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Research Article

Effect of Hypercholesterolemia Patient Serum on The Viability and Characteristics of Mesenchymal Stem Cells

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Abstract

Mesenchymal Stem Cells (MSCs) have regenerative therapeutic potential, including for diseases related to hypercholesterolemia. This study aimed to assess how serum from hypercholesterolemia patients affects the viability and characteristics of MSCs. It also investigated changes in MSC morphology and cytokine secretion levels of TNF-α and IL-10 after 3 days of serum treatment. The study divided into three groups: control 10% FBS, patient serum with cholesterol <200 mg/dL (P1), and patient serum with cholesterol >300 mg/dL (P2). MSCs were exposed serum for 3 days. Cell viability was assessed using MTT assay; morphology was observed microscopically; cytokine levels were measured by ELISA. Statistical analysis used one-way ANOVA with Tukey's test for normal data and Kruskal-Wallis with Dunn's post hoc for non-normal data. Viability was highest in the control group, slightly reduced in P1, and significantly lowered in P2. Morphologically, control and P1 MSCs showed spindle-shaped fibroblast-like cells with high-confluency; P2 MSCs exhibited irregular, less elongated shapes and low-confluency. TNF-α levels were elevated in P1 compared to control and increased in P2. IL-10 levels cytokine secreted by MSCs showed increased in P1 compared to control and elevated in P2. Hypercholesterolemia serum reduced MSC proliferation, influenced morphology, and differentially affected TNF-α and IL-10 secretion.

Keywords: Characteristics; Hypercholesterolemia, Mesenchymal Stem Cells, Petient Serum, Viability

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1 Introduction

Hypercholesterolemia is a lipid metabolism disorder characterised by elevated total blood cholesterol levels. Its prevalence remains high, affecting approximately 35% of the global population, 30% of individuals in Southeast Asia, and 35% of the Indonesian population [1], [2]. This condition represents a major public health concern because elevated cholesterol levels are strongly associated with an increased risk of coronary artery disease, stroke, hypertension, and obesity. Hypercholesterolemia is defined as a total serum cholesterol level exceeding 200 mg/dL [2].

Mesenchymal stem cells (MSCs) are multipotent stem cells capable of promoting tissue repair and maintaining tissue homeostasis through differentiation into multiple cell lineages, while also exhibiting immunomodulatory properties that regulate inflammatory responses. The therapeutic potential of MSCs has attracted considerable interest in the biomedical field, particularly for the treatment of degenerative diseases, autoimmune disorders, and other chronic conditions. However, elevated cholesterol levels may alter the MSC microenvironment. Serum derived from patients with hypercholesterolemia has been reported to influence the phenotype and proliferative capacity of MSCs, thereby potentially reducing their therapeutic efficacy [3]. Investigating the effects of hypercholesterolemia serum is therefore essential, as the therapeutic performance of MSCs depends on their viability, proliferative capacity, differentiation potential, and surface marker profile, all of which are critical for their immunomodulatory and regenerative functions [4]. Previous studies [5], [6], [7] have demonstrated that hypercholesterolemia impairs the regenerative potential of MSCs and increases the risk of mitochondrial dysfunction. Mitochondria play a pivotal role in regulating membrane fluidity and cholesterol permeability [8], [9], [10]. Hypercholesterolemia can disrupt mitochondrial bioenergetics, leading to excessive reactive oxygen species (ROS) production and alterations in the biophysical properties of cellular membranes, as observed in diseases such as Niemann–Pick disease type C, Alzheimer's disease, and non-alcoholic fatty liver disease [10], [11].

In the present study, we investigated the effects of serum obtained from patients with hypercholesterolemia on MSC morphology, viability, cellular characteristics, and the concentration of cytokines secreted by MSCs following three days of treatment. Understanding the impact of hypercholesterolemia serum on MSC biology is essential for developing safer and more effective MSC-based therapeutic strategies.

2 Method

2.1 Study Design

This study was an in vitro experimental study designed to evaluate the effects of serum obtained from patients with hypercholesterolemia on the viability and immunomodulatory characteristics of mesenchymal stem cells (MSCs). Ethical approval was obtained from the Medical/Health Research Bioethics Committee, Faculty of Medicine, Universitas Sultan Agung, under approval number 232/V/2025/Komisi Bioetik. All experiments were conducted at the Stem Cell and Cancer Research (SCCR) Laboratory, Semarang, Indonesia.

2.2 Blood Collection

Blood samples were collected from patients diagnosed with hypercholesterolemia (total cholesterol ≥300 mg/dL) and from normocholesterolaemic individuals (total cholesterol ≤200 mg/dL) who served as the control group. Written informed consent was obtained from all participants, and the study protocol was approved by the institutional ethics committee. Whole blood was collected into plain red-top vacutainer tubes, allowed to clot at room temperature, and centrifuged at 3,000 rpm for 10 minutes. The resulting serum was separated, sterilised by filtration through a 0.22-µm membrane filter, and stored at −20°C until use.

2.3 MSC Culture and Treatment

Mesenchymal stem cells (MSCs) were obtained from the SCCR Laboratory and had been previously characterised according to the criteria established by the International Society for Cell & Gene Therapy (ISCT). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA)

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supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin, and maintained at 37°C in a humidified atmosphere containing 5% CO_2. For the treatment groups, FBS was replaced with 10% patient serum derived either from individuals with hypercholesterolemia or normocholesterolaemic controls. MSCs were seeded into 24-well plates and incubated for 72 hours.

2.4 Cell Viability Assay

Cell viability was assessed using the MTT assay. Following treatment with patient serum, MSCs were incubated with 0.5 mg/mL MTT solution for 4 hours at 37°C. The resulting formazan crystals were dissolved in dimethyl sulfoxide (DMSO), and absorbance was measured at 595 nm using a microplate reader. Cell viability was expressed as a percentage relative to the control MSCs cultured in medium supplemented with FBS

2.5 TNF-α and IL-10 ELISA and Statistical Analysis

The concentrations of TNF-α and IL-10 in the MSC-conditioned medium were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' instructions. Statistical analyses were performed using SPSS version 24.0 (IBM Corp., Armonk, NY, USA). Data normality was assessed using the Shapiro–Wilk test. Comparisons among the three groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for normally distributed data, whereas the Kruskal–Wallis testfollowed by Dunn's post hoc test was applied for non-normally distributed data. Results are presented as mean ±standard deviation (SD), and a p-value <0.05 was considered statistically significant.

3 Results and Discussion

3.1 Patient Demographic Characteristics

A total of four outpatient participants from Essensia Clinic, Semarang, Indonesia, were enrolled in this study. The demographic and baseline clinical characteristics of the participants are summarised in Table 1.

Tabel 1 Demographic and Clinical Characteristics of the Study Participants.

Patient	Age (years)	Sex	Laboratory Analysis				Comorbidities
			Total cholesterol (mg/dL)	Blood Glucose (mg/dL)	Triglycerides (mg/dL)	Uric acid (mg/dL)	
A	55	M	177	102	177	5,6	Diarrhea and gastroenteritis of presumed infectious origin
B	64	M	199	129	93	6,3	Parkinson's disease
C	36	F	301	84	355	5,4	SNH Anxiety and Thyphoid
D	63	F	303	328	287	4,3	SNH Anxiety

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The results presented in Table 1 show that the participants were 36–64 years of age and had diverse underlying clinical diagnoses. According to the 2023 Indonesian Health Survey, the prevalence of coronary heart disease in Indonesia is 0.85%. Furthermore, the World Health Organization (WHO) estimates that approximately 39% of individuals aged over 25 years have hypercholesterolemia, and more than one-third of deaths from ischaemic heart disease and ischaemic stroke are attributable to elevated blood cholesterol levels [12-15]

3.2 MSCs Validation and Characterization

The MSCs used in this study were MSCs passage 5. Passage 5 for storage and time point studies to ensure sufficient cell yield, stable surface marker expression, and avoidance of senescence [16].

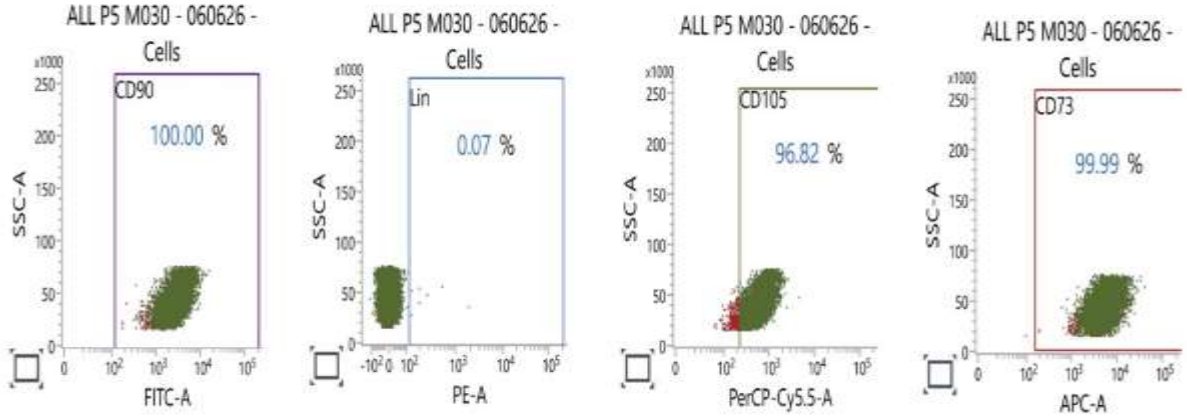

Figure 1 Immunophenotyping characterization of MSCs

Flowcytometry demonstrated that MSC express characteristic positive markers (CD73, CD105, CD90) with expression of hematopoietic or negative markers (CD19, CD45, CD34, HLA-DR). Positive markers included CD73 (99.99%), CD105 (96.82%), and CD90 (100%) for MSC, while the proportion of negative markers did not exceed 0.07%. The minimal criteria for immunotyping umbilical cord-derived mesenchymal stromal cell (UC-MSCs) by flow cytometry require positive expression of CD73, CD90, and CD105 (≥ 95%), and a lack of expression of hematopoietic and endothelial lineage markers including CD14 or CD11β, CD34, CD45, CD19 or CD79α, and HLA-DR (≤ 2%).[17]

3.3 Hypercholesterolemia Patient Serum Reduces MSC Proliferation In Vitro

Hypercholesterolemia patient serum significantly affected the proliferation of MSCs *in vitro*. To evaluate this effect, MSCs were cultured for 72 hours in media supplemented with different serum types. As shown in Figure 1, the control group cultured in medium containing 10% fetal bovine serum (FBS) exhibited the highest proliferation rate (100 ±2%). Treatment with 10% serum from a normocholesterolemia patient (P1; total cholesterol <200 mg/dL) resulted in a modest reduction in MSC proliferation to approximately 93 ±7.6% relative to the control. In contrast, treatment with 10% serum from a hypercholesterolemia patient (P2; total cholesterol >300 mg/dL) produced a markedly greater inhibitory effect, reducing cell proliferation to approximately 66 ±6.6% of the control level.

These findings indicate that the effect of patient serum on MSC proliferation is cholesterol concentration-dependent. Serum from individuals with normal cholesterol levels caused only a slight reduction in cell proliferation, whereas serum from patients with severe hypercholesterolemia significantly inhibited MSC growth, suggesting that elevated cholesterol adversely affects MSC viability and proliferative capacity. The ideal time points for MTT viability and proliferation assays in MSC are 24h, 48h, and 72h [18]. The optimal window for standard proliferation and cytotoxicity test, with the typical doubling time of cultured MSC are 42h and 72h [19].

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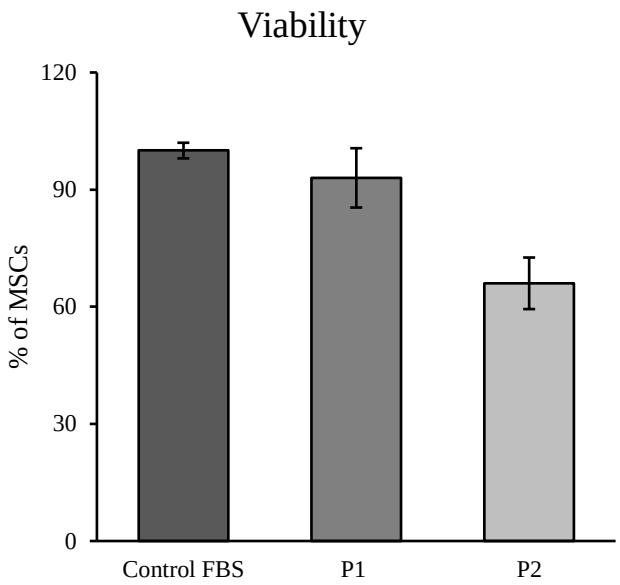

Figure 2 Effect of hypercholesterolemia patient serum on mesenchymal stem cell (MSC) proliferation after 72 hoursof culture. MSCs were cultured in medium supplemented with 10% fetal bovine serum (FBS) (Control), 10% serum from a normocholesterolaemic patient (P1; total cholesterol <200 mg/dL), or 10% serum from a hypercholesterolemia patient (P2; total cholesterol >300 mg/dL). Cell proliferation was expressed as a percentage relative to the control group (set at 100%). Data are presented as mean standard deviation (SD) from three independent experiments (n = 3).

Based on the present findings, MSC proliferation was significantly reduced following exposure to hypercholesterolemia patient serum. This reduction may be attributed to the ability of elevated cholesterol levels to disrupt key intracellular signalling pathways, including Wnt/β-catenin, PI3K/AKT, and MAPK, all of which play critical roles in regulating MSC proliferation, survival, and differentiation. Furthermore, hypercholesterolemia has been shown to increase the production of reactive oxygen species (ROS), leading to oxidative stress and chronic inflammation, which consequently impair MSC viability, proliferative capacity, and differentiation potential [20, 21, 22].

Another potential mechanism is the disruption of membrane lipid homeostasis induced by excessive cholesterol accumulation. Altered membrane cholesterol composition affects membrane fluidity, ion transport, receptor function, and cell signalling, thereby impairing the adhesion, migration, and proliferation of umbilical cord-derived mesenchymal stem cells (UC-MSCs). In particular, hypercholesterolemia modifies the structure and function of caveolae, specialised cholesterol-rich membrane microdomains that regulate receptor activation and signalling complexes involved in the Wnt/β-catenin, PI3K/AKT, and MAPK pathways. These alterations ultimately compromise the regenerative and immunomodulatory functions of MSCs [23, 24, 25].

3.4 Morphological Changes in Mesenchymal Stem Cells (MSCs) Following Exposure to Hypercholesterolemia Patient Serum

The morphological characteristics of MSCs after 72 hours of culture are presented in Figure 2. Under the control condition (10% FBS), MSCs exhibited their typical elongated spindle-shaped, fibroblast-like morphology, with a uniform appearance and high confluency. Similarly, MSCs cultured in medium supplemented with 10% serum from a normocholesterolemia patient (P1; total cholesterol <200 mg/dL) largely maintained their characteristic spindle-shaped morphology and displayed a proliferative pattern comparable to that of the control group, although with slightly lower cell density.

In contrast, MSCs cultured with 10% serum from a hypercholesterolemia patient (P2; total cholesterol >300 mg/dL) exhibited pronounced morphological alterations. The cells appeared less elongated, displayed an irregular cellular morphology, and showed reduced confluency, indicating impaired proliferation and a potential cellular stress response induced by the hypercholesterolemia serum.

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These observations suggest that serum from patients with severe hypercholesterolemia adversely affects both the morphology and proliferative capacity of MSCs *in vitro*.

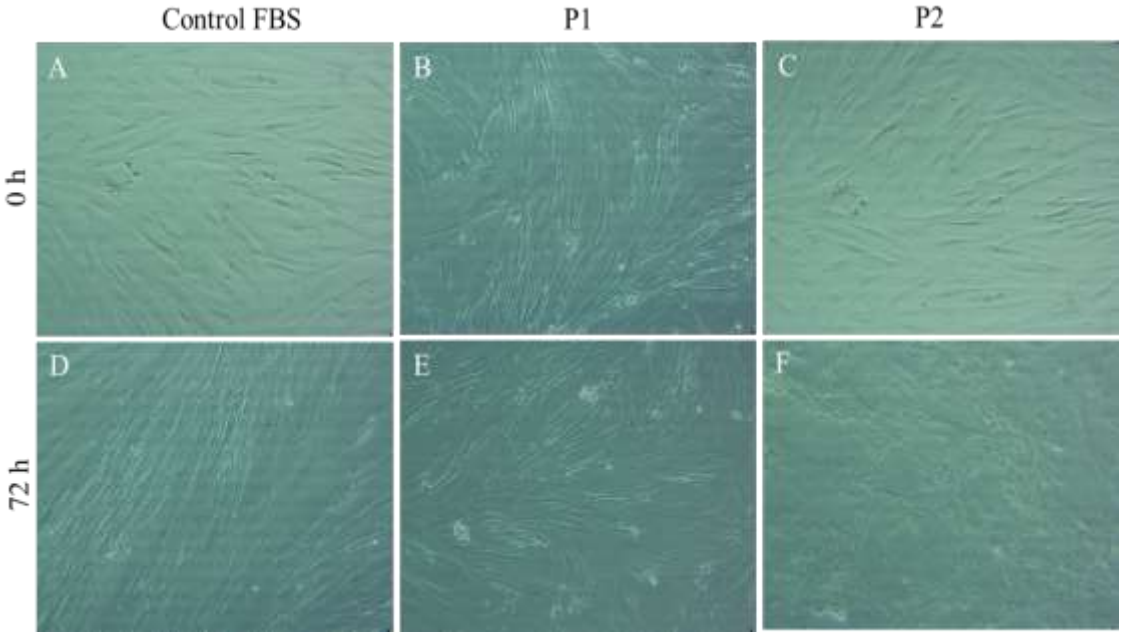

Figure 3 Representative phase-contrast micrographs of mesenchymal stem cells (MSCs) cultured for 72 hours under different serum conditions. (A, D) Control medium supplemented with 10% fetal bovine serum (FBS); (B, E) medium supplemented with 10% serum from a normocholesterolaemic patient (P1; total cholesterol <200 mg/dL); and (C, F)medium supplemented with 10% serum from a hypercholesterolemia patient (P2; total cholesterol >300 mg/dL). MSCs in the control and P1 groups retained their characteristic spindle-shaped, fibroblast-like morphology with good confluency, whereas MSCs in the P2 group exhibited reduced cell density, altered cellular morphology, and decreased proliferation, indicating the detrimental effects of hypercholesterolemia serum on MSC growth. Scale bar = 100 μm.

The observed morphological alterations suggest that exposure to hypercholesterolemia serum adversely affects the cellular microenvironment of MSCs. Elevated cholesterol levels may induce cellular stress, resulting in reduced cell viability, irregular cell morphology, and decreased confluency. These changes are consistent with impaired proliferative capacity and may reflect the onset of cellular senescence. Previous studies have demonstrated that MSCs undergoing oxidative stress-induced senescence exhibit a flattened, enlarged, and irregular morphology, accompanied by a marked loss of proliferative potential [25].

3.5 Effects of Hypercholesterolemia Patient Serum on TNF-α and IL-10 Secretion by MSCs

The concentrations of TNF-α and IL-10 secreted by MSCs following 72 hours of culture with patient serum are presented in Table 2. In the control group cultured in medium supplemented with 10% fetal bovine serum (FBS), the concentration of TNF-α was low (8.3 ±1.7 pg/mL). A significant increase in TNF-α secretion was observed in MSCs cultured with 10% serum from a normocholesterolaemic patient (P1; total cholesterol <200 mg/dL), reaching 448.9 ±293.5 pg/mL ($p < 0.05$ vs. control). In contrast, MSCs treated with 10% serum from a hypercholesterolemia patient (P2; total cholesterol >300 mg/dL) exhibited a significantly lower TNF-α concentration (146.6 ±125.9 pg/mL) than the P1 group, although the level remained significantly higher than that of the control group ($p < 0.05$). Regarding IL-10, MSCs cultured under control conditions secreted 4.3 ±2.1 pg/mL. Treatment with P1 serum resulted in a slight, non-significant decrease in IL-10 production (3.7 ±1.0 pg/mL; $p > 0.05$ vs. control),

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whereas MSCs cultured with P2 serum showed a modest increase (5.1 ±2.3 pg/mL), which was not significantly different from the control group.

Overall, these findings indicate that exposure to patient serum alters the cytokine secretory profile of MSCs. Serum from the normocholesterolaemic patient (P1) markedly enhanced TNF-α secretion, whereas serum from the hypercholesterolemia patient (P2) attenuated TNF-α production while showing a tendency to increase IL-10 secretion. These results suggest a potential shift from a predominantly pro-inflammatory cytokine profile towards a more immunoregulatory phenotype under conditions of severe hypercholesterolemia.

Table 2. Concentrations of TNF-α and IL-10 secreted by mesenchymal stem cells (MSCs) following treatment with patient serum for 72 hours.

Group	TNF-α (pg/mL)	IL-10 (pg/mL)
Control FBS	8,3 ± 1,7	4,3 ± 2,1
P1	448,9 ± 293,5	3,7 ± 1,0
P2	146,6 ± 125,9	5,1 ± 2,3

Data are presented as mean standard deviation (SD) from three independent experiments (n = 3). Based on the present findings, the P2 group, which was exposed to serum from patients with severe hypercholesterolemia, exhibited a reduction in TNF-α secretion. This decrease may be attributed to MSC senescence, a process characterised by reduced cellular metabolism, diminished transcriptional activity, and impaired cytokine production [26,27]. In contrast, the P1 group demonstrated a marked increase in TNF-α secretion. One possible explanation is that one of the patients in the P1 group had elevated random blood glucose (RBG) levels, which may have contributed to a pro-inflammatory state independent of serum cholesterol levels. Hyperglycaemia disrupts normal cellular metabolism and activates inflammatory signalling pathways, resulting in increased production of pro-inflammatory cytokines, including TNF-α and IL-6 [28,29]. Senescent MSCs are known to exhibit reduced proliferative capacity, impaired metabolic activity, and altered cytokine secretion. Furthermore, TNF-α expression is regulated through the NF-κB signalling pathway, and severe hypercholesterolemia can induce mitochondrial dysfunction, leading to dysregulated NF-κB activation. Consequently, prolonged exposure to serum with high cholesterol concentrations may suppress TNF-α expression in MSCs [30]. Conversely, IL-10 secretion showed a tendency to increase in MSCs exposed to hypercholesterolemia serum. This increase may represent a compensatory cytoprotective response, whereby MSCs enhance the production of the anti-inflammatory cytokine IL-10 to preserve cellular homeostasis and counteract cholesterol-induced inflammatory stress [31].

4 Conclusion

In conclusion, exposure of mesenchymal stem cells (MSCs) to serum from patients with hypercholesterolemia adversely affected their viability, morphology, and cytokine secretion. MSCs cultured with hypercholesterolemia serum exhibited reduced proliferative capacity and pronounced morphological alterations, including loss of the characteristic spindle-shaped fibroblast-like appearance and decreased cell confluency, indicating impaired cellular growth and viability. Furthermore, hypercholesterolemia serum altered the immunomodulatory profile of MSCs by suppressing TNF-αsecretion while promoting a relative increase in IL-10 production. These findings suggest that a hypercholesterolemia microenvironment compromises the regenerative and immunomodulatory properties of MSCs and should be considered when developing MSC-based therapeutic strategies for patients with metabolic disorders.

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5 Declaration

5.1 Acknowledgement

The authors gratefully acknowledge Stem Cell and Cancer Research (SCCR) Indonesia for providing the laboratory facilities and technical support that made this study possible.

5.2 Author Contribution

DC conducted the experiments, performed cell culture, and prepared the manuscript. DRA, HH analysed the data and contributed to manuscript preparation. NH supervised the study and provided scientific guidance. YA collected patient samples and assisted with the ethical approval process.

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