Chapter

Progressive Research in the Molecular Mechanisms of Chronic Fluorosis

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Abstract

Long-term excessive intake of fluoride (F) leads to chronic fluorosis, resulting in dental fluorosis and skeletal fluorosis. Chronic exposure to high doses of fluoride can also cause damage to soft tissues, especially when it passes through the blood-brain, blood-testis, and blood-placenta barrier, causing damage to the corresponding tissues. Fluorosis has become a public health problem in some countries or regions around the world. Understanding the pathogenesis of fluorosis is very important. Although the exact mechanism of fluorosis has not been fully elucidated, various mechanisms of fluoride-induced toxicity have been proposed. In this chapter, we will introduce the research progress of the mechanism of fluorosis, focusing on dental fluorosis, skeletal fluorosis, nervous and reproductive system toxicity, and influential factors related to fluoride toxicity (i.e., genetic background, co-exposure with other element). In addition, the application of proteomics and metabolomics in the study of the pathogenesis of fluorosis is also introduced. Currently, there is still no specific treatment for fluorosis. However, since fluorosis is caused by excessive intake of fluoride, avoiding excessive fluoride intake is the critical measure to prevent the disease. In endemic regions, health education and supplement diet with vitamins C, D and E, and calcium and antioxidant compounds are important.

Keywords: chronic fluorosis, fluoride, influential factor, mechanisms, proteomics, skeletal fluorosis

1. Introduction

Fluorine is a highly active gaseous element found widely in nature. Fluoride in small doses is beneficial for preventing dental caries and is commonly used in the prevention of dental caries [1, 2]. However, long-term excessive fluoride intake will affect human health, causing chronic fluorosis. Chronic fluorosis is a systemic disease, high doses of fluoride leads to bioaccumulation in the body, especially hard tissues such as bones and teeth, and primarily harms bones and teeth [3–5]. Besides skeletal and dental damage, excessive exposure to fluoride can also cause other non-phrenological hazards, such as metabolic, structural, and functional damage to

the nervous system [6–12], kidneys [13–16], liver [14, 16–19], cardiovascular system [20–23], and reproductive system [24–26].

Chronic fluorosis is an endemic disease; it is endemic in at least 25 countries across the globe, China and India being the worst affected among them [27]. Most cases of fluorosis were caused by drinking fluorous water. In China, fluorosis is caused by drinking water as well as inhaling combustion fumes of coal being used as an indoor fuel source [28–31]. Guizhou is one of the most severely afflicted areas of endemic fluorosis in China and this occurrence is due to indoor coal burning [30]. Another type of fluorosis is brick tea-type fluorosis, due to fluoride accumulation in brick tea. It is more prevalent in Tibet than in other regions of China [32]. It is also worth noting that chronic exposure to volcanic environments may lead to the exposure of excessive amounts of fluoride [33]. It is estimated that more than 10% of the worldwide population live within the potential exposure range of some active or historically active volcano, either erupting or in a post-eruption phase [34].

In recent years, numerous studies focused on the molecular mechanisms associated with fluoride toxicity [35–39]. Although the underlying mechanisms of chronic fluorosis is still not well understood, the results of the previous studies indicated that fluoride can induce oxidative stress; regulate intracellular redox homeostasis; and lead to mitochondrial damage, endoplasmic reticulum stress, and alteration of gene expression [35–39]. Other mechanisms include enzyme inhibition, induction of apoptosis, cell cycle arrest, etc. [35–39]. This chapter reviews the present research on the potential adverse effects of overdose fluoride on various organisms, summarizes the molecular mechanism of fluorosis, and aims to improve our understanding of fluoride toxicity.

2. Mechanisms of skeletal fluorosis

Fluoride is a cumulative toxin, which accumulates in mineralized tissues, notably in the lattice of bone and tooth crystals. The bones and teeth are recognized as the target organs of fluoride, and bone tends to accumulate this element with age. The main features of the disease are dental fluorosis and skeletal fluorosis. Dental fluorosis is the first visible toxic effect of F exposure, which manifests as pitting of tooth enamel and yellow cracked teeth in adults and in children [37]. Skeletal fluorosis is a metabolic bone disease with osteosclerosis as the major clinical sign, mostly involving bone joints [40]. It results in ligament calcifications, accompanied by osteopenia, osteoporosis, and osteomalacia to varying degrees [40, 41]. Fluorine is a trace element that is incorporated into bone mineral during bone formation [42]. Fluoride substitutes for the hydroxyl group in hydroxyapatite, forming fluorapatite. Bone metabolism includes the process of osteoblasts forming bone and the osteoclasts degrading bone. Fluoride has an effect on bone mineral, bone cells, and bone architecture [42]. Fluoride at physiological levels promotes osteoblast proliferation, increases bone mass, as well as increases osteoblast activity via the up-regulation of markers such as alkaline phosphatase (ALP), bone morphogenetic protein (BMP), and bone gla protein (BGP) [43]. The levels of ALP and BGP were higher in patients with skeletal fluorosis than the control group [44]. However, fluoride may stimulate osteoblastic activity and delay mineralization of new bone [42]. On the other hand, osteoclasts are derived from hematopoietic progenitors in bone marrow and are only responsible for bone resorption. The mechanism associated with the osteoclasts is complicated; some studies showed that high fluoride concentrations may promote the formation of osteoclasts [45], or reduce the number of osteoclasts and decrease their bone resorption ability [46, 47]. Others suggested that fluoride had little effect on the number of osteoclasts and no effect on the osteoclast formation

[48]. Indeed, excessive fluoride intake can destroy the processes of bone formation and resorption, which may lead to bone turnover disorders and result in skeletal fluorosis. Bone turnover is a dynamic balance regulated by osteoblasts and osteoclasts. Excessive fluoride disrupts this balance, influencing the differentiation of osteoblasts and osteoclasts and resulting in the development of bone lesions [49]. This may be related to certain signaling pathways and mechanisms. Fluoride influences bone turnover by regulating certain factors, such as runt-related transcription factor 2 (Runx2) and receptor activator for nuclear factor-κB ligand (RANKL), which act as markers of osteoblasts and osteoclasts [48, 50]. Through the mitogen-activated protein kinases (MAPK) pathway, fluoride mediates gene expression and cell viability. In ameloblasts, fluoride activates the Rho/ROCK pathway. Fluoride can also induce endoplasmic reticulum (ER) stress, leading to protein misfolding [51]. In addition, TGFβ-SMAD signaling regulates expression of essential genes (MMP13, Collagen Type I, Collagen Type VII, Aggrecan, and Biglycans) involved in the formation of extracellular matrix (ECM). Fluoride exposure affects the expression of these genes through TGFβ-SMAD signaling [52]. Additionally, excessive fluoride exposure leads to disturbances of bone homeostasis. c-Fos is known to be essential in bone development by affecting osteoblast and osteoclast differentiation, suggesting that c-Fos might negatively regulate osteoprotegerin (OPG) expression induced by fluoride in osteoblastic cells [53].

Furthermore, collagen and noncollagenous proteins are of significant importance for maintaining the biomechanical integrity of the bone and many bone matrix proteins play important roles in mineralization [42]. Excessive intake of fluoride affects the bone matrix proteins, that is, collagen and noncollagenous proteins, which may be another possible mechanism of skeletal fluorosis [42, 54]. For example, it has been shown that fluoride could inhibit the synthesis of type I collagen and decrease the degree of collagen cross-linking [54–59] or affect other collagen proteins [60–62], and affect the synthesis of proteoglycan [63], and expression of matrix metalloproteinases (MMPs) [54, 64, 65]. Taken together, these studies suggested that exposure to fluoride alters growth, ECM formation, bone mineralization, and skeletal development and induced bone formation and bone resorption, thus leading to the development of fluorosis.

3. Nervous system toxicity

Excessive fluoride may cross the blood-brain barrier and accumulate in the brain, causing dysfunction of the central nervous system (CNS). In recent years, many studies have focused on fluorine neurotoxicity. The central nervous system during development is highly sensitive to the influence of fluorine due to its weakened protective mechanisms [66]. Studies showed that children in high fluoride areas had significantly lower IQ (intelligence quotient) scores than those who lived in low fluoride areas [67, 68]. The results of meta-analyses supported the possibility of adverse effects of fluoride exposures on children's neurodevelopment [67, 69]. In the animal experiments, as exposed to high levels of fluorine, the content of fluorides in the rats' brains was even 220 and 300 times higher than in the control group [70], and fluoride exposure affects the behavior, memory, cognitive and learning ability [71–73]. Dendritic thickening and disappearance, mitochondrial swelling, neuronal endoplasmic reticulum dilation, and impaired hippocampus synaptic interface structure can be observed in the brain of fluoride exposed rats [73]. The numbers of Nissl bodies in neurons in the hippocampus and cortex of brains from both adult rats and their pups with fluorosis were reduced, suggesting an injury of neurons [10]. These data indicate that excessive exposure to fluoride

results in structural and functional damages to the central nervous system, and may significantly hinder the neurodevelopment.

Fluorine neurotoxicity may be associated with oxidative stress, neuroinflammatory and neurotransmitter alterations. Fluorine induces increase in ROS (reactive oxygen species) and lipid peroxidation and decrease in anti-oxidative enzyme activity in neurons and glia, resulting in oxidative stress, which in turn causes cell damage and metabolism disorders [12, 74]. Fluorine causes glial cell activation which is involved in inflammation through producing proinflammatory cytokines. Chronic inflammation in the brain appears to cause neuronal damage [66, 75]. Moreover, fluorine influences the synthesis of neurotransmitters, the activity of enzymes, the expression of receptors, and the plasticity of neurons [76–78]. Therefore, excessive exposure to fluoride results in structural and functional damages to the central nervous system.

Of note, because fluoride can not only cross the blood-brain barrier, but also penetrate through the placenta, fluorine exposure in the prenatal and neonatal periods is dangerous [66, 79]. A recent study showed that during pregnancy and lactation, even at very low concentrations, F exposure may alter parameters of the central nervous system functionality, producing a delay in eye-opening development in the offspring as well as hypoactivity in adult offspring [80]. Further studies will be crucial to elucidate the molecular mechanisms through which F exposure during gestation and lactation trigger neurobehavioral changes [80].

4. Reproductive system toxicity

Research on the effects of fluoride on the reproductive system has been carried out for many years. As early as 1925, Schulz and Lamb reported the reproductive toxicity of fluoride [81]. Fluoride shows adverse effects on the male reproductive system, including spermatogenesis defect, sperm count loss, sperm differentiation, and maturation impairment [82], and increase in chromosomal aberrations in primary testicular cells and the rate of sperm deformity [83]. Interestingly, recent studies showed that exposure to fluoride can alter the BTB (blood-testis-barrier) [84, 85]; fluoride induced structural and functional alterations in the BTB by increasing the expression levels of Arp3 protein with a concomitant increase in the expression levels of IL-1α (interleukin-1α) that led to the reorganization of the highly branching F-actin and the decreased expression of F-actin [25]. A significant increase in the fluoride concentration in the testes of mice that were exposed to sodium fluoride (NaF) has been observed [85]. In addition, ovaries of albino rats treated with high doses of NaF exhibited abnormal ovarian follicles, dilated blood vessels, stromal congestion, and necrotic granulose cells [86].

Cell apoptosis is one early sign of genotoxic damage in mature testis, and plays critical roles in spermatozoa output. Fluoride may induce oxidative stress through the activation of MAPK cascade and Jun N-terminal kinase (JNK, c-Jun) and extracellular signal-regulated protein kinase (ERK) signaling pathway lead to cell apoptosis that includes both intrinsic and extrinsic apoptotic pathways [82]. Fluoride could also cause leakage of potassium ions, thereby reducing sodium and potassium levels in spermatozoa [87]. In addition, higher levels of inflammatory factor such as IL-1α were detected in the testes of NaF-treated rats [25], suggesting that inflammation was involved in the in the toxicity of fluoride to the reproductive system [25, 88]. More recently, a proteomics study analyzed the proteome characteristics of sperm from fluoride-exposed mice, and identified 15 differentially expressed proteins between fluoride-exposed and control groups. Most of them are associated with sperm functions such as sperm motility, maturation, capacitation

and acrosome reaction, lipid peroxidation, detoxification, inflammation, and stability of membrane structure [89]. Another study reported altered MicroRNA (miRNA) expression profiling in sperm of mice induced by fluoride. Sixteen altered miRNAs were identified and they mainly were involved in protease inhibitor activity, apoptosis, ubiquitin-mediated proteolysis, and signaling pathways of calcium, JAK-STAT, MAPK, p53, and Wnt [90]. These findings provide new insights into the mechanism underlying fluoride reproductive toxicity. However, the toxicity mechanism of fluoride on the reproductive system still needs further exploration.

5. Other systems

As mentioned above, excess fluoride uptake affects other organs including liver and kidneys, and cardiovascular system. Liver is the most important detoxification organ in the body. The effect of fluoride on the liver has been widely studied and it has been demonstrated that excessive intake of fluoride causes serious liver damage [14, 16–19].

The kidneys are the main route of F removal from the body, and approximately 60% of the total daily F absorbed is filtered and excreted in urine [91]. The link between fluoride and kidney disease has been known and confirmed for many years [13–16], the toxicity or damage of fluoride to the kidney has been observed in population and experimental animals, including the kidneys of the fetus and suckling mammal [92]. Of note, people on kidney dialysis, patients with reduced glomerular filtration rates, and diabetic mammals are particularly susceptible to fluoride exposure [15].

A rising number of research studies have been carried out on the toxic effect of F in cardiovascular system [20–23, 93]. Fluoride can accumulate in the cardiovascular system, resulting in arterial calcifications, elastic properties of ascending aorta disruption, and ventricular diastolic dysfunction [93].

6. Influence factor

6.1 Genetic susceptibility to fluorosis

Clearly, toxic effects in humans due to chronic fluoride ingestion mainly depend on the total dosage and duration of exposure. However, dose and time alone are not the only factor affecting fluorosis. Some studies have shown the existence of non-responder populations to fluorine [94], while others have shown that some people seem to be very sensitive to fluorine [95, 96]. Animal experiments have observed that three inbred strains of mice (A/J, SWR/J, 129P3/J) displayed variations in the onset and severity of dental/enamel fluorosis with equivalent fluoride exposure [97]. The bone mechanical properties were reduced in the "susceptible strain" (A/J), moderately altered in the "intermediate strain" (SWR/J), and unaffected in the "resistant strain" (129P3/J), suggesting a genetic contribution to the variation in bone response to fluoride content [97]. Fluoride effects on bone formation and mineralization are influenced by genetics [42]. Another study showed that exposure to the same dosage and time, as compared with Wistar rats, the urine fluoride of SD rats was higher while bone and teeth fluoride levels were lower. Meanwhile, dental fluorosis susceptibility of SD rats is higher [98].

Interestingly, the association between genetic polymorphisms in candidate genes and the susceptibility in the development of fluorosis has been well reviewed [27]. Candidate genes associated with human dental fluorosis and skeletal fluorosis are

listed in **Table 1**. Candidate genes in dental fluorosis include BGLAP (Osteocalcin), COL1A2 (Collagen type 1 alpha 2), CTR/CALCR (Calcitonin Receptor), ESR (Estrogen Receptor), and VDR (Vitamin D Receptor). Candidate genes in skeletal fluorosis include MMP-2 (Matrix metallopeptidase2), MPO (Myeloperoxidase), GSTP1 (Glutathione S-transferase pi 1), PRL (Prolactin), and VDR (Vitamin D Receptor). These genes are involved in different functions—BGLAP, ESR, and COL1A2 are related to bone formation and development; VDR and CTR are related to bone formation and metabolism; and PTH and PRL are related to hormones' secretion. GSTP1, MMP, COMT, and MPO are related to detoxifying enzymes, extracellular matrix, cognitive and immune responses, respectively [27]. These results suggest that an individual's genetic background plays a major role in influencing the risk to fluorosis.

6.2 Co-exposure with other element

Co-exposure to other elements is another major factor affecting fluorosis. All of these could complicate the overall toxic response. For example, in geothermal areas, volcanic activity includes CO_2-rich hot springs, steaming vents, hot ground and boiling mud pools that normally contain unusually high concentrations of Li, Rb, Cs, Si, B, As, and F [33]. Thus, chronic exposure to volcanic environments may lead to the exposure of excessive amounts of fluoride and other elements. Interestingly, a recent study indicated that an increase or decrease in various elements (including F, Al, Se, Zn, Cu, Fe, Mo, Mn, B, V, Ca, Mg, and P) in the environment is related to the abnormal levels of the corresponding elements in a fluoride-exposed population [28]. High levels of F, Al, As, Pb, and Cr were a risk factor for dental fluorosis, but not Se, Zn, Cu, B, Ca, and P, which was a protective factor for dental fluorosis [28].

Candidate genes	Polymorphism site (restriction sites or mutational bases)	References
Dental fluorosis		
COL1A2 (Collagen type1 alpha 2)	rs414408 (PvuII) rs412777 (A/C)	[99] [100]
ESR (Estrogen receptor)	rs1256049 (G > A, RsaI) rs2234693 (A > C, XbaI)	[101]
AMBN (Ameloblastin)	rs4694075 (C/T)	[102]
TFIP11 (Tuftelin interacting protein 11)	rs5997096 (C/T)	[102]
TUFT1 (Tuftelin)	rs4970957 (A/G)	[102]
DLX1 (Homeobox protein DLX-1)	rs788173 (A/G)	[103]
DLX2 (Homeobox protein DLX-2)	rs743605 (A/G)	[103]
TIMP1 (Metalloproteinase inhibitor 1)	rs4898 (C/T)	[103]
Skeletal fluorosis		
FRZB1 (frizzled-related protein 1)	rs2242070 (A/G)	[104]
VDR (Vitamin D receptor)	rs2228570 (Fok I)	[105]
GSTP1 (Glutathione S-transferase pi 1)	rs1695 (A/G)	[106]
PRL (Prolactin)	rs1341239	[107]
MMP-2 (Matrix metallopeptidase 2)	rs2287074 (G/A) rs243865	[108]

Table 1.
Candidate genes in dental fluorosis and skeletal fluorosis.

At present, some studies have been reported on the co-exposure of fluorine and arsenic (As) or fluorine and aluminum (Al). Both arsenic and fluoride are ubiquitous in the environment. The co-exposure of fluorine and arsenic is mainly through drinking water [109–111] or burning coal [112, 113]. The latter is a unique type in China, which was attributed to exposure to high levels of As and F in food and breathing As-laden air, caused by polluted food and air due to indoor combustion of coal [112, 113]. The interaction mechanism of these two elements is complicated, which may be independent, synergistic, or antagonistic [114]. A recent study indicated that arsenic may be involved in fluoride-induced bone toxicity through PTH/PKA/AP1 signaling pathway [115]. Arsenic affects the expression of c-Fos, thereby affecting the expression of transcription factor AP1, indirectly involved in fluoride-induced bone toxicity [115]. Another study showed that the joint effect of fluoride and arsenate on the gene expression of ODF (osteoclast differentiation factor) is antagonistic, while the combined effect on the gene expression of OPG is synergistic [116]. Ma et al. reported that As and F can induce the expression of adhesion molecules, chemokines and pro-inflammatory cytokines in rabbit aorta separately, and antagonistic effects were observed on inflammatory response [117]. Fluoride and arsenic, either alone or combined, can decrease learning and memory ability in rats [118].

Combined exposure to fluoride and aluminum is another noteworthy problem related to fluorosis. It mainly occurs through indoor combustion of coal, especially kaolin mixed with coal [119], and high Al content in tea such as brick tea [120]. The interaction mechanism of F and Al is also complicated, may be independent, synergistic, or antagonistic. Aluminum exposure impairs bone formation; inhibition of bone formation by aluminum through different signal transduction pathways has been reported [121]. Exposure to Al is associated with low bone mineral density (BMD) and an increased risk of osteoporosis [121–124]. Fluoride enhances the uptake of aluminum; the simultaneous administration of fluorine and aluminum increased plasma [125], and bone [126] concentrations of aluminum in rats, whereas aluminum suppresses the uptake of fluoride [127]. Decreased bone mineral density was observed in fluorine and aluminum-treated rats [126]. Patients with co-exposure to fluoride and aluminum display with osteomalacia or osteoporosis may be due to fluoride promoting aluminum accumulation in bone, while aluminum inhibits bone formation. However, the vitro study showed that there was a synergistic effect of fluoride and aluminum on the expression of Runx2 and Osterix mRNA in osteoblastic MC3T3-E1 cells, thereby enhance MC3T3-E1 cells proliferation and differentiation [128], and contribute to osteosclerosis. This may explain the different clinical features of skeletal fluorosis, that is, osteosclerosis accompanied with osteomalacia, and osteopenia.

It is worth mentioning that Al is a well-known neurotoxic agent, and it has long since been implicated in the etiopathology of AD [129]. Fluorine and aluminum are able to cross the blood-brain barrier and the placental barrier [66, 130]. They can accumulate in the brain, and fluoride did not affect the accumulation of aluminum in the CNS [131]. It has been reported that increases of microglia activation and inflammatory response were seen in aluminum, fluoride, and a combination of aluminum-fluoride-treated rat brain [132]. Excessive fluoride and aluminum intake induces the progression of cell death which inhibits acetylcholinesterase (AChE) activities and triggers the release of lysosomal and cell cycle proteins in the brain of rats [133]. More recently, Xie et al. found that continuous exposure to fluorine and/or aluminum of mother rats impaired the neurobehavioral reflexes, spatial learning, and memory of offspring rats [134, 135]. The effects of F were obvious, but the effects of Al were slight. There were antagonistic effects between F and Al, with Al reduction in the toxicity of F [135].

In addition, Chinoy et al. reported that simultaneous exposure of the animals to NaF and $AlCl_3$ was associated with an increased toxic effect on gonadal

steroidogenesis, uterine metabolism of carbohydrates, and hypercholesterolemia, as compared with each compound administered separately [136]. Recently, Dong et al. reported that F induced the reduction in testosterone and sperm amount; however, Al had antagonism effects on F and weakened the toxicity of F to some extent [137]. Moreover, fluoride interacts with aluminum to form a fluoro-aluminum complex AlFx (e.g., AlF_3 and AlF_{4-}), which can interact with the G protein (guanine nucleotide-binding proteins) and activated effect or enzymes, providing false information, and amplify the processes of signal transmission [138]. Together, further investigation is needed on the underlying mechanisms by which fluorine and arsenic or fluorine and aluminum induce toxicity.

7. Proteomics and metabolomics applications

Proteomics and metabolomics are useful and powerful tools for clarifying toxicological mechanisms associated with diseases. Proteomics offers the possibility to map the entire proteome of an organism or cells and detect toxic effects at significantly lower doses, as well as faster screening for potential adaptive mechanisms by the use of high-throughput technologies [139]. In particular, during the last 10 years, apart from the gel-based techniques (e.g., 2D-PAGE and 2D-DIGE), gel-free techniques (e.g., stable-isotope labeling or using label-free methods) have been dominating the field of MS-based quantitation in proteomics [140]. This enhances the ability of proteomics to explore disease mechanisms. As mentioned above, proteomics analysis has been used to investigate the toxicity mechanism of fluorine on sperm [89]. Proteomics analysis associated with F-toxicity has also been studied in other tissues including gastrocnemius muscle, kidney, liver, midgut, bone, cells, serum, and urine [18, 54, 62, 68, 141–147]. All of these studies are listed in **Table 2**. As shown in **Table 2**, proteomic techniques 2D-PAGE, LC-MS/MS (liquid chromatography-tandem mass spectrometry), and iTRAQ (isobaric tags for relative and absolute quantification) labeling coupled with LC-MS/MS analysis were employed in these studies. The proteins associated with fluoride exposure were found involved in oxidative stress, ER stress, cell proliferation and apoptosis, mitochondrial-metabolism, tricarboxylic acid (TCA) cycle, unfolded protein response, inflammatory response, etc. These pathways or biological processes have previously been linked to the pathophysiology of fluorosis. The results support the current views on the molecular mechanism of F-toxicity.

Interestingly, Khan et al. evaluated the effects of F on the liver proteome of mice susceptible (A/J) or resistant (129P3/J) to the effects of F. As compared with 129P3/J mice, most of the proteins with fold change upon treatment with lower F concentrate (15 ppm) were increased in the A/J mice, suggesting an attempt of the former to fight the deleterious effects of F. However, upon treatment with 50 ppm F, most proteins with fold change were decreased in the A/J mice, especially proteins related to oxidative stress and protein folding, which might be related to the higher susceptibility of the A/J animals to the deleterious effects of F [18]. These results add light to the mechanisms underlying genetic susceptibility to fluorosis [18].

It is worth mentioning that in our previous comparative proteomic analysis of fluoride treated rat bone [54], 13, 35, and 34 differentially expressed proteins were identified in low-, medium-, and high-dose NaF exposure group. The medium- and high-dose groups shared a more similar protein expression pattern. Most of these proteins belong to collagen proteins, matrix metalloproteinases, proteoglycans (PGs), proteolytic protein, osteoclast-related protein, and myosin proteins, involved in collagen metabolism, bone mass change, mineralization process, dysfunction of the motor cell, and affected osteoblasts and/or osteoclasts, finally, contributing to the pathophysiology of skeletal and chronic fluorosis [54].

Models/materials	Toxic effects	Method	Critical proteins	Pathways/biology processes	References
Calvarial osteoblasts	Cells were treated with NaF	2-DE MALDI-TOF MS	ATP synthase, Dihydropyrimidinase-like 2, Heat shock 70-kDa protein(HSP70), Nucleoside diphosphate kinase, Glutamate oxaloacetate transaminase, Phosphatidylethanolamine binding prote in Proteasome 26S ATPase, Nucleoside diphosphate kinase, Protein disulfide isomerase, Ras-GTPase-activating protein, Thioredoxin, Tubulin, beta	Cell proliferation Nucleotide metabolism Signal transduction Protein oxidative folding Hydrophobic ligands Cell motility	Xu et al. [141]
Urine from the fluoride-treated Wistar rat	Dental fluorosis	2D-PAGE MALDI-TOF-TOF MS/MS	Androgen regulated 20 kDa protein, Aflatoxin B1 aldehyde reductase, alpha-2-μ-globulin	Detoxification Hormone regulation	Kobayashi et al [142]
Kidney (A/J and 129P3/J mice)	Dental fluorosis Kidney impairment Genetic susceptibility	2D-PAGE LC-MS/MS	Twenty five (25), 30 and 32 differentially expressed proteins were successfully identified between different doses of NaF treatment groups and their respective controls.	Metabolic and cellular processes Response to stimuli Development Regulation of cellular processes	Carvalho et al. [143]
Gastrocnemius muscle Streptozotocin-induced diabetes exposed to fluorides	Alter glucose homeostasis and lead to insulin resistance	2D-PAGE LC-MS/MS	78 kDa glucose-regulated protein, Alpha-enolase, Beta-enolase, Gamma-enolase, Gelsolin, Glyceraldehyde-3-phosphate dehydrogenase, Glycerol-3-phosphate dehydrogenase [NAD(+)], Heat shock cognate 71 kDa protein, L-lactate dehydrogenase A chain, L-lactate dehydrogenase B chain, L-lactate dehydrogenase C chain, Malate dehydrogenase, Myosin-3, Myosin-6, Myosin-7, Myosin-8, Myosin-binding protein C_ slow-type, Myosin-binding protein H, Pyruvate kinase isozymes M1/M2	Muscle contraction Carbohydrate catabolic processes Generation of precursor Metabolites and energy NAD metabolic processes Gluconeogenesis	Leite et al. [144]
Femurs, tibiae, and lumbar vertebrae (129P3/J and A/J mice)	Bone architecture Mineral apposition rate Genetic susceptibility	LC-ESI-MS/MS	129P3/J vs A/J mice: Bone morphogenetic protein 1, Bone sialoprotein 2, Collagen alpha-1(I) chain, Collagen alpha-2(I) chain, Exportin-2, NADPH oxidase 4, Protocadherin beta 15, Secreted frizzled-related sequence protein 4 129P3/J vs F-treated 129P3/J mice: Aflatoxin B1 aldehyde reductase member 2, Carbonyl reductase [NADPH] 2, Catenin alpha-2, Chromodomain-helicase-DNA-binding protein	Osteogenesis Osteoclastogenesis	Kobayashi et al. [62]

Models/materials	Toxic effects	Method	Critical proteins	Pathways/biology processes	References
			4, Chromodomain-helicase-DNA-binding protein 7, NADPH oxidase 4, Phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase 2, Protocadherin beta 9 A/J vs F-treated A/J mice: Eukaryotic translation initiation factor 2 alpha kinase 3, Exportin-2, Phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase 1		
Kidney (Wistar rats)	Alteration of renal metabolism	2D-PAGE MALDI-TOF MS	Control vs 10 ppm F: Aldo–keto reductase, Adnylate kinase 3-like 1, Enoyl coenzyme A hydratase, Pyruvate carboxylase, Control vs 5 ppm F: Aldolase B, Endoplasmic reticulum protein 29	Detoxification Metabolism Housekeeping	Kobayashi et al [145]
Hippocampus from rats	Leaning ability Memory	2D-PAGE, MALDI-TOF-MS	5'-AMP-activated protein kinase, Aconitate hydratase, Actin, cytoplasmic2-like isoform 3, Actr2 protein, Beta-actin, Cytosolic aspartate, dehydrogenase [NADP+], and fructose-bisphosphate aldolase C, Dynamin, Fascin, Fructose-bisphosphate aldolase C-B, Gln synthetase, Glycogen phosphorylase, glycoprotein 1 precursor, Lysosome-associated membrane, MHC class I antigen, Mitogen-activated protein kinase 1, Mixture 1: alcohol, N-ethylmaleimide sensitive, Otub1 protein, PDZ and LIM domain prptein 3, Phosphatase 1E isoform 1, Pyruvate carboxylase, Serum albumin, Tropomyosin 1, Ulip2 protein, Voltage-dependent anion-selective channel protein 1, WD repeat-containing protein 1	Biosynthesis of amino acids Carbon metabolism Insulin signaling pathway Phagosome Oxytocin signaling pathway	Pan et al. [146]
Serum from Wistar rats treated by NaF	Dental fluorosis	iTRAQ labeling, NanoLC-MS/MS	A total of 37 differentially expressed proteins were identified in different doses of the NaF treatment group	Complement and coagulation cascade Inflammatory response Complement activation Defense response Wound response	Wei et al. [147]

Models/materials	Toxic effects	Method	Critical proteins	Pathways/biology processes	References
Sperm samples from Kunming mice	Sperm damage	2D-PAGE, MALDI-TOF-MS	Adenylate kinase isoenzyme 1 isoform 2, Aldose reductase-related protein 1 Annexin A13, Annexin A4, Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, Gamma-actin, Inhibitor alpha1 Phosphoglycerate kinase 2, Proteasome (prosome, macropain) subunit, alpha type 3, Serotransferrin precursor, Triosephosphate isomerase	Sperm motility Maturation Capacitation and acrosome reaction Lipid peroxidation Detoxification Inflammation Stability of membrane structure	Sun et al. [89]
Bone samples (Sprague-Dawley rats)	Chronic fluorosis	iTRAQ labeling, NanoLC-MS/MS	Thirteen (13), 35, and 34 differentially expressed proteins were identified in low-, medium-, and high-dose NaF-treated group, respectively. These proteins belong to collagen proteins, matrix metalloproteinases (MPPs), proteoglycans(PGs), proteolytic protein, osteoclast related protein, and myosin proteins	Bone mass change Mineralization process Dysfunction of the motor cell Affected on osteoblasts and/or osteoclasts	Wei et al. [54]
Liver (A/J, 129P3/J mice)	Disturbances in soft tissues Genetic susceptibility	Nano-LC-ESI-MS/MS	Eighty one (81) differentially expressed proteins were identified in the liver of A/J and 129P3/J mice treated with 15 ppm F. One hundred one (101) differentially expressed proteins were identified in the liver of A/J and 129P3/J mice treated with 50 ppm F	Carboxylic acid metabolic process Cellular amino acid metabolic Process Oxidative stress and protein folding might be related to the susceptibility to the deleterious effects of F	Khan et al. [18]
Plasma from Children	Children intelligence Gene polymorphism	2-DE, MALDI-TOF/TOF-MS	Alpha-1-B glycoprotein, Apolipoprotein E precursor, Complement C1s subcomponent precursor, Hemopexin, Immunoglobulin light chain variable region	Cell immunity Metabolism	Zhang et al. [68]

Table 2.
Fluorosis-related proteomics studies reported in the literatures.

Metabolomics can capture low-molecular weight metabolites that are the closest to the phenotype, which is believed to be one of the most powerful techniques to study the metabolic alteration associated with the treatment of environmental toxicants. However, the study on metabolic profile response to fluoride exposure is limited. A recent study carried out a metabonomics study on NaF treated human oral squamous cell carcinoma cells. The results showed that inhibition of the enolase reaction in glycolysis pathway was observed in the early stages of fluoride treatment. In the later stages, gradual increases in the AMP/ATP ratio (a putative marker of apoptosis) and oxidized products (e.g., GSSH, and methionine sulfoxide), and marginal changes in polyamine levels (putative marker of necrosis), were observed [148]. It suggested that the inhibition of enolase reaction and TCA cycle progression at early stage is specific to NaF, whereas the increase of ATP utilization at later stage may be common to apoptotis-inducing agents, but not to necrosis-inducing agents [148].

8. Treatment and prevention of chronic fluorosis

So far, there is no specific treatment for fluorosis. Efforts are being made to reduce the severity of the disease and improve quality of life of affected patients [149]. Medical treatment being used is mainly supplementation of vitamin (Vit) C, D, and E, calcium, antioxidants and treatment of malnutrition [150]. In recent years, some traditional Chinese medicines (TCM) have been developed to treat fluoride-induced bone lesions in China [49]. Treatment options for dental and skeletal fluorosis vary according to the severity of the disease [149]. Methods for treating dental fluorosis include micro/macro abrasion, bleaching, composite restorations, veneers, and full crowns [151]. Treatment of skeletal fluorosis may include surgical processes while treatment of deformity includes use of physiotherapy, corrective plasters, and orthoses (appropriate appliances) [149].

Clearly, chronic fluorosis is mainly caused by excess intake of fluoride through drinking water, food products, air, and industrial pollutants over a long period. Therefore, avoiding excessive intake of the fluoride is essential for the prevention of this disease. Notably, to keep fluoride intake within safe limits, one needs to consider the total daily intake, including fluoride intake from water, food, air, fluoride-rich dental products and drugs. The recommended level for daily fluoride intake is 0.05–0.07 mg F/Kg/day [152, 153]. In China, for people aged 8–16 (including 16-year-olds), the recommended values of the total daily fluoride intake per person is ≤2.4 mg; for those 16 years old, the total daily fluoride intake per person is ≤3.5 mg [154]. In water-borne fluorosis endemic areas, alternative water resources with low fluoride levels or defluorinated water can be used. Coal-burning endemic fluorosis areas need to change the way coal is burned and food is dried. Likewise, it is beneficial for a daily intake of foods, vegetables, and fruits rich in vitamin C, D, and E, calcium, and antioxidants for the prevention of chronic fluorosis in endemic regions [49, 82, 150]. Moreover, health education is a very important aspect for disease management. Knowledge regarding the harmful effects of fluoride and the causes of fluorosis can help people, especially the affected population, pay more attention to their living habits [49, 149]. Furthermore, the identification of candidate genes that affect risk factors is necessary to develop more effective measures to prevent and treat fluorosis [27].

9. Conclusions

The contents of this chapter are reviewed in **Figure 1**. Excess intake of fluoride can cause chronic fluorosis, leading to dental fluorosis and skeletal fluorosis and damage

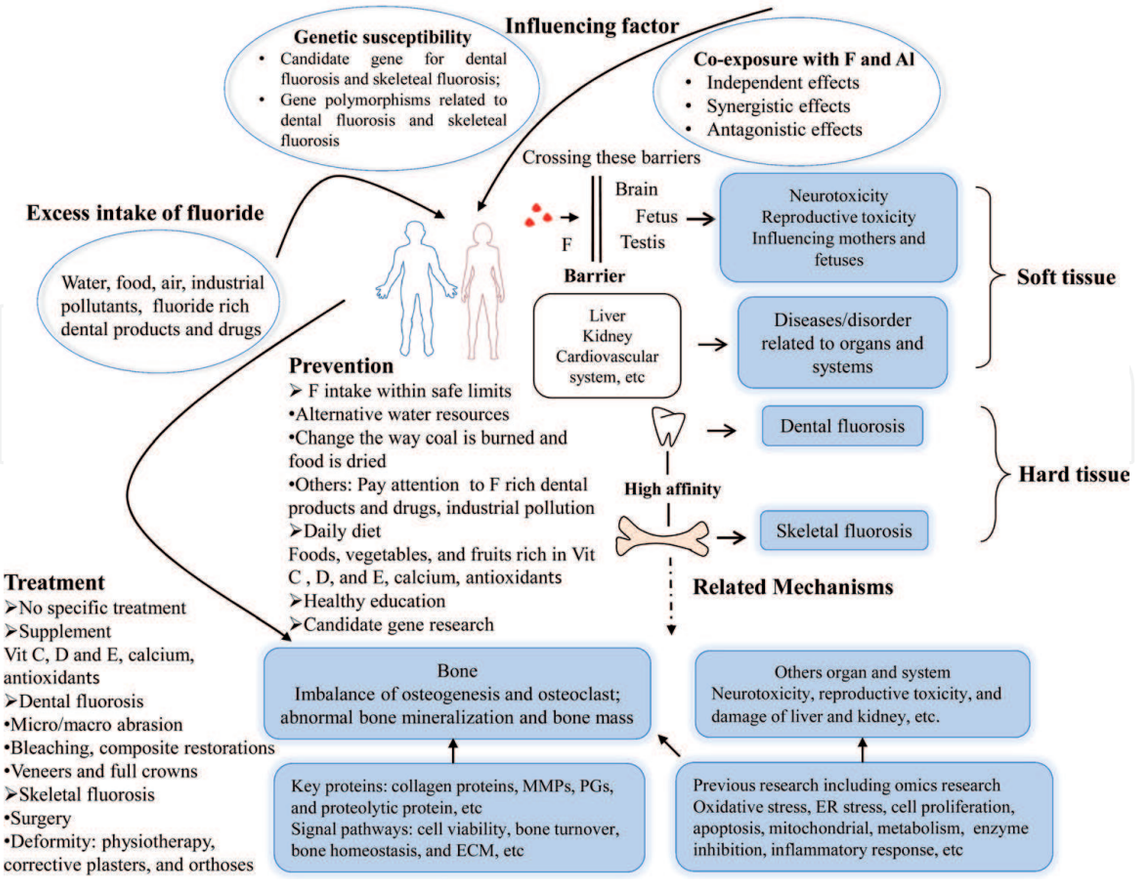

Figure 1.
An overview of the occurrence, influencing factors, pathogenesis, treatment, and prevention of fluorosis.

to nervous system, reproductive system, cardiovascular system, liver, and kidney. The possible mechanisms involved different key proteins and signal transduction pathways associated with the pathogenesis of fluorosis have been proposed. Some high-throughput methods such as proteomics, metabolomics, and transcriptomics have been used in the study of the mechanism underlying development of fluorosis. Genetic factors play a critical role in the pathogenesis of chronic fluorosis. Combined exposure to fluoride with other element such as arsenic or aluminum may result in more complicated adverse health effects than exposure to fluoride or these elements alone. Further research is needed to reveal the interaction between fluorides with these elements with regard to their toxic effects. Clearly, the mechanisms of chronic fluorosis still need further research. Prevention of chronic fluorosis is important and it can be prevented by keeping fluoride intake within safe limits. It is important to consider total exposure (i.e., exposure through air, food, and water) when evaluating adverse health effects of fluoride.

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Conflict of interest

The authors declare that they have no competing interests.

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