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Oxidative stress, inflammation, and apoptosis in Alzheimer's disease associated with HSV-1 and CMV coinfection

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Abstract

Oxidative stress, inflammation, and apoptosis have been reported to influence cognitive function in patients with Alzheimer's disease (AD), particularly those infected with herpes simplex virus type 1 (HSV-1) or cytomegalovirus (CMV). This study aimed to evaluate the effects of viral infection on oxidative stress markers associated with these pathways in AD patients. A total of 100 adults with mild-to-moderate AD were randomly assigned to a double-blind, placebo-controlled clinical trial and categorized into three groups: AD (uninfected), AD with HSV-1, and AD with CMV. The primary outcomes included changes in serum inflammatory markers (IL-1β and TNF-α), blood antioxidant and oxidative stress markers—glutathione peroxidase (GPx), superoxide dismutase (SOD), malondialdehyde (MDA), reactive oxygen species (ROS), and total antioxidant capacity (TAC), as well as the expression levels of apoptosis-related proteins (BAX and BCL-2). Results showed that, compared to the control group, the AD group exhibited significant alterations in inflammatory and oxidative stress markers. CMV infection led to increased antioxidant enzyme activity and decreased serum inflammatory markers relative to the uninfected AD group. However, there were significant differences in ratio BAX/BCL-2 protein expression between the CMV and HSV-1 groups when compared to the AD group. In conclusion, AD patients infected with HSV-1 or CMV demonstrated distinct alterations in inflammatory, oxidative stress, antioxidant profiles, and apoptosis markers, which may have beneficial implications for circulatory biomarkers and potentially cognitive outcomes in AD.

Keywords Oxidative stress, Inflammation, Apoptosis, Alzheimer's disease, HSV-1 infection, CMV infection

Introduction

Alzheimer's disease (AD) is the most prevalent cause of dementia, accounting for approximately 80% of all diagnosed cases worldwide [1]. It is a progressive neurodegenerative disorder characterized by the accumulation of amyloid-β (Aβ) plaques and neurofibrillary tangles, primarily in the medial temporal lobe and neocortical structures regions of the brain essential for memory and cognition [2]. The progressive loss of cognitive function in AD may be attributed not only to intrinsic neurodegeneration but also to external factors such as infections, exposure to toxins, pulmonary and circulatory impairments leading to cerebral hypoxia, tumors, vitamin B12

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deficiency, and other nutritional deficits [3, 4]. Globally, it is estimated that around 50 million individuals are currently living with AD, and this number is expected to triple to approximately 152 million by 2050. The disease imposes a significant burden on individuals, families, and healthcare systems, with global costs exceeding 1 trillion USD annually. Despite ongoing research efforts, there is currently no definitive cure for AD; existing treatments offer only limited symptomatic relief and do not halt disease progression [5, 6].

Herpes simplex viruses type 1 (HSV-1) and type 2 (HSV-2) are widespread pathogens that establish lifelong latent infections in humans [7]. HSV-1 is predominantly transmitted through oral contact, although sexual transmission has become more common in certain regions, particularly in Asia and Western countries [8–10]. HSV-2 is primarily transmitted through sexual contact [11]. These infections often remain latent and asymptomatic, with occasional symptomatic reactivation [12, 13]. Serological testing (seroprevalence) is commonly used to detect exposure to HSV-1 and HSV-2 [14]. These infections are also considered indicators of sexual behavior and may facilitate the transmission of other viruses, particularly HIV. HSV-2 seropositivity, in particular, is a known marker of high-risk sexual behavior and increased vulnerability to HIV infection [15–17]. Unlike bacterial infections, herpesvirus infections persist for life and are unaffected by antibiotic treatment, although transmission patterns and host immune responses can influence their clinical presentation.

Cytomegalovirus (CMV), a member of the *Herpesviridae* family and classified as a β-herpesvirus (HHV-5), is another highly prevalent virus worldwide. It is the largest known herpesvirus infecting humans, with a genome size of approximately 240 kb [18]. It is a member of the *Herpesviridae* family and *Cytomegalovirus* genus [19]. CMV is categorized as β-herpesvirus (HHV-5), considered the largest herpesvirus infecting humans, with a genome approaching 240 kb [20, 21]. CMV infection is nearly universal in certain regions, with prevalence rates reaching close to 100% in Asia and Africa and around 80% in North America and Europe. According to data from the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC), more than 50% of adults in the United States are infected with CMV by the age of 40, and approximately one-third of children acquire the infection by age five [22]. While CMV is typically asymptomatic in healthy individuals, it can cause severe complications in immunocompromised populations, including transplant recipients and patients receiving blood transfusions [23]. CMV disease may present with non-specific symptoms, posing diagnostic challenges and increasing the risk of delayed or incorrect diagnosis and poor outcomes [24]. Thus, CMV infection is reflected as an important health problem in high-risk groups.

HSV-1 has been shown to promote oxidative stress in the central nervous system through microglial activation, resulting in heightened inflammatory responses. In contrast, CMV infection has been associated with resistance to apoptosis triggered by various stimuli, including death receptor signaling and chemotherapeutic agents such as doxorubicin [25, 26]. In this study, we investigated the levels of oxidative stress markers, pro-inflammatory cytokines, and apoptotic proteins in blood samples from AD patients infected with HSV-1 or CMV, aiming to elucidate the potential impact of these viral infections on the molecular mechanisms underlying AD pathology.

Materials and methods

Chemicals

2′,7′-Dichlorofluorescein diacetate (DCFH-DA), 2, 4, 6-tripyridyl-s-triazine (TPTZ), and Thiobarbituric acid reactive substances (TBARS) were purchased from Sigma (Sigma-Aldrich, St. Louis, MO). The salts used to make buffer solutions were of the analytical grade and obtained from Merck (Darmstadt, Germany). TNF-α Assay Kit (Karmaniapars gen co. Iran) and IL-1β Assay Kit (Karmaniapars gen co. Iran) were used for the calculation of inflammatory biomarkers.

Patients

Blood samples were taken from 47 women and 53 men with AD in hospitals in Tehran, Iran. Demographic date and medical records of the subjects mentioned in Table 1. As mentioned in our previous manuscript [27], plasma samples were taken from 100 AD patients. The samples were obtained from the hospitals of Imam Reza at Kermanshah university of medical science, Kermanshah, Iran (KUMS) and Milad at Tehran, Iran. An ethical permission was issued by the KUMS Ethics Committee (IR.KUMS.REC.1379.3525). All patients provided written informed consent. The research proposal for this study and the experimental steps were approved by the KUMS institutional review board.

Groups

For this research, we selected four groups as follows: the control group comprised of 26 individuals, the AD group comprised of 24 individuals, and the AD + HSV-1 and AD + CMV groups, each consisting of 24 individuals, with an equal number of men and women in each group.

Study plan

Samples were collected in sterile tubes and stored at -80 °C until DNA extraction. The DNA was quantitatively and qualitatively evaluated using spectrophotometry and agarose gel electrophoresis. Viruses like HSV-1 and

Table 1 Demographic date and medical records of the subjects

	Control	AD	HSV-1	CMV
Age	70.75 ± 6	71.3 ± 5	69.9 ± 8	72.2 ± 3
Gender	Male = 13 Female = 13	Male = 12 Female = 12	Male = 12 Female = 12	Male = 12 Female = 12
Marital status	Married	Married	Married	Married
Duration of disease	No disease	10 ± 2.3 Months	12 ± 1.02 Months	10 ± 3.9 Months
Underlying disease	History of infectious diseases (*n* = 22) Hallucination (*n* = 45) Anemia (*n* = 6) Blood fats (*n* = 64) Fe deficiency (*n* = 48) Brain stroke (*n* = 10) P (normal), Ca (normal), Na (normal), K (normal) Thyroid (*n* = 24) Insomnia (*n* = 32) Depression (*n* = 53) Heart attack (*n* = 36) Hypertension (*n* = 68) Diabetes (*n* = 27)			

CMV were detected using specific primer pairs. PCR was conducted following viral DNA isolation. Patients with Alzheimer's disease carrying these viruses were found to have different antioxidant and oxidative stress levels. Inflammatory markers and gene expression were also analyzed in the study.

Blood sampling

5 ml blood samples were collected from the right cubital vein in the arm using heparinized vacuum tubes (Venoject) and stored at 4 °C. All experiments were conducted within 24 h of blood sampling [28]. Also, leukocytes were extracted from blood samples (2.5 mL in EDTA) using the osmotic lysis procedure. Total RNA was isolated by the manufacturer's instructions. Afterthat, the concentration of the acquired total RNA was evaluated and converted to cDNA.

Oxidative stress markers

Lipid peroxidation in the serum

Thiobarbituric acid reactive substances (TBARS) were used to measure lipid peroxidation in serum. The reaction mixture contained thiobarbituric acid, phosphoric acid, and serum, which was heated and then n-butanol was added. After centrifugation, the absorbance of the color in the n-butanol phase was measured at 532 nm.

Reactive oxygen species (ROS) formation in the serum

Reactive oxygen species (ROS) levels in the serum were measured using a method outlined by Gupta et al., with some adjustments. Serum samples were homogenized in Tris-HCl buffer and mixed with dichlorofluorescein diacetate before being incubated and assessed for fluorescence intensity. The measurement was carried out using a FLUOstar Omega® microplate reader (λ excitation = 485 nm and λ emission = 525 nm) [29].

Ferric reducing antioxidant power (FRAP) of the serum

The FRAP assay measures the change in absorbance at 593 nm as a blue Fe II-tripyridyl triazine compound forms from a colorless Fe III form due to electron donating antioxidants. The FRAP reagent is prepared by mixing acetate buffer (300 mmol/L acetate buffer; pH 3.6), TPTZ (10 mmol/L in hydrochloric acid 40 mmol/L), and ferric chloride (20 mmol/L). Serum is homogenized in Tris buffer, then added to the FRAP reagent and incubated at 37°C for 5 min. The absorbance of the developed color is measured at 595 nm by an Ultrospec2000 ® spectrophotometer [30].

Antioxidant enzymes levels assay

Superoxide dismutase activity assessment

Blood samples were collected and treated to assess superoxide dismutase (SOD) activity. Hemolyzed red blood cells were obtained by mixing blood with ice-cold water, removing hemoglobin with chloroform and ethanol, and centrifuging the mixture [31]. The clear supernatant was then used for the SOD assay following a specific method based on inhibiting nitro blue tetrazolium reduction. The intensity of the superoxide reaction by SOD was calculated as per McCord and Fridovich's definition [32].

Catalase activity determination

The catalase (CAT) activity in hemolysates was assessed using the Aebi method. Fresh red blood cells provided the hemoglobin solution. Heparinized blood samples were centrifuged, and plasma was removed. Red cells were washed and hemolyzed with distilled water. The breakdown of H2O2 was measured at 240 nm using a

spectrophotometer. CAT activity was reported as the rate constant (k) per gram of hemoglobin, following Aebi's definition [33].

Glutathione peroxidase

Glutathione peroxidase activity was measured in hemolysates following a modified method by Carmagnol et al. [34] based on the original technique of Paglia and Valentine [35]. Hemoglobin was converted into cyanmethemoglobin to prevent pseudoperoxidase reactivity. The GPx activity was determined using a coupled assay system where the oxidation of GSH was coupled to NADPH oxidation catalyzed by glutathione reductase. The reaction mixture included cumene hydroperoxide, GSH, yeast glutathione reductase, NADPH, and KCN in potassium phosphate buffer. GSSG produced by GPx action was reduced by glutathione reductase and NADPH, with the decrease in NADPH concentration recorded at 340 nm. GPx activity was defined as the enzyme amount converting 1 nM of NADPH per minute, expressed as units per gram of hemoglobin [28].

Measurement of pro-inflammatory response

TNF-α Assay Kit and IL-1β Assay Kit were used to evaluate serum TNF-α and IL-1β levels and the results were expressed as pg/ml.

Real-Time quantitative PCR (qRT-PCR)

Total RNA was extracted from frozen leukocytes tissue of Alzheimer's disease patients and stored at -80 °C using a RNA extraction kit. The RNA was then synthesized into cDNA using a Total RNA Extraction Kit and Easy™ cDNA Synthesis kit. Primers for Bax and Bcl-2 (Table 2) were designed and synthesized by Metabion International AG Company (Germany). Quantitative real-time PCR was conducted using SYBR Green Real time-PCR kit on a Step one plus Real Time System. The cycling conditions were as follows: 95 °C for 10 s, 45 cycles of 95 °C for 15 s, 56 °C for 30 s, and 72 °C for 30 s. The gene expression levels of Bax, Bcl-2, and GAPDH were analyzed using Step one software v2.1 and the 2(−ΔΔCt) equation.

Statistical methods

Graph Pad Prism 6 software (Graph Pad Software Inc., San Diego, CA, USA) was used for the statistical analysis.$P<0.05$ was considered to be statistically significant. Data are presented as the Mean ± SD. Data comparison was performed by the one-way analysis of variance with Tukey post-test.

Results

Oxidative stress factors

The results of Fig. 1 indicate that lipid peroxidation levels and free radical production were significantly elevated in the AD, HSV-1, and CMV groups compared to the control group ($p<0.001$). Notably, lipid peroxidation was significantly higher in the HSV-1 group compared to the AD group($p<0.05$), and ROS levels were significantly increased in both the HSV-1 and CMV groups relative to the AD group ($p<0.001$). Furthermore, the results of the FRAP assay demonstrated a significant reduction in total antioxidant capacity in the AD, HSV-1, and CMV groups compared to the control ($p<0.001$).

Antioxidant enzymes activity

As shown in Fig. 2 (a-c), the GPx, SOD, and CAT enzymes activity were significantly decreased ($p<0.05$) in AD and HSV-1 groups, compared to the control group. Additionally, the activity of these antioxidant enzymes were markedly increased ($p<0.05$; SOD and GPX activity and $P<0.001$; CAT activity) in CMV group in comparison with AD group.

Pro-inflammatory factors

TNF-α and IL-1β levels were used to estimate exploratory pro-inflammatory factors in the serum of AD (Fig. 3a and b). As can be observed in Fig. 3, the level of TNF-α and IL-1β increased significantly in the HSV-1 group in comparison with the control group ($p<0.001$). In contrast, CMV group was not significantly changed the TNF-α and IL-1β levels in the serum of AD.

Apoptosis-related proteins expression

As shown in Fig. 4, the ratio of Bax/Bcl-2 protein expression level was significantly increased in the AD group and significantly decreased in the HSV-1 group compared to the control group. Additionally, the data indicate that this apoptotic marker was significantly reduced in the HSV-1 and CMV groups compared to the AD group.

Discussion

Emerging evidence suggests that microorganisms may play a critical role in the progression of Alzheimer's disease (AD). In our previous research, we proposed that latent herpes simplex virus type 1 (HSV-1), residing in

Table 2 Primer sequences for targeted genes

	Forward	Reverse
GAPDH	5′-CTTTTGCGTCGCCAGGTGAA-3′	5′-AGGCGCCCAATACGACCAAA-3′
BAX	5′-GCGACTGATGTCCCTGTCTCC-3′	5′-AAAGATGGTCACGGTCTGCCA-3′
BCL-2	5′-GAACTGTACGGCCCCAGCAT-3′	5′-GGGGCAGGCATGTTGACTTC-3′

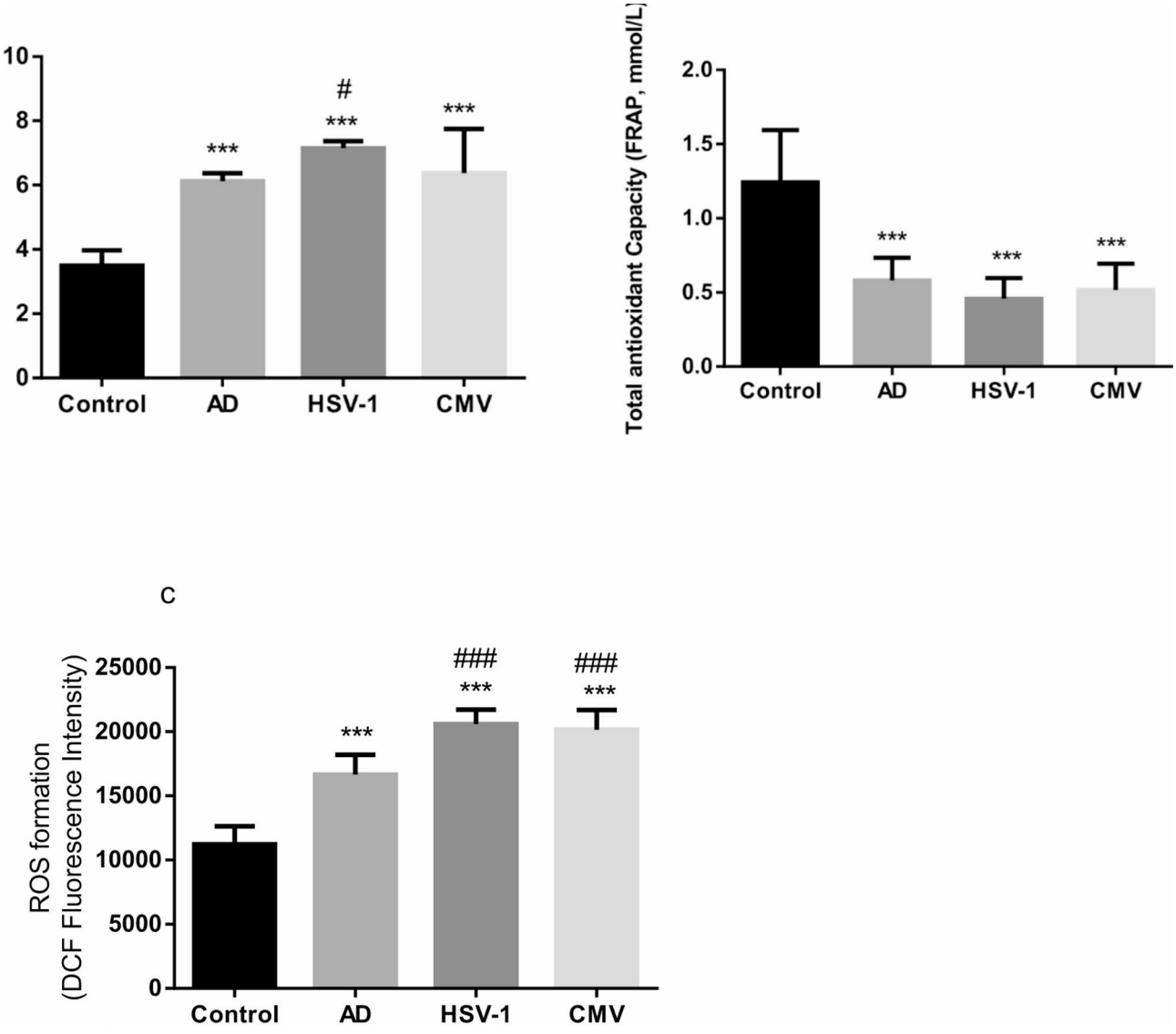

Fig. 1 Markers of oxidative stress in the Serum of control, AD, HSV-1, and CMV groups. Alzheimer's disease (AD), Cytomegalovirus (CMV), Herpes simplex virus types 1 (HSV-1). ROS: Reactive oxygen species; DCF: Dichlorodihydrofluorescein; MDA: Malonyl dialdehyde **(a)** Lipid peroxidation levels, **(b)** Total antioxidant levels, **(c)** ROS production levels. Data are reported as mean ± SD ($n = 100$). **Indicates significantly different as compared with the Control group ($P < 0.01$), ***Indicates significantly different as compared with the Control group ($P < 0.001$), #Indicates significantly different as compared with the AD group ($P < 0.05$)

the trigeminal ganglia, may migrate along neural pathways to reach the limbic system and brain regions particularly vulnerable in AD. The presence of HSV-1 antibodies has been associated with cognitive impairments in children and deficits in reading and visuospatial processing in middle-aged adults. Additionally, cytomegalovirus (CMV)—which is transmitted via bodily fluids—has been shown to elicit heightened IgG antibody responses in older adults compared to younger individuals. Age-related changes in cell-mediated immunity may contribute to subclinical reactivation of CMV in these populations.

Our previous study demonstrated that AD patients co-infected with HSV-1 and CMV exhibit an elevated risk of disease progression, potentially due to enhanced beta-amyloid (Aβ) production. Oxidative stress and immune dysfunction have been implicated as key mechanisms in AD pathogenesis. The present study aimed to further explore these molecular mechanisms in AD patients with concurrent HSV-1 and CMV infections.

Our findings regarding oxidative stress markers in AD patients are consistent with prior studies. AD is known to promote oxidative stress through excessive production of reactive oxygen species (ROS) and a concurrent deficiency in antioxidant defense systems. Huang et al.

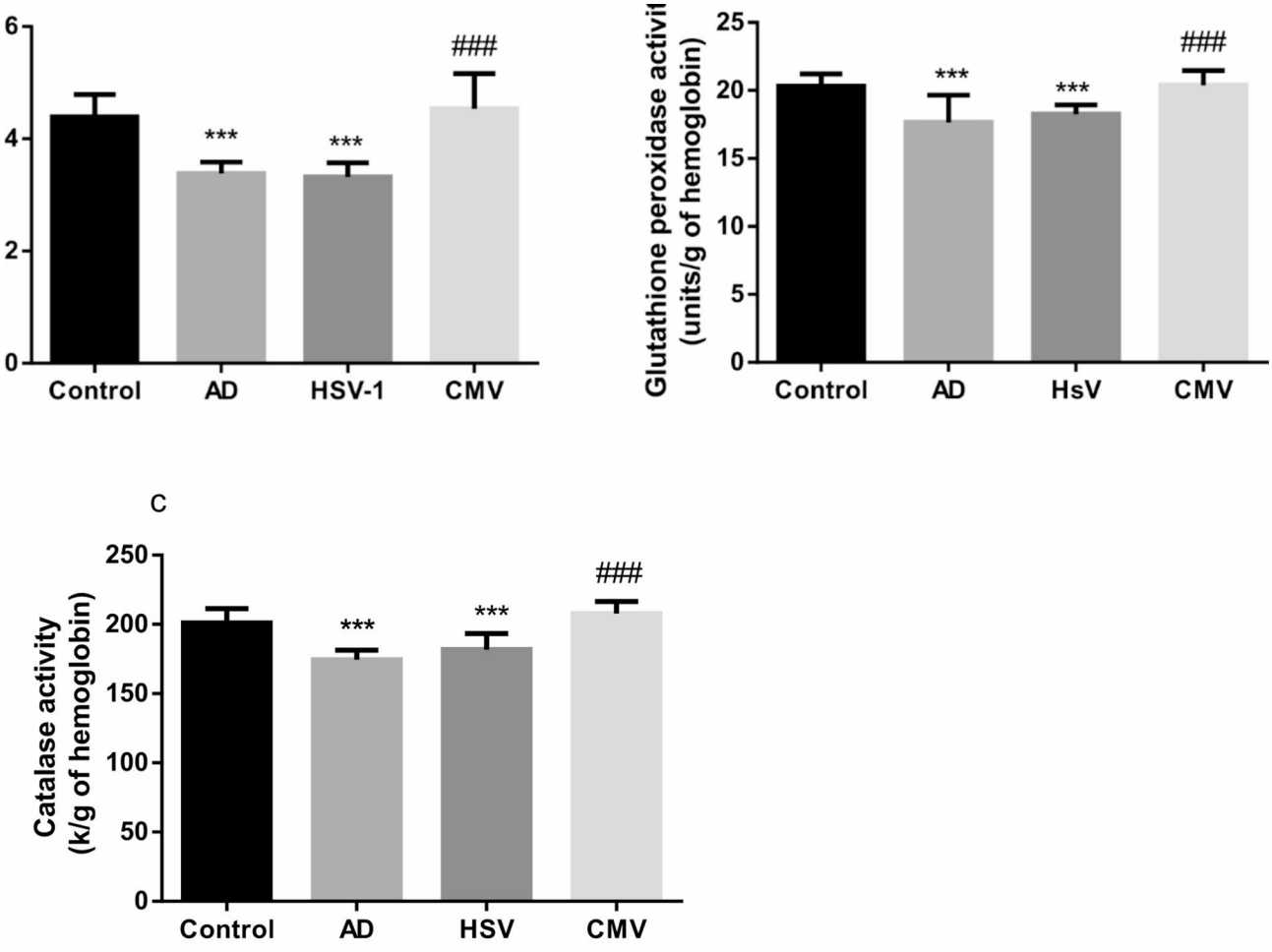

Fig. 2 Antioxidant enzymes activity in the samples of control, AD, HSV-1, and CMV groups. Alzheimer's disease (AD), Cytomegalovirus (CMV), Herpes simplex virus types 1 (HSV-1). **(a)** Superoxide dismutase, **(b)** Glutation peroxidase, and **(c)** Catalase. Data are reported as mean ± SD ($n = 100$). *Indicates significantly different as compared with the Control group ($P < 0.05$), # Indicates significantly different as compared with the AD group ($P < 0.05$). ## Indicates significantly different as compared with the AD group ($P < 0.01$)

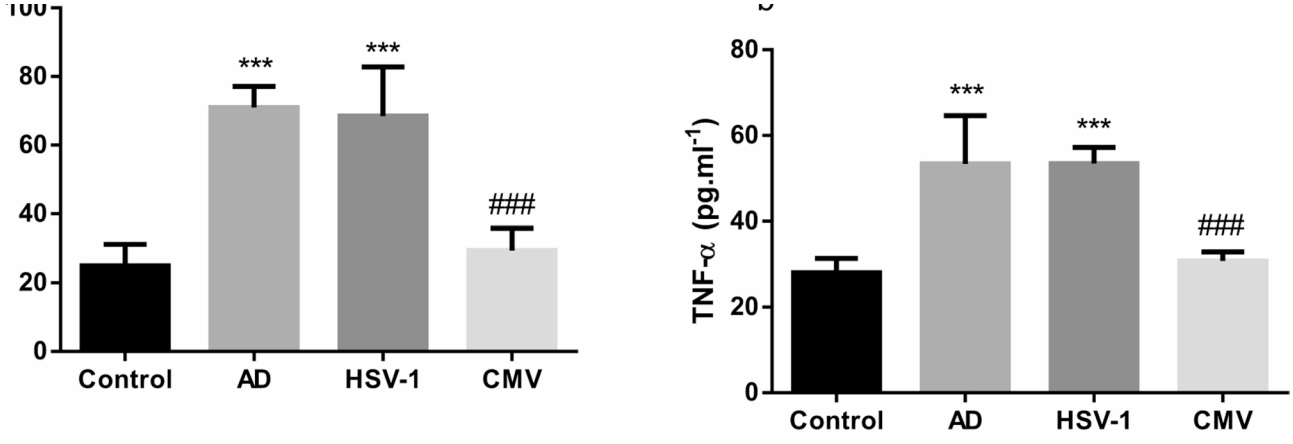

Fig. 3 Pro-inflammation factors in the samples of control, AD, HSV-1, and CMV groups. Alzheimer's disease (AD), Cytomegalovirus (CMV), Herpes simplex virus types 1 (HSV-1). **(a)** IL-1β serum level (ng/ml), **(b)** TNF-α serum level (pg/ml) in the control, AD, HSV-1, and CMV groups. Data are reported as mean ± SD ($n = 100$). Data are reported as mean ± SD ($n = 100$). **Indicates significantly different as compared with the Control group ($P < 0.01$), ***Indicates significantly different as compared with the Control group ($P < 0.0001$), # Indicates significantly different as compared with the AD group ($P < 0.05$), ### Indicates significantly different as compared with the AD group ($P < 0.0001$)

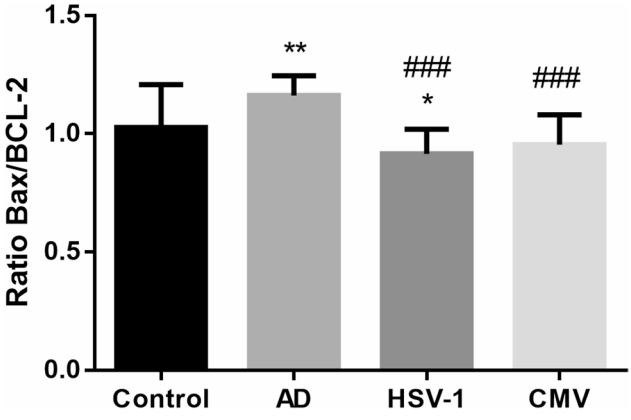

Fig. 4 Apoptotic factors gene expression in the leukocyte of control, AD, HSV-1, and CMV groups. Alzheimer's disease (AD), Cytomegalovirus (CMV), Herpes simplex virus types 1 (HSV-1). Ratio BAX/BCL-2 gene expression in the control, AD, HSV-1, and CMV groups. Data are reported as mean ± SD ($n = 100$). *Indicates significantly different as compared with the Control group ($P < 0.05$). ** Indicates significantly different as compared with the AD group ($P < 0.01$). ### Indicates significantly different as compared with the AD group ($P < 0.001$)

emphasized the critical role of oxidative stress in AD pathophysiology, pointing to the mitochondrial respiratory chain as the primary source of ROS in neuronal tissues [36]. Bhatia et al. further confirmed that mitochondrial dysfunction is a major contributor to ROS generation in AD [37]. Accumulation of Aβ peptides in the brain disrupts mitochondrial membranes and impairs respiration, leading to mitochondrial dysfunction and oxidative stress [38]. Oxidative stress has been proposed not only as a contributor to AD progression but also as a potential initiator of neurodegenerative changes.

Studies have shown that antioxidants can mitigate these effects. Changes in antioxidant enzyme levels and activity within the brain may exacerbate oxidative damage in AD [39]. Our data reveal that increased ROS production and diminished antioxidant capacity are strongly correlated with reduced activity of key antioxidant enzymes. Specifically, lower levels of glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) in AD patients support the presence of oxidative imbalance. These enzymes play pivotal roles in detoxifying ROS and maintaining redox homeostasis. For instance, the interaction of CAT with Aβ peptides may impair hydrogen peroxide breakdown, contributing to oxidative stress. Aβ oligomers not only induce ROS production but also impair the function of cellular proteins, thereby facilitating further Aβ production. Additionally, glutathione (GSH) acts as a major antioxidant, offering protection against Aβ-mediated neurotoxicity. Deficiencies in SOD have been shown to accelerate amyloid aggregation and cognitive decline in AD models, whereas overexpression of mitochondrial SOD (MnSOD) can reduce Aβ levels and prevent neuronal apoptosis. Conversely, SOD deficiency is linked to increased tau hyperphosphorylation in experimental AD models. Collectively, our findings support the hypothesis that viral infections such as HSV-1 and CMV may influence oxidative stress pathways in AD, further exacerbating disease pathology. Continued investigation is necessary to elucidate the precise role of antioxidant enzymes in AD progression and to explore their potential as therapeutic targets.

However, proinflammatory cytokines such as IL-1, IL-6, IL-10, and TNF-α are released at sites of neurodegeneration in the brain, where they interact with damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), both of which are commonly detected in the brains of Alzheimer's disease (AD) patients. These molecular patterns are recognized by pattern recognition receptors (PRRs) [40]. Several studies have identified the pivotal roles of proinflammatory cytokines, particularly IL-1β and TNF-α, in the pathophysiology of Alzheimer's disease (AD). These cytokines are integral to neuroinflammatory responses, especially in the activation of microglia and the progression of amyloid-beta (Aβ)-induced inflammation, which is a hallmark of AD pathology. IL-1β, a key mediator of neuroinflammation, has been shown to exacerbate Aβ accumulation and tau phosphorylation, further accelerating neuronal degeneration and cognitive decline in AD patients [41]. TNF-α, another critical cytokine in the inflammatory response, is strongly linked to synaptic dysfunction and neuronal apoptosis in AD [42]. The upregulation of IL-1β and TNF-α in response to viral infections, such as herpes simplex virus type 1 (HSV-1), further implicates their role in viral-induced neuroinflammation, which may contribute to the progression of AD. Studies have demonstrated that HSV-1 infection in the brain can enhance the activation of microglia and astrocytes, leading to the release of IL-1β and TNF-α, thus perpetuating the inflammatory cycle that exacerbates neurodegeneration [43, 44].

Although IL-1β and TNF-α are also altered in various other infections such as H1N1, influenza, and malaria, their relevance in AD has been extensively validated in the literature. In fact, both cytokines are commonly utilized in studies investigating the role of viral infections in neurodegenerative diseases, further emphasizing their importance in the inflammatory processes associated with AD. For instance, it has been shown that the upregulation of IL-1β and TNF-α in the brains of AD patients is not only a response to Aβ deposition but also reflects the involvement of these cytokines in the neurodegenerative cascade triggered by viral infections [45]. Furthermore, their elevated levels correlate with cognitive decline and the progression of AD symptoms, suggesting that these inflammatory markers may serve as useful biomarkers for monitoring disease progression and evaluating

potential therapeutic interventions. Our results demonstrated a reduction in proinflammatory cytokine levels in the blood of AD patients. Consistent with these findings, previous studies have reported IL-1β overexpression in microglial cells within the AD brain, likely triggered by Aβ plaque deposition, tau phosphorylation, and neurofibrillary tangle formation [37, 46]. Similarly, TNF-α is strongly associated with cognitive decline, neuronal apoptosis, and neurodegeneration in the cortex, hippocampus, and striatum in mild to advanced stages of AD [47, 48].

Recent studies have shown that viral infections and other diseases can disrupt the host's redox homeostasis by increasing reactive oxygen species (ROS) production while reducing antioxidant defenses, thereby exacerbating oxidative stress [49]. In our study, we assessed oxidative stress-related biomarkers—ROS production, malondialdehyde (MDA) levels, total antioxidant capacity (TAC)—as well as the activity of key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), in blood samples of AD patients with concurrent HSV-1 or CMV infections.

Mounting evidence suggests that HSV-1 can invade neural tissues, where it activates microglia, the central nervous system's resident immune cells, leading to excessive oxidative stress and heightened inflammatory responses. These microglia are also major producers of proinflammatory cytokines in response to viral infection [50]. According to Hugo Lövheim and colleagues, while CMV alone is not directly associated with AD onset, its interaction with HSV-1 may influence AD progression by modulating the immune response [51].

CMV infection is known to cause a variety of complications, including retinitis (leading to vision loss), colitis, esophagitis, hepatitis, and encephalitis, all of which involve inflammation and tissue damage. Chronic CMV infection is also linked to elevated ROS levels, contributing to tissue injury and inflammation [52]. Our data corroborate these findings, showing that CMV and HSV-1 infections are associated with heightened oxidative stress in AD patients. Interestingly, AD patients with HSV-1 coinfection showed no significant oxidative differences from the control group, which may be explained by the activation of compensatory antioxidant mechanisms. These seemingly contradictory results suggest that CMV may possess mechanisms to modulate host stress responses, facilitating viral replication while minimizing cellular damage. Notably, ROS have been shown to regulate early CMV gene transcription; however, excessive ROS levels can impair essential cellular functions. Carisa Tilton et al. [53] demonstrated that CMV-infected cells upregulate enzymes involved in glutathione synthesis and utilization, allowing them to neutralize ROS rapidly and maintain redox homeostasis. This adaptation supports the continuation of key signaling pathways, such as mTOR kinase activity, even under oxidative stress conditions. Such viral strategies underscore the importance of Nrf2 activation and glutathione metabolism in sustaining viral survival and replication [53]. In alignment with previous findings, our results indicate a significant reduction in proinflammatory cytokines in CMV-infected individuals compared with those in the AD-only group. However, in patients coinfected with both AD and CMV, no measurable cytokine alterations were observed. In contrast, HSV-1 infection was associated with increased levels of IL-1β and TNF-α relative to controls. These findings suggest that CMV infection may help mitigate inflammatory imbalances in AD, potentially through redox modulation and immune regulation.

Extensive neuronal death via apoptosis is a hallmark of neurodegenerative diseases, including Alzheimer's disease (AD). Apoptotic cell death has been observed in both neurons and glial cells in AD brains [54]. While insufficient apoptosis of immune cells can contribute to autoimmune disorders, excessive apoptosis is also a pathological feature, particularly in neurodegenerative conditions such as AD, Huntington's disease, and Parkinson's disease. Apoptosis is a tightly regulated and complex process involving a cascade of energy-dependent molecular events. Currently, two primary apoptotic pathways have been identified: the extrinsic (death receptor-mediated) pathway and the intrinsic (mitochondrial-mediated) pathway.

Several gene families play critical roles in regulating apoptosis, including caspases, inhibitors of apoptosis proteins (IAPs), the Bcl-2 family, the tumor necrosis factor (TNF) receptor superfamily, and the p53 gene. Within this framework, the pro-apoptotic protein Bax (Bcl-2-associated X protein) initiates a cascade that promotes cytochrome c release from mitochondria, activating caspases and ultimately leading to programmed cell death.

Numerous studies have indicated that certain viruses—including HIV-1, HPV, HTLV-1, and Kaposi's sarcoma-associated herpesvirus (KSHV)—can trigger apoptosis. Herpes simplex virus type 1 (HSV-1) is known to both induce and inhibit apoptosis at various stages of infection, with cell type-specific effects [55]. Cytomegalovirus (CMV), on the other hand, is known to inhibit apoptosis in response to diverse triggers such as Fas and TNFR-1 activation, serum withdrawal, and chemical stressors. Although CMV proteins do not share homology with established anti-apoptotic proteins, ongoing research aims to identify the viral genes responsible for apoptosis suppression [25]. Previous studies have demonstrated that apoptotic pathways are active in AD brain tissue [56]. In addition to neuroinflammation mediated by necroptosis and the NLRP3 inflammasome, significant neuronal

loss in AD also occurs through apoptosis and autophagy [56]. Our data confirmed the role of apoptotic pathways in HSV-1 and CMV infection in Alzheimer's disease.

Limitations

The total antioxidant capacity (TAC) did not show statistically significant changes in certain infected patient groups, this does not necessarily indicate a lack of oxidative stress. Instead, it may reflect a compensatory balance between ROS generation and the antioxidant defense mechanisms that are still functionally active in peripheral blood samples. The lack of significant TAC change may also be due to systemic regulation or the influence of non-enzymatic antioxidants that were not specifically measured in this assay. At this stage, we did not assess the gene expression of specific peroxidases; however, we agree this is a valuable direction for future research and have noted this as a limitation in the revised discussion section.

We did not include peroxidase gene expression profiling in the current study. Our focus was on measuring functional antioxidant enzyme activity (SOD, CAT, GPX) and oxidative stress markers (ROS, MDA, TAC) in peripheral blood samples. We acknowledge the importance of understanding peroxidase gene expression, such as glutathione peroxidase or peroxiredoxins, in ROS detoxification, and it highlighting the need for future investigations into these molecular pathways.

Conclusion

The data obtained from this study suggest that increased reactive oxygen species (ROS) production, lipid peroxidation, and decreased total antioxidant capacity are key mechanisms associated with HSV-1 and CMV infection in Alzheimer's disease. Additionally, alterations in inflammatory cytokines and antioxidant enzyme activity further implicate oxidative stress and immune dysregulation in the pathogenesis of AD in the context of viral coinfection. These findings support the hypothesis that HSV-1 and CMV may contribute to AD development and progression through their combined effects on immune function and oxidative balance.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12985-025-02786-8.

Supplementary Material 1

Author contributions

Forouzan Khodaei and Sepideh Khodamoradi,: Design of the work, the acquisition, analysis, interpretation of data for the work. Fateme Rabee, Taher Mohammadian and Atousa Ferdousi: Drafting the work, revising it critically for important intellectual content, Final approval of the version to be published. Sepideh Khodamoradi: devised the project, the main conceptual ideas and proof outline.

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Data availability

Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of Kermanshah University of Medical Sciences. (IR.KUMS.REC.1379.3525). All participants in this study provided informed consent before blood drawing.

Competing interests

The authors declare no competing interests.

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