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# The role of NOS3-rs1799983 and NOS3-rs2070744 SNP in occurrence of avascular necrosis as a post COVID-19 complication

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**Abstract**
Avascular necrosis (AVN) is a debilitating condition characterized by bone tissue death due to inadequate blood supply, leading to joint dysfunction and collapse. This study investigates the potential association between AVN and COVID-19, focusing on genetic factors such as NOS3 polymorphisms. A total of 180 individuals were included, comprising 120 COVID-19 patients and 60 healthy controls. Clinical, haematological, biochemical, and genetic parameters were assessed. Results revealed significant differences in respiratory and heart rates, haematological counts, and biochemical markers between AVN and control groups. Genetic analysis showed a higher prevalence of the TG genotype and G allele in NOS3 rs1799983 polymorphism among AVN patients. Additionally, NOS3 rs2070744 polymorphism correlated with various clinical parameters, including blood pressure, heart rate, and haematological indices. This study highlights the potential role of genetic factors in predisposing individuals to AVN following COVID-19 infection.

**Keywords** COVID-19, Nitric oxide synthase, Single nucleotide polymorphism, Avascular necrosis

## Introduction

Avascular necrosis (AVN) is a debilitating condition characterized by the death of bone tissue due to poor blood supply, resulting in pain, joint dysfunction, and, ultimately, joint collapse [1]. AVN has been associated with various risk factors, including trauma, corticosteroid use, alcohol consumption, and certain medical conditions [2]. Emerging evidence suggests that viral infections, such as COVID-19, may also predispose individuals to AVN as a post-infectious complication [3–5].

The global COVID-19 pandemic has had profound implications for public health, with millions of individuals affected by the virus worldwide [6]. While COVID-19 primarily manifests as a respiratory illness, increasing reports indicate its involvement in diverse systemic manifestations, including musculoskeletal complications [7]. Avascular necrosis has been identified as one such complication, with reports of its occurrence following COVID-19 infection [8].

Genetic factors are known to play a crucial role in the pathogenesis of AVN, influencing an individual's susceptibility to the condition [9]. Among these factors, single nucleotide polymorphisms (SNPs) in genes related to vascular function and regulation have garnered significant interest. One such gene is nitric oxide synthase 3 (NOS3), which encodes an enzyme involved in the

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production of nitric oxide, a key regulator of vascular tone and endothelial function [10]. Two common NOS3 SNPs, rs1799983 and rs2070744, have been implicated in various vascular disorders, raising the possibility of their involvement in AVN as well [11].

Despite the growing recognition of the potential link between COVID-19 and AVN, the underlying mechanisms remain poorly understood. Furthermore, the contribution of genetic factors, particularly NOS3 SNPs, to the development of AVN in the context of COVID-19 has not been adequately investigated [12]. Therefore, this study aims to elucidate the role of NOS3-rs1799983 and NOS3-rs2070744 SNPs in the occurrence of AVN as a post-COVID-19 complication.

By examining the association between these genetic variants and AVN risk in individuals recovering from COVID-19, we seek to provide insights into the pathogenesis of post-infectious AVN and identify potential genetic markers for risk stratification and targeted interventions. This research has the potential to advance our understanding of the interplay between viral infections, genetic susceptibility, and musculoskeletal health, ultimately informing clinical management strategies for patients recovering from COVID-19.

## Subjects and methods

### Subjects and control samples

A total of 180 subjects of both sexes were included in this study; 120 of them were confirmed with COVID-19 disease with different severity, and 60 were healthy. The patients were selected from confirmed patients with the COVID-19 pandemic in El-Bagour Hospital in the period from March 2021 to December 2021.

**Table 1** ABCD scoring for COVID-19 severity assessment

| Study Variable | Values | Score | |
|---|---|---|---|
| | | 0 | 1 |
| Age (Years) | Young, Elderly | 0–50 | > 50 |
| Blood Test | Leucopenia | No | Yes |
| | Lymphocytes (< 1500 per mm3) | No | Yes |
| | CRP (> 10 mg/L) | No | Yes |
| | LDH (> 250 U/L) | No | Yes |
| | D Dimer (> 0.5 mg/L) | No | Yes |
| Chest X-ray or CT | Ground Glass patchy shadows | No | Yes |
| Comorbidities | COPD/Smoker | No | Yes |
| | Cancer | No | Yes |
| | Hypertension & Chronic heart failure | No | Yes |
| | Chronic renal disease | No | Yes |
| | DM | No | Yes |
| Dyspnea | PR > 30/minute | No | Yes |
| | O2 saturation < 90% | No | Yes |
| Total Score | | | |

**CRP**: c-reactive protein, **LDH**: lactate dehydrogenase, **COPD**: chronic obstructive lung disease, **DM**: diabetes mellitus, **PR**: pulmonary rehabilitation.

### Study design

In this study, participants were categorized into three distinct groups: a control group comprising sixty healthy individuals **(GpI)**, a non-avascular necrosis infected COVID-19 group **(GpII)** consisting of sixty patients, and a post-COVID-19 avascular necrosis group **(GpIII)** also comprising sixty subjects. The study adhered to the principles outlined in the Helsinki Declaration for ethical research. This included obtaining voluntary, informed consent from all participants, ensuring their privacy and confidentiality, minimizing potential risks, and conducting the research with scientific rigor and integrity. Additionally, the study protocol underwent ethical review and approval by the Menoufia University Faculty of Medicine's Ethical Committee for Medical Research (11/2023 BIO12), further demonstrating its commitment to ethical standards.

### Assessment of COVID-19 severity

In this study, the ABCD (Airway, Breathing, Circulation, Defibrillation) severity score specifically designed for COVID-19 was employed. This scoring system incorporates various factors to assess the severity of the condition, as detailed in Table 1. The score was determined based on the presence and severity of these factors according to the established criteria [13].

### Complete blood count (CBC)

In the methodology, an automated hemogram was conducted for all participants. This included the estimation of hemoglobin levels (Hb), the count of white blood cells (WBC), lymphocytes, and platelets (PLT). These parameters were assessed using a Coulter counter model Beckman 750, manufactured by Int, U.S.A., following the same procedures as previously described.

### Determination of biochemical parameters

Biochemical parameters were determined by assessing levels of C-reactive protein (CRP) using the Cromatest Linear Chemicals S.L.U. Barcelona Spain kit, following the manufacturer's instructions and protocols. D-dimer levels were measured according to a previously described method [14], while ferritin levels were determined based on established protocols [15]. Interleukin-6 (IL-6) levels were also assessed, adhering to the manufacturer's instructions for the assay procedure (SHANGHAI KORAIN BIOTECH CO., LTD., Shanghai, China).

### Genotyping of polymorphisms

Genomic DNA was extracted from all frozen gastric tissue samples using Quick-g DNA $^{TM}$ Miniprep Kit, USA (catalogue No D3024) and SNP (single nucleotide polymorphism) assay using TaqMan Allelic Discrimination Technique. The rs2070744 polymorphism was genotyped

using the Primer probe for rs2070744: [VIC/FAM]: CC AGGGCATCAAGCTCTTCCCTGGC[**C**/**T**]GGCTG ACCCTGCCTCAGCCCTAGTC and Primer probe for rs1799983: [VIC/FAM]: CCCTGCTGCTGCAGGCCCC AGATGA[**G**/**T**]CCCCCAGAACTCTTCCTTCTGCCC C.

After PCR amplification, an endpoint plate was read using an Applied Biosystems Real-Time PCR System. The genotyping run results were recorded by the system software on a scatter plot of Allele G versus Allele A. On the plot, each well of the 96-well reaction plate is represented by a distinct point.

## Statistical analysis

Data were entered into the computer and analyzed with the version of the IBM SPSS software package 25.0. The standard deviation, mean, range (minimum and maximum), and median were used to express distributed data. The three groups under investigation were compared using an ANOVA, and pairwise comparisons were made using the Post Hoc Tukey test. To compare the two groups, the Chi-square test was used. At the 5% level, the significance of the results was determined.

## Results

### Clinical characteristics of patients

According to respiratory rate, there was a statistically significant difference between the three studied groups ($p=0.001$), with a higher value in **(GpIII)** followed by the **(GpII)** than **(GpI)**. Our data showed that heart rate was statistically significantly higher among **(GpIII)** than the **(GpII)** as compared to **(GpI)** ($p=0.001$). Mean OS was statistically significantly lower among **(GpIII)** than among **(GpII)** and **(GpI)** ($p=0.001$) (Table 2).

### Hematological and biochemical test numbers and percentages

According to the results, there were low WBC counts ($x10^3$) in **(GpII)** (2.8±0.4) and **(GpIII)** (2.8±0.2) groups with statistically significant differences as compared to **(GpI)** (7.3±1.6) ($p=0.001$). Also, there were low PLT counts ($x10^3$) in **(GpII)** (130.8±13.2) and **(GpIII)** (129±6.3) groups with statistically significant difference as compared to **(GpI)** (293±56) ($p=0.001$) as shown in Table 2.

Our data showed increased levels for D-dimer, CRP, ferritin and IL-6 tests in **(GpII)** and **(GpIII)** compared to **(GpI)**. D-dimer values showed a statistically significant difference between **(GpII)** (1.5±0.2) and **(GpIII)** (1.4±0.1) groups as compared to **(GpI)** (0.2±0.1) ($p=0.001$). However, there was a statistically significant difference between the three studied groups according to CRP, ferritin and IL-6 ($p=0.001$).

### Polymorphism of NOS3

Additionally, it was observed that there was a statistically significant difference in the genotype and allele distributions of the NOS3-rs1799983 polymorphism between the patient and control groups. The TG genotype was higher in **(GpIII)** (60%). The T allele was significantly higher in **(GpI)** (65%), and the G allele was significantly higher in **(GpIII)** (47.5%, Table 3). The odds ratio showed that there was a statistically significant difference in TG ($p=0.020$) and GG ($p=0.009$) genotype and G allele ($p=0.049$) in **(GpIII)** as compared with **(GpI)** (Table 4).

### Comparing COVID-19 patient results of hematological and biochemical parameters according to NOS3-rs2070744

For subjects IN **(GpII)** (Table 5 ), statistically significant differences were noticed in systolic blood and diastolic blood pressures ($p=0.02$ and 0.047) with high value in CC genotype and low value in CT genotype, heart rate ($p=0.044$) with high value in TT genotype and low value in CC genotype, Hb ($p=0.017$) with high value in CT genotype and low value in CC genotype, PLT count ($p=0.031$) with high value in CT genotype and low value in TT genotype. In **(GpIII)** (Table 6), statistically significant differences were observed in temperature, heart rate, lymphocyte count and CRP ($p=0.018$, 0.022, 0.009 and 0.007) with high values in CT genotype and low values in CC genotype, OS, ($p=0.032$) with high value in CC genotype and low value in TT genotype, WBC count and D-dimer ($p=0.011$ and 0.001) with high value in CT genotype and low value in TT genotype, Hb and mortality ($p=0.002$ and 0.001) with high value in CC genotype and low value in CT genotype and finally age ($p=0.045$) with high value in TT genotype and low value in CC genotype. Our results showed a statistically significant difference ($p=0.015$) between avascular necrosis complication and NOS3-rs2070744, with a high value in the CT genotype and a low value in the TT genotype.

### Comparing COVID-19 patient results of hematological and biochemical parameters according to NOS3-rs1799983

For comparing the hematological tests and biochemical parameters to NOS3-rs1799983, a statistically significant difference was observed in **(GpIII)**, statistically significant differences were observed in WBC and PLT counts ($p<0.001$ and 0.044) with high value in the TT genotype and low value in GG genotype (Table 7).

## Discussion

Numerous clinical characteristics of COVID-19 are present, ranging from asymptomatic or mild to severe or serious form [16].

This is the first study that investigated the relationship between the polymorphisms rs2070744 and rs1799983 of

**Table 2** Comparison of the three studied groups according to different variables

| | Group I (n = 60) | Group II (n = 60) | Group III (n = 60) | Test of Sig. | p |
|---|---|---|---|---|---|
| **Respiratory rate** | | | | | |
| Mean ± SD. | 21.7 ± 2.3 a | 25.8 ± 6.4 b | 28.9 ± 7.5 c | H = 42.151* | 0.001* |
| Median (Min. – Max.) | 22 (18–26) | 24 (20–40) | 26 (20–50) | | |
| **Sig. bet. Grps.** | $p_1 < 0.001^*, p_2 < 0.001^*, p_3 = 0.004^*$ | | | | |
| **Heart rate** | | | | | |
| Mean ± SD. | 86.3 ± 4.1 a | 97.5 ± 14.8 b | 100.5 ± 15.3 b | F = 21.489* | < 0.001* |
| Median (Min. – Max.) | 86 (80–94) | 94.5 (80–125) | 97 (76–130) | | |
| **Sig. bet. Grps.** | $p_1 < 0.001^*, p_2 < 0.001^*, p_3 = 0.388$ | | | | |
| **OS** | | | | | |
| Mean ± SD. | 98.6 ± 1 | 98.1 ± 2.9 | 70.2 ± 6.8 | F = 858.218* | < 0.001* |
| Median (Min. – Max.) | 99 (97–100) a | 98 (77–100) a | 72.5 (55–79) b | | |
| **Sig. bet. Grps.** | $p_1 = 0.812, p_2 < 0.001^*, p_3 < 0.001^*$ | | | | |
| **WBC (x10$^3$/ul)** | | | | | |
| Mean ± SD. | 7.3 ± 1.6 a | 2.8 ± 0.4 b | 2.8 ± 0.2 b | F = 448.998* | < 0.001* |
| Median (Min. – Max.) | 7.8 (4.5–10.6) | 2.8 (2–4.1) | 2.8 (2–3.2) | | |
| **Sig. bet. Grps.** | $p_1 < 0.001^*, p_2 < 0.001^*, p_3 = 0.982$ | | | | |
| **PLT(x10$^3$/ul)** | | | | | |
| Mean ± SD. | 293 ± 56 a | 130.8 ± 13.2 b | 129 ± 6.3 b | F = 475.999* | < 0.001* |
| Median (Min. – Max.) | 295 (178–397) | 127 (115–172) | 131 (115–137) | | |
| **Sig. bet. Grps.** | $p_1 < 0.001^*, p_2 < 0.001^*, p_3 = 0.953$ | | | | |
| **D-dimer** | | | | | |
| Mean ± SD. | 0.2 ± 0.1 a | 1.5 ± 0.2 b | 1.4 ± 0.1 b | H = 124.413* | < 0.001* |
| Median (Min. – Max.) | 0.2 (0.1–0.3) | 1.5 (1.1–2.5) | 1.4 (1.1–1.6) | | |
| **Sig. bet. Grps.** | $p_1 < 0.001^*, p_2 < 0.001^*, p_3 = 0.064$ | | | | |
| **CRP (mg/L)** | | | | | |
| Mean ± SD. | 2.2 ± 1.2 a | 64.5 ± 33.7 b | 126.4 ± 45 c | H = 145.721* | < 0.001* |
| Median (Min. – Max.) | 1.9 (1.1–7.2) | 96.0 (24.0–96) | 96 (96–192) | | |
| **Sig. bet. Grps.** | $p_1 < 0.001^*, p_2 < 0.001^*, p_3 < 0.001^*$ | | | | |
| **Ferritin (ng/ml)** | | | | | |
| Mean ± SD. | 29.8 ± 3.3 a | 34.1 ± 2.9 b | 96.3 ± 10.1 c | H = 137.229* | < 0.001* |
| Median (Min. – Max.) | 30 (20–35) | 33.7 (28.3–43.2) | 96 (75–120) | | |
| **Sig. bet. Grps.** | $p_1 < 0.001^*, p_2 < 0.001^*, p_3 < 0.001^*$ | | | | |
| **IL6 (pg/ml)** | | | | | |
| Mean ± SD. | 2.0 ± 0.7 a | 7.8 ± 1.6 b | 11.9 ± 1.2 c | H = 156.426* | < 0.001* |
| Median (Min. – Max.) | 1.9 (1.2–3.4) | 7.5 (4.9–11) | 12.1 (10–14) | | |
| **Sig. bet. Grps.** | $p_1 < 0.001^*, p_2 < 0.001^*, p_3 < 0.001^*$ | | | | |

**SD**: Standard deviation; **$\chi^2$**: Chi square test; **F**: F for One way ANOVA test; **H**: H for Kruskal Wallis test, Post Hoc Test was performed for pairwise comparison between each two groups. **p**: p value for comparing the three studied groups; **$p_1$**: p-value after comparing Group I and Group II; **$p_2$**: p-value after comparing between Group I and Group III; **$p_3$**: p-value after comparing between Group II and Group III. *: Statistically significant at $p \leq 0.05$; Group **I**: Healthy group; Group II: non-avascular necrosis group; **Group III**: post COVID-19 avascular necrosis group

the NOS3 gene and avascular necrosis complications in COVID-19 patients.

In this study, we reported that respiratory rate is often increased in patients diagnosed with COVID-19 when compared with healthy controls. This is comparable to other studies showing a higher respiratory rate in patients with COVID-19 [17, 18].

Compared to uninfected or healthy controls, an increase in heart rate was observed in individuals with COVID-19. On the other hand, consistent differences between the non-avascular necrosis and post-COVID-19 avascular necrosis groups were not observed. These findings come in agreement with the study of Maloberti et al., 2021 [19]. OS showed a significant difference with post-COVID-19 avascular necrosis COVID-19 in comparison with non-avascular necrosis and healthy groups.

Patients with COVID-19 pneumonia may have normal, low, or high leukocyte count. This might also make it easier to monitor the disease's development and facilitate treatment-related decisions. Our data investigated that WBC and PLT counts were statistically significantly lower in the non-avascular necrosis and post-COVID-19 avascular necrosis groups than in the healthy group.

**Table 3** Comparison of the three studied groups according to rs1799983

| | Group I (n = 60) | Group II (n = 60) | Group III (n = 60) | $\chi^2$ | p |
|---|---|---|---|---|---|
| **rs1799983** | | | | | |
| T/T | 30(50.0%) | 23(38.3%) | 15(25%) | 11.181* | 0.025* |
| T/G | 28(46.7%) | 33(55%) | 36(60%) | | |
| G/G | 2(3.3%) | 4(6.7%) | 9(15%) | | |
| $^{HW}p_1$ | **0.123** | **0.085** | **0.058** | | |
| T | 78 (65%) | 67 (55.8%) | 63 (52.5%) | 8.930* | 0.012* |
| G | 42 (35%) | 53 (44.2%) | 57 (47.5%) | | |

$\chi^2$: Chi square test; **p**: p value for comparing between the three studied groups; $^{HW}p_1$: p value of Chi square for goodness of fit for Hardy-Weinberg equilibrium; *: Statistically significant at $p \leq 0.05$; **Group I**: Healthy group; **Group II**: non-avascular necrosis group; **Group III**: post COVID-19 avascular necrosis group

**Table 5** Relation between rs2070744 and different parameters in group II ($n = 60$)

| | rs2070744 | | | Test of Sig. | p |
|---|---|---|---|---|---|
| | C/C (n = 8) | C/T (n = 26) | T/T (n = 26) | | |
| **Systolic blood pressure (mmHg)** | | | | | |
| Mean ± SD. | 123.9 ± 12.52 | 115.2 ± 7.28 | 116.9 ± 5.31 | F = 4.220* | 0.020* |
| Median (Min. – Max.) | 125 (110–137) | 120 (100–125) | 120 (110–125) | | |
| **Diastolic blood pressure (mmHg)** | | | | | |
| Mean ± SD. | 79.63 ± 8.80 | 71.92 ± 10.59 | 76.15 ± 4.96 | F= 3.231* | 0.047* |
| Median (Min. – Max.) | 80 (70–89) | 80 (50–80) | 80 (70–80) | | |
| **Heart rate** | | | | | |
| Mean ± SD. | 89.38 ± 8.03 | 93.15 ± 13.57 | 104.3 ± 14.92 | F = 5.970* | 0.004* |
| Median (Min. – Max.) | 92 (80–97) | 87.50 (80–121) | 98 (86–125) | | |
| **Hemoglobin (gm/dl)** | | | | | |
| Mean ± SD. | 11.06 ± 1.82 | 12.76 ± 1.32 | 12.59 ± 1.45 | F = 4.374* | 0.017* |
| Median (Min. – Max.) | 10 (10–14) | 13 (10–14) | 13 (10–14) | | |
| **PLT(x10$^3$/ul)** | | | | | |
| Mean ± SD. | 127.4 ± 7.25 | 135.9 ± 17.94 | 126.8 ± 5.11 | F = 3.711* | 0.031* |
| Median (Min. – Max.) | 124 (124–145) | 128 (116–172) | 127 (115–147) | | |

**SD**: Standard deviation; **F**: F for One way ANOVA test; **H**: H for Kruskal Wallis test; $\chi^2$: Chi square test; **MC**: Monte Carlo; **p**: p value for Relation between rs2070744 and various parameters; *: Statistically significant at $p \leq 0.05$; **Group II**: non-avascular necrosis group

These findings come in agreement with the study of Xiong et al., 2020 and Zheng et al., 2020 [20, 21].

In regard to the findings of the previous studies, IL-6 has been introduced as a catalyst for the cytokine storm in COVID-19 and is a powerful pro-inflammatory cytokine that can stimulate CRP. This hyperinflammatory condition raises CRP and IL-6 levels, which is consistent with the findings of previous studies. Interleukin-6, interleukin-10 (IL-10) and CRP are substantially linked with the severity of the illness, according to study results by Keddie et al. [22, 23].

Our study found that mean D-dimmer and S-ferritin levels were statistically significantly higher in the positive COVID-19 groups than in the negative group, which agrees with studies of Alkan et al., 2021 and Assar et al., 2023 [24, 25].

NO has a crucial role in preventing RNA and DNA viruses from replicating. NO declines with age and has a potentially fatal effect on COVID-19 infection in older adults, while it is also regarded as a crucial protective component [26]. In our study, there was no significant difference found between healthy and COVID-19 groups according to NOS3-rs2070744 polymorphism in neither genotype nor allele distribution. Even so, the genotype and allele distribution of the NOS3-rs1799983 polymorphism were observed to be statistically significantly different. The TG genotype and the G allele were higher in post COVID-19 avascular necrosis group, while the T allele was higher in healthy controls, while another study reported that there was no significant difference found between COVID-19 and healthy controls [27].

When comparing rs1799983 with different parameters, we observed that WBC and PLT counts in post post-COVID-19 avascular necrosis group were statistically significant differences.

**Table 4** Odds ratio

| | *N* | $p_1$ | $OR_1$ (LL – UL 95%C.I) | *N* | $p_2$ | $OR_2$ (LL – UL 95%C.I) | *N* | $p_3$ | $OR_3$ (LL – UL 95%C.I) |
|---|---|---|---|---|---|---|---|---|---|
| **rs1799983** | | | | | | | | | |
| T/T® | 30/23 | | | 30/15 | | | 23/15 | | |
| T/G | 28/33 | 0.255 | 1.537(0.733–3.224) | 28/36 | 0.020* | 2.571(1.164–5.680) | 33/36 | 0.210 | 1.673(0.749–3.737) |
| G/G | 2/4 | 0.292 | 2.609(0.439–15.503) | 2/9 | 0.009* | 9.0(1.724–46.994) | 4/9 | 0.071 | 3.450(0.898(13.248) |
| **Allele** | | | | | | | | | |
| T® | 78/67 | 0.147 | | 78/63 | 0.049* | | 67/63 | 0.604 | |
| G | 42/53 | | 1.469(0.873–2.471) | 42/57 | | 1.680(1.0–2.823) | 53/57 | | 1.144(0.688–1.901) |

$OR_1$: Odds ratio for Group I and Group II; $OR_2$: Odds ratio for Group I and Group III; $OR_3$: Odds ratio for Group II and Group III; **CI**: Confidence interval; **LL**: Lower limit; **UL**: Upper Limit; ®: reference group; *: Statistically significant at $p \leq 0.05$

**Table 6** Relation between rs2070744 and various parameters in group III (n = 60)

| | rs2070744 | | | Test of Sig. | p |
|---|---|---|---|---|---|
| | C/C (n = 6) | C/T (n = 33) | T/T (n = 21) | | |
| **Age (years)** | | | | | |
| Mean ± SD. | 53.67 ± 4.41 | c ± 10.70 | 66.33 ± 11.77 | F = 3.272* | 0.045* |
| Median (Min. – Max.) | 56 (45–56) | 65 (38–86) | 65 (47–84) | | |
| **Temperature** | | | | | |
| Mean ± SD. | 36.25 ± 0.36 | 37.23 ± 1.12 | 36.69 ± 0.49 | F = 4.316* | 0.018* |
| Median (Min. – Max.) | 36.2 (35.8–36.9) | 36.8 (36–39.6) | 36.9 (36–37.5) | | |
| **Heart rate** | | | | | |
| Mean ± SD. | 91.67 ± 1.97 | 105.64 ± 17.63 | 94.90 ± 9.54 | F = 4.826* | 0.012* |
| Median (Min. – Max.) | 92 (88–94) | 109 (80–130) | 95 (76–115) | | |
| **OS** | | | | | |
| Mean ± SD. | 76.83 ± 0.98 | 69.79 ± 7.11 | 68.81 ± 6.27 | F = 3.667* | 0.032* |
| Median (Min. – Max.) | 77 (75–78) | 72 (59–79) | 70 (55–77) | | |
| **Hemoglobin (gm/dl)** | | | | | |
| Mean ± SD. | 13 ± 0 | 11.24 ± 1.66 | 12.62 ± 1.47 | F = 7.045* | 0.002* |
| Median (Min. – Max.) | 13 (13–13) | 11 (9–14) | 13 (10–14) | | |
| **WBC (x10$^3$/ul)** | | | | | |
| Mean ± SD. | 2.75 ± 0.23 | 2.89 ± 0.21 | 2.70 ± 0.22 | F = 4.940* | 0.011* |
| Median (Min. – Max.) | 2.8 (2.3–3) | 2.9 (2.4–3.2) | 2.80 (2–3) | | |
| **Lymphocytes (10$^3$/ul)** | | | | | |
| Mean ± SD. | 1.47 ± 0.12 | 2.38 ± 3.52 | 1.63 ± 0.14 | H = 9.471* | 0.009* |
| Median (Min. – Max.) | 1.40 (1.40–1.70) | 1.50 (1.30–16) | 1.60 (1.50–1.90) | | |
| **D-dimer (mg/ml)** | | | | | |
| Mean ± SD. | 1.38 ± 0.10 | 1.44 ± 0.14 | 1.29 ± 0.11 | H = 14.868* | 0.001* |
| Median (Min. – Max.) | 1.40 (1.20–1.50) | 1.50 (1.20–1.60) | 1.30 (1.10–1.40) | | |
| **CRP (mg/L)** | | | | | |
| Mean ± SD. | 96 ± 0 | 142.55 ± 48.72 | 109.71 ± 34.42 | H = 9.859* | 0.007* |
| Median (Min. – Max.) | 96 (96–96) | 96 (96–192) | 96 (96–192) | | |

**SD**: Standard deviation; **F**: F for One way ANOVA test; **H**: H for Kruskal Wallis test; **$\chi^2$**: Chi square test; **MC**: Monte Carlo; **p**: p value for Relation between rs2070744 and various parameters; *: Statistically significant at $p \leq 0.05$; **Group III**: post COVID-19 avascular necrosis group

**Table 7** Relation between rs1799983 and various parameters in group III (n = 60)

| | rs1799983 | | | Test of Sig. | P |
|---|---|---|---|---|---|
| | T/T (*n* = 15) | T/G (*n* = 36) | G/G (*n* = 9) | | |
| **WBC (x10$^3$/ul)** | | | | | |
| Mean ± SD. | 2.90 ± 0.19 | 2.85 ± 0.18 | 2.51 ± 0.25 | F = 12.740* | < 0.001* |
| Median (Min. – Max.) | 2.90 (2.40–3.20) | 2.80 (2.40–3.20) | 2.60 (2.0–2.80) | | |
| **PLT(x10$^3$/ul)** | | | | | |
| Mean ± SD. | 131.1 ± 4.99 | 129.3 ± 6.82 | 124.6 ± 4.19 | F = 3.291* | 0.044* |
| Median (Min. – Max.) | 132 (115–136) | 132 (115–137) | 124 (116–130) | | |

**SD**: Standard deviation; **F**: F for One way ANOVA test; **H**: H for Kruskal Wallis test; **$\chi^2$**: Chi-square test; **p**: p-value for Relation between rs1799983 and different parameters; *: Statistically significant at $p \leq 0.05$; **Group III**: post COVID-19 avascular necrosis group

About rs2070744, we noticed that heart rate and Hb were statistically significant differences in both non-avascular necrosis and post-COVID-19 avascular necrosis groups. Systolic, diastolic blood pressure and PLT count were statistically significant differences in the non-avascular necrosis group. In post COVID-19 avascular necrosis group, Age, temperature, OS, WBC count, lymphocyte count, D-dimer and CRP were statistically significant differences.

Long COVID is a complicated, multifactorial condition that has been demonstrated to affect almost all organ systems, including inducing a severe prothrombotic state in both the microvascular and macrovascular levels [28]. There are very few case reports of femoral AVN following a COVID-19 infection [29].

Our study showed that genotypes of the polymorphisms 1,799,983 in post-COVID-19 avascular necrosis had no significant association with the susceptibility of AVN, in agreement with the study of Zhao et al.,2019 showed the association of NOS3 rs1799983 polymorphism with osteonecrosis of the femoral head (ONFH)

and in Chinese Han population, its T allele may be a protective factor against ONFH occurrence [30]. On the other hand, genotypes of the polymorphism rs2070744 had a significant association with avascular necrosis complications.

Patients, after being treated for COVID-19 infection, developed AVN of the femoral head, according to the study of Agarwala, S.R. et al., 2021 [31]. The hypothesis that the use of corticosteroids with COVID-19 raises the risk of AVN while lowering the onset of COVID-19-related respiratory symptoms is supported by a number of case studies. The case study of Kingma T.J. et al., 2022 showed that Long COVID is a complex condition which has contributed to the development of bilateral hip AVN in COVID-19 patients as COVID-19 affect intravascular blood flow causing a hypercoagulable state. In order to prevent catastrophic outcomes like bone collapsing, AVN must be examined early when evaluating anterior hip pain in individuals with a history of COVID-19 infection [32]. Bone loses its smooth shape as a result of AVN, which may cause severe arthritis. Also, other studies evaluated the relationship between AVN and COVID-19 [33].

## Conclusion

In conclusion, our findings suggest a potential link between COVID-19 infection and AVN development, with genetic factors such as NOS3 polymorphisms playing a significant role. The higher prevalence of specific genotypes and alleles among AVN patients underscores the importance of genetic susceptibility in post-infectious complications. Understanding these genetic mechanisms can aid in risk stratification and targeted interventions for AVN patients recovering from COVID-19. Further research is warranted to elucidate the underlying pathophysiological mechanisms and validate these genetic markers in larger cohorts. Ultimately, integrating genetic profiling into clinical management strategies may improve outcomes for individuals predisposed to AVN following viral infections like COVID-19.

### Abbreviations

| | |
|---|---|
| AVN | Avascular necrosis |
| COVID-19 | Coronavirus disease 2019 |
| NOS3 | Nitric oxide synthase 3 |
| SNP | Single nucleotide polymorphism |
| ABCD | Airway, Breathing, Circulation, Defibrillation |
| Hb | Hemoglobin |
| WBCs | White blood cells count |
| PLT | Patelet |
| IL-6 | Interleukin-6 |
| CRP | C-reactive protein |
| ONFH | Osteonecrosis of the femoral head |

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### Author contributions

Conceptualization, E.A.F., E.A.B.; data analysis, E.A.F., E.A.B., D.G., M.A.M; Writing—original draft, E.A.B., D.G., M.A.M; L revised the final version of the manuscript, E.A.F., E.A.B., D.G., M.A.M. All authors have read and agreed to the published version of the manuscript.

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### Data availability

The datasets generated and/or analyzed during the current study are available in the dbSNP repository, with the primary accession code NC_000007.14.

## Declarations

### Ethics approval and consent to participate

This study has been approved by the Menoufia University Faculty of Medicine's Ethical Committee for Medical Research (ethics approval number and date: 11/2023 BIO12). Informed consent was obtained from each patient before taking part. Moreover, all methods were carried out in accordance with relevant guidelines and regulations.

### Consent for publication

Not applicable.

### Conflict of interest

The authors declare no conflict of interest.

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