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ORIGINAL RESEARCH

Anti-tumorigenic factors in predicting the biological behaviour of odontogenic keratocyst - A cross-sectional study

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ABSTRACT

Background: Nowadays, different clinical behaviors of odontogenic cysts, little information about their biological agents, importance of diagnosis, and early diagnosis of these lesions have encouraged the researchers to conduct new studies. Therefore, the study's objectives were to measure alpha tocopherol levels in patients with OKC and compare them to alpha tocopherol levels in serum of healthy, normal individuals
Methods: Twelve randomly chosen healthy people made up Group A, the control group, while 12 patients with an OKC diagnosis that had been histopathologically verified made up Group B. The amount of alpha tocopherol was measured in "mg/mL" units.
Results: When compared to healthy controls (mean ± SD = 12.075 ± 1.6504 mg/mL), the mean vitamin E level was found to be lower (mean ± SD = 5.417 ±1.9244 mg/mL).
Conclusion: OKC patients had lower amounts of vitamin E than normal, healthy people. It can definitely aid in predicting more invasive tendencies of pathologic lesions like OKC.
Keywords: OKC, Tocopherol, Odontogenic cyst

INTRODUCTION

The odontogenic keratocyst (OKC), first described as Philipsen in 1956,[1] is designated as “keratocystic odontogenic tumor” (KCOT) by the World Health Organization (WHO) in 2005 however, it has been reclassified as cyst in the 2017 WHO Classification and is still a debate entity. Any jaw cyst in which significant amounts of keratin were produced was referred to as a "keratocyst." OKC's histology is typical and has been thoroughly described. [1] It has a flat epithelial-fibrous tissue junction that is typically free of epithelial rete ridges, a thin, uniform layer of stratified squamous epithelium with a tendency to separate from the underlying connective tissue capsule, an 8 to 4 cell thick layer of spinous cells with intracellular oedema, and a relatively thin fibrous capsule without an inflammatory cell infiltrate. The OKC is described as "a benign uni or multi cystic, intraosseous tumors of odontogenic origin, with a characteristic lining of parakeratinized stratified squamous

epithelium Toller had proposed in 1967 that OKC would be better recognised as a benign tumour rather than a typical cyst based on clinical behaviour. [2] The word "KCOT" is advised by the WHO since it more accurately describes its malignant nature. [3] An oral and maxillofacial lesion with unique traits like rapid growth, expansion into the surrounding tissues, and high rates of recurrence is known as the KCOT. [4]

The first to call attention to OKC's violent behaviour were Pindborg and Hansen. Because of their clinical characteristics, Toller stated in 1967 that OKC should be treated more like a benign tumour than a typical cyst. In 1984, Ahlfors and others proposed that OKC be recognised as a real benign cystic epithelial neoplasm and recommended altered treatment regimens. Shear extensively studied the aggressiveness of the odontogenic keratocyst before publishing his findings and finally classifying it as a benign cystic tumour. Shear fiercely referred to this cyst as a "keratocystoma." The pathogenetic mechanisms of OKC have been sought to be explained by Regezi and others. They say that a high rate of proliferation, excessive expression of antiapoptotic proteins (bcl2), and expression of matrix metalloproteinase are factors that promote the growth and progression of OKCs (MMPs 2 and 9). The pathophysiology of this cyst has also been attributed to a mutation in the PTCH 1 ("patched") gene[5].

The PTCH gene, which has been located on chromosome 9q22.3–q31, is most likely a tumour suppressor. The so-called Hedgehog (Hh) signalling pathway includes the PTCH1, which is a crucial molecule. PTCH is typically associated with the oncogene SMO ("smoothened") to generate a receptor complex for the SHH ("sonic hedgehog") ligand. Studies on NBCCS and sporadic KCOT have revealed molecular proof that a two-hit mechanism contributes to the pathogenesis of these tumours. These studies show allelic loss at two or more loci of 9q22, which causes the overexpression of TP53 and bcl-1 in the NBCCS. This lends credence to the idea that KCOT is a neoplasm. Additionally, mounting data suggests that the PTCH gene may play a substantial role in the emergence of sporadic KCOT. Furthermore, early findings indicate that genes on 12q are overexpressed and amplified. More p53 is expressed in the epithelial lining of OKC/KOT cysts than any other cyst forms. This overexpression isn't caused by a p53 gene mutation; instead, it's a result of the normal p53 protein being overproduced or stabilising. PTCH2 and SUFU are other genes that may be associated with OKC/KOT. The p16, MCC, TSLC1, LTAS2, and FHIT genes have also shown loss of heterozygosity, according to a small number of publications. These results are useful in explaining OKC's aggressive behaviour [5].

It is crucial to promptly diagnose and treat this cyst due to its unique clinical behaviour, which has an impact on the choice of treatment. Due to the various clinical behaviours of odontogenic cysts, the lack of knowledge about the biological causes of these lesions, the significance of early diagnosis and treatment for prevention or at the very least lowering the risk of recurrence and malignancy changes in these lesions, it appears that new studies are required.

At a very early stage, such as in premalignant lesions, premalignant states, and carcinoma in situ, dietary alternatives can prevent oral cancer. Beta-carotene, provitamin A, vitamin A, vitamin C, vitamin E, Lipoic acid, zinc, selenium, and spirulina are some of these dietary replacements. Antioxidants have the job of removing free oxygen radicals from the body, which stops cells from getting damaged or developing any carcinomatous changes. Antioxidants are a class of chemical substances that can neutralise free radicals and stop their synthesis. In the body, free radicals are oxidants. One unpaired electron in oxidants actively seeks out another electron to stabilise the chemical structure. In their pursuit of an additional electron, they become loose cannons that pelt and obliterate molecules and other cells.

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The comparatively non-toxic antioxidant nutrients beta carotene, alpha tocopherol, and glutathione, as well as different retinoids, are currently the subject of intense investigation because to their potential anticancer properties. It has been demonstrated that the carotenoids and tocopherols can inhibit and prevent carcinogenesis in addition to regressing cancer. Their combined effects have also been demonstrated. Recent research has identified a number of potential mechanisms by which these anti-oxidant nutrients inhibit the growth of cancer cells and kill them through apoptosis (programmed cell death), the induction of cytotoxic cytokines, the regulation of gene expression, blocking the formation of the tumor's vital blood supply, or cellular differentiation.

In vitro studies using various experimental systems have shown that vitamin E (alpha tocopherol) inhibits mutagenesis, carcinogenesis, and suppresses the development of tumours. [6] Four tocopherols, a family of weak antioxidants, make up vitamin E. (alpha , beta , gamma , and delta). The only type of vitamin E that the human body actively maintains is alpha tocopherol. Strong antioxidant alpha tocopherol dissipates free oxygen radicals and prevents the production of cancer-causing nitrosamines. [7]

The forming tumours were smaller and fewer in number in the experimental mice receiving vitamin E, beta-carotene, or glutathione in addition to the carcinogen treatments, and the noteworthy angiogenesis seen in the control animals was not evident in the animals receiving the nutrients.

All tocopherols and tocotrienols have the ability to scavenge lipoperoxyl radicals, making them powerful antioxidants. Since T is the primary form of vitamin E in tissues and insufficient intake of this form causes vitamin E deficiency-related ataxia [8], the majority of vitamin E research has only lately concentrated primarily on T [9]. However, numerous investigations on T supplementation in humans and animals have produced negative results with regard to its protective effect in the prevention or treatment of chronic diseases like cancer and cardiovascular diseases [10, 11]. Contrarily, new mechanistic investigations in conjunction with preclinical animal models have shown that alternative forms of vitamin E, as contrasted to T, appear to have distinct and superior biological features that may be helpful for both the prevention and treatment of chronic diseases. Additionally, new research indicates that some long-chain vitamin E metabolites have anti-inflammatory properties that surpass those of their vitamin precursors. These byproducts could be cutting-edge anti-inflammatory compounds that support the positive effects of vitamin E in vivo.

Therefore, the study's objectives were to measure alpha tocopherol levels in patients with OKC and compare them to alpha tocopherol levels in serum of healthy normal individuals.

METHODS

The study subjects were chosen from a group of patients who sought treatment for oral disorders at the outpatient Department of Oral Medicine, Radiology, and Oral Surgery. These people were split into two separate groups, Group A and Group B, and their ages and sexes were not matched. Twelve randomly chosen healthy people made up Group A, the control group, while 12 patients with an OKC diagnosis that had been histopathologically verified made up Group B. Patients having a history of diabetes, hypertension, jaundice, liver or renal abnormalities, or history of other systemic diseases without a history of tobacco chewing were excluded from enrolment.

Patients in the study and control groups had their veins punctured to obtain a sample of blood (5 ml), which was then transferred into a presterilized vial without the addition of ethylene diamine tetra acetic acid. The collected samples were then centrifuged at 3000 rpm for 10 min to separate the serum, and they were then sent to a lab where the alpha tocopherol levels

in the serum were determined using the "liquid chromatography tandem mass spectrometry" method, a potent, precise, and quantitative analytical method based on the series coupling of mass spectrometers to analyse complex mixtures. The amount of alpha tocopherol was measured in "mg/mL" units.

Data so collected was tabulated in an excel sheet, under the guidance of statistician. The means and standard deviations of the measurements per group were used for statistical analysis (SPSS 22.00 for windows; SPSS inc, Chicago, USA). For each assessment point, data were statistically analyzed using one way ANOVA. Difference between two groups was determined using t test and the level of significance was set at $p < 0.05$.

The statistical analysis for the present study was done by applying the following formulae:

1. Mean: The mean (or average) is the most popular and well known measure of central tendency. It can be used with both discrete and continuous data, although its use is most often with continuous data. The mean is equal to the sum of all the values in the data set divided by the number of values in the data set. So, if we have n values in a data set and they have values x1, x2, ..., xn, the sample mean, usually denoted by $\bar{x}$ (pronounced x bar), is:

$$\bar{x} = \frac{(x_1 + x_2 + \cdots + x_n)}{n}$$

This formula is usually written in a slightly different manner using the Greek capitol i.e.:

Sample Mean	Population Mean
$\bar{x} = \frac{\Sigma x}{n}$	$\mu = \frac{\Sigma x}{N}$

where Σx is sum of all data values

N is number of data items in population

n is number of data items in sample

2. Standard deviation: the standard deviation (SD, also represented by the lower case Greek letter sigma σ or the Latin letter s) is a measure that is used to quantify the amount of variation or dispersion of a set of data values. A low standard deviation indicates that the data points tend to be close to the mean (also called the expected value) of the set, while a high standard deviation indicates that the data points are spread out over a wider range of values.

$$\sigma = \sqrt{\frac{\Sigma\ (x - \bar{x})^2}{n}}$$

σ = lower case sigma
Σ = capital sigma
$\bar{x}$ = x bar

3.

3. t test: A student t-test is any statistical hypothesis test in which the test statistic follows a Student t-distribution under the null hypothesis. It can be used to determine if two sets of data are significantly different from each other. It is most commonly applied when the test statistic would follow a normal distribution if the value of a scaling term in the test statistic were known. When the scaling term is unknown and is replaced by an estimate based on the data, the test statistics (under certain conditions) follow a Student's t distribution.

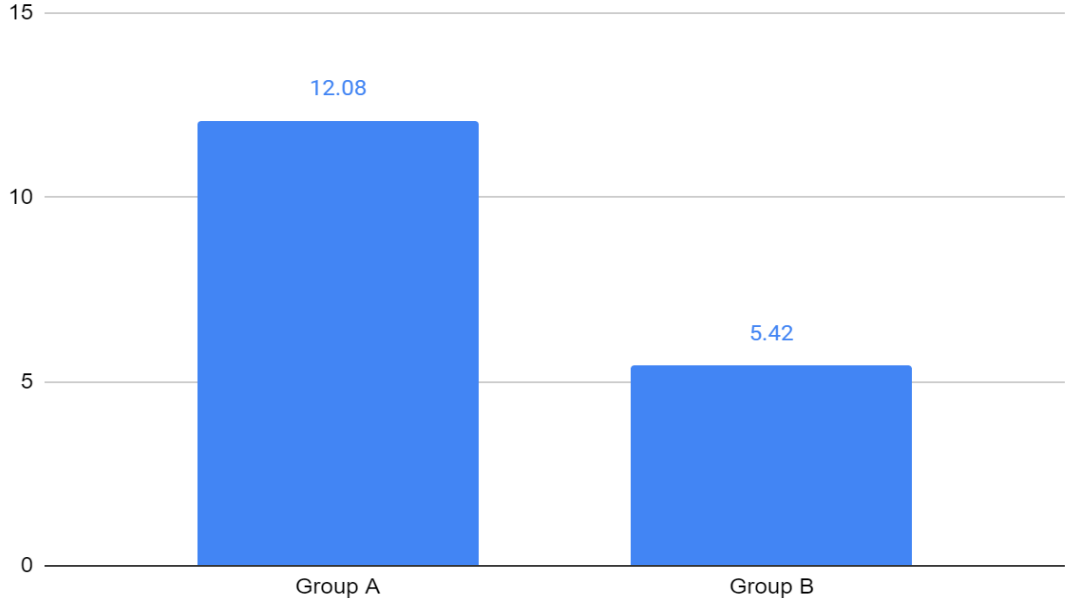
RESULTS

A total of 24 samples, 41.2 % women and 58.8% men with a mean age of 35.24 ± 10.6 years were evaluated in this study. To evaluate the data, an independent sample t test was performed. In OKC cases, lower serum vitamin E levels were discovered. When compared to healthy controls (mean ± SD = 12.08 ± 1.92 mg/mL), the mean vitamin E level was found to be lower (mean ± SD = 5.42 ±1.65 mg/mL). The difference in means is d = 22, with a 0.738 standard error. Statistics showed that the serum vitamin E level had decreased ($P < 0.05$) (table 1 and graph 1).

Table 1: Mean values of Vitamin E levels in Group A and Group B

Group	N	Mean ± SD (mg/mL)	T	Df	P value
Group A	12	12.08 ± 1.92	-9.098	22	0.000
Group B	12	5.42 ± 1.65			

Graph 1: Mean values of Vitamin E levels in Group A and Group B



DISCUSSION

In contrast to other cysts, the main factor in the growth of OKC is proliferation of the cyst's epithelial lining under the induced impact of ectomesenchyme. This distinguishes OKC from the more common radicular and dentigerous cysts in terms of growth process and biologic behaviour.

OKCs have a significant propensity for postoperative recurrence (30–60%) [12]. The causes of this could be satellite tiny cysts in the connective tissue wall of the OKC or an inadequate removal of the vacuole.

In our study, when the levels of alphatocopherol were assessed and compared among OKC and normal healthy individuals, serum Vitamin E levels were found to be decreased in OKC cases. Mean Vitamin E level was found to be decreased (mean ± SD = 5.417 ±1.924mg/mL)

as compared to healthy controls (mean ± SD = 12.075± 1.650 mg/mL). The reduction in serum Vitamin E level was statistically significant ($P < 0.001$). The reduction in levels of OKC.

Based on earlier research, it is possible to establish the following useful connection. When vitamin E is applied to oral tumours, the generation of endogenous prostaglandins by the tumour cells may be inhibited, which compromises prostaglandin feed-back regulation of cancer cell growth and proliferation. [13] It has been demonstrated that cyclooxygenase and the prostaglandin E2 (PGE2) that it produces enhance the development of cancer cells and encourage tumour angiogenesis. [14] By lowering the formation of peroxynitrite, vitamin E reduces the activity of cyclooxygenase in macrophages from aged mice, according to in vitro research by Beharka AA, Wu D, Serafini M, and Meydani SN. [15]

Only a few small-scale clinical investigations have been carried out to look into potential positive benefits of T in humans, in contrast to T which has been explored in numerous big clinical trials. Pro-inflammatory IL-6 and C-reactive protein (CRP) were reduced in hemodialysis patients who supplemented with T, but not T alone [16], while IL-6 was reduced without having an impact on CRP in patients who combined T and DHA [17]. In the meantime, patients with chronic renal disease who received T or T supplementation had less contrast-induced kidney injury [18]. According to a recent study, T dramatically decreased the likelihood of multiple sclerosis relapse and decreased the chance of sustained disability advancement in individuals with multiple sclerosis when paired with rich n-3 fatty acids [19]. A study found that T or mixed tocopherols increased blood pressure without affecting cytokines or endothelium-dependent and independent vasodilation, whereas another found that T or mixed tocopherols increased blood pressure without affecting cytokines or endothelium-dependent and independent vasodilation in diabetic patients[20].

Some of the effects of various nutrients, including butyrate, biotin, lipoic acid, garlic organosulfur compounds, vitamin E metabolites, folate, vitamin B12, vitamin B1, polyphenols, flavonoids, phytoestrogens, sulforaphane/isothiocyanates, vitamin A, fat, and selenium, on epigenetics have been reviewed. [21] Studies by Lod et al. suggest that epigenetic changes have an impact on oral health and may influence both disease risk and treatment response. [22]

In light of all the effects that alpha-tocopherol has on the elements involved in the etiopathogenesis of OKC, alpha-tocopherol may play a role in assisting with the epigenetic modification required to stop the spread of OKC and suppress the existing tumour, as suggested by numerous earlier in vitro studies.

CONCLUSION

In our investigation, it was discovered that OKC patients had lower amounts of vitamin E than normal, healthy people. This may be indicative of a potential in vivo relationship between vitamin E and OKC. Additionally, by affecting the variables involved in the etiopathogenesis of OKC, increasing vitamin E consumption may aid in lowering the chance of recurrence in OKC.

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