Assessing Alpha-Tocopherol Levels in Patients with Keratocystic Odontogenic Tumor: A Cross-sectional Study

Abstract

**Aims and Objectives:** A keratocystic odontogenic tumour (KCOT) is a benign uni- or multicystic, intraosseous tumour of odontogenic origin, with a characteristic lining of parakeratinized stratified squamous epithelium and potential for aggressive, infiltrative behaviour. Various studies in hamsters showed that, alpha-Tocopherol, which is an active biological form of Vitamin E, is a potent antioxidant known to inhibit tumour formation and also regression of established tumours. So, the aim of the present pilot study was to assess the levels of Alpha-Tocopherol (Vitamin E) in Patients with KCOT and compare them with Vitamin E levels in normal healthy individuals. **Materials and Methods:** A sample of 20 individuals were taken and Alpha Tocopherol levels in serum were assessed. Independent sample t test was used to analyse the data. Serum Vitamin-E levels were found to be decreased in KCOT cases. **Results:** Mean Vitamin-E level was found to be decreased (mean ± S.D. = 10,549.34 +/- 2494.21 ng/mL) as compared to healthy controls (mean ± S.D. = 13,982.42 +/- 2178.02 ng/mL). The reduction in serum vitamin E level was statistically significant (P < 0.05). **Conclusion:** The reduction in Vitamin E levels in KCOT patients might be suggestive of the possible interrelation between Vitamin E and KCOT invivo. Also, increase in intake of Vitamin E might help in reducing the risk of recurrence in KCOT by reducing the dysregulation of Cyclin D1 and Down-Regulation of mutant p53.

**Keywords:** Epigenetics, keratocystic odontogenic tumor, tumor suppression, Vitamin E

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, and is defined as “a benign uni- or multi-cystic, intraosseous tumour of odontogenic origin, with a characteristic lining of parakeratinized stratified squamous epithelium and potential for aggressive, infiltrative behavior.” Based on clinical behavior, Toller in 1967 had suggested that OKC may best be regarded as a benign neoplasm rather than a conventional cyst.[2] The WHO recommends the term “KCOT” as it better reflects its neoplastic nature.[3] The KCOT is a relatively common oral and maxillofacial lesion with specific characteristics such as rapid growth, extension into the surrounding tissues, and high rates of recurrence.[4]

Vitamin E (alpha-tocopherol) is found to inhibit mutagenesis and carcinogenesis and suppress tumor formation in different experimental systems in vitro.[3] Vitamin E includes a family of light antioxidants, four tocopherols (alpha-, beta-, gamma-, and delta-). Alpha-tocopherol is the only form of Vitamin E that is actively maintained in the human body. Alpha-tocopherol is a potent antioxidant that neutralizes free oxygen radicals and inhibits carcinogenic nitrosamine formation.[6]

Aim

The aim of this study was to assess alpha-tocopherol levels in patients with KCOT and to compare them with alpha-tocopherol levels in serum of normal healthy individuals.

Need for the study

Previous studies have demonstrated the role of Vitamin E in the prevention and inhibition of cancers. Antitumorigenic properties of Vitamin E were confirmed in an in vitro study based on dimethylbenz(a) anthracene (DMBA)-induced tumors. A thorough research in all online and offline databases showed that there were no in vivo studies correlating Vitamin E levels and KCOT.
KCOT is considered to be a locally destructive and highly recurrent tumor, this study was done to observe the possible correlation between alpha-tocopherol (Vitamin E) levels and KCOT, for example, Vitamin E with its antitumorigenic properties might help in reducing the rate of recurrence of KCOT.

Materials and Methods

The study participants (sample size, n = 20) were selected from a population of patients who presented to the outpatient Department of Oral Medicine and Radiology and Oral Surgery for evaluation and management of oral diseases. These individuals were divided into two groups, Group A and Group B, and were not age and sex matched. The Group A or the control group consisted of ten randomly selected healthy individuals and Group B consisted of ten patients with histopathologically confirmed diagnosis of KCOT. The exclusion criteria for enrollment included patients with a history of diabetes, hypertension, jaundice, and liver or kidney disorders or history of other systemic diseases with no history of tobacco chewing. Blood samples (5 ml) were collected from both the study and control group patients by venous arm puncture under aseptic precautions and transferred into a presterilized vial, without ethylenediaminetetraacetic acid. The collected samples were then subjected to centrifugation at 3000 rpm for 10 min to separate serum and sent to laboratory, where the alpha-tocopherol levels were assessed in the serum using “liquid chromatography tandem mass spectrometry” method, as it is a powerful, quantitative, precise, analytical technique, based on coupling mass spectrometers together in a series to analyze complex mixtures. Alpha-tocopherol levels were recorded in “ng/mL” units [Table 1].

Results

Independent sample t-test was used to analyze the data. Serum Vitamin E levels were found to be decreased in KCOT cases [Figure 1]. Mean Vitamin E level was found to be decreased (mean ± standard deviation [SD] =10,549.34 ± 2494.21 ng/mL) as compared to healthy controls (mean ± SD = 13,982.42 ± 2178.02 ng/mL) [Table 2]. The difference of means d = 3433.077, standard error = 1047.133 [Figure 2]. The reduction in serum Vitamin E level was statistically significant (P < 0.05).

Discussion

The WHO has reclassified KCOT as a tumor based on several factors, such as their locally destructive behavior, high recurrence rate, basal epithelial layer showing proliferation and budding into the underlying connective tissue in the form of daughter cysts, and presence of mitotic figures in the suprabasal layers of the lesional epithelium. Genetically, patched (PTCH), a tumor suppressor gene that is involved in both syndrome associated and sporadic KCOTs, occurs on chromosome 9q22.3 – q31. Normally, PTCH forms a receptor complex with the oncopgene smoothened (SMO) for the sonic hedgehog (SHH) ligand. PTCH binding to SMO inhibits growth signal transduction. SHH binding to PTCH releases this inhibition. If normal functioning of PTCH is lost, the proliferation stimulation effects of SMO are permitted to predominate. Pathogenesis of syndrome associated and sporadic KCOTs involves a “two-hit mechanism” with allelic loss at 9q22, by which tumor suppressor gene gets inactivated. In KCOTs, this leads to dysregulation of the oncoproteins cyclin D, and p53. [7]

Many in vivo studies conducted previously, based on the influence of Vitamin E in tumor and carcinogenesis, have established a link between Vitamin E, tumors, and cancer. Studies done by Shklar et al. in hamsters showed that the application of Vitamin E significantly inhibited DMBA-induced tumor formation and led to regression of established oral tumors. [8] Vitamin E because of its antioxidant properties is receiving growth and attention in the prevention of precancerous lesions. [9] As a part of the cellular membrane, it is characterized as potent antioxidant. Vitamin E as an essential nutrient has many roles that include free radical scavenging, inhibition of cancer cell growth/differentiation, cytotoxicity, inhibiting mutagenicity and nitrosamine formation, and inhibition of DNA and RNA protein synthesis in tumor cells. [10] In addition to these, alpha-tocopherol (biologically active form of Vitamin E) has been found to inhibit mutagenesis and carcinogenesis in vitro and to suppress tumor formation in different experimental systems.

In our study, when the levels of alpha-tocopherol were assessed and compared among KCOT and normal healthy individuals, serum Vitamin E levels were found to be decreased in KCOT cases. Mean Vitamin E level was found to be decreased (mean ± SD = 10,549.34 ± 2494.21 ng/mL) as compared to healthy controls (mean ± SD = 13,982.42 ± 2178.02 ng/mL). The reduction in serum Vitamin E level was statistically significant (P < 0.05). The reduction in levels of KCOT

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

| Group A (ng/ml) | Group B (ng/ml) |
|----------------|----------------|
| 14149.5        | 9468.13        |
| 12523.5        | 15750          |
| 14858          | 11777.3        |
| 16713          | 7858           |
| 15613.6        | 8693           |
| 10989.6        | 8858           |
| 17193          | 13457          |
| 12061          | 8738           |
| 11415          | 11751          |
| 14308          | 9743           |
suggests a possible interrelation between alpha-tocopherol levels and KCOT.

The following practicable link can be established based on previous studies. Vitamin E application to oral tumours could result in inhibition of endogenous Prostaglandin production by tumour cells, and therefore compromised Prostaglandin feed-back control of cancer cell growth and proliferation.\(^{[11]}\) Cyclooxygenase and its derived prostaglandin E\(_2\) (PGE\(_2\)) have been shown to stimulate the growth of cancer cells and promote tumor angiogenesis.\(^{[12]}\) In vitro studies by Beharka AA, Wu D, Serafini M, Meydani SN have shown that Vitamin E inhibits cyclooxygenase activity in macrophages from old mice by reducing peroxynitrite production.\(^{[13]}\) In vitro studies by El Attar have shown that inhibition of PGE\(_2\) production by vitamin E succinate results from inhibition of the activities of both phospholipase A\(_2\) and cyclooxygenase. There is ample evidence for the effectiveness of PGs in controlling cell replication. Several cell lines derived from human malignant tumors have been reported to be inhibited by PGA, PGD, and PGJ, invitro as well as in vivo.\(^{[11]}\) PGA\(_2\) downregulates cyclin D\(_1\) expression by decreasing cyclin D\(_1\) mRNA stability.\(^{[14]}\) Thus, by inhibition of endogenous PG production, tumor proliferation can get compromised, along with downregulation of cyclin D\(_1\), which gets deregulated in KCOT.

Studies provide a mechanistic insight into the regulation of tumor suppressor protein SMAR1 by a cancer therapeutic PGA\(_2\), that leads to repression of cyclin D\(_1\) gene.\(^{[15]}\) Alpha-tocopherol is a potent antioxidant that neutralizes free oxygen radicals and inhibits two carcinogenic nitrosamine formation. They may inhibit cancer development through several mechanisms such as stimulation of wild-type p53, downregulation of mutant p53, activation of heat shock proteins, and an antiangiogenic effect mediated by blockage of transforming growth factor alpha.

The effect of several nutrients, such as folate, Vitamin B\(_{12}\), Vitamin B\(_{1}\), polyphenols, flavonoids, phytoestrogens, sulforaphane/isothiocyanates, Vitamin A, fat, and selenium, and dietary components, such as butyrate, biotin, lipoic acid, garlic organosulfur compounds, and Vitamin E metabolites, on epigenetics has been reviewed to some extents.\(^{[16]}\) According to studies done by Lod et al., epigenetic modifications affect the oral health and may contribute not only to disease susceptibility but also to response to treatment.\(^{[17]}\)

Considering all the effects of alpha-tocopherol on the factors related to the etiopathogenesis of KCOT [Figure 3], alpha-tocopherol might have a possible role in contributing to the epigenetic modification needed to prevent the recurrence of KCOT and to suppress the already existing tumor as suggested by many previous studies done in vitro.

**Conclusion**

Many previous in vitro studies showed that the application of Vitamin E significantly inhibited tumor formation and also led to regression of established oral tumors. Although the status of KCOT as a tumor is now debatable, it is

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### Table 2: Mean and mean difference values of control group and study group

| Group | N | Mean (SD) | Mean Difference (95%CI) | t | df | P |
|-------|---|-----------|-------------------------|---|----|---|
| A     | 10| 13,982.42 (2178.02) | -3433.0770 (-5633.0230, -1233.1310) | -3.27 | 18 | 0.0042* |
| B     | 10| 10,549.34 (2494.21)  |                           |     |    |   |

*P<0.05 statistically significant, P>0.05 nonsignificant
nevertheless, locally destructive and highly recurrent. In our study, the levels of Vitamin E were found to be reduced in patients with KCOT when compared to normal healthy individuals. This might be suggestive of the possible interrelation between Vitamin E and KCOT in vivo. Furthermore, increase in intake of Vitamin E might help in reducing the risk of recurrence in KCOT by influencing the factors involved in the etiopathogenesis of KCOT.

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Conflicts of interest
There are no conflicts of interest.

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