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Mini Review

# Dental Fluorosis and Oxidative Stress

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## Abstract

Fluoride is an effective caries prophylactic, but at high doses can also be an environmental health hazard. Acute or chronic exposure to high fluoride doses can result in dental enamel and skeletal and soft tissue fluorosis. Fluorine is one of the most important milestones in the history of dentistry due to its undeniable anti-cariogenic properties. Fluoridation occurs in several ways: in the fluoridation of public water supply, added to table salt, prescribed in drops; in topical applications; in community mouthwash programs; in dentifrices; among others. However, its adverse effects are studied and known when its ingestion reaches levels of chronic or acute toxicity and when fluorosis develops. The amount of fluoride used varies according to the region, taking into account the amount of fluoride present in water, food, and other beverages, plus some habits such as tea consumption and cooking and dehydrating foods with coal-rich in fluorides. In the environment, fluoride is abundant in rocks, groundwater, and soils, originating in soils. Increased concentrations of this element in soils are also a result of the use of groundwater for irrigation and/or increased capillarity of the ground. The state of Zacatecas (Central Noth) in México has high level of fluor in it soil, due the variety of minerals due to all the mines surrounding the area. Dental fluorosis is manifested as mottled, discolored, porous enamel that is susceptible to dental caries. Studies with various *in vitro* and *in vivo* systems attempt to understand which cellular damage the fluorides can cause. Among these studies, some authors suggest that fluoride can increase the production of free radicals acting on the oxidative degradation of lipids and promoting changes in cell membrane, leading to the formation of cytotoxic products and consequently cell death. In addition, studies seek answers about the epigenetic or DNA repair system mechanisms, which are still not explained. Some of these studies relate the exposure time and amount of fluorides, animal and human cells, and animals and populations exposed to high fluoride levels with the expression of various genes. This mini review wants to establish the link between dental fluorosis and oxidative stress, such as develop the molecular aspects that fluoride induces cell stress, including endoplasmic reticulum stress and oxidative stress, which leads to impairment of ameloblasts responsible for dental enamel formation.

***Keywords:*** *Dental Fluorosis; Oxidative Stress; Fluoride; Enamel Formation*

## Introduction

Fluoride is of great importance in the prevention and treatment of dental caries. Nonetheless, excessive chronic fluoride exposure from drinking water, contaminated dust, and fumes leads to endemic fluorosis, which mainly involves teeth and the skeleton, renal toxicity,

epithelial lung cell toxicity, and reproductive toxicity. Fluoride is a naturally occurring mineral that protects against tooth decay. The Centers for Disease Control and Prevention (CDC) recommends public water fluoridation at an optimal fluoride concentration of 0.7 ppm in order to prevent dental caries [1].

Several fluoride belts have been identified: one running through Turkey, Iraq, Iran, Afghanistan, India, Northern China, and Thailand, and another one stretching from Syria through Jordan, Egypt, Libya, Algeria, Sudan and Kenya. In the Americas, some regions have high fluoride content in groundwater, including some southern areas of the United States and Northern Mexico [2].

The most common risk management strategy for local authorities is to monitor the fluoride concentrations in the drinking water, as this, is been assumed to be the predominant way of exposure. Health risk assessments (HRAs) have become a hot issue topic since the 1990s. HRAs consider a risk value as a numerical index for quantifying the magnitude and possibility of health hazards caused by harmful elements [3].

In some regions, artificial fluorides used to fluoridate community water supplies (mostly at around 1 mg/l) include silicofluoride compounds (sodium silicofluoride and hydrofluosilicic acid) and sodium fluoride (NaF). At neutral pH, silicofluoride is dissociated to silicic acid, fluoride ion, and hydrogen fluoride (HF). The primary benefit associated with fluoride supplementation is linked to the potential to reduce the risk of dental caries due to the cariostatic effects of fluoride. Even in the past, fluoride was considered an essential element. But, these days, there is a lack of consensus as to the role of fluoride in human nutrition and optimal development and growth [4].

The daily intake fluoride recommendation for primary prevention of fluorosis is 0.05 to 0.07 mg F/Kg/day. However, there are consequences among children if fluoride concentration is more than 1.5 to 4 mg/L, which is higher than WHO recommendation known as dental fluorosis. Skeletal fluorosis can occur if the fluoride concentration is in the range of 4 to 10 mg/L. The most common sources of ingested fluoride are fluoridated drinking water, toothpaste, supplements, and formula for children. The children at the age of 1 to 4 years old is at high risk. The risk of fluorosis subsequently decreases at around 8 years of age and it is highly prevalent among children below this age who is exposed to high fluoride [5].

Human health risk assessment of toxic agents is a systematized process used to estimate the nature and probability of adverse health outcomes in people who may be exposed to these agents present in environmental media currently, in the past, or in the future. The risk assessment paradigm involves a problem formulation, hazard identification and characterization (in this case, $F^-$), exposure assessment, and risk characterization [6].

Fluoride exerts diverse cellular effects in a time (concentration) and cell-type-dependent manner. The main toxic effect of fluoride in cells consists of its interaction with enzymes. In most cases, fluoride acts as an enzyme inhibitor, but fluoride ions can occasionally stimulate enzyme activity. The mechanisms depend on the type of enzyme that is affected; at micromolar levels is considered an effective anabolic agent because it promotes cell proliferation [1].

Fluoride harbors various cellular effects and excessive fluoride may cause oxidative stress, inhibit protein secretion and transport induce inflammatory response and interfere with cell proliferation and migration which could be fluoride concentration and/or cell type dependent. However, the underlying mechanisms of these cellular functions are less clear [7].

## Dental fluorosis (DF)

Since the major manifestations of fluorosis are dental and skeletal fluorosis, researchers have long focused on the pathology of bone and tooth tissues. In the past 5 years, new progress has been made in research into the pathogenesis of dental and skeletal fluorosis. In

addition, researchers have begun to pay more attention to the effects of fluoride on other organs and systems in the body. The largest body of research in recent years has investigated the mechanism of action of fluoride on non-skeletal tissue. Dental fluorosis is the earliest specific clinical manifestation of endemic fluorosis. The pathological changes mainly occur in enamel, but dentin and cementum are also involved. In recent years, research into the pathogenesis mainly focused on fluoride interference with protein secretion of ameloblasts, resulting in amelogenin hydrolysis and removal delay, and differences in susceptibility to fluoride due to individual genotypes [8].

Dental fluorosis is a developmental disturbance of the enamel layer of the tooth characterised by hypomineralised subsurface enamel caused by exposure to high levels of fluoride during tooth development and presents clinically as enamel opacities. The mechanism of fluorosis has been related to alteration of the enamel mineralisation process caused by a delay in the removal of amelogenins at the early-maturation stage of enamel formation [9]. In deciduous teeth, it occurs during the embryonic phase, while in permanent teeth, it occurs primarily in children aged 2 - 8 years old. The more important sources of fluoride intake are from food and water, as well as from toothpaste, which contains added fluoride. Fluoride is also being added to different materials, including fluoride varnish, fluoride foam and dental resin to prevent the occurrence of dental caries. All these methods increase the morbidity of dental fluorosis. The incidence of dental fluorosis is currently a problem worldwide, although the prevalence of dental fluorosis varies in different countries [10].

## Pathogenesis of dental fluorosis

Dental fluorosis is a sign of fluoride toxicity and can range from very mild to severe. Clinically, it is characterized by staining and pitting of the teeth. In more severe cases, the entire enamel may be damaged and lost.

During formative stage, intake of fluoride content drinking water enamel formation is disturbed and hypo-mineralised at its maturation. The risk of enamel fluorosis is lowest when exposure takes place only during the secretory stage, but highest when exposure occurs in both secretory and maturation stages. Enamel is produced by specialized epithelial cells called ameloblasts. The chief function of an ameloblast is to support the growth of a single hydroxyl-apatite enamel rod by secreting scaffold proteins during the secretory stage, in which the rods growth in length and then removing these proteins during the maturation stage, in which the rods thicken [11].

Enamel crystals develop in specialized extracellular compartments modulated by the activities of epithelial cells, known as ameloblasts, during the secretory and maturation stages of enamel development. Ameloblasts coordinate the transport of ions required for the growth of crystal. Rather than strengthening the bonds between enamel crystals, excessive fluoride disrupts mineralization, resulting an uneven, holey, pitted enamel with white opaque surfaces and hypomineralization. The mechanisms by which fluoride causes DF are complex. Variables affecting the impact of fluoride include its concentration, duration of exposure, and whether fluoride intake occurs during the formative (or secretory) or mineralizing (or maturation) stages of enamel development. It may also have a genetic component given the variable impact of excessive fluoride intake [12].

Fluorosis occurs when fluoride interacts with mineralizing tissues, causing alterations in the mineralization process. In dental enamel, fluorosis causes subsurface hypomineralization or porosity, which extends toward the dentinal-enamel junction as severity increases. This subsurface porosity is most likely caused by a delay in the hydrolysis and removal of enamel proteins, particularly amelogenins, as the enamel matures. At the early maturation stage, the relative quantity of amelogenin protein is increased in fluorosed enamel in a dose-related manner. This appears to result from a delay in the removal of amelogenins as the enamel matures. Although, definite mechanism of dental fluorosis is yet to be confirmed, current knowledge on ameloblast function during enamel formation may help to understand the same. Previously it was demonstrated that fluoride induces phosphorylation of the alpha subunit of eukaryotic initiation factor 2 (eIF2α) ribosomal components, which significantly decreases protein synthesis. This occurs during the maturation stage of development when proteins are normally removed from the hardening enamel [13].

## Mechanisms linked oxidative stress in dental fluorosis

Oxidative stress, as an important mode of fluoride toxicity, which might be responsible for several mechanisms undelaying dental fluorosis.

Oxidative stress is a pathway which excessive oxidant challenge causes damage to biomolecules, maintenance of a physiological level of oxidant challenge, termed oxidative eustress, essential for governing life processes through redox signaling. Redox balance is maintained by prevention, interception, and repair, and concomitantly the regulatory potential of molecular thiol-driven master switches such as Nrf2/Keap1 or NF-κB/IκB is used for system-wide oxidative stress response. Non-radical species such as hydrogen peroxide ($H_2O_2$) or singlet molecular oxygen ($0^0$), and free-radical species, perform major second messenger functions. Chemokine-controlled NADPH oxidases and metabolically controlled mitochondrial sources of $H_2O_2$ as well as glutathione- and thioredoxin-related pathways, with powerful enzymatic back-up systems, responsible for fine-tuning physiological redox signaling [14].

Studies with various *in vitro* and *in vivo* systems attempt to understand which cellular damage the fluorides can cause. Among these studies, some authors suggest that fluoride can increase the production of free radicals acting on the oxidative degradation of lipids and promoting changes in the cell membrane, leading to the formation of cytotoxic products and consequently cell death. In addition, studies seek answers about the epigenetic or DNA repair system mechanisms, which are still not explained. Some of these studies relate the exposure time and amount of fluorides, animal and human cells and animals and populations exposed to high fluoride levels with the expression of various genes [15].

Excessive fluoride can induce oxidative stress in ameloblasts, and the fluoride-induced reactive oxygen species (ROS) production causes oxidative damage to mitochondria and DNA, leading to activation of SIRT1/autophagy via ROS-mediated JNK (c-Jun N-terminal kinase (JNK) pathway is one of the major signaling cassettes of the mitogen-activated protein kinase (MAPK) signaling pathway) signalling. In addition, excessive fluoride can induce apoptosis of ameloblasts. The expression of and CHOP in ameloblasts increases with the increase of fluoride concentration. It is speculated that apoptosis induced by the endoplasmic reticulum stress pathway may play a role in the occurrence of dental fluorosis. High-fluoride partially activates the FasL, p-ERK and p-JNK signalling pathways7 of ameloblasts, leading to increased expression of apoptotic genes, indicating that oxidative stress is closely associated with apoptosis in dental fluorosis. Fluoride can also induce apoptosis by increasing the phagocytic activity of mature ameloblasts, and the Bcl-2 signalling pathway is involved in this process. Furthermore, there is evidence that autophagy is involved in dental fluorosis. Other study observed that fluoride increased expression of Beclin1, which is required for autophagosome formation, and decreased the expression of mTOR, an autophagy-related complex, indicating that autophagy is involved in dental fluorosis [16].

Previous studies have confirmed that fluoride exposure induces oxidative stress, activating apoptotic pathway in cementoblasts and ameloblasts. Superoxide dismutase isozymes (SODs), an important family of the oxidative stress system, are essential to protect the body from effects of $O_2^-$, including SOD1, SOD2 and SOD3. SOD1, also called Cu/Zn-SOD (almost exclusively found in intracellular cytoplasmic spaces). SOD2 (Mn-SOD) and SOD3 (extracellular Cu/Zn-SOD) can all be found in serum. It has been reported that mutations in genes might be associated with changes in phenotypes and mutations in *SOD2*, and *SOD3* genes appear to be associated with changes in serum SOD activity. The SOD activity is believed to be involved in several fluoride-induced health effects, including reproductive damage, neural impairment, and hepatic anomalies. A study of Li., *et al*. (2017) indicated that SOD activity also played an important role in the mechanism of NaF-induced ameloblast apoptosis. Therefore, SOD activity might correlate with the DF status [17].

Another mechanism which involves oxidative stress in dental fluorosis is apoptosis, also called programmed cell death, is the main cause of tissue injury and cell damage in endemic fluorosis. Many studies have reported that apoptosis is activated in several cell types,

such as cementoblasts, ameloblasts, osteoblasts, odontoblasts, and gingival fibroblasts, after treatment with fluoride, demonstrating that apoptosis was activated in regular cells after fluoride exposure via the extrinsic (death receptor) apoptotic pathway [18].

## Conclusion

As previously stated, there are various approach mechanisms in relation to the pathophysiology of dental fluorosis, as well as toxicological mechanisms, which are finally molecular mechanisms that involve different signaling pathways, among the involving oxidative stress pathways. Perhaps further advances in molecular technology are needed to somehow reverse the pathological conditions of dental fluorosis and systemic fluorosis, finding molecular key points for a better approach to the disease.

## Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Author Contributions

All authors contributed to the writing of the manuscript and approved the submitted version.

## Bibliography

1. Barbier O., *et al*. "Molecular mechanisms of fluoride toxicity". *Chemico-Biological Interactions* 188.2 (2010): 319-333.

2. Zepeda-Zepeda MA., *et al*. "Diagnosis of Dental Fluorosis Using Micro-Raman Spectroscopy Applying a Principal Component-Linear Discriminant Analysis". *International Journal of Environmental Research and Public Health* 18.20 (2021): 10572.

3. Yousefi M., *et al*. "Spatial Distribution Variation and Probabilistic Risk Assessment of Exposure to Fluoride in Ground Water Supplies: A Case Study in an Endemic Fluorosis Region of Northwest Iran". *International Journal of Environmental Research and Public Health* 16.4 (2019): 564.

4. Suzuki M., *et al*. "Fluoride induces oxidative damage and SIRT1/autophagy through ROS-mediated JNK signaling". *Free Radical Biology and Medicine* 89 (2015): 369-378.

5. Shahroom N., *et al*. "Interventions in management of dental fluorosis, an endemic disease: A systematic review". *Journal of Family Medicine and Primary Care* 8.10 (2019): 3108-3113.

6. Farías P., *et al*. "Fluoride Exposure through Different Drinking Water Sources in a Contaminated Basin in Guanajuato, Mexico: A Deterministic Human Health Risk Assessment". *International Journal of Environmental Research and Public Health* 18.21 (2021): 11490.

7. Tian Y., *et al*. "Vitamin E and lycopene reduce coal burning fluorosis-induced spermatogenic cell apoptosis via oxidative stress-mediated JNK and ERK signaling pathways". *Bioscience Reports* 38.4 (2018): BSR20171003.

8. Wei W., *et al*. "The pathogenesis of endemic fluorosis: Research progress in the last 5 years". *Journal of Cellular and Molecular Medicine* 23.4 (2019): 2333-2342.

9. Di Giovanni T., *et al*. "Iterventions for dental fluorosis: A systematic review". *Journal of Esthetic and Restorative Dentistry* 30.6 (2018): 502-508.

10. Ji M., *et al*. "How pH is regulated during amelogenesis in dental fluorosis". *Experimental and Therapeutic Medicine* 16.5 (2018): 3759-3765.

11. Bronckers AL., *et al*. "The impact of fluoride on ameloblasts and the mechanisms of enamel fluorosis". *Journal of Dental Research* 88.10 (2009): 877-893.

12. Aulestia FJ., *et al*. "Fluoride exposure alters Ca2+ signaling and mitochondrial function in enamel cells". *Science Signaling* 13.619 (2020): eaay0086.

13. Mohanta A and Mohanty PK. "Dental fluorosis-revisited". *Biomedical Journal of Scientific and Technical Research* 2 (2018): 2243-2247.

14. Sies H., *et al*. "Oxidative Stress". *Annual Review of Biochemistry* 86 (2017): 715-748.

15. Garcia A., *et al*. "Fluorosilicic acid induces DNA damage and oxidative stress in bone marrow mesenchymal stem cells. Mutation research". *Genetic Toxicology and Environmental Mutagenesis* 861-862 (2021): 503297.

16. Wei W., *et al*. "The pathogenesis of endemic fluorosis: Research progress in the last 5 years". *Journal of Cellular and Molecular Medicine* 23.4 (2019): 2333-2342.

17. Du Y., *et al*. "Effects of SNPs in SOD2 and SOD3 interacted with fluoride exposure on the susceptibility of dental fluorosis". *International Journal of Hygiene and Environmental Health* 239 (2022): 113879.

18. Ni J., *et al*. "Fluoride resistance in fibroblasts is conferred via reduced susceptibility to oxidative stress and apoptosis". *FEBS Open Biology* 10.3 (2020): 362-370.

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