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Original Paper

Association of IL4 (Rs2243250) Gene Variant and Mycoplasma Pneumoniae Infection with Asthma Susceptibility in an Iraqi Population

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Key Words
Interleukin-4 • Rs2243250 • Single Nucleotide Polymorphism • Asthma • Mycoplasma pneumoniae

Abstract
Background/Aims: Asthma is a multifactorial disease influenced by both genetic and environmental factors. This study aimed to investigate the association between the IL4 gene polymorphism (rs2243250) and asthma susceptibility, along with serum IL-4 levels. Additionally, it explored *Mycoplasma pneumoniae* infection as a potential risk factor for asthma. ***Methods:*** A total of 118 individuals were enrolled, including 60 asthma patients and 58 healthy controls. Genotyping for IL4 rs2243250 was performed using allele-specific PCR (AS-PCR). Previous *Mycoplasma pneumoniae* infection was assessed serologically, and serum IL-4 levels were measured using ELISA. ***Results:*** No significant differences were observed between groups in terms of age, sex, or residence. Smoking (OR: 7.85, P = 0.001) and family history of asthma (OR: 5.33, P = 0.004) were identified as significant risk factors. *Mycoplasma pneumoniae* infection was significantly more prevalent in asthma patients (41.7%) than in controls, with a strong association with asthma risk (OR: 8.75, P < 0.0001). Genotype frequencies of rs2243250 differed significantly: CC (36.7% vs. 68.9%), CT (41.7% vs. 24.2%), and TT (21.6% vs. 6.9%) in patients versus controls, respectively. The T allele was more frequent among patients (42.5%) than controls (18.97%), increasing asthma risk (OR: 3.16, P = 0.0001). Both CT (OR: 3.25) and TT (OR: 5.91) genotypes were strongly associated with asthma. Moreover, individuals with the TT genotype had significantly higher serum IL-4 levels (P < 0.001). ***Conclusion:*** The IL4

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rs2243250 polymorphism is associated with increased asthma susceptibility and elevated serum IL-4 levels in the Iraqi population. *Mycoplasma pneumoniae* infection also appears to be a significant contributing factor. Larger-scale studies are warranted to confirm these findings and further explore the role of this infection in asthma pathogenesis.

Introduction

Asthma is a prevalent, complex, and chronic inflammatory disease that affects individuals of all ages and imposes a considerable burden on healthcare systems [1]. It is characterized by variable airflow limitation and persistent airway inflammation, manifesting as intermittent respiratory symptoms such as wheezing, shortness of breath, chest tightness, and coughing [2]. Globally, asthma affects approximately 300 million people, resulting in an estimated 250, 000 deaths annually [3] .By 2025, this number is projected to rise to 400 million cases [4]. In Iraq, asthma accounts for approximately 200, 000 hospitalizations or emergency department visits each year. Neighboring countries report prevalence rates of 5.6% in Saudi Arabia and 8.5% in Kuwait [5, 6].

Asthma arises from a combination of genetic susceptibility to atopy and increased bronchial responsiveness to environmental stimuli. This pathophysiological process involves the activation of airway cells—including eosinophils, T cells, mast cells, macrophages, neutrophils, epithelial cells, fibroblasts, and bronchial smooth muscle cells—which release various pro-inflammatory cytokines and mediators [7, 8]. Numerous risk factors contribute to asthma development, including genetic predisposition, maternal smoking during pregnancy, prenatal nutrition, antibiotic use, mode of delivery, family size and birth order, exposure to environmental smoke, socioeconomic status, viral infections [9], and certain occupational exposures [10].

The etiology of asthma is multifactorial, involving genetic, environmental, and immunological components. Infections caused by *Mycoplasma pneumoniae* (Mp), a pathogen commonly associated with pneumonia and bronchitis, have been implicated in the initiation and exacerbation of asthma, particularly in genetically predisposed individuals [11, 12].

Interleukins 4 (IL-4) and 13 (IL-13) are key mediators in type 2 asthma, although their roles are complex. IL-4 promotes T-helper 2 (Th2) cell differentiation, immunoglobulin class switching, and eosinophil recruitment. IL-13 acts synergistically with IL-4 to enhance IgE production, induce nitric oxide synthesis, and drive airway remodeling involving goblet cells, fibroblasts, and smooth muscle cells. Both cytokines activate shared signaling pathways by binding to receptor complexes containing the IL-4Rα subunit, triggering receptor dimerization and downstream pathological effects [13].

Asthma's clinical presentation, severity, and treatment response vary widely, partly due to underlying genetic polymorphisms. Single nucleotide polymorphisms (SNPs), in particular, contribute to this variability. Understanding these genetic predispositions is crucial for improving asthma management. Advances in asthma genetics have enhanced our knowledge of the disease's pathogenesis, diagnosis, and potential therapeutic targets [14].

Among the genes implicated in asthma susceptibility, IL-4 has been extensively studied. However, findings regarding the association between IL-4 gene polymorphisms and asthma risk remain inconsistent [15, 16]. The IL-4 gene is located on chromosome 5q31–q33 [17], and its promoter variant C-590T (rs2243250) has been suggested to increase gene expression, elevate plasma IgE levels, and worsen asthma symptoms.

This study aims to investigate the association between the IL-4 rs2243250 polymorphism and asthma susceptibility and to evaluate its impact on serum IL-4 levels in individuals with allergic asthma. Additionally, we explore the potential role of prior *Mycoplasma pneumoniae* infection as a contributing risk factor for asthma.

Materials and Methods

Study Design and Participants

This case-control study was conducted from January to August 2021 at the Chest and Respiratory Disease Center in Al-Hillah, Babylon, Iraq. A total of 60 patients with clinically diagnosed asthma (20 males and 40 females), aged 20–64 years, were recruited. The diagnosis was confirmed by consultant pulmonologists according to internationally accepted criteria. The control group consisted of 58 age- and sex-matched healthy individuals (25 males and 33 females) with no history of asthma, chronic respiratory disease, autoimmune conditions, or recent respiratory infections. Controls were recruited from the general population and hospital staff after completing a health screening questionnaire. Participants who had received immunosuppressive therapy or antibiotics within the previous month were excluded from both groups. The sample size was determined based on the availability of eligible participants during the study period, as well as by referencing similar previous studies in the field.

Blood and Serum Collection

Five milliliters of venous blood were collected from each participant under aseptic conditions. Of this, three milliliters were placed in plain tubes for serum separation, while two milliliters were collected in EDTA tubes for DNA extraction. All samples were stored at –20 °C until analysis to preserve integrity and ensure reliable laboratory results.

Measurement of IL-4 Cytokine

Serum levels of interleukin-4 (IL-4) were measured using an enzyme-linked immunosorbent assay (ELISA) with a commercial kit (Diaclone, France), following the manufacturers protocol.

Detection of Previous Mycoplasma pneumoniae Infection

To evaluate prior exposure to *Mycoplasma pneumoniae*, serum IgG levels were assessed using the Mycoplasma pneumoniae IgG Human ELISA Kit (Abcam, UK). According to the manufacturer's guidelines, samples with IgG concentrations above 10 U/mL were considered seropositive, indicating past infection.

SNP Genotyping

Genomic DNA was extracted from whole blood using the Qiagen DNA extraction kit, following the manufacturer's protocol. DNA concentration and purity were assessed by measuring absorbance at 260/280 nm using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA). DNA samples were stored at –20 °C until genotyping analysis.

Allele-specific PCR (AS-PCR) was used to detect the IL-4 gene polymorphism rs2243250, with primers designed according to [18] as detailed in Table 1 .The AS-PCR protocol followed the method described by [19]. Each 25 µL PCR reaction contained 12.5 µL of Master Mix (Promega, USA), 1 µL of each primer, 30 ng of genomic DNA, and nuclease-free water. PCR amplification was performed using a Bio-Rad thermal cycler under the following conditions: initial denaturation at 95 °C for 2 minutes, followed by 30 cycles of denaturation at 95 °C for 30 seconds, annealing at 65 °C for 50 seconds, and extension at 72 °C for 30 seconds, with a final extension at 72 °C for 5 minutes. Amplified products were visualized on a 1.5% agarose gel (Promega, USA) stained with ethidium bromide.

Statistical Analysis

Serum cytokine levels are presented as mean ± standard deviation (SD). Data normality was assessed before conducting one-way ANOVA to compare group means. Genotype and allele frequencies were calculated by direct counting, and deviations from Hardy–Weinberg equilibrium (HWE) were evaluated using Pearson's chi-squared test. Genotype distributions are expressed as percentages. Associations between SNP genotypes and

Table 1. Primers used in the study

Gene	SNPs	Primer sequence 5′ to 3′	Amplicon(bp)	Reference
IL-4 accession numbers: 3565 GRCh38.p14 (NC_000005.10)	rs2243250 (C>T)	Forward Primer (Wild type) CACCTA AAC TTG GGA GAA CAT TGT C	248	[18]
		Forward Primer (Mutant type) CACCTA AAC TTG GGA GAA CAT TGT T		
		Common Reverse Primer GAA TTT GTT AGT AAT GCA GTC CTC C		

asthma risk were analyzed using odds ratios (ORs) with 95% confidence intervals (CIs). A p-value < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 23, WinPepi version 11.65, and an online HWE calculator.

Results

Demographic Characteristics and Risk Factors Associated with Bronchial Asthma

The study included 60 patients diagnosed with bronchial asthma and 58 healthy controls. Demographic data are presented in Table 2. The mean age did not differ significantly between the patient group (40.36 ± 11.10 years) and the control group (42.02 ± 10.53 years; $P = 0.642$). Among patients, 20 (33.3%) were male and 40 (66.7%) were female, while the control group comprised 25 males (43.1%) and 33 females (56.9%). No statistically significant differences were found between the groups in terms of age ($P = 0.642$), gender ($P = 0.274$), or place of residence ($P = 0.474$), indicating proper matching for a case-control design. However, smoking and a positive family history of asthma were identified as significant risk factors. Smokers were 7.85 times more likely to develop asthma (OR: 2.17–28.44; $P = 0.001$), and individuals with a family history of asthma were 5.33 times more likely to be affected (OR: 1.67–17.03; $P = 0.004$) as shown in Table 3.

The association between prior Mycoplasma pneumoniae infection and disease status was assessed in both patients and controls, as shown in Fig. 1. The figure demonstrates that M. pneumoniae infection was significantly more prevalent among patients (25/60, 41.7%) compared to controls (8/58, 13.8%). The calculated odds ratio was 8.75 (95% CI: 3.53–21.64; p < 0.0001; Table 4), indicating a strong and statistically significant association between *M. pneumoniae* infection and the patient group.

Association of IL-4 Gene Single-Nucleotide Polymorphism (rs2243250 C>T) with Susceptibility to Bronchial Asthma

Genotypic and allelic frequencies were analyzed to assess the association between the IL-4 rs2243250 polymorphism and susceptibility to bronchial asthma. The genotype distribution among healthy controls was in accordance with Hardy-Weinberg equilibrium ($P > 0.05$), as presented in Table 5. A significant difference in genotype frequencies was observed between the control group (CC: 68.9%, CT: 24.2%, TT: 6.9%) and the bronchial asthma group (CC: 36.7%, CT: 41.7%, TT: 21.6%) (Figure 2). The T allele was significantly

Table 2. Demographic Characteristics of Bronchial Asthmatic Patients and Controls. n: number of cases; SD: standard deviation; †: independent samples t-test; ¥: Chi-square test; NS: not significant at P > 0.05; S: significant at P < 0.05

Characteristic	Patients (n = 60)	Control (n = 58)	P-value
Age (years)			
Mean ± SD	40.36 ± 11.10	42.02 ± 10.53	0.642† (NS)
Gender, n (%)			
Male	20 (33.3%)	25 (43.1%)	0.274¥ (NS)
Female	40 (66.7%)	33 (56.9%)	
Residence, n (%)			
Urban	37 (61.7%)	32 (55.2%)	0.474¥ (NS)
Rural	23 (38.3%)	26 (44.8%)	

Table 3. Risk Factors Associated with Bronchial Asthma. OR: Odd ratio;CI: Confidence interval

Risk Factor	Patients		Controls		OR (95% CI)	P-value
	Yes	No	Yes	No		
Smoking, n (%)	18 (30%)	42 (70%)	3 (5.2%)	55 (94.8%)	7.85 (2.17–28.44)	0.001 (S)*
Family History of Asthma, n (%)	17 (28.3%)	43(71.7%)	4 (6.9%)	54 (93.1%)	5.33 (1.67–17.03)	0.004 (S)*

more frequent in asthma patients (42.5%) compared to controls (18.97%) (OR = 3.16, *P* = 0.0001) (Fig. 3, Table 5). Both the CT (OR: 3.25; 95% CI: 1.41–7.49; *P* = 0.005) and TT (OR: 5.91; 95% CI: 1.72–20.33; *P* = 0.004) genotypes were significantly associated with an increased risk of bronchial asthma.

Fig. 1. Distribution of Previous Infection of M.pneumoniae among study groups.

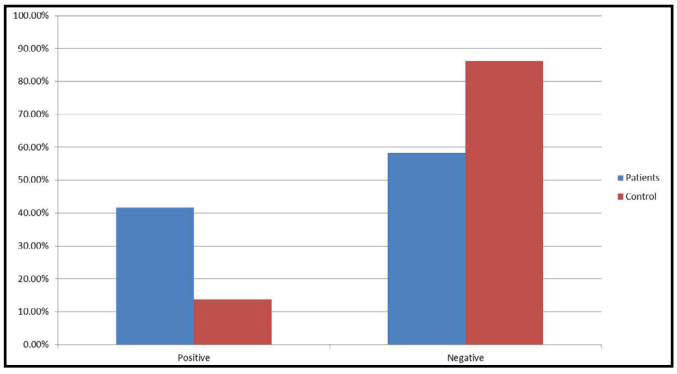


Table 4. Association of Prior M.pneumoniae Infection with Asthma Risk

Groups study	Positive	Negative	OR	(95% CI)	P-value
Patients (n = 60)	25 (41.7%)	35(58.3%)	8.75	(3.53 - 21.64)	< 0.0001*S
Control (n = 58)	8 (13.8%)	50(86.2%)			

Fig. 2. Genotype frequency among study groups.

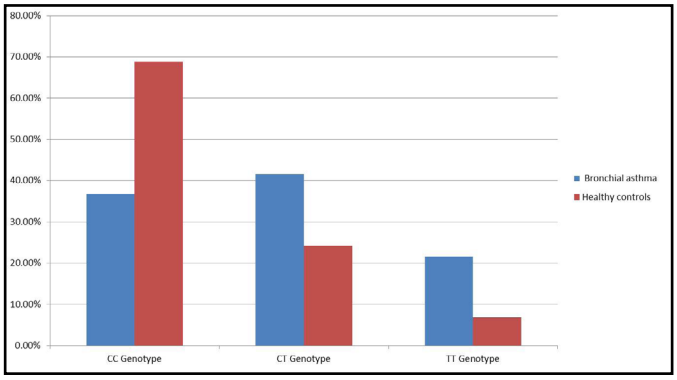
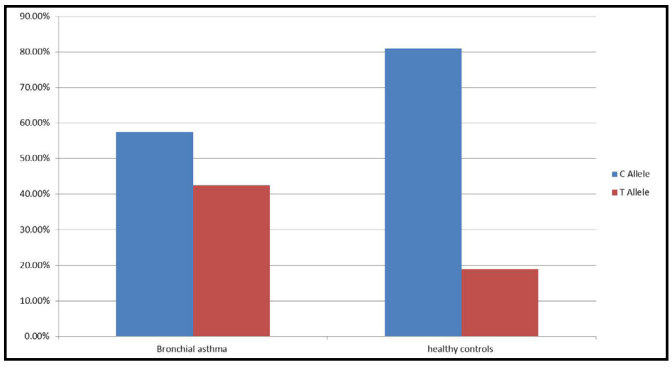


Fig. 3. Wild type and Mutant allele frequency among study groups.



Impact of IL-4 (rs2243250 C>T) Gene Polymorphisms on Serum Levels of IL-4

The association between the IL-4 gene polymorphism—specifically the rs2243250 variant, characterized by a C>T substitution—and serum IL-4 levels in patients with bronchial asthma is presented in Fig. 4. This figure illustrates how this genetic variation may influence IL-4 concentrations in the serum of affected individuals. The analysis demonstrated a statistically significant effect of the IL-4 (rs2243250) polymorphism on serum IL-4 levels ($P < 0.001$). Patients with the TT genotype showed the highest IL-4 concentrations, significantly greater than those observed in individuals with the TC and CC genotypes ($P < 0.001$).

Table 5. Association between IL-4 (rs2243250 C>T) genotypes and bronchial asthma risk and allele frequency distribution. OR: odds ratio; CI: confidence interval; S: significant at $P < 0.05$

SNPs	Genotype	Bronchial asthma (n=60)	HC (n=58)	OR (95%CI)	P-value
rs2243250 C>T	CC	22 (36.7 %)	40 (68.9%)	Reference	
	CT	25 (41.7%)	14 (24.2 %)	3.25(1.41 - 7.49)	0.005*S
	TT	13 (21.6%)	4 (6.9%)	5.91(1.72 - 20.33)	0.004*S
Hw-P		0.253	0.102		
Allele frequency	C	69 (57.5%)	94 (81.03%)	Reference	
	T	51 (42.5%)	22 (18.97%)	3.16(1.75 -5.69)	0.0001*S

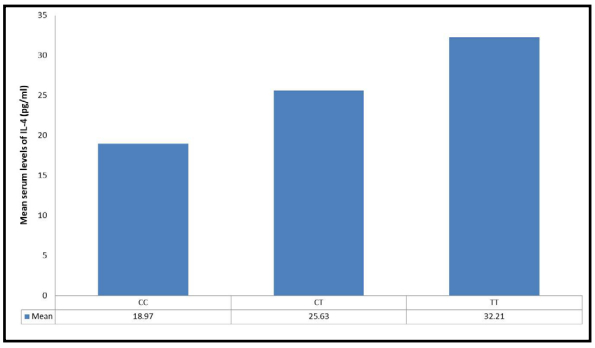


Fig. 4. Association between IL-4 gene polymorphism (rs2243250 C > T) genotypes and serum levels of IL-4 among bronchial asthma patients.

Discussion

This case-control study investigated the association between the IL-4 gene single nucleotide polymorphism (SNP) rs2243250 and susceptibility to bronchial asthma, along with its relationship to serum IL-4 levels in allergic asthma patients. Participants were statistically matched for age, gender, and residence. The asthma patient group included 66.7% females, consistent with findings by [20], who reported a 65% female predominance in asthma cases.

Asthma prevalence and severity are influenced by age and sex. While boys are more commonly affected during childhood, adult women experience higher prevalence and more severe forms of the disease. This disparity is influenced by hormonal changes, genetic predisposition, sociocultural factors, and differential treatment responses. Hormonal fluctuations during puberty, menstruation, and pregnancy are associated with asthma development in women. Additionally, gene-environment interactions contribute to disease outcomes [21].

Smoking was found to significantly increase asthma risk; individuals who smoked were 7.85 times more likely to develop asthma compared to non-smokers. This elevated risk aligns with existing literature, which suggests that tobacco exposure disrupts pulmonary immune responses, promotes Th2-dominated inflammation, and increases infection susceptibility [22, 23]. Even minimal exposure exacerbates asthma symptoms and increases the frequency of attacks [24]. A family history of asthma also increased disease risk by 5.33 times. Asthma's polygenic nature and strong familial aggregation suggest heritability estimates between 60% and 80% [25].

Patients exhibited a significantly higher prevalence of prior *Mycoplasma pneumoniae* infection (41.7%) compared to controls (13.8%), suggesting that *M. pneumoniae* may contribute to increased susceptibility in affected individuals, either as a risk factor or a triggering event (OR = 8.75, $p < 0.0001$). This observation aligns with earlier epidemiological studies reporting a higher incidence of *M. pneumoniae* infection among asthmatic individuals compared to non-asthmatic controls [26, 27, 28].

The immunomodulatory properties and associated inflammatory responses elicited by *M. pneumoniae* may exacerbate or initiate immune dysregulation, contributing to the observed clinical manifestations. Further investigation is warranted into mechanisms such as cytokine dysregulation, molecular mimicry, and persistent infection [29]. *M. pneumoniae* is frequently associated with asthma exacerbations and is known to increase hospitalization and emergency room visits during outbreaks. Although the precise mechanisms remain unclear, its role in asthma pathogenesis likely involves epithelial damage, mucus hypersecretion, and airway inflammation [30].

Guo et al. (2024) further explored the association between *M. pneumoniae* and the onset or worsening of asthma, highlighting the interplay between host genetic susceptibility and infectious agents in asthma pathogenesis. Their findings underscore the need to consider both genetic and environmental factors when examining asthma etiology and exacerbation. The high prevalence of *M. pneumoniae* infection in asthma patients underscores the importance of further research to clarify its role in disease progression and to inform therapeutic or preventive strategies [31].

Genetic polymorphisms in inflammatory mediator genes, particularly cytokines, play a significant role in disease susceptibility [32]. In the present study, a significant difference in the distribution of IL-4 rs2243250 genotypes and alleles was observed between asthma patients and healthy controls. Specifically, the CT (heterozygous) and TT (homozygous mutant) genotypes were significantly associated with an increased risk of bronchial asthma. Individuals carrying the T allele were found to have a 3.16-fold higher likelihood of developing asthma compared to those carrying the C allele.

Several studies have suggested a link between the IL-4 -590 C>T polymorphism and asthma risk. Vercelli (2008) reported a strong association between the T allele and asthma severity in British patients [33], while Neelofar et al. (2017) found that the T allele was associated with asthma and elevated serum IgE levels in children. These findings are consistent with our results, which also demonstrate a significant association between the T allele and asthma [24]. Zhang et al. (2019) similarly reported a correlation between the IL-4 -590 C>T polymorphism and bronchial asthma in Uyghur children, noting increased IgE levels and decreased FEV1, suggesting that the T allele may have a pathogenic role in this population [35].

Despite this evidence, the association between the IL-4 -590 T allele and asthma susceptibility remains debated. Polymorphisms in the promoter region of the IL4 gene have been linked to increased asthma risk, as IL-4 plays a central role in asthma pathophysiology. IL-4 promotes the differentiation of naïve T cells into Th2 cells, inhibits Th1 responses, and stimulates B cell proliferation and IgE production by enhancing the expression of MHC class II, CD40, CD23, and FcεRI. It also promotes endothelial proliferation and upregulates vascular cell adhesion molecule-1 (VCAM-1), facilitating the inflammatory cascade associated with asthma. Notably, several asthma phenotypes are associated with IL4 gene polymorphisms [17].

Analysis of IL4 rs2243250 also revealed a significant influence on serum IL-4 levels. Individuals with the TT genotype exhibited significantly higher cytokine levels compared to those with TC or CC genotypes. Rosenwasser et al. (1995) first implicated the C>T polymorphism at position -590 in the IL4 promoter in asthma development, showing that the T allele was associated with elevated IgE levels. This single nucleotide polymorphism enhances IL4 gene expression by increasing transcription factor binding, contributing to elevated IL-4 production and stronger inflammatory responses [36]. Numerous studies support the role of high IL4-590C/T gene expression in bronchial asthma [37, 38], although some have reported conflicting results [39]. These discrepancies may reflect differences in study design, sample size, population genetics, or geographic factors.

Study Limitations

This study has several limitations. The relatively small sample size and the fact that the study was confined to a single geographic area may limit applicability to other regions

or ethnic groups. Potential confounding factors, such as environmental exposures (e.g., air pollutants, allergens), socioeconomic conditions, and other unmeasured genetic variants, may also have influenced the outcomes.

Clinical and Public Health Implications

Despite these limitations, the study highlights important associations between genetic variation, infection history, and asthma risk. Genotyping of IL-4 rs2243250 may aid in identifying individuals at heightened risk of developing asthma and inform targeted therapeutic strategies. From a public health perspective, strategies to prevent *M. pneumoniae* infection, particularly among genetically susceptible individuals, may reduce asthma onset or severity. These findings support the integration of genetic screening and infection control into comprehensive asthma management and prevention programs.

Conclusion

The IL4 rs2243250 variant is significantly associated with increased asthma susceptibility and elevated serum IL-4 levels in the Iraqi population. However, larger, more diverse studies are needed to validate these findings. Additionally, the strong association observed between prior *Mycoplasma pneumoniae* infection and asthma highlights its potential clinical significance and justifies further investigation into its role in disease pathogenesis and as a possible target for therapeutic or preventive interventions.

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Author Contributions

All authors made equal contributions to the study design, data collection, analysis, manuscript preparation, revision, and final approval of the submitted version.

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Ethical Approval

This study received ethical approval from the Babylon Health Directorate (Approval No. 68, dated 21/01/2021). Verbal informed consent was obtained from all participants.

Disclosure Statement

The authors declare no conflicts of interest related to this work. The content of this paper has been written without assistance of AI-based services.

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