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Bacterial, Viral and Fungal Infection Factors in Aetiophathogenesis of Alzheimer's Disease

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Alzheimer's disease (AD), the most common type of senile dementia, is an age related progressive neurodegenerative disease that affects more than 20 % people over the age of 80. It is predicted that over 35 million people will have AD by the year 2050 world-wide[1]. Neurodegenerative disorders in AD patients leads to progressive cognitive dysfunctions that includes language, memory, judgement, decision-making and orientation to physical surroundings[2,3]. It has been estimated that 5% of the population older than 65 years is affected by Alzheimer's disease[4,5] .The prevalence doubles approximately every 5 years beyond age 65[6] and some studies suggest that nearly half of the people aged 85 years and older suffer from this devastating disorder[1,7].

Great progresses have been made in understanding the pathological basis of the disease, but the knowledge about the mechanism(s) involved in triggering the onset of AD is still very limited. Among the different hypotheses that have been presented over the years, the possibility of involvement of infectious agents in the etiopathogenesis of AD is being debated for a long time[8]. In this communication the findings of research on two pathogen, one virus (Herpes simplex virus type 1, HSV-1) and one bacteria (*Helicobacter pylori*) as etiopathological agent of AD is reviewed. Although various pathogens are implicated in the pathogen hypothesis for AD, HSV-1, *H. pylori* were chosen as a high proportion of world population are chronically infected by these pathogens. In addition, fungus as an etiological agent is also included as it a newly reported possible cause of AD.

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Herpes simplex virus type 1(HSV-1)

In 1982, HSV-1 was first proposed as a probable candidate viral etiologic agent of AD and it is one of the better-known infectious agents in AD[9]. HSV-1 is double-stranded DNA (dsDNA) herpes virus, a neurotrophic, neuroinvasive member of the family Herpesviridae which can establish life-long latency in central nervous system (CNS) tissues. Following infection, the virus replicates at the site of infection and enter sensory neurons via their termini that innervate the infected dermatome. The latent virus genome is stably retained within sensory neurons and is characterized by repression of all viral lytic genes. In response to a variety of diverse stimuli e.g. illness, stress, or exposure to sunlight the virus can periodically reactivate to resume virus replication and produce infectious virus[10].

HSV-1 came into focus as an etiological of AD as many pathological characteristics induced by the virus closely resemble to that observed in AD patients. Several observations links HSV-1 infection with AD. These include (a) the earliest and most severely affected regions of the brain affected in herpes simplex encephalitis (HSE) are the same as those similarly affected in AD[11], (b)

HSE leads to certain long-term after-effects such as cognitive decline and memory loss which are highly characteristic of AD, (c) in both diseases headache and seizure is common feature and (d) pathology of HSE brain resembles that of AD[12]. Many clinical changes noted in AD patients such as brain cell atrophy, altered gene expression, inflammation, immunological aberrations, amyloidogenesis, cognitive deficits are also observed as consequences of infection by various pathogens including HSV-1[13, 14, 15]. The brains of AD sufferers have two characteristic neuropathological features: (a) insoluble aggregates of amyloid –â protein (Aâ) leading to formation of amyloid plaques and (b) intraneuronal neurofibrillary tangles, composed of hyperphosphorylated Tau protein[16]. Numerous other conditions, among which oxidative stress [17] and inflammation, due to microglia activation and astrocytosis, may concur to produce the structural and functional alterations typically found in AD brain[18].

Abundant clinical and epidemiological data support the role of HSV-1 as a risk factor for development of dementia which cumulatively led to consideration it as the prime candidate for pathogen induced AD. Molecular evidences show that HSV-1 infection of neuronal and glial cells results in the formation of the characteristic abnormal molecule of AD (α-amyloid) and a decrease of the amyloid precursor protein (APP). Moreover, it has been demonstrated recently that HSV-1 can modulate host homeostatic process by interfering with host autophagy thus influencing the process of controlling elimination / turnover of cytoplasmic components, protein aggregates and damaged organelles[19]. Interference with this vital homeostatic process has been suggested to be involved in the process of deposition of amyloid plaques in the neurons[18].

In addition, HSV-1 infection also leads to abnormally phosphorylated, AD-like tau (P-tau), the main component of neurofibrillary tangles[18]. Another compelling piece of evidence about HSV-1 as an etiological agent of AD came from experiments in animal models. In murine model, it has been shown that the host protein Amyloid-â protein is composed of 40 to 43 amino acids which is generated from amyloid precursor protein (APP), by the proteolytic cleavage, which is a ubiquitous transmembrane protein involved various cellular processes such as neuronal protein trafficking along the axon, neurite outgrowth and synaptogenesis, transmembrane signal transduction, Ca2+ signaling and cell adhesion[20, 21].

HSV-1 infection may have profound effects on the host intracellular pathways leading to activation/inactivation of several signaling molecules and kinases involved in APP metabolism. Several studies demonstrated that HSV-1 affects APP processing leading to abberent Aâ 40 and Aâ 42 accumulation in human neuroblastoma and glioblastoma cells *in vitro*[22].

HSV-1 binding to neuronal membrane induced membrane depolarization leading to increased neuronal excitability and triggering action potentials leading to a cascade of cellular events leading to increased intracellular Ca2+ signaling. This Ca2+ signaling causes marked intracellular Ca2+ entry from extracellular medium and Ca2+ release from intracellular stores resulting in Ca dysregulation triggering neurodegeneration, which is a hallmark of AD[23, 24, 25]. HSV-1infection induces a significant accumulation of Aâ 42 inside neurons and this effect is depended on activation of Ca2+ signaling process [23]. HSV-1 infection also induced Ca2+-mediated APP phosphorylation which is a key event critically involved in APP processing and Aâ formation [26].

Experimental studies have shown that HSV-1 is a strong risk factor for Alzheimer's disease in the brains of possessors of the type 4 allele of the apolipoprotein E gene (APOE-epsilon4), and that â-amyloid, the main component of plaques, accumulates in HSV1-infected cell cultures and mouse brain. In addition, amyloid â accumulation in HSV-1-infected cells and mouse brain, suggest that this virus is a major cause of amyloid plaques and hence probably a significant etiological factor in Alzheimer's disease[27].

Neuroinflammation and several markers of neurodegeneration including Tau hyperphosphorylation was observed following reactivation of HSV-1 in the brain of asymptomatically infected mice [28]. When present in AD brains, HSV-1 DNA is primarily located within amyloid plaques [29]. Whether or not HSV-1 reactivation contributes to activation of proneurodegenerative pathways leading to AD

may largely depend on several host factors, including genetic predisposition but studies have shown that when present in AD brains, viral DNA was primarily located within amyloid plaques [22].

Examination of CNS and trigeminal ganglia of HSV-1 infected mice for markers of inflammation and neurodegeneration showed that reactivation of virus from the latent phase was accompanied by concurrent upregulation of Toll-like receptors (TLRs) and interferon á and â indicating onset of neuroinflammatory process [28]. Upregulation of HSV-1 activates TLRs 2 and 4 in mouse astrocyte cultures and in microglial cells were also noted following activation of HSV-1[30, 31]. In addition, phosphotau and caspase-3-cleaved tau, which indicate early neurodegenerative processes, were also upregulated. Taken together these data provide additional support to the hypothesis that HSV-1 in brain reactivates recurrently, thereby promoting neuroinflammation by triggering TLRs activation, thus conferring a risk of neurodegeneration as seen in AD[28].

Examination of serum anti-HSV1 IgG titres of patients with mild AD and measurement of cortical gray matter volumes by MRI showed that higher levels of antibody was more frequent in patients than age matched healthy controls which correlated positively with cortical bilateral temporal and orbitofrontal gray matter volumes. No such correlation was observed when the same serum samples were analyzed for Cytomegalo virus (CMV), another closely related member of herpes virus family, antibody; thus providing support to the hypothesis that HSV-1 infection as an etiological agent of AD[32].

Studies have shown that intravenous immunoglobulin infusion (IVI) in mice with HSV-1 encephalitis led to prevention of death and decreased the number of HSV-1 in latently infected trigeminal ganglia[33]. In a retrospective case control study designed to determine usefulness of IVI of immunoglobulin showed the use of intravenous immunoglobulin was associated with reduced risk of AD[34]. In addition, pilot studies reported overall stability on neurocognitive scores and trends toward improvement in some areas in IVIg receiving patients with mild form AD [35, 36].

Positivity for anti-HSV IgM, a sign of reactivated infection, was found to almost double the risk for AD, whereas the presence of anti-HSV IgG antibodies did not affect the risk indicating that there is a link between infections of herpes simplex virus and the risk of developing Alzheimer's disease[37]. Formation of these molecules is inhibited by treatment with the antiviral agent acyclovir (ACV), which prevents viral DNA replication indicating that HSV-1 is an etiological agent of Alzheimer's disease[38]. Taken together these findings suggest that HSV-1 plays a key role in neurodegeneration in the host and has emerged as one of the most compelling theory of infectious etiology of AD.

Among the different hypotheses that have been presented over the years, the possibility of involvement of infectious agents in the etiopathogenesis of several chronic diseases, including AD, is being debated for long time. Later, it has been demonstrated that HSV-1 induces the formation of the characteristic abnormal molecules of Alzheimer's disease (AD) brains, â-amyloid, and abnormally phosphorylated, AD-like tau (P-tau). Formation of these molecules is inhibited by treatment with the antiviral agent acyclovir (ACV), which prevents viral DNA replication indicating that HSV-1 is an etiological agent Alzheimer's disease[38, 39].

Apolipoprotein E (ApoE), a major component of very-low density lipoproteins, may play an important modulatory function in the CNS. The human APOE gene has three common alleles, å2, å3, and å4, which encode the ApoE2, ApoE3, and ApoE4 protein isoforms, respectively[40]. APOE4 has been identified as a risk factor for AD in many studies, irrespective of race or geographical locations. However, it is not sufficient for development of the disease[41, 42]. The incidence of AD is not higher in individuals with HSV-1 DNA or the APOE4 allele alone, but it is highest in the carriers of this allele who also has HSV-1 DNA in the CNS [43]. However, many genes and proteins other than ApoE, interact with HSV-1 genome and regulate its life cycle, providing additional support to the widely debated synergy between host and pathogens in causing AD-like brain damage[18, 44].

Studies have shown that carriage of APOE increases HSV-1 load in the brain and the spread of virus is lower APOE knock out mice and correlation was observed between APOE expression and HSV-1 DNA concentration in the brain[45]. Cumulatively, the findings of the studies

reviewed here provide excellent support for HSV-1 being an agent for development of AD by directly activating intracellular pathways leading to typical AD molecular hallmarks. However, absolute proof of HSV-1 as a causative agent of AD would require successful prevention of the disease by vaccination against the virus, or reduction of disease progression by antiviral treatment as no direct human experiment cannot be performed. The human CNS (especially in aged individuals) is under constant assault by a variety of bacterial, viral and fungal pathogens as innate immune system and physiological often become compromised as people age[14].

In summary, the studies reviewed in this manuscript indicate that greater attention should be paid to HSV-1 as important agents among the pathogen factors contributing to AD pathogenesis. Currently, there is no curative therapy for AD and only some symptoms are ameliorated. So it is important to understand the etiology of AD in order to design and implement necessary treatment to reduce the loss of neurons[8,14].

Helicobacter pylori

H. pylori is a spiral-shaped, microaerophilic, Gram-negative bacteria with a chronic infection affecting 10-30% of the population of developed countries and 80-90% of the population of developing countries[46]. It is primarily associated with various upper gastrointestinal disorders such as chronic gastritis, peptic ulcer disease, and gastric cancer. In addition, *H. pylori* is also implicated in the pathogenesis of several extra-digestive diseases, including atherosclerosis[47], chronic respiratory disease[48], and idiopathic thrombocytopenic purpura[49].

Experimental evidences linking *H. pylori* infection in the pathogenesis of AD have emerged in recent years.Using enzyme-linked immunosorbent assay (ELISA)[50] analysis of serum and cerebrospinal fluid (CSF) of 27 patients showed significantly higher mean concentrations of anti-*H. pylori* immunoglobulin G antibodies in patients with AD. Analysis of serum levels of anti-*H. pylori* antibodies in patients with vascular dementia also revealed higher levels of anti IgA and IgG in comparison to controls [51]. Furthermore, histological analysis gastric mucosal biopsy sample showed higher prevalence *H. pylori* in patients with AD in comparison to control patients without AD who had iron deficiency anemia[52].

H. pylori infection is closely associated with stomach diseases, and also to a lesser extent, non-gastrointestinal disorders arising from blood vessel dysfunction. These disorders can contribute towards the weakening of the blood-brain barrier, which is an important risk factor for Alzheimer's disease[53, 54]. Due to sharing of epitope (molecular mimicry) with the human host, *H. pylori* induces irregular humoral and cellular immune responses that cross-react with components of nerves and thus contribute and perpetuate the apoptotic neural tissue damage and apoptosis observed in AD[53]. *H. pylori* influences the pathophysiology of AD by promoting platelet and platelet–leukocyte aggregation; releasing various pro-inflammatory and vasoactive substances; developing crossmimicry with host antigens; producing reactive oxygen metabolites and circulating lipid peroxides; influencing the apoptotic process and increasing, through induction of atrophic gastritis, homocysteine, which contributes to vascular disorders implicated in endothelial damage and neurodegeneration[53].

Another hypothesis assumes the roles of histidine rich protein HPN secreted by *H. pylori* in neurodegeneration, as this protein can cross the blood-brain barrier and directly increases the accumulation Aâ[55]. Clinical studies on European population demonstrated a greater incidence of *H. pylori* infection among patients with AD compared with healthy subjects. These researches were confirmed histopathologically[56] and increased levels of antibodies against *H. pylori* IgG and IgA[51, 57]. In addition, an improvement in the cognitive function was observed following successful eradication therapy for *H. pylori* [53]. Anti-*H. pylori* therapy consists of mixture of two or more antibacterial agents (clarithromycin, amoxicillin, bismuth subsalicylate, metronidazole and tetracyclin). Using several antibiotics increases the eradication rates and reduces the risk of development of resistance. As GIT is the primary site of infection for *H. pylori*, a proton pump inhibitor (PPI) or histamine receptor inhibitor (H2 blocker) is combined with antibiotics to prevent the degradation of the drugs in acidic environment[58, 59]. However, it is possible that the

increased incidence of infections caused by *H. pylori* among patients with Alzheimer's disease is caused by the low level of hygiene among patients with advanced dementia[57]. Similar epidemiological studies, which used urease test, conducted in Japan, showed non-significant differences in the incidence rates of *H. pylori* infection among patients with AD compared to healthy subjects[54].

Genetic, epigenetic and environmental factors contribute to the clinical phenotype of neurological diseases such as AD. The role of *H. pylori* as a directly participating infectious agent as a causative factor in AD is under investigation but ample evidences support this notion that in susceptible individuals *H. pylori* infection may influence the diseases process of AD[52,53]. Further studies involving in vivo and in vitro experiments are necessary to establish unequivocally *H. pylori* as an infectious agent for AD. For treatment option, eradication of the pathogens using anti-infective agents may prove useful. But as many anti-infective drugs possess little or no blood brain barrier (BBB) permeability, agents which increase BBB may be included. Another significant aspect is the role of general inflammation, neuroinflammation in particular. As in addition to bacterial / viral / fungal agents, various components of these agents are also reported to aggravate the symptoms of AD, inflammation process itself (caused by any infectious agent or their products / component) should also be given due importance as a physiologic process leading to AD. So, anti-inflammatory drugs in conjugation ant-infective drugs can be considered as a treatment option for reversing or slowing down the loss of cognitive function observed in AD patients[57,58,59].

Disseminated mycoses

Disseminated mycoses is emerging as a new candidate risk factor for development of AD. Disseminated mycosis has been detected in the blood serum of AD patients, fungal proteins and DNA detected in the brains of AD patients indicating infection of the brain[60]. Several evidences support the hypothesis that AD can be caused by fungal infections. These include presence of fungal proteins, polysaccharides and DNA in the peripheral blood, presence of fungal DNA protein and fungal (1,3)-â-glucan in the brain samples of AD patient and detection of fungi by anti-fungal antibody in the brain sections of diseased patients with AD. Mixed fungal infections such as *Saccharomyces cerevisiae*, *Malassezia* spp, and *Penicillium* spp can be present in a single patient and the actual fungal species present may vary from patient to patient. As amyloid peptide has potent antifungal activity, it is hypothesized that fungal infection can serve as a trigger for synthesis and export of amyloid peptides which appear as deposits observed in the CNS. Moreover, amyloids are associated with the surface structures of fungi which may aid in their organization and promote higher order assembly of myeloid[61]. Elevation of inflammatory cytokines both in CNS and peripheral blood is noted in AD patients due to possible fungal infection and this inflammation is implicated in observed vascular dystrophy found in AD patients. Inflammatory cytokines are elevated not only in the CNS but also in peripheral blood, probably as a consequence of disseminated fungal infection. And importantly, the symptoms of AD were markedly reversed following treatment with antifungal agents. Amphotercin B is a drug of choice for systemic mycosis, however; other antifungal used in such infections include; itraconazole, liposomal amphotericin B, voriconazole, caspofungin, micafungin, posaconazole anidulafungin, and Fluconazole [62]. Taken together, these results suggest the presence of disseminated mycoses in blood serum from AD patients.

CONCLUSION

Research findings reviewed here show that no single infectious agent has been linked conclusively as the causative agent of AD. It is possible that various neurotrophic pathogens may have promoting role in AD depending upon host factors such as status of immunity and genetic constitution. Alternatively, neuropathology of AD may increase susceptibility of affected area to infection by neurotrophic pathogens. Preventive and therapeutic strategies for AD based on eradication of infectious agents need to be evaluated in clinical trials. Studies are need to determine whether late-life cognitive decline is linked to susceptibility chronic infection; how antibiotic therapy can control AD and disease progression and whether CNS infection precedes neuropathology observed in AD or vice versa[8, 63]

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