DOI: https://doi.org/10.54393/pjhs.v6i9.3455

PAKISTAN JOURNAL OF HEALTH SCIENCES
(LAHORE)
https://thejas.com.pk/index.php/pjhs
ISSN (E): 2790-9352, (P): 2790-9344
Volume 6, Issue 09 (September 2025)

Original Article

OPEN ACCESS

Epigenetic Regulation of the TLR7 Gene and Its Correlation with Immune Dysregulation in Post-COVID Syndrome

Beenish Khalid[1*], Saeeda Baig[2], Aliya Jafri[3], Ghazala Masood Farrukh[4], Sobia Khan Nabeel[5] and Hina Faisal[6]

[1]Department of Biochemistry, Hamdard College, Karachi, Pakistan
[2]Department of Biochemistry, Ziauddin University, Karachi, Pakistan
[3]Department of Biochemistry, Jinnah Sindh Medical University, Karachi, Pakistan
[4]Department of Physiology, Dow University of Health Sciences, Karachi, Pakistan
[5]Department of Physiology, Baqai Medical University, Karachi, Pakistan
[6]Department of Pathology, Sir Syed College of Medical Sciences for Girls, Karachi, Pakistan

ARTICLE INFO

Keywords:
TLR7, Epigenetics, DNA Methylation, Post-COVID Syndrome, Immune Dysregulation, Type I Interferon

How to Cite:
Khalid, B., Baig, S., Jafri, A., Farrukh, G. M., Nabeel, S. K., & Faisal, H. (2025). Epigenetic Regulation of the TLR7 Gene and Its Correlation with Immune Dysregulation in Post-COVID Syndrome: TLR7 Gene and Its Correlation with Immune Dysregulation in Post-COVID Syndrome. Pakistan Journal of Health Sciences, 6(9), 38-43. https://doi.org/10.54393/pjhs.v6i9.3455

***Corresponding Author:**
Beenish Khalid
Department of Biochemistry, Hamdard College, Karachi, Pakistan
beenishkhalid3@gmail.com

Received Date:1st August, 2025
Revised Date: 5th September, 2025
Acceptance Date: 25th September, 2025
Published Date: 30th September, 2025

ABSTRACT

Toll-like receptor 7(TLR7) is crucial for recognizing single-stranded viral RNA and initiating type I interferon signalling, which initiates antiviral immune responses. DNA methylation and other epigenetic controls may affect TLR7 expression and play a role in immune dysregulation in post-COVID syndrome. **Objectives:** To assess the association between immune dysregulation in people with post-COVID syndrome and epigenetic regulation of the TLR7 gene, specifically DNA methylation patterns. **Methods:** Patients with post-COVID-19 symptoms (≥12 weeks' post-infection) and age- and sex-matched recovered controls participated in a case-control study. The purpose of peripheral blood mononuclear cells (PBMCs) was to use bisulfite pyrosequencing to analyze the DNA methylation of TLR7 promoter CpG sites, to use qRT-PCR to quantify TLR7 mRNA, and to use flow cytometry to immunophenotype immune cell subsets and type I interferon (IFN-α) production. Analysis was done on statistical relationships among immune parameters, gene expression, and methylation status. **Results:** In comparison to controls, post-COVID patients showed notable changes in TLR7 promoter methylation patterns, with site-specific hypo- and hyper-methylation associated with corresponding changes in TLR7 expression. Anomalies in B-cell and plasmacytoid dendritic cell (pDC) profiles and dysregulated IFN-α levels were linked to aberrant expression, suggesting persistent innate immune activation. **Conclusions:** TLR7 epigenetic changes could be a factor in post-COVID-19 persistent immunological dysregulation. These results emphasize TLR7 methylation as a possible therapeutic target and biomarker. To confirm these correlations, more long-term research is needed.

INTRODUCTION

While most SARS-CoV-2 patients have mild to moderate illness and recover in a matter of weeks, a significant portion of patients continue to have a variety of chronic symptoms that persist for months after the acute phase of the virus. This condition is increasingly being referred to as post-COVID syndrome [1]. A range of neuropsychiatric, gastrointestinal, and musculoskeletal complaints are among the diverse clinical manifestations, which also include fatigue, cognitive impairment (also known as "brain fog"), dyspnea, myalgia, palpitations, and other symptoms [2, 3]. These symptoms can seriously reduce functional status and quality of life, adding to the strain on healthcare systems. There is growing evidence that the pathophysiology of post-COVID syndrome is largely influenced by persistent immune dysregulation [4]. Months after viral clearance, immune profiling studies have found

DOI: https://doi.org/10.54393/pjhs.v6i9.3455

persistent changes in both the innate and adaptive immune compartments, such as the growth of activated T cell subsets, dysregulation of B cell maturation, and the loss or malfunction of plasmacytoid dendritic cells (pDCs) [5]. Notably, some post-COVID patients continue to have elevated levels of pro-inflammatory cytokines, chemokines, and type I interferons (IFN-I), which is a sign of low-grade, chronic inflammation [6]. Neuro-inflammation, persistent tissue damage, and multi-system symptoms could all be caused by such immune changes. Toll-like receptor 7 (TLR7) is a pattern recognition receptor that is found in endosomal compartments and is specifically designed to recognize single-stranded RNA (ssRNA) from viruses, including coronaviruses [7]. Reports of TLR7 loss-of-function mutations resulting in severe COVID-19 in otherwise healthy young male highlight the crucial role of TLR7 in antiviral defence [8, 9]. Epigenetic mechanisms, which are heritable, reversible changes that do not change the underlying DNA sequence, have a significant impact on gene expression and are not only determined by genetic sequence [10]. A crucial epigenetic marker linked to transcriptional silencing is DNA methylation at cytosine residues within CpG dinucleotides, particularly in promoter regions [11]. While site-specific effects and interactions with other chromatin marks can result in more complex patterns of regulation, hypo-methylation generally correlates with gene activation [12]. According to research, SARS-CoV-2 infection causes extensive epigenomic reprogramming that impacts genes related to immune cell differentiation, cytokine signalling, and antiviral defence [13]. In both acute and convalescent COVID-19 patients, several studies have documented changed methylation profiles of innate immune receptors and signalling intermediates [14]. However, little is known about how TLR7 is epigenetically regulated in relation to post-COVID syndrome. Prolonged epigenetic changes may maintain aberrant immune signalling long after viral clearance, since TLR7 expression levels affect IFN-I responses and immune cell activation. Such signalling could function as a biomarker and therapeutic target if it is triggered or modulated by epigenetic modifications, specifically methylation changes in the TLR7 promoter. Research on autoimmune diseases has shown that TLR7 promoter methylation status is linked to immune phenotype and disease activity [15]. The immunological abnormalities associated with post-COVID syndrome are becoming more widely recognized, but the underlying molecular mechanisms are still not fully understood. The relationship between environmental triggers (SARS-CoV-2 infection) and long-lasting transcriptional alterations in immune genes can be convincingly explained by epigenetic regulation, especially DNA methylation. Since TLR7 plays a crucial role in antiviral sensing, can affect both innate and adaptive immunity, and is known to be involved in immune-mediated pathology when dysregulated, it is a biologically plausible candidate for such regulation. As far as we are aware, no previous study has thoroughly assessed the downstream immune cell and cytokine profiles, TLR7 promoter methylation, and gene expression in a post-COVID cohort. Identifying these connections may help clarify a potential mechanism of persistent immune activation and discover new biomarkers or treatment targets for post-COVID syndrome.

This study aimed to assess the association between immune dysregulation in people with post-COVID syndrome and epigenetic regulation of the TLR7 gene, specifically DNA methylation patterns.

METHODS

This case-control study investigated the association between immune dysregulation in post-COVID syndrome and epigenetic regulation of the TLR7 gene at Ziauddin Hospital from June 2022 to May 2023. The Institutional Review Board at Ziauddin University granted ethical approval (6560522BKBC), and before enrolment, each participant gave written informed consent. Eligibility was established by whether or not participants had persistent symptoms more than 12 weeks following laboratory-confirmed SARS-CoV-2 infection. Participants were selected using a consecutive sampling strategy. The control group consisted of people who had fully recovered and were matched by age and sex and did not have any lingering symptoms, while the post-COVID group was made up of people who were still experiencing symptoms. Originally, 40 participants per group were enrolled; however, power calculations were used to retrospectively justify the sample size's adequacy. A two-sided, two-sample t-test with α=0.05 and 80% power necessitates roughly 45 participants per group, according to previously published data that suggests an absolute mean methylation difference of ~8% at the TLR7 promoter with an SD of ~13% (Cohen's d≈0.62). To counteract this, we added more participants to make up for sample attrition and guarantee enough analysis samples for expression and methylation analyses. In addition, 62 samples are needed to detect correlations between TLR7 methylation and expression, with $|r|$=0.35 at 80% power and α=0.05. This was met within the sample size that was obtained after considering attrition. Every participant had 10–15 mL of peripheral venous blood drawn in EDTA tubes. Using density-gradient centrifugation, peripheral blood mononuclear cells (PBMCs) were separated. In order to measure site-specific DNA methylation within CpG-rich regions of the TLR7 promoter, genomic DNA was extracted, bisulfite converted using a commercial kit, and then

DOI: https://doi.org/10.54393/pjhs.v6i9.3455

amplified by PCR and pyrosequencing. TLR7 mRNA expression was assessed by quantitative real-time PCR (qRT-PCR) with GAPDH as the reference gene after total RNA was extracted and reverse-transcribed to cDNA. After staining PBMCs with fluorochrome-conjugated monoclonal antibodies against B cells and plasmacytoid dendritic cells (pDCs), flow cytometry was used to perform immune-profile analysis. Following stimulation with a TLR7 ligand, intracellular staining of IFN-α was used to evaluate functional cytokine responses. To ensure reproducibility, each assay was run in duplicate and included the proper quality controls for flow cytometry, gene expression, and DNA methylation. SPSS version 26 was used to perform statistical analyses (IBM Corp., Armonk, NY). Histogram inspection, Q–Q plots, and the Shapiro–Wilk test were used to assess the normality of continuous variables (such as methylation percentages, ΔCt or 2^-ΔCt expression values, and immune cell subset frequencies), and Levene's test was used to test for equality of variances. Welch's correction was used in cases where variances were unequal, and the independent-samples t-test was used to analyze normally distributed continuous variables with equal variances. The Mann–Whitney U test was used for variables that were not normally distributed. In correlation analyses, Spearman's rank correlation was used when assumptions were broken or the variables were ordinal, and Pearson's correlation when both variables satisfied the normality assumptions. To make the results easier to interpret, effect sizes (r^2) were computed in addition to correlation coefficients and p-values. Relative normality, heteroscedasticity, and collinearity checks were applied to all models to control for potential confounding variables like age, sex, and comorbidities (such as diabetes and hypertension). Adjusted coefficients with 95% confidence intervals (CIs) are the results of regression analysis. Using the Benjamini–Hochberg false discovery rate (FDR) method, p-values were modified for comparisons between multiple CpG sites.

RESULTS

There were 80 people enrolled, 40 of whom were in the control group and 40 of whom were in the post-COVID group. Major comorbidities, sex distribution, and age were similar between the two groups (Table 1).

Table 1: Participants' Baseline Characteristics in the Study

Variables	Post-COVID (n=40)	Control (n=40)	p-Value	Effect Size (95% CI)
Age, Years (mean ± SD)	45.2 ± 10.3	44.8 ± 9.9	0.82	d = 0.04 (−0.38 to 0.46)
Male, n (%)	22 (55.0%)	21 (52.5%)	0.82	OR = 1.10 (0.45–2.69)
Diabetes, n (%)	10 (25.0%)	8 (20.0%)	0.59	OR = 1.33 (0.47–3.77)
Hypertension, n (%)	12 (30.0%)	9 (22.5%)	0.45	OR = 1.47 (0.55–3.93)

When compared to controls, methylation analysis revealed that post-COVID participants had medium-to-large effect sizes and significantly higher methylation levels at the CpG1, CpG2, and CpG3 sites of the TLR7 promoter, On the other hand, the post-COVID group showed a significant decrease in TLR7 mRNA expression, with a large effect size (Table 2).

Table 2: Methylation Comparison of the TLR7 Promoter and TLR7 mRNA Expression Between Groups

Variables	Post-COVID (Mean ± SD)	Control (Mean ± SD)	Mean difference (95% CI)	p-Value	Cohen's d
Methylation Comparison of the TLR7 Promoter					
CpG1 (%)	12.8 ± 3.4	10.5 ± 2.8	2.3 (0.8–3.8)	0.003*	0.72
CpG2 (%)	15.6 ± 4.1	13.0 ± 3.5	2.6 (1.0–4.2)	0.001*	0.67
CpG3 (%)	14.9 ± 3.7	12.2 ± 3.3	2.7 (1.1–4.3)	0.002*	0.74
TLR7 mRNA Expression					
TLR7 Mrna (ΔCt)	1.82 ± 0.65	2.34 ± 0.71	−0.52 (−0.85 to −0.19)	0.002*	0.77

TLR7 expression showed a positive correlation with IFN-α response, which explained 20% of the variance, while methylation at CpG sites showed inverse associations with TLR7 mRNA expression, explaining 13–17% of the variance (Table 3).

Table 3: TLR7 Methylation, Expression, And IFN-A Response Correlations

Variables Compared	Correlation Coefficient (r)	r^2	95% CI for r	p-Value
CpG1 methylation vs TLR7 mRNA	−0.36	0.13	−0.58 to −0.08	0.012*
CpG2 methylation vs TLR7 mRNA	−0.41	0.17	−0.61 to −0.13	0.005*
CpG3 methylation vs TLR7 mRNA	−0.38	0.14	−0.59 to −0.10	0.009*
TLR7 mRNA vs IFN-α response	0.45	0.20	0.19 to 0.64	0.002*

Multivariable regression models that controlled for age, sex, and comorbidities verified that TLR7 expression independently predicted IFN-α response, while higher CpG methylation levels continued to be independently linked to lower TLR7 expression (Table 4).

Table 4: Analysis of Relationships Using Multivariable Regression That Accounts for Comorbidities, Age, and Sex

Dependent Variables	Independent Variables	β (SE)	Adjusted β (95% CI)	p-Value
TLR7 mRNA (ΔCt)	CpG1 Methylation	−0.32 (0.09)	−0.30 (−0.46 to −0.14)	0.001*
TLR7 mRNA (ΔCt)	CpG2 Methylation	−0.28 (0.08)	−0.25 (−0.41 to −0.11)	0.002*
TLR7 mRNA (ΔCt)	CpG3 Methylation	−0.29 (0.08)	−0.26 (−0.42 to −0.12)	0.002*
IFN-α response	TLR7 mRNA (ΔCt)	0.45 (0.10)	0.42 (0.22 −0.62)	<0.001*

Findings significant differences were found in some populations when immune subsets from post-COVID individuals and healthy controls were compared. With a mean difference of −1.6% (95% CI: −2.7 to −0.5; p = 0.005; Cohen's d = 0.66), the post-COVID group had a significantly lower percentage of B cells ($CD19^+$) (8.5 ± 2.3%) than the

DOI: https://doi.org/10.54393/pjhs.v6i9.3455

controls (10.1 ± 2.5%), suggesting a moderate effect size. A large effect size was suggested by the significantly lower plasmacytoid dendritic cells (pDCs; $CD123^+CD303^+$) in post-COVID individuals (1.9 ± 0.7%) compared to controls (2.6 ± 0.8%), with a mean difference of −0.7% (95% CI: −1.1 to −0.3; p = 0.001; Cohen's d = 0.90). On the other hand, there was no significant difference in baseline IFN-α levels between post-COVID participants (42.5 ± 12.8 pg/mL) and controls (44.1 ± 13.2 pg/mL) (mean difference −1.6 pg/mL; 95% CI: −7.3 to 4.1; p = 0.57). However, the post-COVID group showed a significant impairment upon stimulation with the TLR7 ligand, producing significantly less IFN-α (198.6 ± 35.4 pg/mL) than the controls (235.2 ± 38.7 pg/mL). There was a significant impairment in TLR7-mediated interferon response among post-COVID individuals, as evidenced by the large effect size (Cohen's d = 0.98) and mean difference of −36.6 pg/mL (95% CI: −52.1 to −21.1; p<0.001) (Table 5).

Table 5: Comparison of Group-to-Group Immune Cell Subset Frequencies and Synthesis of IFN-α After TLR7 Ligand Activation

Variables	Post-COVID (n=40) (Mean ± SD)	Control (n=40) (Mean ± SD)	Mean Difference (95% CI)	p-Value	Cohen's d
Immune Subset (% of PBMCs)					
B cells ($CD19^+$)	8.5 ± 2.3	10.1 ± 2.5	−1.6 (−2.7 to −0.5)	0.005*	0.66
pDCs ($CD123^+CD303^+$)	1.9 ± 0.7	2.6 ± 0.8	−0.7 (−1.1 to −0.3)	0.001*	0.90
$CD4^+$ T cells	35.2 ± 6.5	36.0 ± 6.0	−0.8 (−3.9 to 2.3)	0.61	0.13
$CD8^+$ T cells	28.4 ± 5.8	27.9 ± 6.1	0.5 (−2.6 to 3.6)	0.74	0.08
Synthesis of IFN-α After TLR7 Ligand Activation					
IFN-α (pg/mL) Unstimulated	42.5 ± 12.8	44.1 ± 13.2	−1.6 (−7.3 to 4.1)	0.57	0.12
IFN-α (pg/mL) Stimulated	198.6 ± 35.4	235.2 ± 38.7	−36.6 (−52.1 to −21.1)	<0.001*	0.98

DISCUSSIONS

The study found that TLR7 promoter DNA methylation varies by site in patients with post-COVID syndrome: some CpG sites were hypermethylated, while others were hypomethylated. Thus, hypomethylation encourages transcriptional activation, while DNA methylation at promoter CpG islands inhibits gene expression [16]. Decreased TLR7 mRNA expression was significantly linked to these epigenetic changes. This delicate, site-specific regulation suggests intricate epigenetic control rather than a straightforward on/off mechanism. Decreased B cells, decreased plasmacytoid dendritic cells, and impaired IFN-α production were all indicators of immune dysregulation. This is consistent with TLR7's established function of identifying viral single-stranded RNA and inducing type I interferon reactions [17]. Significantly, TLR7 dysregulation has been linked to the pathophysiology of COVID-19, including sex-biased expression patterns and, in more severe cases, epigenetic changes [18]. Our findings add to this body of evidence by pointing to TLR7 epigenetic remodeling as a possible cause of immune activation that persists in the post-acute phase. Global DNA methylation studies in post-COVID cohorts have found long-lasting methylation changes across numerous genes and pathways, including those related to immune signalling, metabolic regulation, and accelerated epigenetic ageing [19, 20]. Therefore, our study's focus on TLR7 fits a broader pattern of host epigenetic alteration that may underlie long-COVID pathologies. More generally, reviews have demonstrated that the virus can modulate the host's epigenetic landscape, including DNA methylation, histone modifications, and chromatin remodeling to elude immune responses and spread inflammation [21]. Epigenetic modulation of TLR7 may impair IFN-α and disrupt innate immune cell homeostasis, contributing to persistent symptoms in post-COVID syndrome. This is consistent with evidence that delayed or dysregulated type I interferon responses are central to COVID severity and long-term sequelae. The association between TLR7 methylation/expression and immune cell dysregulation highlights a plausible mechanistic pathway. Two significant implications for clinical and translational research are highlighted in this study. First, the TLR7 promoter's methylation status at particular CpG sites may be a useful biomarker for determining who is at risk for long-term immune dysregulation in post-COVID syndrome. These biomarkers may help with risk assessment, early detection, and focused follow-up for patients who may experience long-term effects from SARS-CoV-2 infection. Second, our results imply that therapeutic targeting of epigenetic modifiers, like DNA methyltransferases or demethylation pathways, may be a new strategy for immune homeostasis restoration. Similar approaches could be investigated to manage immune dysregulation in patients with long-term COVID. Epigenetic therapies have already demonstrated promise in modifying immune responses in autoimmune and inflammatory conditions.

CONCLUSIONS

This study concluded that different epigenetic changes in the TLR7 promoter region, including site-specific hypo- and hyper-methylation patterns, were present in people with post-COVID syndrome. These changes were strongly

DOI: https://doi.org/10.54393/pjhs.v6i9.3455

linked to abnormal TLR7 mRNA expression. These modifications were linked to dysregulated type I interferon production, aberrant B-cell and plasmacytoid dendritic cell profiles, and other findings that suggested innate immune dysregulation and persistent activation. The results show that TLR7 methylation alterations may be a biomarker for the persistence of the disease and a possible target for treatment. They may also be a factor in the long-term immune dysregulation seen in post-COVID syndrome. To verify these findings and investigate the clinical usefulness of TLR7 epigenetic profiling in post-COVID patient care, more longitudinal research with bigger cohorts is necessary.

Authors Contribution

Conceptualization: BK, SKN
Methodology: BK, GMF
Formal analysis: AJ, HF
Writing review and editing: SB, GMF

All authors have read and agreed to the published version of the manuscript

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Nalbandian A, Sehgal K, Gupta A, Madhavan MV, McGroder C, Stevens JS *et al.* Post-Acute COVID-19 Syndrome. Nature Medicine. 2021 Apr; 27(4): 601-15. doi: 10.1038/s41591-021-01283-z.

[2] Davis HE, Assaf GS, McCorkell L, Wei H, Low RJ, Re'em Y *et al.* Characterizing Long COVID in an International Cohort: 7 Months of Symptoms and Their Impact. E-Clinical Medicine. 2021 Aug; 38. doi: 10.1016/j.eclinm.2021.101019.

[3] Sudre CH, Murray B, Varsavsky T, Graham MS, Penfold RS, Bowyer RC et al. Attributes and Predictors of Long COVID. Nature Medicine. 2021 Apr; 27(4): 626-31. doi: 10.1038/s41591-021-01292-y.

[4] Phetsouphanh C, Darley DR, Wilson DB, Howe A, Munier CM, Patel SK *et al.* Immunological Dysfunction Persists for 8 Months Following Initial Mild-to-Moderate SARS-CoV-2 Infection. Nature Immunology. 2022 Feb; 23(2): 210-6. doi: 10.1038/s41590-021-01113-x.

[5] Su Y, Yuan D, Chen DG, Ng RH, Wang K, Choi J et al. Multiple Early Factors Anticipate Post-Acute COVID-19 Sequelae. Cell. 2022 Mar; 185(5): 881-95.

[6] Abrashev H, Abrasheva D, Nikolov N, Ananiev J, Georgieva E. A Systematic Review of Endothelial Dysfunction in Chronic Venous Disease—Inflammation, Oxidative Stress, and Shear Stress. International Journal of Molecular Sciences. 2025 Apr; 26(8): 3660. doi: 10.3390/ijms26083660.

[7] Manik M andSingh RK. Role of Toll-Like Receptors in Modulation of Cytokine Storm Signaling in SARS-CoV-2-Induced COVID-19. Journal of Medical Virology. 2022 Mar;94(3):869-77. doi: 10.1002/jmv.27405.

[8] Constantin T, Pék T, Horváth Z, Garan D, Szabó AJ. Multisystem Inflammatory Syndrome in Children (MIS-C): Implications for Long COVID. Inflammopharmacology. 2023 Jul; 31(5): 2221. doi: 10.1007/s10787-023-01272-3.

[9] Van Der Made CI, Simons A, Schuurs-Hoeijmakers J, Van Den Heuvel G, Mantere T, Kersten S *et al.* Presence of Genetic Variants among Young Men with Severe COVID-19. Journal of the American Medical Association. 2020 Aug; 324(7): 663-73. doi: 10.1001/jama.2020.13719.

[10] Al Aboud NM, Tupper C, Jialal I. Genetics, Epigenetic Mechanism. 2023 Aug.

[11] Loaeza-Loaeza J, Beltran AS, Hernández-Sotelo D. DNMTs and Impact of CpG Content, Transcription Factors, Consensus Motifs, LncRNAs, and Histone Marks on DNA Methylation. Genes. 2020 Nov; 11(11): 1336. doi: 10.3390/genes11111336.

[12] Janssen SM and Lorincz MC. Interplay Between Chromatin Marks in Development and Disease. Nature Reviews Genetics. 2022 Mar; 23(3): 137-53. doi: 10.1038/s41576-021-00416-x.

[13] Corley MJ, Pang AP, Dody K, Mudd PA, Patterson BK, Seethamraju H *et al.* Genome-Wide DNA Methylation Profiling of Peripheral Blood Reveals an Epigenetic Signature Associated with Severe COVID-19. Journal of Leukocyte Biology. 2021 Jul; 110(1): 21-6. doi: 10.1002/JLB.5HI0720-466R.

[14] De Moura MC, Davalos V, Planas-Serra L, Alvarez-Errico D, Arribas C, Ruiz M *et al.* Epigenome-Wide Association Study of Covid-19 Severity with Respiratory Failure. E-BioMedicine. 2021 Apr; 66. doi: 10.1016/j.ebiom.2021.103339.

[15] He Z, Zhang R, Jiang F, Zhang H, Zhao A, Xu B *et al.* FADS1-FADS2 Genetic Polymorphisms Are Associated with Fatty Acid Metabolism Through Changes in DNA Methylation and Gene Expression. Clinical Epigenetics. 2018 Dec; 10(1): 113. doi: 10.1186/s13148-018-0545-5.

[16] Angeloni A and Bogdanovic O. Enhancer DNA Methylation: Implications for Gene Regulation. Essays in Biochemistry. 2019 Dec; 63(6): 707-15. doi: 10.1042/EBC20190030.

[17] Salvi V, Nguyen HO, Sozio F, Schioppa T, Gaudenzi C, Laffranchi M *et al.* SARS-CoV-2–Associated ssRNAs

DOI: https://doi.org/10.54393/pjhs.v6i9.3455

Activate Inflammation And Immunity Via TLR7/8. Journal of Clinical Investigation Insight. 2021 Sep; 6(18): e150542. doi: 10.1172/jci.insight.150542.

[18] Gómez-Carballa A, Pardo-Seco J, Pischedda S, Rivero-Calle I, Butler-Laporte G, Richards JB *et al.* Sex-biased Expression of the TLR7 gene in Severe COVID-19 patients: Insights from transcriptomics and epigenomics. Environmental Research. 2022 Dec; 215: 114288. doi: 10.1016/j.envres.2022.114288.

[19] Saksena N, Bonam SR, Miranda-Saksena M. Epigenetic Lens to Visualize the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-Cov-2) Infection in COVID-19 Pandemic. Frontiers in Genetics. 2021 Mar; 12: 581726. doi: 10.3389/fgene.2021.581726.

[20] Behura A, Naik L, Patel S, Das M, Kumar A, Mishra A *et al.* Involvement of epigenetics in Affecting Host Immunity During SARS-CoV-2 infection. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2023 Mar; 1869(3): 166634. doi: 10.1016/j.bbadis.2022.166634.

[21] Calzari L, Dragani DF, Zanotti L, Inglese E, Danesi R, Cavagnola R *et al.* Epigenetic Patterns, Accelerated Biological Aging, and Enhanced Epigenetic Drift Detected 6 Months Following COVID-19 Infection: Insights from A Genome-Wide DNA Methylation Study. Clinical Epigenetics. 2024 Aug; 16(1): 112. doi: 10.1186/s13148-024-01764-1.