrophages. Genetic polymorphisms in the IL-9 gene can modulate its expression, thereby influencing cytokine-mediated inflammatory pathways relevant to T2DM pathophysiology [4,5]. Previous studies have shown associations between cytokine gene variants and susceptibility to metabolic diseases, but data on IL-9 polymorphisms remain limited, especially in the Indian population, which exhibits genetic heterogeneity and a high prevalence of diabetes [6,7].

This study investigates the distribution of IL-9 gene polymorphisms in T2DM patients from Chhattisgarh, correlates these genotypes with serum IL-9 levels and glycemic parameters, and aims to clarify IL-9's role as a biomarker and potential therapeutic target in diabetes management.

Materials and Methods

Study Design and Setting: A cross-sectional observational study was conducted at Pt. Jawaharlal Nehru Memorial Medical College, Raipur, over one year. Ethical approval was obtained from the institutional ethics committee, and written informed consent was collected from all participants.

Sample Size and Participants: The study enrolled 160 patients diagnosed with T2DM based on American Diabetes Association criteria. Patients aged 30 years and above were included. Those with type 1 diabetes, gestational diabetes, acute infections, autoimmune disorders, or on immunosuppressants were excluded to reduce confounding inflammatory factors.

Gender-wise Distribution: The study cohort comprised 92 males (57.5%) and 68 females (42.5%).

Gender	Number	Percentage (%)
Male	92	57.5
Female	68	42.5
Total	160	100

DNA Extraction: Peripheral venous blood (5 mL) was collected aseptically in EDTA tubes. Genomic DNA was extracted using the Qiagen QIAamp DNA Blood Mini Kit following the manufacturer's protocol. This kit employs silica-membrane technology to yield high purity DNA suitable for downstream PCR applications. DNA quality and quantity were assessed by spectrophotometry (260/280 nm ratio).

Primer Design and PCR Amplification: Primers targeting the IL-9 gene polymorphic region were designed based on the NCBI reference sequence using Primer3 software. Primers were synthesized commercially by a certified oligonucleotide provider.

- Forward primer: 5'-TGT TGG TGG AGA GAA TGC TG-3'
- Reverse primer: 5'-GCA CAG GAA GGA TGA GGT TG-3'

PCR amplification was carried out in a 25 µL reaction mixture containing 50 ng genomic DNA, 10 pmol of each primer, dNTPs, Taq DNA polymerase, $MgCl_2$, and buffer. Cycling conditions included an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 seconds, annealing at 58°C for 30 seconds, extension at 72°C for 45 seconds, and a final extension at 72°C for 7 minutes.

The PCR products were subjected to restriction fragment length polymorphism (RFLP) analysis using the enzyme that cuts at the polymorphic site to differentiate AA, AG, and GG genotypes.

Serum IL-9 Measurement: Serum was separated by centrifugation and stored at -80°C until analysis. IL-9 concentrations were measured using a commercially available ELISA kit according to the manufacturer's instructions. All samples and standards were assayed in duplicate. The assay sensitivity was 2 pg/mL.

Blood Sugar Measurement: Fasting blood glucose was measured by the glucose oxidase-peroxidase method using an automated analyzer calibrated daily for accuracy.

Statistical Analysis: Data were analyzed using SPSS version 25. Continuous variables were presented as mean ± standard deviation (SD). Group comparisons were made using one-way ANOVA and post hoc Tukey tests. Correlations between serum IL-9 and fasting blood sugar were analyzed by Pearson's correlation. A p-value <0.05 was considered statistically significant.

Results

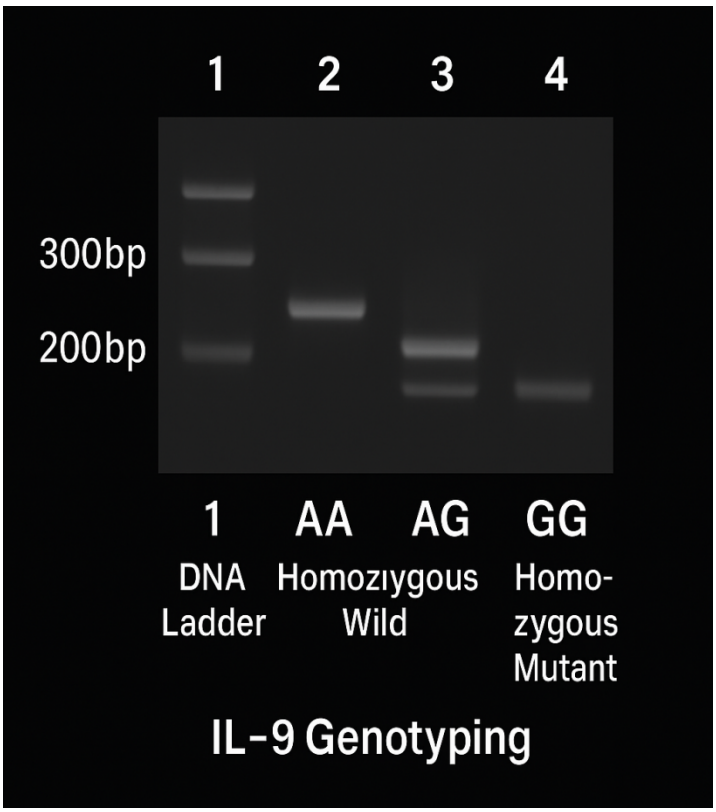
Demographic and Clinical Characteristics: The mean age of participants was 52.8 ± 9.4 years. Males accounted for 57.5% of the study population.

Genotype Distribution and Serum IL-9 Levels: Genotyping identified three IL-9 polymorphisms:

Genotype	Number of Patients	Percentage (%)	Mean Serum IL-9 (pg/mL) ± SD	Mean Fasting Blood Sugar (mg/dL) ± SD
AA	72	45.0	10.8 ± 3.9	140 ± 25
AG	68	42.5	13.1 ± 4.2	155 ± 30
GG	20	12.5	17.5 ± 5.0	175 ± 35
Total	160	100	—	—

Statistical analysis demonstrated significant differences in serum IL-9 and fasting blood sugar levels across genotypes ($p < 0.001$), with the GG genotype showing the highest levels.

Correlation Analysis: A positive correlation was observed between serum IL-9 concentration and fasting blood glucose ($r = 0.41$, $p < 0.01$), indicating that higher IL-9 levels associate with poorer glycemic control.



Electrophoresis Gel Image

- **Lane 1:** DNA Ladder (100 bp)
- **Lane 2:** Homozygous Wild-type (AA) — single band at 300 bp
- **Lane 3:** Heterozygous (AG) — bands at 300 bp and 200 bp
- **Lane 4:** Homozygous Mutant (GG) — single band at 200 bp
- **Lane 5:** Negative Control

Discussion

This study elucidates the role of IL-9 gene polymorphisms in modulating serum IL-9 concentrations and glycemic status among T2DM patients. The AA genotype was predominant, consistent with other Indian cohorts, suggesting this as the wild-type allele in this population.

Significantly elevated IL-9 levels in GG genotype carriers imply that this polymorphism increases IL-9 expression, potentially augmenting inflammatory processes that exacerbate insulin resistance and hyperglycemia. The positive correlation between IL-9 and fasting glucose supports this inflammatory-metabolic link.

The use of the Qiagen DNA extraction kit ensured the isolation of high-quality DNA critical for reliable PCR amplification. The primers designed specifically for the IL-9 gene polymorphism provided clear differentiation of genotypes via PCR-RFLP, confirmed by gel electrophoresis banding patterns [8,9].

Our findings align with previous reports associating cytokine gene variants with diabetes susceptibility and progression [10,11]. However, limitations include the cross-sectional design and relatively small sample size, warranting longitudinal studies with functional assays to validate causality [12].

Conclusion

The AA genotype of the IL-9 gene polymorphism is most common among T2DM patients in this region. However, the GG genotype correlates with increased serum IL-9 levels and higher fasting blood glucose, highlighting its potential impact on diabetes pathogenesis via inflammatory mechanisms. These results suggest that IL-9 gene polymorphisms could serve as biomarkers and therapeutic targets in T2DM management.

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