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Detection and Effect of Fluoride Level in the Neoplastic Tissue of Conventional Osteosarcoma Sub-Variants

Abdullatef Nureddin[1@], Mohammed Ayoop[2] and Adel Abdulazem[2]

[1]Department of Oral Surgery, Oral Medicine and Oral Pathology, Faculty of Dentistry, University of Tripoli - Libya.
[2] Department of Oral Pathology, Faculty of Dentistry-Ain Shams University, Cairo- Egypt.

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ABSTRACT

Conventional osteosarcoma is a primary intramedullary high grade malignant tumour in which osteoid is synthesized by malignant cells even in small amount. WHO sub-classified conventional osteosarcomas into three sub-variants osteoblastic, chondroblastic and fibroblastic depends on the neoplastic tissue produced by the malignant osteoblasts. However, fluoride level in drinking water shows great variation between the costal-western region of Libya (mean fluoride level 1.38ppm) and Cairo-Egypt (fluoride level 0.4ppm). Therefore, the aim of this study is to detect analysis and compare the fluoride level in the neoplastic tissue of osteosarcomas from fluoridated community (Libyan cases) and non-fluoridated community (Egyptian cases) as a case-control retrospective study to evaluate the effect of fluoride on the malignant osteoblasts. 20 cases of osteosarcoma from each community in a formalin-fixed paraffin embedded specimens and serial sections from the paraffin blocks for detection of fluoride level in tumor tissue were included in this study.

The fluoride detection was determined using a combined Fluoride Ion Selective Electrode according to the Orion method for fluoride detection after calibration with fluoride standards. The result of this study showed that in Libyan cases, the mean of fluoride level in tumor tissue was 0.93 ppm with std. deviation 0.11 and histopathologically, 94% of osteosarcomas were ostoblastic, 6% chondroblastic and no fibroblastic variant was seen. In contrast to Egyptian cases, the mean of fluoride level in tumor tissue was 0.0095 ppm with std. deviation 0.0070 and histopathologically, 61% of cases were osteoblastic, 22% chondroblastic and 17% fibroblastic. Analysis of variance used as a pair-wise test performed at the significance showed *P* value = 0.001 between two results. It is concluded from this study that fluoride level and the osteoblastic variants of osteosarcoma are significantly higher in areas where fluoride concentration in drinking water is higher than the normal limit.

Keywords - *Osteosarcoma;* Osteoid *osteoblastic; Chondroblastic; Fibroblastic; Fluoridated; Ion-Selective Electrode.*

INTRODUCTION

Osteosarcoma is the most common primary malignant bone tumor, by definition, an osteosarcoma is a neoplasm in which osteoid is synthesized by malignant cells even in small amount.[1] Conventional osteosarcoma accounts for 90 % of all osteosarcomas. It is usually begins in the medullary canal and then penetrates the cortex and invades the adjacent soft tissues.[2]

WHO classified osteosarcoma into many sub-variants that differ in the histopathological features depends on the tissue produced by malignant cells into (osteoblastic, chondroblastic and fibroblastic).[3]

Osteosarcoma, usually affects children and young adults with estimated incidence of 4-5 per million population, and appears to be there is no significant association with ethnic group or race, moreover, the neoplasm is extremely rare in young children. It most frequently occurs in the second decade of life with some 60% of patients under the age of 25 years, although 30% of osteosarcomas occur in patients over 40 years of age.[4] Although, many osteosarcomas have predisposing factors such as Paget's disease of bone, prior radiation therapy, osteoblastoma, osteochondroma, fibrous dysplasia, osteomyelitis, endoprosthesis implantation, Rothmund-Thomson syndrome and Li-Fraumeni syndrome, which all have been linked with osteosarcoma, but the precise aetiology remains unknown.[5-9]

Osteosarcoma affects males more frequently than females in a ratio of 3:2 especially in patients under the age of 20 years.[10] Pain with or without a palpable mass, tenderness, or swelling rarely occurring for more than a few months, are the usual presenting symptoms.

Pain is usually deep, boring and severe; other findings may include limitation of normal function, oedema, localized warmth, tellangectasias and pathological fracture.[11,12]

In the jaws, osteosarcoma is slightly more common in the mandibular than maxilla. The tumours presents as rapidly enlarging swelling that may be accompanied by pain, numbness of the lip, trismus, and displacement and loosening of teeth. Nasal obstruction and symptoms

@ Author to whom correspondence should be addressed Dr. Abdullatef Nureddin, Tel.: +218914319554 ; Email: nureddinabdullatef@yahoo.com

referable to the eye may also be features of maxillary tumours.[13,14]

Radiographically, the lesion may be purely osteoblastic, osteolytic or mixed lytic/blastic lesion. The lesion may appear as a poorly circumscribed medullary radiolucencey with mottled areas of radiodense and cortical bone destruction with a focally mineralized soft tissue mass. When the cortical plates are perforated, the periosteum is raised and the tumour may extend into the surrounding soft tissue to produce the classical sun-ray appearance.[15,16]

Gross examination of osteosarcomas is often showing a large tumor, usually over 5 cm, fleshy or hard in consistency which may contain osteoid, fibrous tissue or cartilage. It frequently perforated the cortex and associated with a soft tissue mass. Some osteoblastic variety may appear grey-tan or pumice-like in colour, while others become, sclerotic and more yellow-white. Chondroblastic variety tends to be white to tan in colour, and variably calcified with a fish-flesh or rope-like cut surface.[17]

Osteosarcoma is immunoreactive with antibodies to smooth muscle acting and may be immunoreactive for cytokeratin. Osteosarcoma, usually has a diffuse moderate to strong intra-cytoplasmic staining for CD99. Osteocalcin and osteonectin have sometimes been used to highlight osteoid.[18]

Cytogenetic studies of osteosarcomas, showed that the most, if not all, osteosarcomas have clonal chromosomal aberration either numerical or structural. Multiple clones are common and diploid ploidy pattern by DNA cytofluorometry has been reported to be a poor prognostic sign.[19]

Although, no specific translocation or other diagnostic structural aberration has been assigned to conventional osteosarcoma. However, there is recurrent involvement of certain chromosomal regions such as 1p11-13, 1q11-12, 1q21-22, 11p14-15, 14p11-13, 15p11-13, 17p and 19q13 are most frequently affected by chromosomal structural aberration and the most common imbalances are +1, -6q, -9, -10, -13 and -17 as well as cytogenetic manifestations of gene amplification are frequently seen.[20,21]

Moreover, chromosome arms 3q, 13q, 17p, and 18q are most frequently involved in loss of heterozygosity (LOH), as the LOH incidence is high at 3q26.6-26.3. Some patients may be genetically predisposed to develop osteosarcoma. For example, patients who have hereditary retinoblastoma have a several hundred-folds increase in the incidence of osteosarcoma.[22]

However, it seems that there are a conflicting data regarding the association between fluoride exposure and the incidence of bone tumours in mankind as well as in animals. Several studies have been pointed out the possible carcinogenic action of fluorides in bone, kidney, bladder, oral mucosa, pharyngeal, white blood cells and uterine.

One tumours presented as the greatest plausibility as a potential tumour target due to deposition of fluorides within the bone as well as the mitogenic effect of fluorides on osteoblasts, therefore it is biological plausibility that exposure to fluorides during skeletal growth may associate with subsequent development of osteosarcoma or fluoride may either increase or modify the incidence rate of osteosarcoma.[23]

MATERIALS AND METHODS

I. *Collection of cases*

Fourthy cases of a formalin-fixed, paraffin-embeded specimens of osteosarcomas were included in this study.

Twenty cases of osteosarcomas collected from the costal-western region of Libyan territories. The histopathological reports, slides, paraffin blocks and clinical data were retrieved from archives of Pathology Department of Sabratah Tumor Institute, Tripoli Central Hospital and Tripoli Medical Center.

All the cases have been diagnosed between the years 1998 and 2008 respectively. The cases that have not mentioned histopathologically the type of neoplastic tissue produced by tumor cells (osteosarcoma sub-variant) were excluded from this study.

The samples of osteosarcomas collected from this region regarded as cases because the mean of fluoride concentration in public drinking water is about 1.38 ppm. It is about two times above the normal limit of 0.6-0.7 ppm that recommended by WHO while osteosarcomas that collected from Cairo, where the fluoride concentration in public drinking water (Nile River) is lower than the normal limit (mean 0.4 ppm) of that recommended by WHO, regarded as controls.

However, on the other hand, twenty cases of osteosarcomas were collected from Pathology Departments of Cairo Hospitals (Ain Shams Hospital and National Cancer Institute). All the cases (controls) have been diagnosed as osteosarcoms between years 2006 and 2009. Any cases that histopathologically have not mentioned the sub-variant were excluded from this study.

In order to confirm the diagnosis of all the cases and determine the histological variant of the cases included in this study. Two slides from each osteosarcoma were re-stained with haematoxyline and eosin and re-examined by two pathologists from Ain Shams University.

All the cases from both communities were further subdivided reclassified and tabulated according to the histopathological sub-variant (osteoblastic, chondroblastic or fibroblastic variants).

II. *Detection of fluoride level in tumor tissues*

Fluoride detection in tissue tumor samples was performed in the Micro-analysis Center, Faculty of Science, Cairo University.

Serial sections from each paraffin block were taken as a sample for fluoride detection in the tissue. Each sample was kept in clean-dry container. Equal weight of all the samples was adjusted using electronic balance-model ESJ-200, capacity 205g, min 0.0001g, S/N EM 4436.

The fluoride detection was determined using a combined fluoride Ion Selective Electrode (Orion Series-Orion Model 92-01 and an Orion Model 407 A Meter) according to the Orion method for fluoride detection after calibration with fluoride standards.[24]

III. *Statistical assessment*

Data was collected and tabulated using Microsoft Excel 2003 and then statistically analyzed using SPSS version 15. Analysis of variance (ANOVA) will be performed and when it yielded a significant result, post-hoc Bonferroni test will be used as a pair-wise test. All statistical analyses will be performed at the significance.

RESULTS

A. *Histopathological findings*

All osteosarcomas cases and controls from both communities included in this study are tabulated according to the community, diagnosis and the histopathological sub-variant (Tables 1 and 2).

In Libyan cases the histological variants of osteosarcomas present only in 16 cases out of total number 20 cases. It is interested that in this region where the level of fluoride in public drinking water is above the normal limit,15 out of 16 of osteosarcomas have a histopathological sub-variant

Table 1: Osteosarcoma cases from Libya and Egypt with the histopathological sub-variants.

No. of Libyan cases	Diagnosis	Histopathological variant	No. of Egyptian cases	Diagnosis	Histopathological variant
1	Osteosarcoma	-	1	Osteosarcoma	Fibroblastic
2	Osteosarcoma	Osteoblastic	2	Osteosarcoma	-
3	Osteosarcoma	Osteoblastic	3	Osteosarcoma	Osteoblastic
4	Osteosarcoma	-	4	Osteosarcoma	Osteoblastic
5	Osteosarcoma	Osteoblastic	5	Osteosarcoma	-
6	Osteosarcoma	-	6	Osteosarcoma	Osteoblastic
7	Osteosarcoma	Osteoblastic	7	Osteosarcoma	Osteoblastic
8	Osteosarcoma	Osteoblastic	8	Osteosarcoma	Chondroblastic
9	Osteosarcoma	Osteoblastic	9	Osteosarcoma	Osteoblastic
10	Osteosarcoma	Osteoblastic	10	Osteosarcoma	Chondroblastic
11	Osteosarcoma	Osteoblastic	11	Osteosarcoma	Osteoblastic
12	Osteosarcoma	Osteoblastic	12	Osteosarcoma	Fibroblastic
13	Osteosarcoma	Chondroblastic	13	Osteosarcoma	Fibroblastic
14	Osteosarcoma	-	14	Osteosarcoma	Osteoblastic
15	Osteosarcoma	Osteoblastic	15	Osteosarcoma	Osteoblastic
16	Osteosarcoma	Osteoblastic	16	Osteosarcoma	Osteoblastic
17	Osteosarcoma	Osteoblastic	17	Osteosarcoma	Osteoblastic
18	Osteosarcoma	Osteoblastic	18	Osteosarcoma	Osteoblastic
19	Osteosarcoma	Osteoblastic	19	Osteosarcoma	Chondroblastic
20	Osteosarcoma	Osteoblastic	20	Osteosarcoma	Chondroblastic
Total 20 cases			Total 20 cases		

Table 2: Osteosarcomas variants

Osteosarcoma subvariant	Osteoblastic	Chondroblastic	Fibroblastic	Total
Libyan cases	15	1	-	16
Egyptian cases	11	4	3	18

Table 3: Fluoride level in tumor tissue

	Total number	F-range in tissue ppm	Mean Ppm	Std. Deviation	Std. Error Mean
Libyan cases	20	0.002-0.33	0.93	0.11	0.02
Egyptian cases	20	0.0015-0.015	0.0095	0.0070	0
	40				

of osteoblastic type, which represent of about 94% , while about 6% of cases are of chondroblastic type and no fibroblastic type determined in Libyan cases. Osteoblastic variant (production of osteoid by tumor neoplastic cells predominate the histopathological sub-types.

In Egyptian cases two cases out of 20 were excluded from this study. However, In contrast to the Libyan cases where the fluoride level in public drinking water below the normal limit of that recommended by WHO, eleven cases are histologically osteoblastic which represent about (61%), four cases chondroblastic (22%) and three cases (17%) fibroblastic.

B. *Evaluation of fluoride in tumor tissues*

A summary of the results of fluoride level in all the cases of tissue tumors (Table 3).

As shown in Table 3. Fluoride level in tumor tissue in Libyan cases rage between (0.002 – 0.33 ppm) with mean of 0.93 ppm, standard deviation 0.11 while in Egyptian cases the fluoride level in tumor tissue range between (0.0015 – 0.015 ppm). With a mean of 0.0095 ppm and standard. Deviation 0.0070. It is observed in this study that, it is a significantly clear the fluoride level in tumor tissue in Libyan cases are higher than that observed in the Egyptian cases. When comparing the means, Std. deviation of fluoride level in the matrix of the neoplastic tissue of osteosarcomas of Libyan cases versus Egyptian cases, statistical analysis revealed a statistically significance difference (*P* value = 0.001).

DISCUSSION

Approximately 99% of ingested fluoride is deposited in the skeleton with about 50% of the daily ingested fluoride being deposited directly into bone and fluoride appears to have the potential to initiate or promote cancers, particularly of the bone, but the evidence to date is tentative and mixed. Biologically, the link between fluorides in tap water and bone cancer in childhood period is highly plausible.[25]

This study is conducted in two different countries, where there is a different fluoride concentration in drinking water and designed to measure and compare the fluoride level in the malignant soft tissue osteoid that is the main product of osteosarcomas.

The Paraffin blocks of osteosarcomas cases were collected from the costal-western districts of Libya as the fluoride level in natural drinking water is higher than the limits that recommended by WHO of 0.6-0.7ppm for hot climate countries.[26] The other cases of osteosarcoma as a controls were collected from Cairo-Egypt, where the fluoride level in tape water is lower than of that recommended by WHO.

However, there is no study has conducted in this aspect before, therefore, this study may be considered as the first leading research to find out the percent of fluoride uptake and the effect of fluoride on the proliferation of malignant cells as fluoride is confirmed a mitogen agent in humans that increases the proliferation of osteoblasts.[23, 27]

It is estimated that the fluoride level was significantly higher in Libyan cases than those of Egypt which reflected a higher consumption of fluoride in drinking water. The range was between 0.002-0.33 ppm with a mean 0.93 ppm and standard deviation 0.1, while in Egyptian cases fluoride level range between 0.0015-0.015 ppm with a mean 0.0095 and standard deviation 0.0070.

It is also more interestingly in this study that our analysis showed the histological variation or subtypes of osteosarcoma depending on the type of extra-cellular matrix produced by tumor tissue.[3] in relation to the fluoride level in drinking water and in tumor tissue.

Libyan cases showed significantly a higher proportion of osteoblastic variant 94% comparing to those of Egypt 61%. This finding may suggest that fluoride may promote osteoblasts to produce osteoid rather than chondroid or fibroblastic tissue in malignat osteoblasts, since the fluoride is well documented that it increases proliferaion of osteoblasts.[28]

However, there is no study conducted in this aspect before, a very recent study published case-control study lends evidence that fluoride exposure may cause osteosarcoma and other types of bone cancer[29], who found that the average serum fluoride level of osteosarcoma cases was twice as higher as the bone cancer cases and 3.5 times as higher than in the controls.

Furthermore, several studies have demonstrated fluoride to be genotoxic to mammalian cells that it induces DNA breaks and chromosomal aberration in bone -forming cells where osteosarcoma arises.[30- 32]

Two ecological studies that found an association in age groups less than twenty years of age.[33,34] Another study conducted in New Jersey also demonstrated an increase in osteosarcoma incidence rates but for mlaes less than 20 years of age who lived in fluoridated areas compared to those living in non-fluoridated areas. [34]

Bassin (2006) also reported that there was an association between increase in fluoride level in tap water and osteosarcoma.

Bassin's study measured the risk of osteosacoma before age 20 years based on exposures to fluoride in drinking water during each year of age in childhood. The methodology employed is rigorous and fluoride levels in tap water for each study participant were confirmed for each year of exposure during childhood. The analysis shows significantly elevated risks of bone cancer in boys exposed to fluoridated water during a window of vulnerability, from ages five through ten, with a peak risk associated with exposure at seven years of age.

The high level of natural fluoride in drinking water in certain parts of the world such as in the costal-western part of Libya that surrounded by Naphosa mountain, may be derived from the solvent action of water on rocks and soils of the earth's crust, and the porosity of the rocks and soil through which the water passes.[35] But in Cairo the main source for drinking water is the Nile river through the governmental public water supply. The level of fluoride is

lower than the normal limit (0.41 ppm).

Depending upon fluoride concentration and exposure time, fluoride affects the in vitro-determined activities of many cellular enzymes. 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.[36]

Fluoride inhibits acid phosphatase activity in osteoblastic cells, induces an increases in tyrosin phosphorylated protein, and activate G protein leading to cell proliferation.[37] 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.[38]

Recently it is 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.[39]

Fluoride also increases activity of peroxidases and catalases due to inhibition of androgenesis regulator enzymes which may cause adverse effects on testicular activity. Increases level of alkaline phosphatase osteocalcin and serum calcium which are both cosidered as a markers for osteoblastic activity.[40- 42]

CONCLUSION

Fluoride level in tumor tissue and the osteoblastic variants in Libyan cases are significantly higher than those of Egyptian cases. Higher fluoride level than the normal limit in public drinking water affects the malignant osteoblasts to produce osteblastic neoplastic tissue rather than chondroblastic or fibroblastic tissue.

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