INTERACTION BETWEEN PRO-INFLAMMATORY CYTOKINES AND NOS3 GENE EXPRESSION IN POST-COVID-19 INDIVIDUALS

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Abstract

Objective

To investigate the molecular mechanisms of endothelial dysfunction in post-COVID individuals by analyzing NOS3 gene expression and its association with pro-inflammatory cytokine profiles (IL-6, IL-18, TNF-α), thereby identifying potential biomarkers of early vascular impairment.

Methodology

This case-control study involved 240 participants: COVID-positive diabetics (Group 2), COVID-positive prediabetics (Group 1), COVID-negative diabetics (Group 4), COVID-negative prediabetics (Group 5), and healthy controls (Group 3). Blood samples were analyzed for biochemical parameters, cytokine levels (IL-6, IL-18, IL-1β, TNF-α) using the ELISA method, and NOS3 gene expression using RT-PCR, with expression levels normalized to GAPDH and calculated using the $2^{-\Delta\Delta Ct}$ method. Statistical analysis was performed using Stata 17.0.

Result

The study showed that COVID-positive prediabetic and diabetic groups had the highest inflammatory and NOS3 levels, while COVID-negative non-diabetics had the lowest. ROC analysis identified TNF-α (AUC 0.85, $p < 0.001$) and IL-6 (AUC 0.84, $p < 0.001$) as the strongest classifiers. Scatter plots demonstrated significant positive correlations between NOS3 expression and IL-1β, IL-18, IL-6, and TNF-α (all $p < 0.01$). Logistic regression confirmed IL-18 (adjusted OR 10.30, $p < 0.01$), IL-6 (adjusted OR 4.05, $p < 0.05$), and TNF-α (adjusted OR 9.89, $p < 0.01$) as independent predictors, while IL-1β was not significant ($p > 0.05$).

Conclusion

Our study demonstrates a novel inflammatory–endothelial interaction in prediabetes, with elevated IL-1β, IL-18, IL-6, TNF-α, and NOS3 expression, most pronounced in COVID-positive groups. Notably, this is the first evidence of NOS3 upregulation in prediabetes, suggesting early endothelial adaptation to inflammatory stress, with IL-18, IL-6, and TNF-α emerging as strong predictors of NOS3 dysregulation. These findings highlight IL-6 and TNF-α as potential early biomarkers and position NOS3 as a novel indicator of endothelial dysfunction, offering new insight into immuno-metabolic pathways that link inflammation, glycemic status, and vascular risk.

Keywords: Endothelial dysfunction; NOS3 gene expression; post-COVID-19; Prediabetes; Pro-inflammatory cytokines; Vascular risk.

1 Introduction

The global covid-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (sars-cov-2), has transitioned from an acute infectious outbreak to an ongoing public health concern, with many recovered individuals experiencing a spectrum of long-term complications collectively termed post-covid condition (pcc) or "long covid" [1]. Although initially recognized as a respiratory illness, sars-cov-2 is now known to exert profound effects on the vascular endothelium, resulting in microvascular inflammation, thrombosis, and widespread endothelial injury [2]. Importantly, individuals recovering from covid-19, particularly those with underlying metabolic conditions such as prediabetes, frequently show persistent vascular and metabolic abnormalities [3]. While the acute stage of infection is defined by a severe cytokine surge and systemic inflammation, accumulating evidence suggests that chronic low-grade inflammation and sustained endothelial dysfunction are key drivers of the diverse and prolonged symptoms associated with post-covid condition (pcc).

At the molecular level, endothelial dysfunction is strongly linked to reduced nitric oxide (no) availability, an essential vasodilator synthesized by endothelial nitric oxide synthase (enos), encoded by the nos3 gene. Located on chromosome 7q35–36 and spanning about 21 kilobases, the nos3 gene plays a vital role in maintaining vascular function [4] dysregulation of nos3 expression or impairment of enos activity has been reported in both prediabetic and post-covid-19 individuals, indicating that metabolic disturbances and viral infection may act together to promote persistent endothelial injury and elevate cardiovascular risk [5]

Beyond impaired no signaling, sars-cov-2 infection triggers a pro-inflammatory response that often extends into the post-acute phase and mirrors the inflammatory profile seen in post-covid individuals. Both conditions are characterized by elevated circulating cytokines, including interleukin-6 (il-6), interleukin-18 (il-18), tumor necrosis factor-alpha (tnf-α), and interleukin-1beta (il-1β). These inflammatory mediators drive endothelial activation, dysregulate nos3 expression, disrupt enos function, and ultimately promote vascular inflammation and dysfunction [6,7]

Understanding the link between nos3 gene expression and pro-inflammatory cytokine profiles in post-covid individuals is essential. Detecting distinct molecular patterns may improve the diagnosis of endothelial dysfunction, allow risk stratification for long-term cardiovascular complications, and guide the development of targeted therapies. This study focuses on these molecular markers to enhance understanding of the mechanisms underlying post-covid condition (pcc) and to support better clinical management in the future.

2 Methodology

Study design

This case-control study was conducted to investigate the molecular mechanisms underlying endothelial dysfunction in individuals with prediabetes and post-covid diabetic changes, with a specific focus on nos3 gene expression and circulating pro-inflammatory cytokines. The study was conducted by the ethical

standards outlined in the declaration of helsinki (1964) and its subsequent amendments. Ethical approval was obtained from the institutional ethics committee of genetika (iecg) (ref. No: 07/2024/iecg).

Study area and population

A total of 240 participants were recruited for the study and were categorized into the following groups:

- **Cases (120):** individuals with a history of confirmed covid-19 (rt-pcr positive) who are currently diagnosed with prediabetes and diabetes.
- **Controls (120):** 60 age- and sex-matched healthy individuals, along with 30 covid-negative prediabetic participants and 30 covid-negative diabetic participants.

The case subjects were recruited from hridayalaya heart and robotics research centre, located in thiruvananthapuram, kerala. The control subjects were recruited from various medical camps conducted by hridayalaya heart and robotics research centre in collaboration with genetika. All laboratory analyses, including molecular investigations, were conducted at genetika, centre for advanced genetic studies, thiruvananthapuram.

Inclusion criteria

Participants included in the study were adults aged between 20 and 60 years. Eligibility required a documented history of sars-cov-2 infection, confirmed by either rt-pcr or antigen testing. All participants were diagnosed with prediabetes and diabetes according to the american diabetes association (ada) criteria, which include one or more of the following parameters:

Prediabetes

- **Fasting blood glucose (fbg):** 100 to 125 mg/dl
- **Hba1c:** 5.7% to 6.4%

diabetes

- **Fasting blood glucose (fbg):** ≥126 mg/dl
- **Hba1c:** ≥6.5%

Participants in the control group had no history of sars-cov-2 infection and demonstrated normal glucose regulation, defined as fasting plasma glucose (fpg) < 100 mg/dl and hba1c < 5.7%. Additionally, they were age- and sex-matched with the individuals in the case group.

Exclusion criteria

- History of cardiovascular diseases, stroke, or chronic kidney disease
- Presence of autoimmune, infectious, or chronic inflammatory diseases
- Use of anti-inflammatory, immunosuppressive, or antioxidant supplements/medications
- Current smokers or individuals with alcohol/substance abuse
- Pregnant or lactating women
- Individuals with any malignancy or undergoing radiation/chemotherapy

- Age below 20 or above 60 years

Data gathering

A detailed collection of demographic and clinical data was conducted through personal interviews using a pre-structured questionnaire. This included information on socio-demographic characteristics, anthropometric measurements, lifestyle factors, and family history of diabetes. Written informed consent was obtained from all participants before their inclusion in the study.

Sample collection and preparation

Fasting venous blood samples (6–8 ml) were collected from each participant using standard aseptic techniques. The samples were distributed into edta tubes and plain vacutainers. Serum was separated from the plain tubes by centrifugation and used for the analysis of inflammatory cytokines. Blood collected in edta tubes was utilized for molecular analysis, including rna extraction, cdna synthesis, and quantitative real-time pcr (qrt-pcr) to assess nos3 gene expression.

Laboratory investigations

Blood samples were analyzed for biochemical parameters following standardized laboratory protocols. Levels of inflammatory markers, including il-6, il-18, il-1β, and tnf-α, were measured using enzyme-linked immunosorbent assay (elisa) kits obtained from origin diagnostics & research, kerala, india. All assays were performed in strict accordance with the manufacturer's instructions to ensure the accuracy, reliability, and reproducibility of the results.

Genetic analysis

Rna isolation and cdna synthesis

Rna was isolated using an rna extraction kit, quantified using a biospectrometer, and then reverse-transcribed into cdna using a cdna synthesis kit.

Real-time pcr (rt-pcr)

Nos3 gene expression was analyzed using rt-pcr with specific primers (forward: 5'-gaaggcgacaatcctgtatggc-3' and reverse: 5'-tgttcgagggacaccacgtcat-3'). Gapdh was used as the internal reference housekeeping gene. The primer sequences were forward 5′-ccatggagaaggctgggg-3′ and reverse 5′-caaagttgtcatggatgacc-3′. The rt-pcr was performed on a cfx opus 96 real-time pcr system. A 20 µl pcr reaction mixture was prepared, consisting of 2x real-time pcr master mix, primers, cdna, and nuclease-free water. The thermal cycling conditions included an initial pre-denaturation at 95°c for 5 minutes, followed by 30-40 cycles of denaturation at 94°c for 1 minute, annealing at 56°c for 1 minute, and extension at 72°c for 1 minute. A final extension at 72°c for 10 minutes was performed, followed by melt curve analysis. Gene expression levels were normalized to the housekeeping gene, and relative expression was calculated using the ($2^{-\delta\delta ct}$) method.

Statistical analysis

Descriptive statistics summarized molecular and biochemical data. Independent t-tests were used to compare normally distributed variables between groups. Roc analysis assessed biomarker diagnostic utility via auc, sensitivity, and specificity. Logistic regression was used to analyze the associations between nos3 gene expression ($2^{-\delta\delta ct}$) and clinical parameters. Bootstrap methods were employed

to obtain robust p-values when normality assumptions were violated. All analyses were performed using stata 17.0.

3 Results

The study employs a case–control design, comprising a total of 240 participants, of whom cases were recruited from hridayalaya heart and robotics research centre and controls from medical camps.

The inflammatory profiles varied across all five groups, as summarized in **table 1**. Across the five groups, cytokine and nos3 profiles showed clear differences. Groups 1 and 2 had the highest levels of il-1β (11.74 and 13.14 pg/ml), il-18 (132.1 and 140.9 pg/ml), il-6 (7.67 and 8.00 pg/ml), tnf-α (27.8 and 28.6 pg/ml), along with elevated nos3 expression (1.36 and 1.52), indicating strong inflammatory and endothelial activation. Group 3 showed the lowest values for all markers-il-1β (4.83 pg/ml), il-18 (104.4 pg/ml), il-6 (2.07 pg/ml), tnf-α (13.0 pg/ml), and nos3 (0.94)-suggesting reduced activation. Groups 4 and 5 demonstrated intermediate levels of both cytokines and nos3, with group 5 values (il-1β 6.99 pg/ml, il-18 112.8 pg/ml, il-6 4.94 pg/ml, tnf-α 18.3 pg/ml, nos3 1.03) closer to baseline compared to groups 1 and 2.

The roc curve illustrates the relationship between sensitivity and specificity, with curves closer to the top-left corner indicating higher diagnostic accuracy. Among the five markers, tnf-α showed the best performance (auc 0.85), with high sensitivity (94.2%) and good specificity (69.2%), making it the strongest classifier. Il-6 also performed well (auc 0.84), balancing sensitivity (87.5%) and specificity (70%). Il-1β (auc 0.81) and nos3 (auc 0.80) showed good accuracy, with il-1β offering balanced sensitivity and specificity, while nos3 was stronger in ruling out false positives. Il-18 (auc 0.74) was the weakest but still moderately useful. Overall, tnf-α and il-6 emerged as the most reliable markers, supported by roc curve patterns in **figure 1** (roc curve).

Figure 2 (scatter plot) illustrates the association between il-1β levels and nos3 gene expression in prediabetic and diabetic individuals. In both groups, the scatter plots show a clear positive trend, indicating that higher il-1β concentrations are linked to elevated nos3 expression. The fitted regression lines further reinforce this association, displaying consistent upward slopes.

The association between il-18 levels and nos3 gene expression in prediabetic and diabetic individuals is shown in **figure 3** (scatter plot). The plots demonstrate a clear positive correlation, where higher il-18 levels correspond to increased nos3 expression. This suggests that il-18 may significantly influence endothelial gene regulation in both metabolic states, potentially linking inflammatory activity to vascular dysfunction.

Figure 4 (scatter plot) illustrates the relationship between il-6 levels and nos3 gene expression in prediabetic and diabetic individuals. In both groups, the scatter plots show a clear positive trend, indicating that higher il-6 concentrations are linked to elevated nos3 expression. The fitted regression lines further reinforce this association, displaying consistent upward slopes.

Figure 5 shows the scatter plot depicting the association between tnf-α and nos3 gene expression among covid-positive prediabetic and diabetic individuals.

Figure 5 (scatter plot) shows the relationship between tnf-α levels and nos3 gene expression in the case groups. Both groups display a clear positive linear trend, with higher tnf-α levels corresponding to increased nos3 expression. The clustering of data points, especially in the prediabetic group, is relatively tight around the regression line, indicating a strong and consistent association. These results highlight tnf-α as a key inflammatory marker that may influence endothelial gene regulation in individuals with glucose abnormalities.

Table 2 (logistic regression analysis) presents the unadjusted and adjusted odds ratios (ors) with 95% confidence intervals for the association between elevated levels of inflammatory markers and the likelihood of developing the condition, likely prediabetes, or a related metabolic disorder. Individuals with il-18 levels above 100 pg/ml had significantly higher odds of the condition compared to those with levels ≤100 pg/ml. The unadjusted or was 17.11, and even after adjusting for confounding variables, the or remained significant at 10.30, indicating a strong independent association. For il-6, those with levels above seven pg/ml showed increased risk, with an unadjusted or of 10.11 and an adjusted or of 4.05, suggesting il-6 is also an independent predictor. Tnf-α levels above 25 pg/ml were similarly associated with higher odds, with an unadjusted or of 19.52 and an adjusted or of 9.89, confirming its strong and independent association with the condition. In contrast, il-1β levels above 6.5 pg/ml did not show a significant association. The unadjusted or was 1.72, and the adjusted or dropped to 0.33, indicating that il-1β is not an independent predictor in this context. In summary, elevated levels of il-18, il-6, and tnf-α are significantly and independently associated with increased risk, whereas il-1β does not show a meaningful association after adjustment.

4 Discussion

This study demonstrates significantly elevated levels of il-1β, il-18, il-6, tnf-α, and nos3 gene expression in prediabetic and diabetic individuals, highlighting early activation of inflammatory pathways and endothelial dysfunction. Although previous studies have independently addressed the roles of pro-inflammatory cytokines in insulin resistance and metabolic syndrome [8,9] or endothelial dysfunction in type 2 diabetes [10], the present study uniquely integrates these components to elucidate their combined influence in prediabetes, a connection not previously explored in this context.

The inflammatory and endothelial profiles demonstrated a clear gradient across the groups, reflecting the combined influence of infection, glycemic status, and metabolic imbalance. Il-1β levels varied across groups (table 1). The highest concentrations were observed in group 2 (13.14 ± 6.39 pg/ml), a finding consistent with ippolito [11], who attributed elevated cytokine levels in covid-19 and post-covid-19 patients to newly developed hyperglycemia when compared with normoglycemic individuals. Group 1 also showed elevated levels (11.74 ± 5.25 pg/ml), indicating infection-driven il-1β activation. While earlier studies reported elevated il-1β levels in covid-19 subjects [12], our findings demonstrate, for the first

time, significant il-1β activation in covid-positive prediabetic individuals, suggesting that even early dysglycemia amplifies infection-driven inflammatory responses. Group 3 maintained baseline values (4.83 ± 2.52 pg/ml), reflecting normal immune regulation. Intermediate levels were noted in groups 4 (9.30 ± 6.19 pg/ml) and 5 (6.99 ± 3.06 pg/ml). This aligns with the prior studies by alfadul et al, [13] which have demonstrated that il-1β is markedly increased in patients with t2dm and contributes to chronic low-grade inflammation driving disease progression.

Il-18 levels showed a graded distribution across the groups (table 1). Group 2 demonstrated the highest concentration (140.9 ± 32.8 pg/ml), reflecting synergistic cytokine amplification in covid-positive diabetics, consistent with abdullah et al,[14] who reported that higher levels of pro-inflammatory cytokines, especially il-6 and il-18, are closely linked to poorer outcomes in t1d subjects with covid-19. Group 1 followed (132.1 ± 32.8 pg/ml), highlighting il-18's role in viral-induced cytokine release. While earlier studies reported elevated il-18 levels in covid-19 subjects [15], our findings demonstrate, for the first time, significant il-18 activation in covid-positive prediabetic individuals, indicating that even early dysglycemia heightens infection-driven inflammatory responses. In contrast, group 3 (104.4 ± 13.5 pg/ml) remained at physiological baseline, indicative of immune homeostasis. In the non-infected groups, both prediabetics (group 4: 117.2 ± 30.4 pg/ml) and diabetics (group 5: 112.8 ± 14.6 pg/ml) exhibited modest il-18 elevations, aligning with earlier studies reporting higher il-18 levels in prediabetic individuals and significantly increased levels in t2dm subjects [16,17].

Il-6 levels demonstrated a clear stepwise gradient across the groups (table 1). Group 2 recorded the highest concentration (8.00 ± 3.33 pg/ml), reflecting inflammatory amplification in covid-positive diabetics, consistent with findings by zheng et al, [18] who reported that covid-19 subjects with diabetes showed higher levels of il-2, il-6, il-10, and ifn-γ compared to those without diabetes or with impaired fasting glucose. Group 1 followed closely (7.67 ± 2.80 pg/ml), showing a pronounced infection-driven cytokine response, which aligns with the findings by bonyek-silva et al, [19] who found that prediabetes is associated with an increased risk of severe covid-19 and elevated serum il-6 levels, suggesting il-6 as a potential biomarker for severe covid-19 in these subjects. In contrast, group 3 (2.07 ± 1.35 pg/ml) remained at physiological baseline, confirming immune homeostasis in healthy controls. Among the non-infected diabetics, group 4 (5.52 ± 2.77 pg/ml) suggested a stable but chronic inflammatory state, whereas group 5 (4.94 ± 2.54 pg/ml) indicated subclinical inflammation. This is in consistent with earlier studies reporting that diabetic subjects have elevated il-6 levels, indicating a chronic inflammatory state driven by metabolic and immune dysregulation, and that higher il-6 levels are associated with an increased risk of developing type 2 diabetes [20,21].

Interestingly, tnf-α levels showed a distribution pattern similar to il-6 (table 1). Elevated tnf-α levels in covid-positive diabetics (group 2: 28.6 ± 7.7 pg/ml) reflect the combined effects of hyperglycemia and infection, in agreement with singh et al, [22] who also reported higher tnf-α in covid-19 subjects with diabetes. Covid-

positive prediabetics (group 1: 27.8 ± 7.9 pg/ml) showed a clear rise in tnf-α, reflecting viral-induced inflammatory activity. While prior work highlighted elevated tnf-α in covid-19 subjects lu et al, [23] our study is the first to demonstrate significant tnf-α activation in covid-positive prediabetics, suggesting that even early dysglycemia amplifies infection-driven inflammation. Healthy controls-maintained baseline levels (group 3: 13.0 ± 4.0 pg/ml). Among the non-infected cohorts, diabetic individuals (group 4: 20.7 ± 9.9 pg/ml) showed persistently elevated tnf-α, reflecting chronic inflammation. This agrees with saeed et al, [24] who reported that higher tnf-α levels in t2dm subjects may contribute to the disease's development and pathogenesis. Moderate elevation of tnf-α was observed in prediabetic individuals (group 5: 18.3 ± 8.3 pg/ml), consistent with metabolic inflammation. These results corroborate the observations of ali and zaidi, [25] who noted that higher tnf-α levels in prediabetics reflect a heightened inflammatory response, potentially instrumental in the transition to overt diabetes.

Endothelial function, measured through nos3 levels, showed a graded distribution across the groups (table 1). Among the study groups, covid-positive diabetics (1.52 ± 0.56) exhibited the highest nos3 levels, followed by covid-positive prediabetics (1.36 ± 0.42), suggesting infection-driven endothelial activation. Importantly, the resilience seen in covid-positive prediabetics and the inflammation-related nos3 elevation in diabetic individuals are novel observations not previously reported in the literature. Healthy controls-maintained baseline activity (group 3: 0.94 ± 0.09), reflecting preserved vascular homeostasis. Among the non-infected cohorts, diabetic individuals (group 4: 1.15 ± 0.21) showed intermediate but elevated nos3 expression, consistent with earlier reports of upregulated nos3 in diabetes linked to hyperglycemia and insulin resistance [26]. Prediabetic individuals (group 5: 1.03 ± 0.15) also demonstrated intermediate expression; however, to our knowledge, there are no prior studies documenting nos3 expression in prediabetes, making this a novel finding that highlights potential early endothelial alterations before the onset of overt diabetes. Further studies are warranted to validate these findings and explore their mechanistic underpinnings and long-term cardiovascular implications.

Utilizing roc curve analysis (figure 1), this study establishes a combined biomarker approach for the early detection of prediabetes. Among the markers evaluated, il-6 demonstrated the highest diagnostic accuracy, closely followed by tnf-α, underscoring their prominent roles in early metabolic inflammation. Conversely, nos3 and il-1β exhibited moderate discriminatory power, while il-18 showed limited diagnostic relevance. These findings highlight il-6 and tnf-α as potential frontline biomarkers for identifying individuals at risk of progressing to overt diabetes.

The present study demonstrates a consistent positive association between pro-inflammatory cytokines (il-1β, il-18, il-6, and tnf-α) and nos3 gene expression in covid-positive prediabetic and diabetic individuals, as shown in (figures 2–5). The scatter plots consistently show that as levels of these cytokines increase, nos3 expression also rises, suggesting that inflammatory stress may trigger endothelial

activation through nos3 upregulation in the early stages of metabolic dysfunction. These findings highlight a distinct inflammatory-endothelial interplay in covid-positive prediabetic individuals, suggesting that increased nos3 expression may serve as an early indicator of endothelial adaptation in response to inflammation.

Logistic regression analysis (table 2) revealed that elevated levels of il-18, il-6, and tnf-α were significantly and independently associated with increased odds of nos3 upregulation, indicating a strong inflammatory influence on endothelial nitric oxide synthase activity. Specifically, il-18 levels greater than 100pg/ml showed the strongest association, with an adjusted odds ratio (or) of 10.30, followed by tnf-α levels greater than 25 pg/ml, with an or of 9.89, and il-6 levels greater than 7pg/ml, with an or of 4.05. In contrast, il-1β >6.5 pg/ml did not demonstrate a significant independent association with nos3 expression, as the adjusted or dropped to 0.33. The discrepancy may reflect differences in disease stage or modulation by post-covid inflammatory pathways. Overall, these results identify il-18, il-6, and tnf-α as significant independent predictors of nos3 dysregulation. This suggests that nos3 expression may serve as a dynamic endothelial marker responsive to inflammatory activity in the prediabetic stage, particularly among individuals with a history of covid-19 infection.

Strengths and limitations

The major strengths of this study lie in its comprehensive approach, as it simultaneously evaluated multiple pro-inflammatory cytokines (il-1β, il-18, il-6, tnf-α) together with the endothelial function marker nos3, providing an integrated perspective on immunometabolic and vascular alterations. The inclusion of distinct groups covid-positive diabetics, covid-positive non-diabetics, non-infected diabetics, prediabetics, and healthy controls enabled meaningful comparisons across infection and metabolic states. Moreover, the combined use of biochemical assays for cytokines and molecular analysis of nos3 expression allowed cross-validation of inflammatory and endothelial activity. Importantly, the findings are not only consistent with previous literature but also highlight novel interactions between inflammation and endothelial regulation, particularly in covid-positive diabetic individuals.

However, certain limitations must be acknowledged. Being case-control in nature, the study can establish associations but not causality. Although the overall sample size was adequate, subgroup stratification may reduce power for detecting smaller effects. Residual confounding from factors such as medications, obesity, or other comorbidities could not be fully excluded. Cytokine measurements are also subject to biological variability, including circadian and stress-related influences. Finally, the lack of longitudinal follow-up limits insights into the persistence or progression of observed changes beyond the studied timeframe.

5 Conclusion

This study highlights a novel inflammatory–endothelial interaction in prediabetes, particularly in the context of covid-19. Elevated levels of il-1β, il-18, il-6, tnf-α, and nos3 expression were observed in prediabetic and diabetic individuals, with covid-positive groups showing the most pronounced alterations.

Importantly, this is the first evidence of nos3 upregulation in prediabetes, suggesting early endothelial adaptation to inflammatory stress before overt diabetes develops. The consistent positive association between pro-inflammatory cytokines and nos3 expression underscores the role of inflammation in driving endothelial activation. Logistic regression identified il-18, il-6, and tnf-α as strong independent predictors of nos3 dysregulation, reinforcing their pathogenic significance. These findings establish il-6 and tnf-α as potential frontline biomarkers for early detection of prediabetes and position nos3 as a novel marker of early endothelial changes. Collectively, this integrated analysis provides new insight into the immuno-metabolic pathways linking inflammation, endothelial dysfunction, and glycemic status, with implications for early risk stratification and prevention of diabetes-related vascular complications.

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ТАБЛИЦЫ

Table 1. Groupwise Comparison of Inflammatory and Endothelial Biomarkers Between Case and Control Groups

Variable	Group 1 (Min–Max)	Group 1 (Mean ± SD)	Group 2 (Min–Max)	Group 2 (Mean ± SD)	Group 3 (Min–Max)	Group 3 (Mean ± SD)	Group 4 (Min–Max)	Group 4 (Mean ± SD)	Group 5 (Min–Max)	Group 5 (Mean ± SD)
IL-1β (pg/mL)	2.21 – 25.7	11.74 ± 5.25	2.55 – 28.5	13.14 ± 6.39	1.35 – 12.3	4.83 ± 2.52	2.03 – 19.9	9.30 ± 6.19	1.68 – 11.5	6.99 ± 3.06
IL-18 (pg/mL)	84.5 – 240.6	132.1 ± 32.8	82.8 – 196.3	140.9 ± 32.8	80 – 132.3	104.4 ± 13.5	75.6 – 163.4	117.2 ± 30.4	88 – 135.2	112.8 ± 14.6
IL-6 (pg/mL)	1.20 – 15.6	7.67 ± 2.80	1.19 – 15.3	8.00 ± 3.33	0.69 – 6.39	2.07 ± 1.35	1.06 – 9.65	5.52 ± 2.77	1.17 – 8.05	4.94 ± 2.54
TNF-α (pg/mL)	10.5 – 48	27.8 ± 7.9	10.5 – 44.8	28.6 ± 7.7	4.6 – 23.4	13.0 ± 4.0	7.8 – 38.4	20.7 ± 9.9	5.6 – 30.2	18.3 ± 8.3
NOS3	0.685 – 3.158	1.36 ± 0.42	0.674 – 3.251	1.52 ± 0.56	0.763 – 1.142	0.94 ± 0.09	0.82 – 1.46	1.15 ± 0.21	0.72 – 1.30	1.03 ± 0.15

Group 1 – COVID-positive prediabetics (n = 60); Group 2 – COVID-positive diabetics (n = 60); Group 3 – COVID-negative non-diabetics (n = 60); Group 4 – COVID-negative diabetics (n = 30); Group 5 – COVID-negative prediabetics (n = 30). Values are presented as minimum–maximum ranges and mean ± standard deviation (SD).
Abbreviations: IL-6: Interleukin-6; IL-18: Interleukin-18; IL-1β: Interleukin-1 Beta; TNF-α: Tumor Necrosis Factor-alpha; NOS3: Nitric Oxide Synthase 3 (endothelial NOS).

Table 2. Logistic Regression Analysis of Inflammatory Markers Associated with Post-COVID Endothelial Dysfunction

Variable	**Category**	**Unadjusted OR [95% CI]**	**Adjusted OR [95% CI]**
IL-18	≤100 pg/mL (ref)	1.00	1.00
	>100 pg/mL	17.11 [4.59 – 63.77]***	10.30 [1.93 – 55.03]**
IL-1β	≤6.5 pg/mL (ref)	1.00	1.00
	>6.5 pg/mL	1.72 [0.68 – 4.35]	0.33 [0.07 – 1.59]
IL-6	≤7 pg/mL (ref)	1.00	1.00
	>7 pg/mL	10.11 [4.20 – 24.35]***	4.05 [1.09 – 14.95]*
TNF-α	≤25 pg/mL (ref)	1.00	1.00
	>25 pg/mL	19.52 [7.45 – 51.13]***	9.89 [2.53 – 38.71]**

Logistic regression analysis presenting unadjusted and adjusted odds ratios (OR) with 95% confidence intervals (CI) for NOS3 gene dysregulation among COVID-positive individuals. Reference categories are indicated as (ref). The adjusted model includes all listed variables.

Statistical significance: $p < 0.05$ (), $p < 0.01$ **(),** $p < 0.001$ **()**. An OR > 1 indicates increased odds of NOS3 dysregulation, while an OR < 1 suggests a potential protective association.

РИСУНКИ

Figure 1. Roc curve of nos3 and inflammatory markers in post-covid endothelial dysfunction.

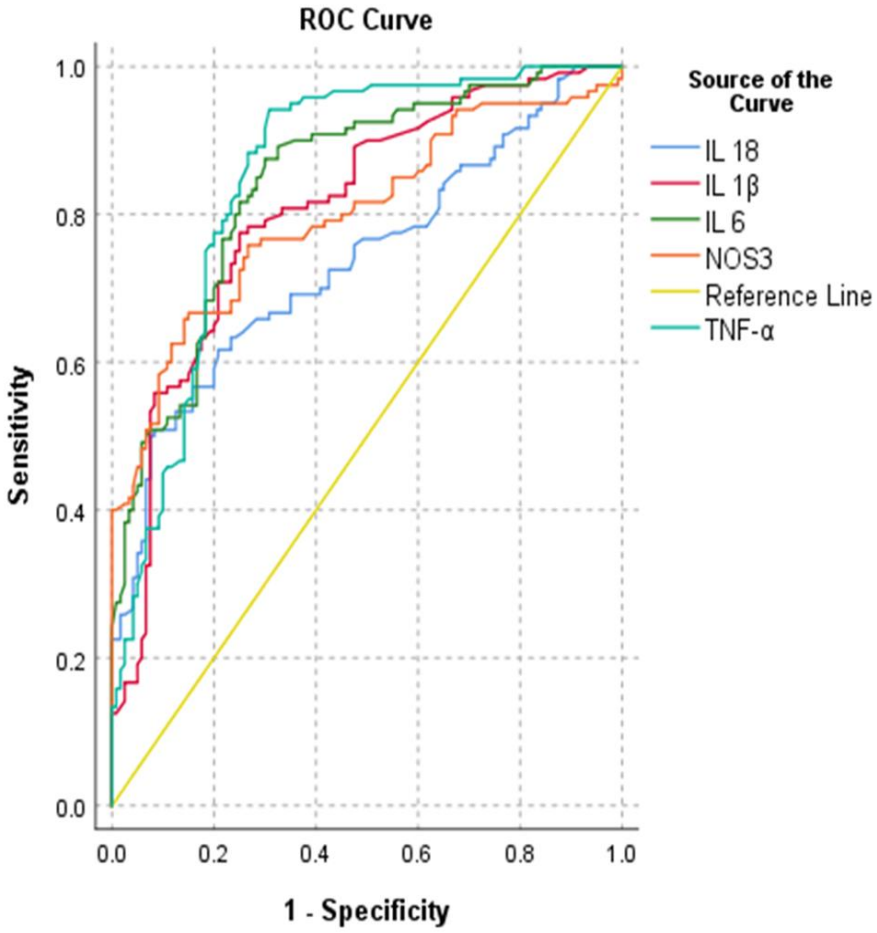

Figure 2. Scatter plot showing the association between il-1β and nos3 gene expression among covid-positive prediabetic and diabetic individuals

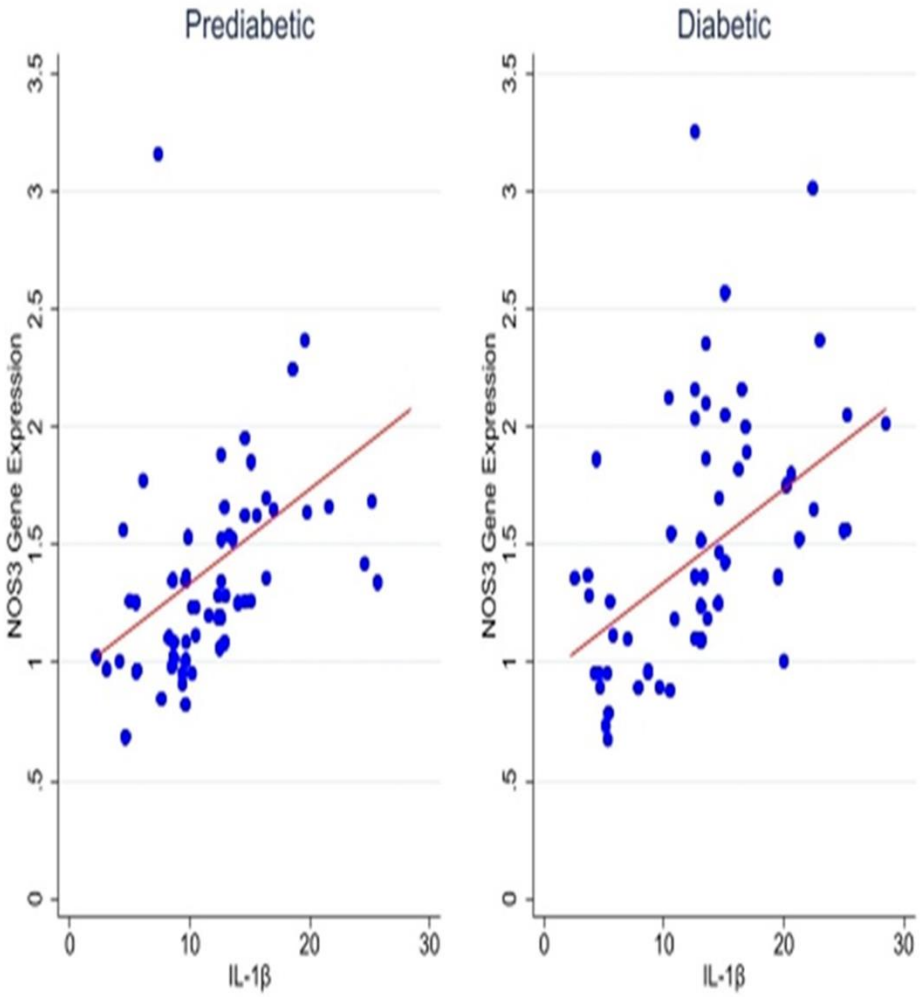

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Figure 3. Scatter plot showing the association between il-18 and nos3 gene expression among covid-positive prediabetic and diabetic individuals

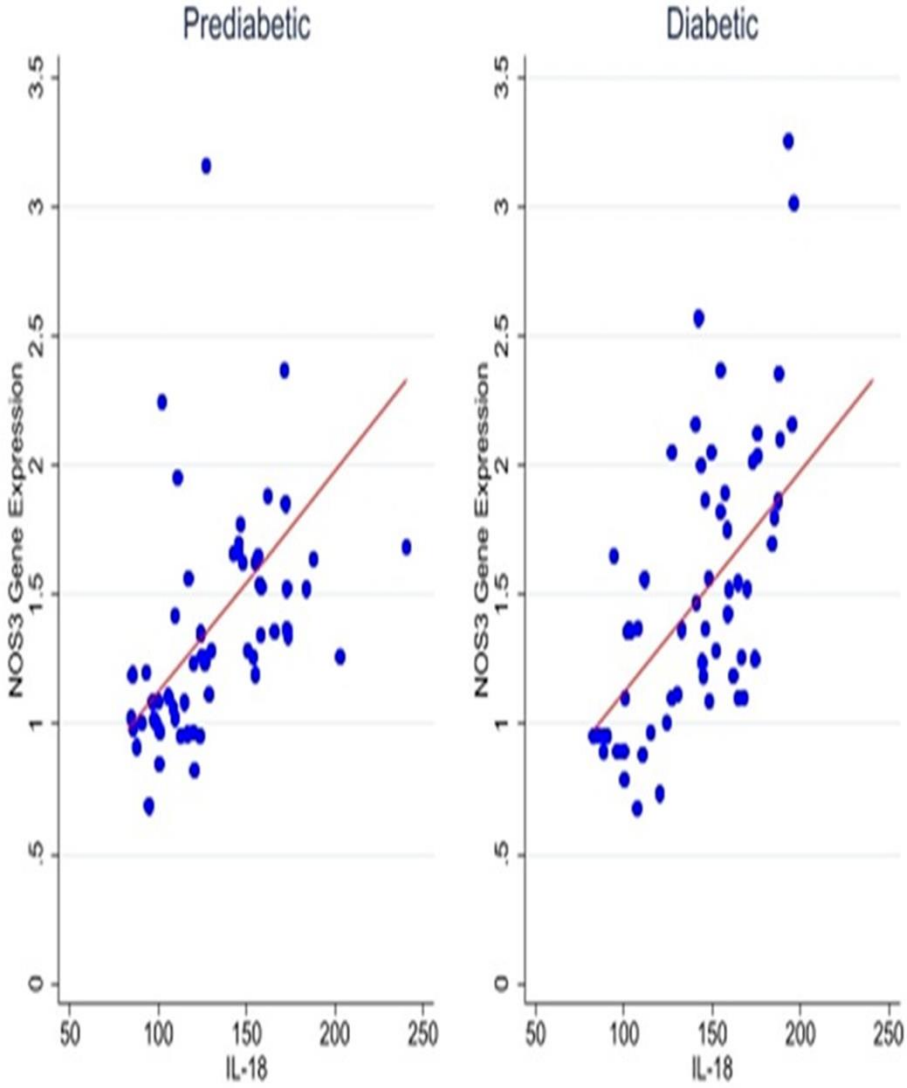

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Figure 4. Scatter plot showing the association between il-6 and nos3 gene expression among covid-positive prediabetic and diabetic individuals

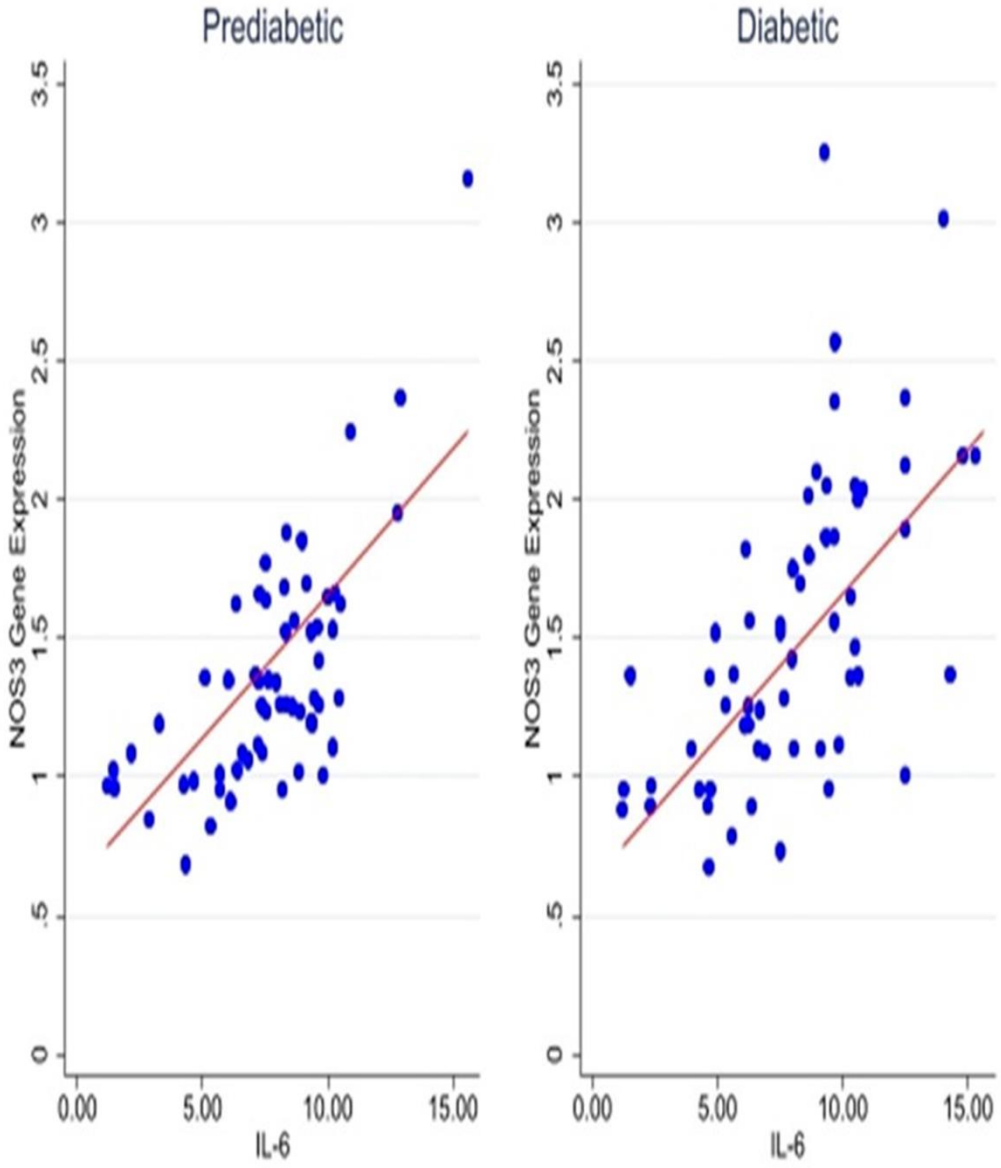
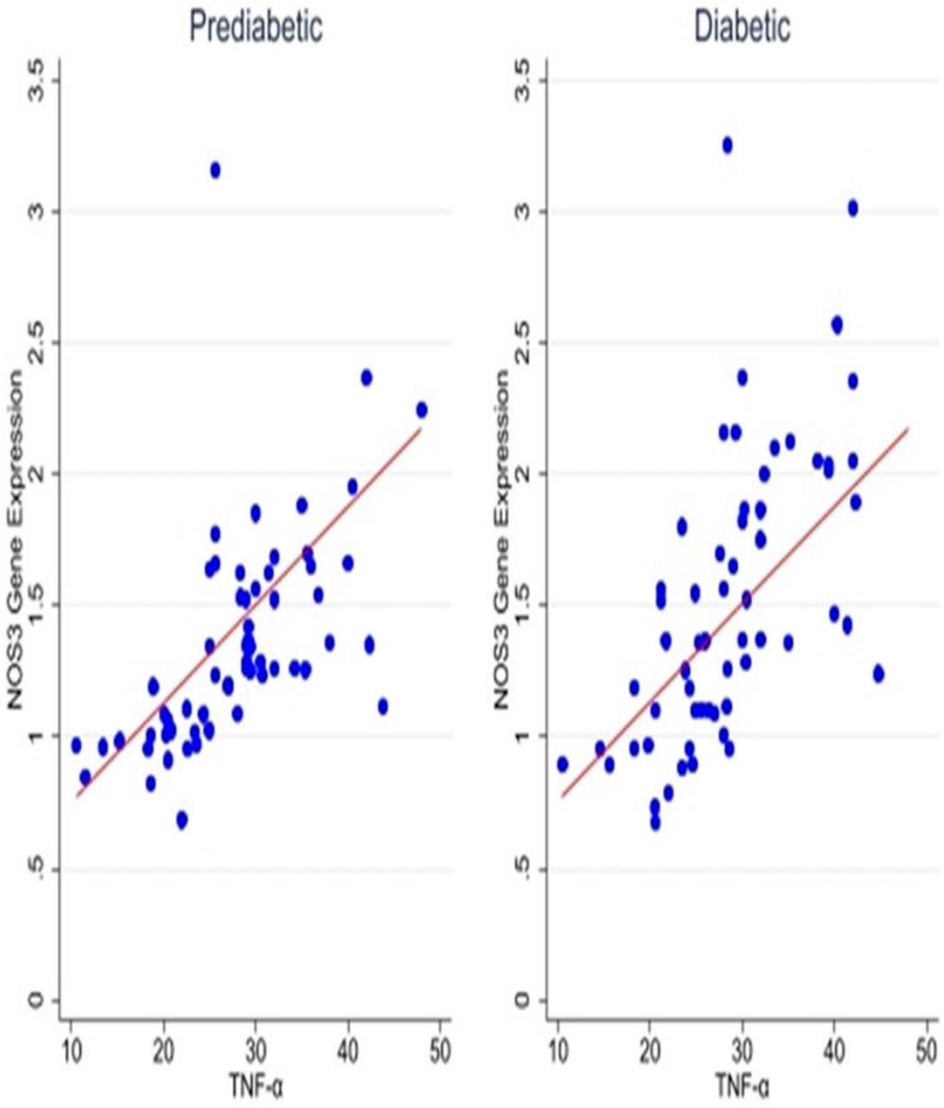


Figure 5. Scatter plot showing the association between tnf-α and nos3 gen

expression among covid-positive prediabetic and diabetic individuals



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Блок 3. Метаданные статьи
INTERACTION BETWEEN PRO-INFLAMMATORY CYTOKINES AND NOS3 GENE EXPRESSION IN POST-COVID-19 INDIVIDUALS

Сокращенное название статьи для верхнего колонтитула:
CYTOKINE- NOS3 INTERACTION IN POST-COVID-19

Keywords: Endothelial dysfunction; NOS3 gene expression; post-COVID-19; Prediabetes; Pro-inflammatory cytokines; Vascular risk.

Оригинальные статьи.
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Количество рисунков – 5.
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