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Review of Literature
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Anabolic action, Genotoxicity and Carcinogenicity of Fluoride Review of Literature

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Abstract

Organic and inorganic compounds containing the element fluorine are called fluorides. Fluorides are usually released to the air from volcanoes and industry then carried by the wind and rain to nearby water, soil and food sources. Its compounds often have properties that range from potent toxins such as sarin to life saving pharmaceutical such as efavirenz and from refractory materials such as calcium fluoride to the highly reactive sulfur tetrafluoride. This literature review may bring data forward for education in this aspect to prevent water fluoridation and to support defluoridation in the areas where the level of fluoride in public drinking is higher than the normal limits of 0.6-0.7 ppm for hot climate countries and 1.2 ppm for cool climate countries as recommended by WHO.

Keywords: *Fluorine; Drinking water; Water fluoridation; Defluoridation; WHO*

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Introduction

Fluorine is the lightest member in the halogen group and is one of the most reactive and electronegative of chemical elements which means that it has a strong tendency to acquire a negative charge. Fluorine have been used in the past to help molten metal flow, hence the name, which derives from Latin verb Fluere, meaning to flow. Because, fluorine is the most reactive of all elements and no chemical substance is capable of freeing fluorine from any of its compounds, for this reason fluorine does not occur free in the nature [1].

Naturally, Fluorides represent about 0.06-0.09% of earth crust and found in a significant levels in a wide variety of minerals including fluorspar, rock phosphate, cryolite apatite, mica, and hornblene. Therefore, waters with high fluoride levels usually found at the foot of high mountains and in areas where the sea has made geological deep fluoride belts on land including one that stretches from Syria through Jordan, Egypt, Libya through Algeria and Morocco as well as Sudan and Kenya. Another stretches from Turkey through Iraq, Iran, Afghanistan, Northern Thailand and China as well as southern parts of USA, southern Europe and southern parts of USSR and Japan [2].

Inorganic compounds of fluoride, including sodium fluoride (Na), stannous fluoride (SnF2) are used in tooth paste, fluoride solutions and fluoride gels for topical use. Fluoride tooth paste is considered to be the most single and widely used to prevent dental caries. Fluorides also added to some municipal water supplies, a process called water fluoridation which is normally accomplished by adding sodium

fluoride, fluorosilicic acid (H 2SiF6), or sodium fluorosilicate (Na 2SiF6) to the public water supply to reduce the incidence of dental caries at the levels of 1 ppm [3].

Fluoride's effect depends on the total daily intake of fluoride from all sources. About 70-90% of ingested fluoride is absorbed into the blood, where it distributes throughout the body. In infants 80-90% of absorbed fluoride is retained, with the rest exerted mostly via urine therefore the critical period of exposure is between ages one and four years. In adults about 60% is retained. About 99% of retained fluoride is stored in bone, teeth and other calcium-rich areas, where excess quantities can cause fluorosis. Adverse effect of fluorides can be prevented by monitoring all sources of fluoride by a process called defluoridation when the fluoride level present above the normal limit of about 0.7 ppm [4].

Fluoride in drinking water

Fluoride has been described as an essential element needed for normal development and growth of animal and extremely useful for human beings. Fluoride is abundant in the environment and the main source of fluoride to humans is drinking water, in which it is the typically the largest single contributor to daily fluoride intake [5].

Fluoride is a naturally occurring toxic mineral present in drinking water and causes yellowing of teeth. Fluorspar, cryolite and fluorapatite are the naturally occurring minerals, from which fluoride finds its path to ground water through infiltration. Fluoride is found in all natural waters at some concentration. Seawater typically contains about 1.3 ppm while rivers and lakes generally exhibit concentrations of less than 0.5 ppm. In ground waters, however, low or high concentrations of fluoride can occur, depending on the nature of rocks and occurrence of fluoride-bearing minerals and fluorite solubility [5]

Fluoride is also found in drinking water as an additive to provide public health protection from dental caries (artificial water fluoridation). In practice, most fluorine added to drinking water is in the form of fluosilicic acid, fluorosilicic acid or the sodium salt (sodium fluosilicate), collectively referred to as fluorosilicates. For some smaller water system, fluoride is added as sodium fluoride. In water, fluorocilicates dissociate to form fluoride ion, hydrofluoric acid and silicic acid, while incomplete dissociated of silico fluorides is attributed to increase up take of lead and elevated lead plasma level in children [3-5].

In tropical countries like Saudi Arabia, the appropriate level of fluoride concentration in drinking water as recommended by World Health Organization (WHO) should be 0.6-0.7 ppm, while in cool climate countries where the consumption of water is relatively low is 1.2 ppm. Actual intakes of fluoride from drinking water by individual depend on their individual water intakes, the source or sources of that water, and the use of home water purification or infiltration systems and the disease condition, such as diabetes millet us which characterized by high water intake and high urine volume level. Fluoride content in bottled water varies considerably with brand or source, with packaging date for a given brand, and the information given on the lables or provided by manufacturer [5,6].

Food and beverages

A part from drinking water direct or indirect consumption, the most important foods in terms of potential contribution to individual fluoride exposure commercial beverages such as juice and soft drinks, grapes and grape products prepared with fluoridated water [6].

Hossny, reported that fluoride concentration in mother's milk ranging from 0.002 to 0.01 mg/L, and others reported that the fluoride level in breast milk of mothers who regularly consumed drinking water with low levels of fluoride was between 0.017 and 0.006 ppm. Infant formula contains an average of 0.24 ppm, while the estimated infant fluoride intakes ranged from 0.0039 mg/kg/day for a 6-12 month old infant [7,8].

Leaf tea including black and green tea contain high levels of fluoride, 0.95-1.41 mg/L in black tea sticks, 0.7-2.44 mg/L in black tea granules and 1.15-6.01 mg/L in black tea bags. Therefore, fluoride excess should be considered in all patients with a history of excessive tea consumption [9].

However, fluoride concentration in various food categories were reported, ranging up to 2.1 mg/Kg for soup, and 1.15 mg/Kg for beverages; the highest single items were cooked veal 1.2 mg/Kg, canned fish 4.6 mg/Kg. Shellfish 3.4 mg/Kg, cooked wheat cereal 1 mg/Kg, and tea 5 mg/Kg, while in fruits and vegetables normally have low fluoride concentration ranging between 0.1 to 0.4 mg/Kg [10].

Recently a huge amount of fluoride in coal has been released into indoor environments by the combustion of coal and fluoride pollution. Combustion of coal and coal bricks is the primary source of gaseous and aerosol fluoride and these forms of fluoride can easily enter exposed food products such as corn, chilies and potatoes. Adding to that the contaminated food can reach human through grazing sheep [11].

Dental products

The multiple use of fluoride for dental caries prevention is clearly increasing. It is a common practice to use fluoride in a variety of delivery systems, including dentifrices, pediatric supplements, and professional or self-applied topical solutions or gels as well as dental restorative materials. These dental products may contain fluoride in concentrations as high as 12,300 ppm. Fluoridated dentifrice include toothpaste, powders, liquids, gels, mouth rinses, NaF tablets, drops, and lozenges, although the amount of fluoride actually swallowed by an individual depends on the amount of toothpaste used, the swallowing control of the person, particularly young children and the frequency of toothpaste used. It is estimated that the amount of fluoride ingested by children aged between 3-12 years range between 14-269 ppm. [12,13]

Absorption, Distribution and Excretion of Fluoride

Upon oral administration of NaF, the ingested fluoride is absorbed rapidly and extensively from gastrointestinal tract. The majority of fluoride is absorbed in duodenum and jejunum. The rate of gastric absorption is inversely related to the pH and gastric contents. In an acidic stomach, fluoride is converted to hydrogen fluoride and overall absorption is reduced by calcium and certain other cations and by elevated plasma fluoride level [14].

In contrast, MFP is not absorbed in stomach, does not react with hydrochloric acid, and is hydrolysed by alkaline phosphatase to release free fluoride ions for rapid absorption in the duodenum. Therefore, hyper fluoremia can be seen in chronic intestinal failure due high fluoride intake in beverages that ingested to compensate for stool losses. Two types of fluorine are found in human plasma: inorganic and organic, remarkably, the amount of organic fluorides in serum is generally greater than the amount of inorganic fluorides. However, fluoride removal from plasma occurs by calcified tissue uptake and urinary excretion. Approximately 99% of the body burden of fluoride retained in bone, calcified cartilage and teeth, where the fluoride ion is exchanged for hydroxyl groups in the hydroxyapatite molecule to form fluoroapatite [14,15].

The calcified clearance of fluoride from plasma in children is higher than in adults due to the greater surface area of the loosely organized crystallites in the developing calcified tissue during growth. Excretion of fluoride is mainly via urine, fluoride not absorbed by GIT is found in feces. Renal clearance of fluoride depends on pH and glomerular filtration rate. At low pH, more HF is formed, promoting reabsorption of fluoride. Fluoride is infiltrated freely in the kidney and the amount of renal fluoride clearance depends on glomerular infiltration retained blood fluoride concentration. Therefore, urinary excretion rates of fluoride may give more correct picture of fluoride exposure than fluoride concentrations in urine and valuable predictor of bone mineral content response during fluoride therapy for osteoporosis [16].

Acute and chronic Toxicity

Fluoride is an essential trace element that has protective effects against bone mineral loss. However, it becomes toxic at higher doses and induce some adverse effects on a number of physiological functions. Symptoms of acute oral fluoride intoxication in humans include sever nausea, vomiting, hyper salivation, abdominal pain, and diarrhea. In severe or fatal cases, these symptoms are followed by systemic hypocalcaemia, convulsions, cardiac arrthymias, cardiovascular collapse and coma. Acute toxic doses range from 1 to 5 mg/Kg and doses exceeding 15 to 30 mg/Kg may be fatal [17].

The mechanism of toxicity involves the combination of the fluoride anion with the calcium ions in the blood to form insoluble calcium fluoride, resulting in hypocalcaemia; calcium is indispensable for the function of nervous system, and the condition can be fatal. Treatment may involve oral administration of dilute calcium hydroxide or calcium chloride to prevent further absorption, and injection of calcium gluconate to increase the calcium level in the blood [18].

Chronic exposure to excessive fluoride is known to cause dental fluorosis and skeletal fluorosis in humans. Other effects, including hypersensitivity reactions, renal insufficiency, osteo-renal syndrome, repetitive strain injury, birth defects and neurological manifestation have also been reported.Chronic exposure to fluoride also reported to cause haematological effects such as anemia, eosinophilia, and dysplastic changes on granulocytes in the bone marrow as well as acquired osteosclerosis, gastrointestinal symptoms, weight loss, lower extremity pain, and sress fractures of the lower extremities [19].

Dental Fluorosis

Several epidemiological studies clearly demonstrated the relationship between dental fluorosis (mottling of enamel) in humans and the level of fluoride in drinking water. Dental fluorosis is a reflection of fluoride exposure prior to eruption of teeth due to increase fluoride concentration in the extar cellular fluid surrounding the tooth during its development, while the degree of fluorosis is dependent on the total fluoride dose, time and duration to fluoride exposure. However, concentrations of fluoride in drinking water of about 1 ppm are associated with a lower incidence of dental fluorosis, particularly in children, whereas excess intake of fluoride in fluoridated water or prolonged use of fluoride supplements, such as fluoride tablets, early use of fluoride tooth paste, another dietary fluoride supplements and prolonged use of infant formula can result in dental fluorosis [13-20].

The prevalence and severity of dental fluorosis increase with increasing fluoride concentration in drinking water. The prevalence of objectionable fluorosis is less than 1% when the level of fluoride in drinking is below 0.3 ppm and approximately 1 to 2% at the level of 1 ppm. Sever dental fluorosis is only consistently observed at level exceeding 2.5 ppm [20].

At the individual level, another factors such as body weight, nutritional factors, genetic factors, rate of skeletal growth and remodeling are also important, while blood group O (ABO) phenotypes appeared to be a marker of resistance to fluoride exposure. Other dental defects of excessive fluoride intake including fibrosis of the pulp, alveolar osseous metaplasia and delay the eruption of permanent teeth [21,22].

Skeletal Fluorosis

Skeletal fluorosis is a chronic metabolic bone and joint disease caused by chronic exposure to high doses of fluoride either through water or rarely from foods of endemic areas. Skeletal fluorosis has several stages: two preclinical symptomatic stages characterized by slight radio graphically detectable increases in bone mass; early symptomatic stage characterized by sporadic pain and stiffness of joints and osteosclerosis of the pelvis and vertebral column; a second clinical phase associated with chronic joint pain and arthritic symptoms and slight calcification of the ligaments. These features may mimic the diagnosis of seronegative arthritis [23].

Crippling skeletal fluorosis characterized by marked limitation of the joint movements, considerable calcification of ligaments, crippling deformaties of the spine and major joints, muscle wasting and neurological defects associated with compressing of the spinal cord. Endemic skeletal fluorosis has been reported predominantly in tropical countries with fluoride concentrations of more than 4 ppm in drinking water [23].

Effects on cellular enzymatic activities

The toxic action of fluoride resides in the fact that fluoride ions acts as enzymatic poisons, inhibiting or increase enzyme activities and ultimately interrupting metabolic process such as glycolysis and protein synthesis. Fluoride inhibits acid phosphatase activity in osteoblastic cells, induces an increases in tyrosin phosphorylated protein, and activate G protein leading to cell proliferation [18-24].

However, the effect of fluoride on osteoblasts have been related mainly to its ability to evoke the activation of G-proteins and the inhibition of phosphotyrosine phosphatases, leading to an intracellular increase of tyrosine phosphorylation and activation of the mitogenic-activated protein kinase pathway, leading to osteoblastic proliferation [25].

Recently, concluded that fluoride causes relase of lactate dehydrogenase in the extra-cellular medium of human osteoblasts (index of cytotoxicity), accumulation of intracellular malonyldialdehyde (index of lipoperioxidation) and the increase in the glutathione consumption. Furthermore, fluoride inhibited the pentose phosphate oxidative pathway and the glucose-6-phosphate dehydrogenase activity in particular, through the oxidative inhibition of glucose-6-phosphate dehydrogenase. These changes cause oxidative damage to the osteoblasts that provide a new mechanism to explain fluoride ions toxicity [26].

Fluoride also increases activity of peroxidases and catalases due to inhibition of androgenesis regulator enzymes which may cause adverse effects on testicular activity and increases level of alkaline phosphatase, osteocalcin and serum calcium which are both considered as a markers for osteoblastic activity. Fluoride decreased super oxidase (SOD), glutathione peroxidase (GSH-PX). Although fluoride directly interfered with the synthesis of carbonic anhydrase by the enamel forming cells, rather than being directly involved in the crystal formation [9-17, 26].

Anabolic action of fluoride

The rate of bone formation is controlled, for the most part of skeleton, by the rate of bone resorption, as osteoblastic bone formation increases or decreases, there is a compensatory increase or decrease in the rate of osteoclastic bone resorption. This relationship between osteoblast & osteoclast which is responsible for the maintaining of steady-state skeletal mass in adults [27].

Osteoblasts arise from osteoprogenitor cells located in the periosteum and the bone marrow which express the master regulatory transcription factor Cbfa1/Runx2. Osteoprogenitors are induced to differentiate under the influence of growth factors, in particular the bone morphogenetic proteins (BMPs), fibroblast growth factor (TGF), platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-beta) may promote the division of osteoprogenators and potentially increase osteogenesis. Once osteoprogenators starts to differentiate into osteoblasts, they begin to express a range of genetic markers including Osterix, CO11, BSP, M-CSF, ALP, osteocalcin, osteopontin, and osteonectin. The principal products of the mature osteoblast are type I collagen (90% of protein in bone), the bone specific vitamin-K dependent proteins, osteocalcin and matrix Gla protein, the phosphorylated glycoproteins including bone sialoproteins I & II, osteopontin and osteonectin, proteoglycans and alkaline phosphatase [28].

The cell responsible for bone matrix resorption is the osteoclast, a large motile, multinucleated cell located on bone surfaces tightly associated with the calcified matrix. There is much evidence supporting the view that osteoclasts are formed by fusion of mononuclear cells derived from haematopoietic stem cells in bone marrow [28]. Osteoclasts actively synthesized lysosomal enzymes, in particular the tartarte resistant isoenzyme of acid phosphatase and cysteine-proteinases such as the cathepsins that are capable of degrading collagen. Systemic agents, important in regulating osteoclastic bone resorption, are parathyroid hormone, 1, 25, di-hydroxy vitamin D3 and calcitonin [29].

However, the demonstration by Farley, that fluoride stimulate the proliferation of primary chicken bone cells has provided the first conclusive evidence that fluoride acts directly on bone cells to exert its anabolic action, with the optimal mitogenic dose of 10 micro-M levels , similar to the effective serum fluoride levels (i.e, basal level at 5-10 micro-M and peck level at 30 micro-M) [30]. Subsequently, the anabolic action of fluoride on osteoblasts is confirmed by a number of laboratories on bone cells of various species including humans [31].

However, fluoride interacts with mineralized tissues in number of ways, at low doses, the fluoride may be passively incorporated into mineral, stabilizing it against dissolution. This one of the mechanisms by which fluoride reduces the incidence of dental caries, but at higher doses, the fluoride may alter the amount and structure of the tissue. Investigators conclude that fluoride exposure increased

the birth rate of new osteoblasts, but at high concentrations there was an independent toxic effect on the cells that blocked full manifestation for the increase in skeletal mass [32].

Moreover, the target bone cells for fluoride action at anabolic doses whereby the fluoride stimulates osteogenic cell proliferation are not clearly established, some studies suggest that osteoblast precursors are more sensitive to fluoride action than mature osteoblasts. The effect of fluoride on osteoblasts number and activity in patients with skeletal fluorosis showed increase in the production of osteoblasts with concomitant increase in a toxic effect of fluoride at the cell level which led to increase in bone volume, an increase in width of cortical plate and an increase in porosity [33,34].

However, fluoride, at mitogenic doses, acts directly on bone cells to stimulate several osteoblastic activities, i.e, synthesis of alkaline phosphatase, collagen. Fluoride at mitogenic dose also increases transient calcium uptake and sodium-dependent phosphate transport in isolated bone cells in vitro. Kleinsasser & Qu showed that fluoride stimulate osteoblast proliferation in a biphasic fashion with the optimal mitogenic concentration being 10 mumol/L (35-37).

This finding suggests that multiple pathways might be activated. It is possible that low, sub toxic doses do stimulate proliferation, but at higher doses other pathways responsible for decreasing proliferation or increasing apoptosis might become activated. This thinking suggested that fluoride might have multiple effects on osteoblasts characterized by the following:

1. The mitogenic dose of fluoride that needed to stimulate bone cell proliferation is low (10-100 mumol/L).
2. Studies *in vivo* revealed that the osteogenic action of fluoride is skeletal tissue specific and the mitogenic action of fluoride is also specific for cells of skeletal origin.
3. The in vitro bone cell mitogenic action of fluoride requires the presence of the bone cell growth factor, such as insulin-like growth factor (IGF)-I or transforming growth factor TGF beta.
4. Fluoride potentiates the mitogenic actions of bone cell growth factors, such as IGF-I both *in vivo* and *vitro*.
5. The *in vitro* bone cell mitogenic action of fluoride is sensitive to changes in the concentrations of inorganic phosphate in culture medium.
6. Fluorides act primarily on osteoprogenator cells and/or undifferentiated osteoblasts rather than the highly differntiated mature osteoblasts.
7. The bone cell mitogenic action of fluoride is associated with increases in tyrosine phosphorylation status of several cellular proteins, including MAP kinase MAPK (35-39).

Lau have proposed that the molecular mechanism for the mitogenic action of fluoride on bone cells, involves the stimulation of the MAP kinase (MAPK) mitogenic signal transduction pathway through inhibition of a fluoride–sensitive phosphotyrosine phosphatase (PTP) [37].

Kawase and Reed have postulated that fluoride at milli molar concentrations stimulates the proliferation of L-929 fibroblasts by activating protein kinase C (protein serine kinase) through a heterotrimeric GTP-binding protein (G protein). Although, the bone cell mitogenic activity of fluoride may be mediated by enhancing the cell sensitivity to TGF-Beta (a bone cell growth factor) [39].

Lau & Bay link, proposed a two competing models of the osteo genic action of fluoride, one involves the inhibition of an osteoblastic fluoride sensitive phosphotyrosine phosphatase (PTP) and the other involves the G1/0 protein mediated activation of protein tyrosine kinases (PTKs). In this review at the cellular level, loss of normal function of oncogene products consistent with a role in the control of cellular proliferation and differentiation in the process known as signal transduction. Signal transduction is a complex multistep pathway from the cell membrane, through the cytoplasm to the nucleus, involving a variety of types of proto-oncogene product involved in positive and negative feedback loops necessary for accurate cell proliferation and differentiation [40].

The MAPK signaling pathway has at least four tyrosine phosphorylation regulatory points. The growth factor receptor, ras GAP, Raf, and MAPK in which dephosphorylation of one or more of these signaling proteins may be mediated by fluoride sensitive PTPs and that fluoride inhibits the activity of one or more fluoride sensitive PTPs in osteoblasts causing an inhibition of tyrosine dephosphorylation of one or more of these four signaling proteins of the MAPK pathway. As result, the overall of tyrosine phosphorylation level of these signaling proteins rises which leads to prolongation of the mitogenic signal initiated by a bone cell growth factor, resulting in the enhancement of the growth factor mediated stimulation of proliferation and/or differentiation of osteoblasts, which supported by evidence of [41].

This model is tenable and attractive because it accounts for a six characteristics of the bone cell mitogenic activity of fluorides.

1. The inhibitory doses of fluoride for the fluoride sensitive PTP in osteoblasts are in the same low micromolar dose range that stimulate bone cell proliferation and bone formation *in vitro* and *in vivo*.
2. The fluoride sensitive PTP is unique to cells of skeletal origin is compatible with the observations of the skeletal specificity of the mitogenic action of fluoride.
3. The fluoride–dependent inhibition of dephosphorylation of cellular phosphotyrosine proteins can increase their overall tyrosine phosphorylation level, it is effective only when the basal level of phosphorylation has been increased in response to activation of protein tyrosine kinase (PTK). Thus, the optimal mitogenic action of fluoride would require the presence of bone cell growth factor to increase the basal tyrosine phosphorylation of cellular proteins.
4. Because the mitogenic actions of bone cell growth factors, such as IGF-1, are mediated by direct activation of the PTK activity of their corresponding receptor and because the mitogenic actions of fluoride are presumed to be mediated by an inhibition of phosphotyrosine dephophorylation, it follows that fluoride should interact with PTK-activating growth factor to promote bone cell proliferation and bone formation.
5. Fluoride can act, in coordination with divalent cations, as a transition state analogue of inorganic phosphate which is a potent inhibitor of most, if not all, PTPs.
6. The osteoprogenitor cells and less differentiated bone cells produce more growth factors and contain more of this fluoride sensitive PTP 32 than the more differentiated osteoblasts. Therefore the osteoproginator cells are the preferred target cells for fluoride.

Genotoxicity and Carcinogenicity of Fluoride

Fluoride's ability to cause genetic damage is considered an important indicator of cancer-causing potential. Many studies have investigated and found positive evidence of fluoride's genotoxicity. Notable among these is the study of Mihashi and Tsutsui who reported that sodium fluoride was mutagenic to rat cortical bone, the same tissue in which osteosarcoma forms [42].

Fluoride may be genotoxic, but when the concentration of fluoride in the water is 1 ppm, it is generally considered to be safe. Therefore, levels of fluoride present in a number of widely used dental products, such as fluoride-containing toothpaste appear to be potentially mutagenic. Since fluoride is increasingly being used as a drug and contamination of the total environment by fluoride emission and solid wastes from industry is a growing problem [43].

Fluoride lacked mutagenic activity in microbial systems, (Ames test, Saccharomyces cerevisiae and rat hepatocytes, but produce mutations in mouse lymphoma cells at cytotoxic concentrations. Chromosomal aberrations were not increased in bone marrow or testicular cells of mice exposed to drinking water containing either 50 mg/l fluoride for five generations or 100 mg/l fluoride for 6 weeks. However, increased aberrations (breaks or gaps) have been reported in vitro [44-46].

Increased aberrations sister chromatid exchange activity was not observed in a variety of *in vivo* and *in vitro* cell system. Negative results have also been reported in the mouse bone marrow micronucleus test. Fluoride was noted both to increase chromosomal aberrations and to have no effect on unscheduled DNA synthesis in cultured human and rodent cells. Chromosomal alterations can include changes in chromosome number and aberrations of the chromosomes before DNA synthesis or chromatids (after DNA synthesis), chromatid gaps and micronuclei which are DNA-containing bodies derived from chromosomal material that is left behind during mitosis[46-48].

Many studies have demonstrated that fluoride is genotoxic to mammalian cells, eliciting chromosomal aberration and genetic mutations. Fluoride induced primarily chromatid gaps and chromatid breaks, indicating that the rodent cells are responsive in G2 stage of the cell cycle. In contrast, studies with synchronized human cells indicated that S phase was the most sensitive [49,50]. Although, another recent study demonstrated a higher level of fluoride in the serum from osteosarcoma patients [51].

However, if fluoride does have a cell cycle-specific effect, it could be expected that differences in the cell treatment and harvest protocols could lead to conflicting results for the induction of chromosome aberration. Gene mutations were produced in cultured rodent and human cells in the majority of studies. Unfortunately, a number of the *in vitro* and *in vivo* cytogenetic studies are of questionable utility because of the protocols used, the quality of the responses reported, or the interpretations of the data.

The conflicting results in the *in vivo* cytogenetic studies are difficult to reconcile. There are reports of increased chromosomal aberrations in rat bone marrow and testes, but other studies, using similar protocols and dose ranges, have reported no induced chromosome damage.

Notable among studies of fluoride genotoxicity is study of Mihashi & Tsutsui [42] who reported that sodium fluoride was genotoxic to rat cortical bone, the same tissue in which osteosarcoma forms. Importantly, ape and human cells have shown greater susceptibility to fluoride's mutagenic effects than rodent cells. These findings suggest that humans may be more susceptible to fluoride's genotoxic properties, and subsequently more susceptible to a potential carcinogenic effect [52].

However, in the majority of studies that were reported to be positive, there were high background frequencies, or the investigators reported categories of nuclear or chromosome damage that are difficult to interpret. Interestingly, many of the positive results were obtained when anaphase cells were scored, whereas similar treatment protocols in other laboratories yielded negative results when metaphase cells were the only cell type examined. It is difficult, without additional data, to determine the reasons for finding chromosome breaks in anaphase, but not in metaphase cells.

Since fluoride acts as an anabolic agent for bone cells and that its osteogenic properties are mediated primarily by an increase in osteoblastic proliferation, as well as most of the fluoride is deposited in actively mineralizing areas of bone, cartilage, and the mitogenic action of fluoride and genotoxicity action. Therefore, it is biologically plausible that widespread exposure to fluoride during critical periods of growth may play a role in the aetiology of OS, but still there are conflicting data regarding the association between fluoride exposure and the incidence of osteosarcoma [53]. Several animal studies have been coducted to found an evidence that fluoride exposure may increase osteosarcoma formation [53-55], while other studies have found no association or inconclusive results including between fluoride and osteosarcoma [56-58].

It is concluded that if certain assumption is made for daily fluoride intakes from the three main biggest contributors of fluoride (food, water and dental products), a specific values may be estimated to determine the range of total fluoride consumption by an individual. Therefore, it is essential to put in mind the possible health risks associated with fluoride exposure. In hot climate countries such as Libya where the main fluoride level in drinking water is high 1.32 ppm [59], it would advisable and recommended to place a national drinking water standard of WHO limit of 0.6 to 0.7 ppm.

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