

Oxidative Stress, Epigenetic, Neuroinflammation and Alzheimer's Disease

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ABSTRACT

The state of oxidative stress is present in a number of neurodegenerative diseases as Parkinson's, Alzheimer's and Multiple Sclerosis, among others. However, oxidative stress state can be produced by endogenous and exogenous factors; among the latter, environmental pollution plays an important role. It is widely proven that the ozone present in the lower layers of the atmosphere, generated as a product of the photochemical pollution of air, causes health problems in densely populated cities since it is able to induce a chronic oxidative stress state. In animal experiments, the subjects are exposed to low doses of ozone for 4 hours during one or two months. The experiments show that the oxidative stress caused by ozone causes a progressive neurodegeneration state with the presence of beta-amyloid insoluble in the hippocampi of rats exposed to this gas. The results are similar to what occurs in Alzheimer's disease. The progressive neurodegeneration process is accompanied by the loss of regulation of the inflammatory response. Although there are multiple studies on the matter, it is still unclear how environmental changes cause disturbances in the expression of genes, modifying inflammatory response. One of

the epigenetic changes reported for Alzheimer's disease is the hypomethylation together with the hyperacetylation of some genes coding for enzymes of the amyloidogenic pathway. The enzymes are overexpressed and promote the accumulation of insoluble beta amyloids such as 1-42. Therefore, the chronic oxidative stress state is able to generate posttranslational disturbances acting at different levels of cell pathways. It also directly modifies or alters the epigenetic regulation on the expression of genes involved in the disease.

Keywords: Pollution; Oxidative stress; Epigenetic; Alzheimer Disease; Beta-amiloide 1-42

INTRODUCTION

Alzheimer's Disease

Alzheimer's disease is the most common cause of dementia and represents a serious public health problem. At early stages, this disease is clinically characterized by the presence of small failures in episodic memory. The pattern of memory loss is relatively specific since patients start to quickly forget recent learning while they keep the oldest information [1]; as a result, a time gradient that affects remote memory is created. This situation applies for both verbal and visual information and, later on, it affects language: the patients have difficulty naming objects, causing pauses and circumlocutions [2]. In later stages, the understanding of figurative language is affected in relation to a deficit in the ability of abstraction. Alzheimer's patients show orientation problems, especially when they leave their usual environmental [3]. Along with that, there is a process of molecular and biochemical alterations which gradually and irreversibly progress to become severe failures which affect the daily activities of the patient.

Neuropathological characteristics of Alzheimer's disease

The neuropathological characteristic of Alzheimer's disease is the formation of senile plaques by the deposition of beta amyloid peptides ($A\beta$) and the formation of neurofibrillary tangles by the hyperphosphorylation of Tau protein. Under normal phosphorylation conditions, Tau protein works as a microtubule stabilizer and modulates neurotransmitter transport in vesicles inside neuronal axons [4]. A state of hyperphosphorylation of Tau protein produces paired helical filaments that limit neurotransmitter transport, hampering neuronal communication [5]. For its part, the generation of $A\beta$ peptide takes place through the processing of the beta Amyloid Precursor Protein (**APP**), which is a membrane integral protein whose function is yet to be known. APP is processed through two different pathways: the non-amyloidogenic and the amyloidogenic pathway. In the non-amyloidogenic pathway, APP is processed by enzymes α and γ secretase, producing an APP intracellular domain and a soluble N-terminal fragment called p3 [6]. In contrast, the participants in the amyloidogenic pathway are the enzymes with beta secretase activity known as BACE-1 (beta site APP cleaving enzyme 1) and a presenilin complex with γ secretase activity (PSN1 and PSN2). The enzyme BACE cleavage on APP generates a soluble APP truncated peptide ($sAPP\beta$) and a residual fragment of 99 amino acids (C99) which is processed.

In consequence, the $A\beta$ peptide and the Amyloid Intracellular Domain (**AICD**), which is bound to the plasmatic membrane, are generated [7]. Around 90% of $A\beta$ peptides has a size from 1 to 40 while the rest is 1-42. However, the formation of beta amyloid peptide 1-42 is favored during Alzheimer's disease [8]. The $A\beta$ peptide 1-42 has a neurotoxic potential since it easily forms aggregates with existing $A\beta$ peptides or membrane lipids and alters cytoskeleton proteins which lead to synaptotoxicity within neurons [9]. The increase in the formation of peptide 1-42 gives rise to its intracellular accumulation and insoluble plaques in the extracellular space [10].

There are a number of factors capable of inducing an increase in the functioning of the synthesis pathway whose final result is the formation of insoluble beta-amyloid. Nevertheless, among these factors, the oxidative stress state is the most relevant as has been proven in animal models using healthy Wistar rats without any factor other than the chronic exposure to low doses of ozone. The ozone causes a chronic oxidative stress state and is able to generate changes in the synthesis pathway of the peptide $A\beta$, causing intraneuronal accumulation of $A\beta$ 1-42 in the hippocampus and the frontal cortex [11] (Figure 1).

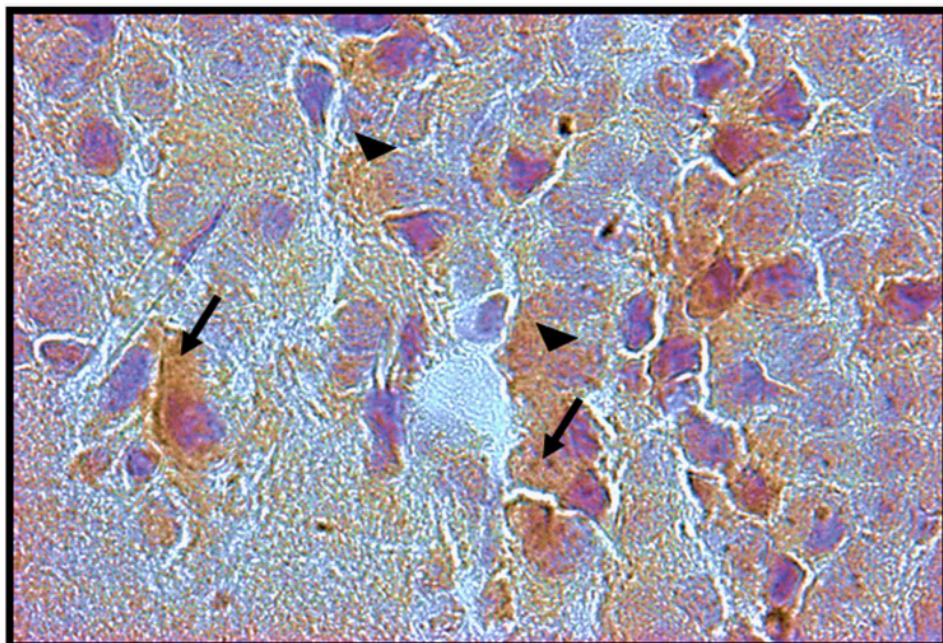


Figure 1: Microphotography showing oxidative stress effect in the Wistar rat hippocampal, specifically in the dentate gyrus neurons. The experimental groups of rat were exposed chronically to low doses of ozone for 4 h daily (0.25ppm) (40X). The arrows indicate the intracellular accumulation of β -Amyloid 1-42 and triangles show the extracellular accumulation of β -Amyloid 1-42.

Pollution, Oxidative Stress and Alzheimer's disease

Air pollution in big cities poses a serious public health problem since it is associated with the growth of both acute disease and chronic-degenerative diseases [12]. It has been widely proven that, on the days of greater air pollution, there is an increase in the number of consultations the emergency services provide. This increase is related to pulmonary, cardiovascular and autoimmune diseases, among others [13-15]. It is also linked to the exacerbation and complication of chronic-degenerative diseases [12]. On the other hand, the increase of ozone pollution, which is composed by the photochemical pollution of the air, has been closely related with diseases that affect the Central Nervous System (**CNS**) [16,17].

The air pollution is composed by: biological material (spores and other molecules), complex mixes of chemical substances as ozone, carbon monoxide, sulfur oxides, nitrogen oxides, and methane; and volatile organic compounds as benzene, toluene and xylene. In addition, metals such as: lead, manganese, vanadium and iron, which are products of both natural and anthropogenic sources [18].

Depending on the diameter of its particles, Particulate Matter (**PM**) is classified in: PM 10, PM 2.5 and PM 0.1 [19]. The particles with the smallest diameter may be more harmful for the health because they are able to deeply penetrate the alveoli, enter the blood flow and reach the brain [20]. PMs have been related to cardiovascular disease and asthma; additionally, they cause the exacerbation of the chronic obstructive pulmonary disease [21-23]. They have also been related to cancer and have been found in brain cells taking part of inflammatory response [25,26].

Pollution affects health through a number of ways, one of which is the production of oxidative stress. It has been demonstrated that acute oxidative stress activates antioxidant defenses leading the organism to a redox balance [27]. However, the chronic exposure to low doses of pollutants has a deleterious effect depending on the time of exposure [28]. Studies concerning this type of exposure have proven that the antioxidant systems fail, that the oxidative effect in lipids, proteins, carbohydrates and DNA is cumulative and that there is a loss of the regulation of inflammatory response among the effects caused by pollution [28-30]. Oxidative stress is commonly modulating the response of the immune system; therefore, the loss of the redox balance causes disturbances in the signaling of response of such system.

The chronic loss of the redox balance causes the following alterations (Figure 2):

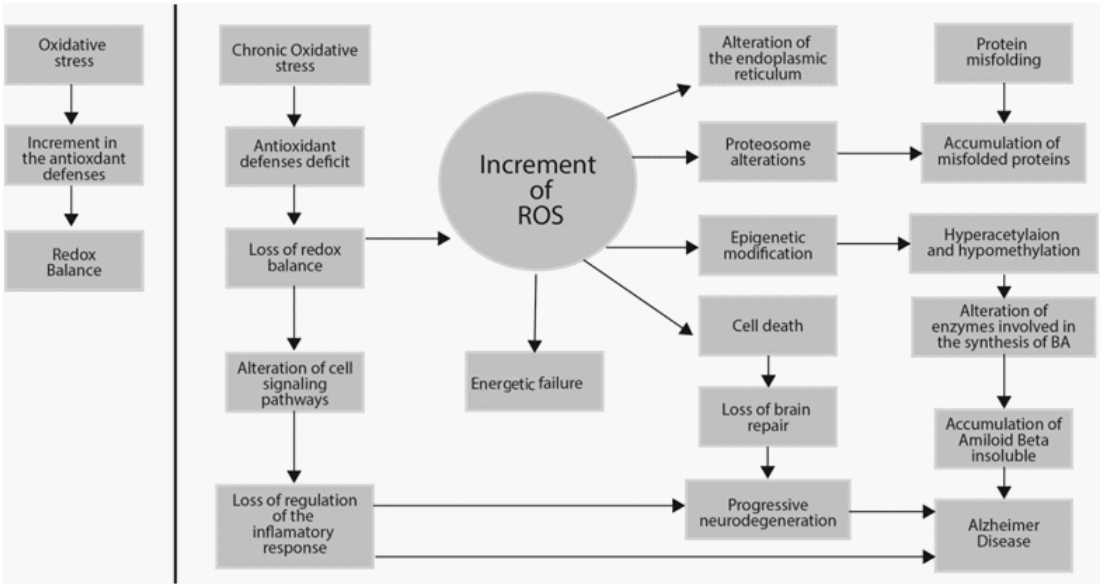


Figure 2: Diagram showing the effects of oxidative stress on the principal's alterations that are present in Alzheimer's disease.

1. The direct oxidation of biomolecules by reactive oxygen species (ROS), which cause disturbances at membrane level and in the different organelles of the cell [31].

2. Changes in the intracellular and extracellular signaling, which leads to the alteration of the metabolic cell pathways and posttranslational alterations that are present in several neurodegenerative diseases. For instance, endoplasmic reticulum stress causes protein misfolding, the disturbances of the proteasome that induce misfolded protein accumulation as in the case of Alzheimer's disease [32,33].

3. The chronic loss of regulation of the inflammatory response, that makes the response go from self-limited and repairing to being unable to limit itself, becoming an inflammatory response which damages the organism. This response can create a vicious cycle between oxidative stress and inflammation that is chronically maintained and promotes the advance of the disease [34].

There is a body of evidences that proves the redox state modulates the immune system through a series of mechanisms in which the ROS participate. This posttranslational regulation is lost when the redox balance is lost. The ROS activate membrane kinases which phosphorylate transcription factors such as NFk-B. This allows their translocation to the nucleus and the transcription of inflammatory response cytokines [35].

4. There exists a change in cytokine expression that maintains the loss of regulation of the inflammatory response. During this change the glial cells as astrocytes and microglia show

phenotypic changes. The astrocytes multiply and provide gliosis response during which they increase in number and add to their processes (Figure 3).

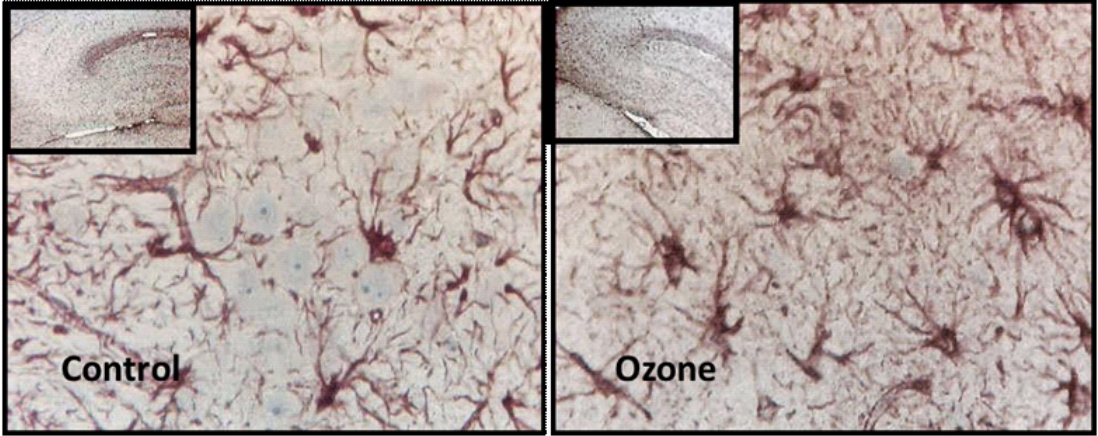


Figure 3: Micrography showing the effect of oxidative stress on astrocytes hippocampal dentate gyrus of Wistar rats chronically exposed (60 days for 4 h daily) to low doses of ozone (0.25 ppm) (40X). Note the changes in number (box (4X)) and morphology of these cells.

The microglial cells change their phenotype as well, activating first, and then turning into macrophage microglia [36].

5. The less clear aspect is probably constituted by the changes oxidative stress produces on the expression of genes involved in metabolic pathways (epigenetic); for example, the hypomethylation and the hyperacetylation reports in the genes of the amyloidogenic pathway in Alzheimer’s disease patients [37].

Alzheimer’s Epigenetic

From the 100% of the cases of Alzheimer’s disease, less than 5% have family or inherited origins. Nowadays, we recognize mutations in three genes linked to this disease: beta Amyloid Precursor Protein (**APP**) [38], Presenilin-1 (PSN1) and Presenilin-2 (PSN2) [39]. There are also numerous polymorphisms associated to the disease among which we can highlight those found in the sequence coding for APOE4 [40], ABCA7 [41], CD33 [42], CR1 [43], CD2AP [44], PICALM [45] and SORL [46].

On the other hand, the sporadic cases of Alzheimer’s represent more than 95% of the total number of cases. This leads us to suppose that the etiology of the disease can be explained by factors added to the DNA sequence modifications. That is why this part can be attributed to epigenetic mechanisms [47].

Epigenetic means “above genetics” and represents the main mechanisms that modulate gene expression in response to the interaction with the environment. The epigenetic mechanisms

include DNA methylation, the remodeling of the chromatin and the regulation by small RNA without causing changes in the DNA sequence [48], these epigenetic mechanisms can explain how different phenotypes can be observed from a same genotype.

DNA methylation

This mechanism is usually associated to gene silencing. It is the binding of a methyl group at the fifth position of the cytosine nucleotide, followed by a guanine nucleotide (dinucleotides CpG) in mammal cell DNA. The dinucleotides CpG are clustered in regions called CpG islands; their content is at least 50% cytosine and guanine nucleotides [49]. It is estimated that there exist 23 million CpG sites in the human genome [50]. Under normal conditions, the methylation mechanism is carried out in different cell processes among which we find: embryonic development, chromosomal stability, silencing of portable elements, allele-specific expression of imprinted genes and the inactivation of an X chromosome in females [51]. Cytosine methylation produces a modification upon the DNA, inhibiting the transcription factors and polymerases. This prevents the methylated gene from transcribing. Additionally, the activation of the Methyl-CpG-Binding Domain (**MBD**) is promoted by the methylated DNA preventing transcription [52]. DNA methylation is mediated by enzymes known as DNA methyltransferases (**DNMT**) which catalyze the transference of a methyl group from a S-adenosyl l-methionine (**SAM**), a cytosine nucleotide. This enzyme family is composed by five elements, of which DNMT1, DNMT3a and DNMT3b have transferase activity. DNMT3a and DNMT3b are associated with novo methylation, while DNMT1 seems to be in charge of maintaining DNA methylation during replication [53].

Histone modification

The nucleosome consists of 147 base pairs wrapped around an octamer of histones, composed of two copies of the histones H2A, H2B, H3 and H4. Every nucleosome is connected by “linker DNA” and linker histones as H1 [54]. Chromatin can be in a transcriptional inactivation state when it is found compact in the nucleosomes (heterochromatin) or in either active or relaxation state (euchromatin) when dissociated from the nucleosome. The tails of these histones can show modifications that regulate the activation or inhibition of the transcription through a number of mechanisms, among which we find: methylation, acetylation, phosphorylation, sumoylation and ubiquitylation [55]. Out of these, methylation and acetylation are the most studied posttranslational modifications on the tails of histones. Methylation is carried out in lysine residues and is both associated to relaxation (marks in residues H3K4, H3K9, H3K36 and H3K79) [56] and chromatin condensation in residues H3K27 and H4K20 [57]. The methylation of histone tails is regulated by protein lysine methyltransferases (PKMTs), protein arginine methyltransferases (PRMTs) and a donor of methyl groups (SAM). For its part, acetylation is carried out in lysine residues in histone tails by Histone Acetyltransferase enzymes (**HATs**). It is commonly associated to chromatin relaxation leading to transcriptional activation. In contrast, histone de-acetylation is regulated by deacetylase enzymes (**HDACs**) [58].

Micro RNAs

There are many small non-protein coding RNAs among which we include: small nuclear RNAs (**snoRNAs**), microRNAs (**miRNAs**) and short interfering RNAs (**siRNAs**) that regulate genetic expression at different levels [59]. The miRNAs are approximately 22 nucleotides long and bind to the untranslated regions 3' (3'-UTR) of messenger RNAs. Depending on the degree of complementarity, they can mediate the translational inhibition or the degradation of around 60% of all the genes [60]. The siRNA are another type of small RNA which are 20-25 nucleotides long and regulate the posttranscriptional genetic silencing [61].

Global DNA methylation analysis has been performed in specific brain regions of postmortem specimens of monozygotic twins. One of the twins died due to AD, while the other died from prostate cancer; both of them died at around 70 years of age. The study showed that the levels of global DNA methylation were lower in the twin with Alzheimer's when compared to the mentally healthy twin [62]. Another study performed by the same research team compared postmortem samples of AD patients and samples from healthy subjects. The study found a reduction not only of methylation in neurons of the temporal cortex but also of factors that maintain methylation as MBD2 and MBD3 [63]. Other studies have proven the hypomethylation of genes involved in the posttranslational processing of the Amyloid Precursor Protein (**APP**). That is the case of the promoter gene coding for the transmembrane protein 59 (TMEM59) which is 7.4% less methylated in AD patients [64].

The neurodegeneration process involves the loss of regulation of the inflammatory response as has already been stated. Regarding this, the levels of methylation in pro-inflammatory molecules as iNOS, IL-1 and NFT α have been studied. These studies show that the promoter regions of genes coding for these proteins are hypomethylated in patients with Alzheimer's Disease. In consequence, there is the loss of regulation over the expression of these enzymes, which contributes to the inflammatory process during the disease [65].

Recent works have proven that, in samples from AD patients, there is a loss in the H3K18/K23 mark of acetylation [66] and an increase in both histone H3 tail phosphorylation [67] and protein levels of HDAC2 [68]. It has also been proven that the use of HDAC protein inhibitors promotes the decrease of APP production as well as the formation of senile plaques in transgenic mouse models for Alzheimer's disease [69].

As for small non-protein coding RNAs, several research groups have demonstrated the altered expression of miRNAs in brains of AD patients including miRNA 9, 29, 155, 107, 146a, 181, 34 and 106, most of which participate in the modulation of the expression or processing of APP by the enzyme with beta-secretase activity (BACE-1) [70].

Different studies propose a strong correlation between the reactive species that generate an oxidative stress state and Alzheimer's disease because evidence of the changes in the redox state

of patients with Alzheimer's has been found. The presence of proteins, lipids and DNA damaged by oxidative stress and the alteration in the expression of the antioxidant system in postmortem samples of patients support this proposal [71].

One of the groundbreaking works that points out the influence of the environment on amyloid genesis was performed using cynomolgus monkeys exposed to lead at early stages of their life, while the brain samples were obtained from the subjects at adult age. The study proved that the levels of messenger RNA of APP as well as the levels of $A\beta$ peptide were elevated [72]. This suggests that the exposure to pollutants during brain development determines APP regulation, leading to the formation of senile plaques at old ages [73].

The presence of ROS by endogenous and exogenous factors has become relevant because of the regulating role of CpG islands the species have. This is because the guanine nucleotide of the islands is particularly susceptible to oxidation, which causes the reduction of affinity at the binding site of MBD changing the methylation patterns [74]. It has been stated that the promoter region of protein-coding genes involved in the generation of beta amyloid have 65 sites of methylation for APP and 36 at the BACE-1 promoter. The presence of these sites leads us to assume there is an epigenetic regulation of the generation of beta peptides. Together with the regulation of oxidative stress on the methylation mechanisms, it has been found that the addition of hydrogen peroxide to neuroblastoma cells causes a decrease in the methylation and increases the acetylation of histones. This hyperacetylation together with the hypomethylation results in the increase of APP, BACE-1 and PSEN1 transcription [37]. This produces $A\beta$ peptides which, due to their neurotoxic and pro-oxidant properties, generate cell damage and contribute to pathogenesis and Alzheimer's development [75].

Epigenetic studies of Alzheimer's disease are currently a fertile ground since the results found as of today are not strong enough. The only clear signal is probably the fact that Alzheimer's patients show changes in the methylation of their genome, which lead to the generation of beta peptide. However, the effects of these changes on the physiopathology of the disease remain unclear. The heterogeneity shown in the epigenetic studies related to Alzheimer's disease might exist because of the use of postmortem and peripheral blood samples of patients, which does not allow the researchers to determine whether the modifications found appeared before or during the disease or if they are caused by the disease itself. In addition, the number of samples is small; they reach 100 in a very few studies and are 15 in most of them with an equal or lower number of controls. Therefore, the epigenetic mechanisms involved in the development of the pathology are yet to be determined (Figure 4).

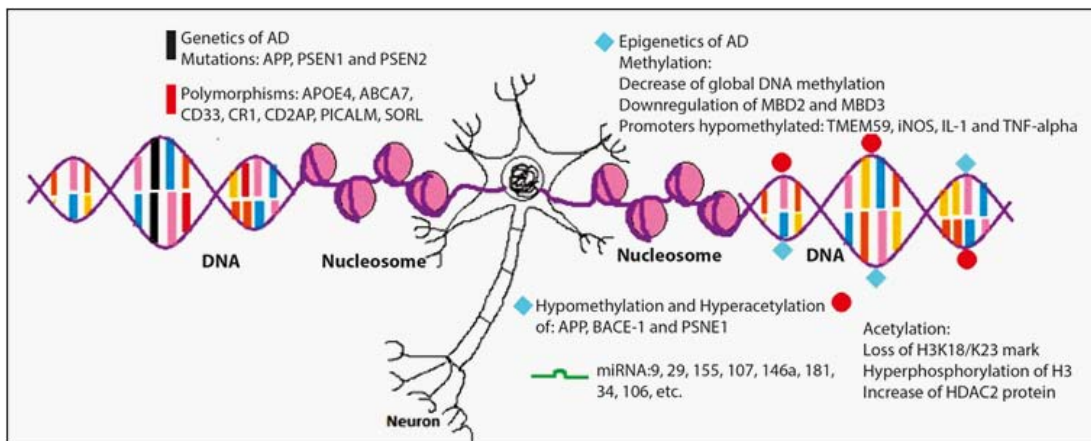


Figure 4: Genetic and epigenetic modifications found in Alzheimer Disease.

CONCLUSION

Oxidative stress is a relevant factor in the development and maintenance of the neurodegenerative disease since it acts at posttranslational and pretranscriptional levels. Thus, the study of epigenetic modifications might contribute to determine the role of environment and the effect of pollution on the progression of Alzheimer's disease. The study might help us understand the physiopathology of the disease and find new markers for an early diagnosis.

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