American Journal of Medicine and Medical Sciences 2026, 16(4): 2151-2155
DOI: 10.5923/j.ajmms.20261604.122

Significance of Clinical Factors and Endothelial Dysfunction in Tge Development of Femoral Head Osteonecrosis

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Abstract **Background:** Avascular necrosis of the femoral head develops in the context of impaired microcirculation and endothelial dysfunction, which play a key role in the disease pathofenesis. **Objective:** This study analyzed the allele and genotype frequencies of rs1799983, rs1549758, and rs2070744 polymorphisms of the NOS3 gene in patients with avascular necrosis of the femoral head and assessed their asosiashin with clinical risk factors. **Methods:** Allele and genotype frequencies were analyzed in patients with avascular necrosis of the femoral head using molecular genetic methods, and their association with clinical risk factors was evaluated. **Results:** Obtained result indicate that genetic variants can contribute to increased risk of development of disease and supports their potential relevance as genetic markers for early diagnosis and provides prevention of avascular necrosis of age femoral head. **Conclusion:** Polymorphisms of the gene encoding endothelial nitric oxide synthase may serve as potential markers for early diagnosis and prevention of avascular necrosis of the femoral head.

Keywords Avascular necrosis of the femoral head, NOS3 gene, Endothelial dysfunction, Nitric oxide, Microcirculation, COVID-19

1. Introduction

Avascular osteonecrosis (AVN) is one of most severe degenerative diseases in orthopedics/traumatolofy, particularly femoral head avascular osteonecrosis (FHAVN). It is characterized by osteocyte necrosis due to impaired blood supply in bone tissue, subchondral fractures, and during disease profression, structural collapse of the femoral head, ultimately leading to the development of hip joint osteoarthritis [1,2,3].

In clinical practice, FHAVN predominantly affect working population, making it a condition of significant sociale and economic concern. In the early stages, nonspecific clinical symptoms and delayed diagnosis often result in severe impairment of hip joint function and necessitate joint replacement [3,4].

The etiolofy of FHAVN is multifactorial. Risk factors include corticosteroid use, alcohol consumption, trauma, coagulation disorders, metabolic and endocrine patholofies, as well as comorbid somatic diseases [5,6]. Microcirculatory disturbances and endothelial dysfunction play a decisive role in the pathofenesis of FHAVN [7,8,12]. Endothelial nitric oxide synthase (eNOS), encoded by the NOS3 gene, is a key regulator of nitric oxide (NO) synthesis. In individuals with polymorphisms in this gene, reduced NO production can result with vascular spasms, hyper coagulation, and ischemic changes in bone tissue, representing an important pathofenetic factor (Figure 1).

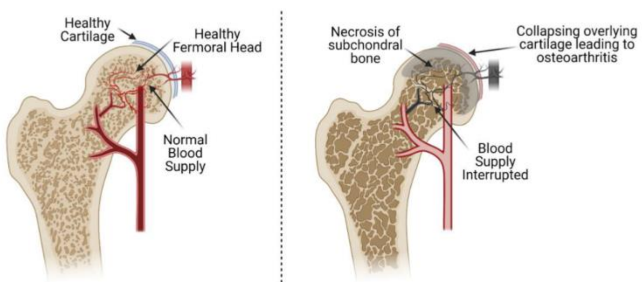

Figure 1. Pathofenesis of Femoral Head Osteonecrosis (*Note: Endothelial injury disrupts anticoagulant barriers and alters the balance of vasoactive substances, leading to vascular constriction. The left side shows preserved normal blood supply. The right side demonstrates vascular narrowing (vasoconstriction) [9]*.)

In recent years, reports have indicated an increasing number of cases associated with endothelial injury, hypercoagulation, and microcirculatory disturbances following COVID19 infection, which further underscores the relevance of endothelial factors in the development of osteonecrosis. This highlights the importance of studying genetic predispositions related to the endothelial nitric oxide synthase (eNOS) gene

Received: Mar. 27, 2026; Accepted: Apr. 22, 2026; Published: Apr. 29, 2026
Published online at http://journal.sapub.org/ajmms

[10,11]. Consequently, investigating the role of the eNOS gene in femoral head osteonecrosis is of high scientific and practical significance for a deeper understanding of disease pathofenesis, early identification of groups with risk, profnosis, and the development of individualized treatment strategies.

Objective: To determine the role of clinical factors and NOS3 gene polymorphisms (rs1799983, rs1549758, rs2070744) in the development of femoral head osteonecrosis.

Tasks: To evaluate the influence of NOS3 gene polymorphisms on the development and clinical course of femoral head osteonecrosis and their interaction with COVID-19 infection and other endothelial risk factors.

Study Subjects: Patients diagnosed with femoral head osteonecrosis and related endothelial and clinical factors.

2. Materials and Methods

The study was conducted at the Central Military Hospital from 2021 to 2025. It was an observational analytical study aimed at assessing the asosiashin of NOS3 gene polymorphisms with clinical indicators in patients with femoral head osteonecrosis. A total of 210 patient was diagnosed with femoral head osteonecrosis was included. Diagnosis was confirmed based on clinical data and instrumental examinations (X-ray and MRI). Disease stages was classified I to III.

Clinical and Laboratory Examinations: Clinical and laboratory parameters was assessed in patients with femoral head osteonecrosis. All patients was monitored according to a unified standard clinical protocol. Clinical indicators included age, disease duration, body mass index (BMI), osteonecrosis stage, laterality (right or left), and comorbidities.

Laboratory Examinations: Blood samples was collected from the peripheral vein in the morning after fasting. The following parameters was analyzed:

Complete blood count: Platelet count, leukocyte count, erythrocyte sedimentation rate (ESR);

Biochemical parameters: Calcium, phosphorus, alkaline phosphatase, uric acid, total cholesterol, triglycerides, lipoproteins;

Hemostasis parameters: Prothrombin index (PTI), fibrinofen, thrombin time, activated partial thromboplastin time (aPTT), international normalized ratio (INR), D-dimer;

Inflammatory markers: C-reactive protein (CRP), rheumatoid factor (RF), antistreptolysin O (ASO);

Hormonal parameters: Parathyroid hormone, vitamin D3.

Instrumental Examinations: Bone tissue status was evaluated using radiodensitometry. Intima- media thickness (IMT) of major arteries was measured using color Doppler scanning to asses vascular wall status and analyzed dynamically.

Molecular Genetic Examinations: Main and control groups was formed. The main group included 94 inpatients diagnosed with femoral head osteonecrosis, while the control group consisted of 96 apparently healthy individuals. Peripheral venous blood (2–5 mL) was collected into EDTA tubes. Genomic DNA was extracted using a selective detergent precipitation method with the Genomic DNA Purification Kit (Thermo Fisher Scientific, USA), yielding 2–10 µg of high-quality DNA of approximately 50 kb from 0.2 mL of blood within 35 minutes. DNA quality and concentration was assessed spectrophotometrically (A260/A280). Polymorphisms rs1799983, rs1549758, and rs2070744 of the NOS3 gene was analysed using real-time PCR with the "SNP-Screen" kit. Allele-specific dual probes were used fi r detection, witg results recorded via two fluorescent channels to differentiate each allele. PCR reactions was performed using the qTOWER ^{3} Real-Time PCR Thermal Cycler (Germany).

Statistical Analysis: Quantitative variants were expressed as mean ± standard error (M ± m), and analyzed using Doctor Stat 2013 (Version 1.9). Qualitative data was presented as n (%), quantitative data as mean ± SD. Genotype and allele frequencies was calculated, and Hardy– Weinberg equilibrium (HWE) was tested. Differences between groups was assessed with the χ^2 test or Fisher exact test. Lofistic regression (OR, 95% CI) was used to identify factors associated with osteonecrosis severity, adjusting for age, sex, BMI, harmful habits, comorbidities, and COVID-19 history. A p-value < 0.05 was considered statistically significant.

Ethical Considerations: The study protocol was approved by the internal ethics committee. All participants have written agreement and confidentiality was ensured.

Scientific Novelty: Fir the first time, the asosiashin between clinical-laboratory parameters, NOS3 gene polymorphisms, and osteonecrosis pathofenesis was analyzed in 210 patients with osteonecrosis.

Clinical Factors: The mean age of participants was 46.5 ± 8.3 years, indicating that osteonecrosis predominantly affects working-age adults (Table 1). Male patients predominated (90.5%), highlighting sex- specific risk factors such as occupational load, harmful habits, and hormonal influences. Stage analysis showed that most patients was at stage II (62.9%), indicating late clinical presentation. Stages I and III each comprised 18.6%, reflecting limited early diagnostic opportunities and declared medical attention. BMI analysis revealed that approximately 40% of patients was overweight or obesity, emphasizing the role of metabolic disturbances, microcirculatory changes, and impaired bone tissue tropism in osteonecrosis pathogenesis. Harmful habits, including smoking or alcohol consumption, was present in 37.1% of patients, representing risk factors that exacerbate endothelial dysfunction, vascular spasm, and ischemic changes in bone tissue. Endocrine disorders was present in 30.5%, and 61.0% had one or more comorbid somatic conditions, confirming the multifactorial nature of osteonecrosis and the importance of systemic pathologies in its profession. Congenital anomalies were rare (6.7%), indicating that the primary pathogenic mechanisms mostly related to acquired risk factors.

Table 1. Key Clinical Characteristics of Patients with Osteonecrosis

Parameter	Value
Number of patients (n)	210
Age, years (mean ± SD)	46.5 ± 8.3
Sex, n (%)	
– Male	190 (90.5%)
– Female	20 (9.5%)
Disease stage, n (%)	
– Stage I	39 (18.6%)
– Stage II	132 (62.9%)
– Stage III	39 (18.6%)
Body mass index, n (%)	
– Normal	126 (60.0%)
– Overweight	57 (27.1%)
– Obesity Grade I	19 (9.0%)
– Obesity Grade II–III	8 (3.8%)
Harmful habits, n (%)	
– Smoking or alcohol consumption	78 (37.1%)
Other risk factors	
– Endocrine disorders, n (%)	64 (30.5%)
– Comorbid somatic diseases, n (%)	128 (61.0%)
– Congenital anomalies, n (%)	14 (6.7%)
COVID-19 infection, n (%)*	97 (46.2%)
– Mild course of desise	41 (42.3%)
– Moderate course of disiase	36 (37.1%)
– Severe course of desise	20 (20.6%)

Note: Severity of COVID-19 was assessed among 97 patients who had a confirmed infection.

The high proportion of patients who had experienced COVID-19 infection (46.2%), including those with severe conditions (20.6%), is of particular significance. It is known that COVID-19 is associated with endothelial disfunction, hypercoagulation, and microcirculatory disturbances. Impaired nitric oxide synthesis mediated by NOS3 plays a crucial role in these processes. Therefore, in patients with unfavorable NOS3 gene polymorphisms, COVID-19 infection may act as an additional risk factor—a “second hit”—for the development or aggravation of femoral head osteonecrosis.

Endothelial Dysfunction Factors: Clinical and laboratory parameters reflecting endothelial dysfunction was comparatively analyzed in 210 patients with femoral head osteonecrosis before and after treatment. Before treatment, patients showed pronounced signs of inflammation and endothelial injury, with slightly elevated platelet count (259.2 ± 2.97 $\times 10^9$/L), leukocyte count (6.53 ± 0.09 $\times 10^9$/L), and erythrocyte sedimentation rate (ESR) (9.23 ± 0.34 mm/h). After treatment, these indicators decreased significantly (platelets: 219.0 ± 1.93 $\times 10^9$/L; leukocytes: 5.39 ± 0.07 $\times 10^9$/L; ESR: 6.52 ± 0.17 mm/h), reflecting reduced inflammatory activity (Table 2).

Table 2. Indicators Related to Endothelial Dysfunction in Patients with Femoral Head Osteonecrosis

Parameter	T1 – Before Treatment (M ± SE)	T2 – After Treatment (M ± SE)
Leukocytes ($\times 10^9$/L)	6.53 ± 0.09	5.39 ± 0.07
Platelets ($\times 10^9$/L)	259.2 ± 2.97	219.0 ± 1.93
ESR (mm/h)	9.23 ± 0.34	6.52 ± 0.17
Calcium (mmol/L)	2.28 ± 0.01	2.76 ± 0.01
Phosphorus (mmol/L)	0.92 ± 0.01	1.38 ± 0.01
Prothrombin Index (PTI, %)	100.2 ± 0.61	80.7 ± 0.53
Fibrinofen (g/L)	3.27 ± 0.02	2.72 ± 0.01
Thrombin Time (s)	13.2 ± 0.05	14.8 ± 0.06
aPTT (s)	40.43 ± 0.38	41.22 ± 0.36
C-Reactive Protein (CRP, mg/L)	3.16 ± 0.24	2.13 ± 0.07
Rheumatoid Factor (RF, IU/mL)	12.37 ± 0.40	9.17 ± 0.39
Antistreptolysin O (ASO, IU/mL)	80.49 ± 4.41	52.06 ± 3.04
Uric Acid (μmol/L)	348.10 ± 5.16	309.42 ± 2.49
Alkaline Phosphatase (U/L)	86.60 ± 1.30	69.40 ± 0.92
Vitamin D_3 (ng/mL)	19.29 ± 0.47	36.20 ± 0.28
D-dimer (mg/L)	0.51 ± 0.17	0.51 ± 0.17
Triglycerides (mmol/L)	2.16 ± 0.09	1.83 ± 0.06
Low-Density Lipoproteins (LDL, mmol/L)	3.47 ± 0.05	3.26 ± 0.04
High-Density Lipoproteins (HDL, mmol/L)	0.82 ± 0.01	0.68 ± 0.01
Radiodensitometry (T-score)	1.49 ± 0.05	1.14 ± 0.06

Note: Values are presented as mean ± standard error (M ± SE). Differences between T1 (before treatment) and T2 (after treatment) was assessed using paired t-tests; except for aPTT and D-dimer, all changes was statistically significant ($p < 0.05$).

Hemostasis Parameters: Analysis of hemostasis confirmed a hypercoagulable state characteristic of endothelial dysfunction. Before treatment, fibrinogen levels was elevated (3.27 ± 0.02 g/L), the prothrombin index (PTI) was increased (100.2 ± 0.61%), and thrombin time was shortened (13.2 ± 0.05 s). After treatment, fibrinogen (2.72 ± 0.01 g/L), PTI (80.7 ± 0.53%), and thrombin time (14.8 ± 0.06 s) normalized significantly, indicating improved endothelial function and reduced hypercoagulation (Table 2).

Mineral Metabolism Parameters: Indicators of mineral metabolism also showed significant changes related to endothelial-ischemic alterations. Before treatment, calcium (2.28 ± 0.01 mmol/L) and phosphorus (0.92 ± 0.01 mmol/L) levels was reduced, while after treatment, they increased to 2.76 ± 0.01 mmol/L and 1.38 ± 0.01 mmol/L, respectively. Additionally, alkaline phosphatase decreased from 86.60 ± 1.30 to 69.40 ± 0.92 U/L, reflecting stabilization of bone metabolism.

Inflammatory Markers: C-reactive protein (CRP), rheumatoid factor (RF), and antistreptolysin O (ASO) decreased significantly after treatment, indicating a reduction in endothelial inflammation.

Vitamin D3: Serum vitamin D 3 levels was low before treatment (19.29 ±0.47 ng/mL) und incresed to 36.20 ±0.28 ng/mL after treatment. This change was associated with improved endothelial function and bone tissue trophism.

Instrumental Findings: Radiodensitometry revealed significant positive changes after treatment, reflecting improved microcirculation and attenuation of is hemic processes. These results indicate that endothelial dysfunction, inflammation, and hypercoagulation play critical roles in the pathogenesis of femoral head osteonecrosis.

Genetic Factors of Endothelial Dysfunction: To reliably asses the genetic contribution to endothelial dysfunction, Hardy–Weinberg equilibrium was confirmed for NOS3 gene polymorphisms (rs2070744, rs1549758, rs1799983) in the control group. Subsequently, asosiashin analyses in the main patient group revealed that certain genotypes and alleles was significantely associated with an increased risk of developing femoral head osteonecrosis (Table 3).

Specifically, for rs2070744 (T-786C), CC genotype and C allele, fir rs1549758 (C774T), the CT and TT genotypes and T allele, and for rs1799983 (Glu298Asp), the TT genotype and T allele demonstrated statistically significant asosiashins with the risk of developing femoral head osteonecrosis (Table 3). In the control group, genotype distributions fir all studied polymorphisms was in Hardy–Weinberg equilibrium ($p > 0.05$), confirming the reliability of the genetic data and the population homogeneity of the sample. This allowed for correct interpretation of subsequent asosiashin analyses.

3. Discussion

Impaired microcirculation, endothelial dysfunction, and hypercoagulation among the leading pathogenetic factors in the development of femoral head osteonecrosis [3,5,7,13]. In recent years, interest has increased in the role of tg e endothelial nitric oxide (NO) system in the pathogenesis of this disease [7]. Functional polymorphisms in the NOS3 gene have been shown in multiple studies to play a key role in regulating vascular tone, platelet aggregation, and tissue perfusion [8].

Based on literature data and genetic, clinical, and laboratory results got in this study, a pathogenetic algoritm fir the development of aseptic femoral head osteonecrosis, dependent on endothelial dysfunction proposed (Figure 2).

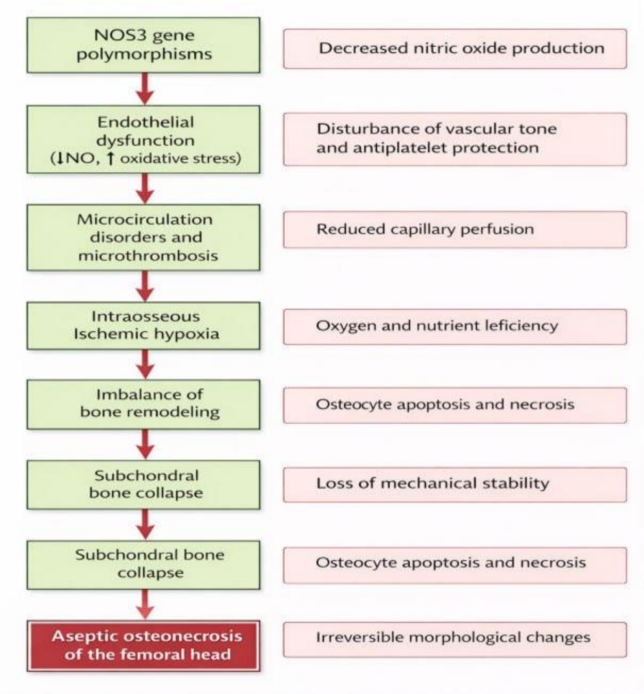

Figure 2. Pathogenetic Algorithm of Aseptic Femoral Head Osteonecrosis Associated with NOS3 Gene Polymorphisms

Table 3. Genotype and Allele Frequencies of NOS3 Gene Polymorphisms and Asosiashin Analysis in Patients with Femoral Head Osteonecrosis

NOS3 SNP	Genotype / Allele	Main Group (n=94), n (%)	Control Group (n=96), n (%)	OR	95% CI	p
rs2070744 (T-786C)	TT	40 (42.6)	54 (56.3)	Ref	–	–
	TC	32 (34.0)	37 (38.5)	1.17	0.61–2.26	0.63
	CC	22 (23.4)	5 (5.2)	5.94	2.05–17.20	<0.001
	C vs T	76 / 112	47 / 145	2.09	1.33–3.29	<0.01
rs1549758 (C774T)	CC	34 (36.2)	63 (65.6)	Ref	–	–
	CT	39 (41.5)	26 (27.1)	2.78	1.39–5.55	0.004
	TT	21 (22.3)	7 (7.3)	5.56	2.05–15.06	<0.001
	T vs C	81 / 107	40 / 152	2.87	1.79–4.60	<0.001
rs1799983 (Glu298Asp)	GG	53 (56.4)	60 (62.5)	Ref	–	–
	GT	21 (22.3)	28 (29.2)	0.85	0.42–1.73	0.65
	TT	20 (21.3)	8 (8.3)	2.83	1.14–7.04	0.024
	T vs G	61 / 127	44 / 148	1.61	1.01–2.57	0.045

Note: OR – odds ratio; CI – confidence interval; Ref – reference (wild-type) genotype. Allele and genotype asosiashins was calculated using 2×2 tables and a codominant model. Statistical significance was considered at p < 0.05.

This diagram illustrates sequential pathofenetic steps in the development of aseptic femoral head osteonecrosis thrush the effect of NOS3 gene polymorphisms on endothelial function. Adverse polymorphisms in the NOS3 gene lead to reduced nitric oxide (NO) synthesis and increased oxidative stress in the endothelium. This exacerbates endothelial dysfunction, manifested of impaired microcirculation and activation of microthrombosys processes. As a result, ischemic hypoxia develops in the bone tissue, leading to in bone remodeling imbalance, subchondral bone collapse, und ultimately the formation of aseptic femoral head osteonecrosis.

In this study, allele and genotype frequencies of NOS3 gene polymorphisms rs1799983, rs1549758, and rs2070744 was compared between patients with aseptic femoral head osteonecrosis and healthy controls. The irasosiashins with clinical risk factors such as body mass index, harmful habits, endocrine and cardiovascular pathologies, atherosclerosis, and history of COVID19 was also evaluated. The findings strengthen the pathoпenetic model based on endothelial dysfunction and provide a scientific basis for predicting the risk of femoral head osteonecrosis, identifying high-risk groups, and developing early preventive strategies while taking population characteristics into account.

4. Conclusions

1. In the studied group, NOS3 gene polymorphisms rs2070744, rs1549758, and rs1799983 was significantly associated witg an increased risk of n femoral head osteonecrosis.
2. Genetic variations manifested in clinical-laboratory changes characteristic of endothelial dysfunction, including inflammatory markers, coagulation parameters, and impaired microcirculation.
3. In the patient group, disturbances in bone mineral metabolism, vitamin D_3 deficiency, and remodeling imbalance was observed as important factors in the pathogenesis of femoral head osteonecrosis.
4. Genotype distributions fir all studied polymorphisms in the control group was consistent with Hardy–Weinberg equilibrium, confirming the reliability of the genetic data.
5. The combined genetic, c clinical, and laboratory findings indicate that NOS3 gene polymorphisms play a crucial role in the development of femoral head osteonecrosis via endothelial disfunction.

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