The Evaluation of The Chemopreventive Effect Of B-Carotene (A-Viton®) On Oral Premalignant Lesions Through The Estimation Of The Expression Of P53 Levels

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Article Info

Article history:
Received: 04/19/2023
Revised: 05/22/2023
Accepted: 06/25/2023

Keywords:
P53 PROTEIN
BETACAROTENE
VITAMIN A
ORAL DYSPLASIA WITH PREMALIGNANT LESIONS

ABSTRACT

INTRODUCTION: Lesions such as leukoplakia, dysplastic leukoplakia, erythroplakia, oral smucuous fibrosis, dysplastic lichenoid lesions, and oral lichen planus are all included in the category of oral premalignant lesions, or OPL. It has been observed that 17% of oral premalignant lesions (OPL) will progress into malignant lesions during the first seven years after diagnosis, making proper identification and treatment of OPLs crucial. Any changes to the p53 protein cause it to accumulate in the nuclei of cells; this can be important for the development of cancer, including oral premalignant lesions and oral malignant lesions. METHODS: Fifteen patients with mild to severe dysplasia oral premalignant lesions were identified both histologically and clinically. They received chemopreventive therapy for four months, consisting of betacarotene (Vit. A). Both before and after therapy, the amounts of p53 protein immunostaining were assessed. RESULTS: The average P53 optical staining density in pretreatment specimens was 59.78 ± 10.22, while in post-treatment instances it was 39.74 ± 3.36; this indicates that the pre-treatment cases had a greater value than the the post-treatment cases. There was a 0.0003 p-value. Consequently, the difference is seen as statistically significant when compared statistically. CONCLUSION: It is discovered that there is an inverse relationship between the expression of P53 protein in OPL and the histological and clinical responses to betacarotene supplementation.

1. INTRODUCTION

Premalignant lesions are defined as benign lesions that have altered clinically and/or histopathologically and are more likely to develop into malignancy after diagnosis or to reveal tiny cancerous foci [1].

The World Health Organization (WHO) convened a workshop in 2005 to deliberate on several classifications and terminologies that are intended to differentiate premalignant diseases from precancerous lesions.

The use of the term "potentially malignant disorders (PMD)," which refers to lesions with a possibility of developing into malignancy either at the time of diagnosis or at a later time, was concluded. [2]. Male to female incidence of potentially malignant illnesses (PMD) is 1:1. These disorders primarily afflict people between the ages of 50 and 69. Nonetheless, certain research has indicated that younger individuals may be impacted by 1-5%.

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Among these risk factors are alcohol consumption, tobacco chewing, and smoking. According to a study by Thomas et al., patients who chewed tobacco and drank alcohol showed several precancerous lesions, but those who smoked tobacco showed no abnormalities at all. Along with immunodeficiency or hereditary disorders, viral infection with the human papillomavirus (HPV) is one of the major risk factors.[5,6] A white plaque of dubious danger that excludes other recognized illnesses that do not represent an increased risk of cancer is known as oral leukoplakia. A) Leukoplakia can occur in non-smokers as well as in smokers and can be homogenous or non-homogenous [2]. B) Oral Erythroplakia: This condition is described as “A fiery red patch that cannot be diagnosed as any other disease by a clinical or pathological means.” It has a 14–50% malignant transformation rate, which is extremely high[7]. C) Fibrosis of the oral submucous. Additionally, it has a significant malignant transformation rate of 7–30%, which is typically observed in Asians, particularly Indians [8]. D) Cheilitis Actinica: It is regarded as a PMD that typically affects the lower lip and is brought on by sun exposure. Symptoms typically include erythema, edema, scaling, thickening, and grey-white plaques on the epithelium. It displays a 1.4–36% elevated probability of malignant transformation. Early detection of the presence of a premalignant lesion is particularly important. Hematoxylin and eosin staining, which is followed by microscopic analysis and the identification of cytological and architectural changes, which are referred to as epithelial dysplasia, is one of the most trustworthy methods of assessment. Hyperplasia, mild, moderate, severe, and carcinoma in situ are the several classifications for epithelial dysplasia, based on the degree of cytological and architectural changes occurring, either sequentially from the lower third of the epithelium to the complete thickness [1].

Carotenoids’ ability to function as provitamin A is their primary role. β carotene, β cryptoxanthin, and α carotene are the most wellknown provitamin carotenoids; together, they account for 35% of the vitamin A content in humans [12]. In addition to playing a significant role in cell growth and proliferation, vitamin A and its derivatives have also been shown in some studies to target immune mechanisms, such as immunemediated T cell poteniation that leads to T cell-mediated cytotoxicity, natural killer cell activation, and macrophage stimulation. Because they reduce angiogenesis and impede the growth of tumor cells, carotenoids like betacarotene and other derivatives have been employed in the treatment and prevention of precancerous and cancerous lesions. They also play a significant role in clinical oncology [13, 14]. Betacarotene’s antioxidant properties are also demonstrated by its ability to mitigate the cellular damage caused by reactive oxygen species. This is mostly due to the lipophilic properties of beta-carotene, which are found in cell membranes and lipoproteins [15].

In higher species, p53 has a function in preventing the development of tumors, while the other two proteins are involved in normal biological processes [16]. Throughout time, p53 has shown to be a reliable marker for both the diagnosis and prognosis of malignant tumors. This precision was attained because, even in early lesions, 50% of human cancers exhibit atypical p53 presentation; these transitional abnormalities can identify the type and extent of cell stress [17].

Based on the data previously presented in this context, the purpose of this study was to assess the an tumor effect of betacarotene, one of the available chemopreventive therapies, and to demonstrate its function in inhibiting the vascularization and epithelial dysplasia of premalignant lesions. Additionally, p53 was identified as a significant predictor of the outcome of this biological treatment.

2. MATERIALS AND METHODS
STUDY SETTING AND DESIGN
It was a cross-sectional design performed under the outpatient flow in department of oral medicine and radiology department Government Dental College And Hospital Kadapa Ysr University Of Health Sciences, Vijayawada

SAMPLE SIZE AND SAMPLING CRITERIA
The sample size was estimated by using G power software version 3.1.9.4 from a previous resource and estimated as 20. A convenience sampling method was implied in the study.

Analysis: A priori: Compute required sample size
Input: Tail(s) = Two
Effect size d = 2.7005009
α err prob = 0.05
Power (1-β err prob) = 0.95
Allocation ratio N2/N1 = 1

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EXCLUSION CRITERIA
Patients who were smokers, had systemic illnesses, or had taken medicine within the three months before the trial were not allowed to participate.

INCLUSION CRITERIA
In this study, fifteen patients with various oral premalignant lesions took part. They had no medical needs. They were chosen from Cairo University's Faculty of Medicine's Oncology Outpatient Clinic. The following treatments were performed on each of the chosen patients:

THERAPY INTAKE
This came in the form of 50000 I.U. carotenoid (β-carotene) Avitoin® capsules. It was recommended that each patient take two capsules once a day, either before or after meals. For four months, this routine was followed. Prior to and following treatment, pictures of the lesions were obtained. Additionally, biopsies were performed both before and after treatment, first to confirm the diagnosis and then to assess each case's development by comparing the levels of p53 protein in the tissues before and after therapy.

BIOPSY PROCEDURE
After identifying the afflicted location, a ring block anesthetic was applied surrounding the lesion, a voiding direct anesthetic infiltration into the surrounding tissues. A double wedge incisional biopsy with a breach of around 2 mm was carried out. The sample was quickly preserved in 10% neutral formalin so that it could be processed in paraffin blocks using the standard procedure. Every paraffin block was sectioned into five micron-thick sections, some of which were immunostained and others of which were stained with H&E. It was supplied liquified as a cell culture with supernatant that had been dialyzed with 0.05 mol/L Tris/HCl to a pH of 7.2 and contained 15 mmol/L NaN3, prepared for staining methods. We repeated these steps twice for each example. Once before therapy began and once after therapy ended.

Examination of the Biopsy:
- H&E sections were inspected under a microscope to identify and validate the existence of epithelial dysplasia and to determine its severity, i.e., mild, moderate, or severe. There were 15 instances in the study (7 men and 8 females). In order to assess the prognosis and the impact of beta-carotene on the dysplastic lesions, they were also investigated after treatment.
- Sections that had been immunostained were analyzed using the well-known "Leica Q Win 500" program on a computer system (England). P53's immunoreactivity was assessed by measuring the area optical density in the impacted epithelial layers in each specimen using a measuring frame every ten fields and an objective lens size of 40, which resulted in a total magnification of 400 after gray calibration. The regions that displayed the strongest and most consistent nuclear or perinuclear staining were the ones that were calculated; any additional cytoplasmic staining was disregarded.

DATA ANALYSIS
Descriptive Data" (mean and standard deviation were obtained) were included in the data analysis. Pre- and post-treatment p53 protein optical density were compared and evaluated for significance using paired student T-tests. P was found to be 0.0003, a very statistically significant value. Additionally, the mean value of p53 positive nuclei in pre- and post-therapy biopsies was assessed using the Paired Student's Test. This number, which had a p-value of 0.0197, was highly statistically significant.

Additionally, the number of p53 nuclei in each specimen and the mean value of p53 optical density were correlated using Pearson's correlation coefficient. (The correlation value is one).

3. RESULTS

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Ten males and ten ladies, ages 35 to 68, participated in this study. The following was how the cases were clinically presented: Ten examples of leukoplakia, erythroplakia, and speckled leukoplakia, a mild dysplasia. A clear clinical improvement was evident in four of these modest cases, or 37.5% as evidenced by the lesion's reduced size, decreased keratinization, and even the resolution of white spots in some areas. However, six of the patients (67.5%) did not exhibit any clinical improvement. Eight out of the ten cases exhibited a noticeable improvement in their clinical condition following the administration of medication. The other ten instances had mild dysplasia, manifesting as erythroplakia and verrucous leukoplakia.

The photos that were taken for each instance were very helpful in demonstrating the clinical improvement of each case both before and after treatment. They also played a significant role in securing the patient’s pleasure and demonstrating the effectiveness of the treatment. (Figure 1A)

![A Photograph showing a speckled leukoplakia of the buccal mucosa which was asymptomatic (pre-treatment).](image)

Microscopic analysis of H&E specimens prior to treatment revealed a lower third-only distribution of basal and parabasal epithelial hyperplasia, which was indicative of moderate dysplasia. At the cytological level, there was only slight nuclear pleomorphism and negligible cellular atypia. Rarely did mitosis occur, and when it did, it was also seen in the basal one-third (Fig. 2A).
Following therapy for the same moderate case, microscopic analysis of the H&E specimens revealed thinner epithelium layers and significantly shorter rete pegs. With dwindling mitotic figures, the basal hyperplasia in the basal 1/3 layer was less noticeable (Fig. 3A).

The following was revealed by the statistical analysis of the P53 antibody's immunoreactivity before and after treatment: Prior to treatment, the mean P53 optical staining density was (49.68±8.24) as opposed to (29.64±4.36) in the cases who underwent treatment; the value was higher in the former than in the latter. There was a 0.0003 p-value. Consequently, the difference is seen as statistically significant when compared statistically (Table 1).
TABLE 1 REPRESENTS THE DISTRIBUTION OF STUDY PARAMETERS AMONG THE STUDY GROUPS MEAN VALUES

<table>
<thead>
<tr>
<th></th>
<th>Before Treatment</th>
<th>After Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean optical density</td>
<td>49.76±8.07</td>
<td>29.64±4.36</td>
</tr>
<tr>
<td>Minimum optical density</td>
<td>38.78</td>
<td>24.84</td>
</tr>
<tr>
<td>Maximum optical density</td>
<td>63.18</td>
<td>46.14</td>
</tr>
</tbody>
</table>

In cases of mild dysplasia, P53 positive nuclei exhibiting brown immunostaining were primarily found in the basal cell layer; in situations of moderate dysplasia, they were found in the prickle cell layer. In 10 high-power (x400) fields for each patient, the number of positive nuclei was determined both before and after treatment, and the mean was computed. As stated in Table (2): Before therapy, there were 13.36±8.16 more p53 immunoreactive nuclei on average than after treatment (1.06±0.39). The p-value for the Paired Student’s T-test was 0.001. This difference meets standard requirements for statistical significance when (P≤0.05).

TABLE 2. SUMMARY OF FINDINGS IN THE BEFORE AND AFTER TREATMENT GROUP COMPARISON

<table>
<thead>
<tr>
<th>Mean Optical density</th>
<th>Before</th>
<th>After</th>
<th>Difference between mean</th>
<th>95% confidence interval</th>
<th>T value</th>
<th>Standard error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.36±8.16</td>
<td>1.06±0.39</td>
<td>8</td>
<td>1.17 to 15.64</td>
<td>3.807</td>
<td>2.569</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>15.26±7.32</td>
<td>1.32±0.25</td>
<td>7.5</td>
<td>1.08 to 12.36</td>
<td>2.908</td>
<td>3.231</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

Table(2): Mean number of p53 positive nuclei before and after treatment and statistical significance of the difference.

A statistically significant correlation (correlation coefficient = 1) was found between the mean p53 optical density and the number of p53 positive nuclei before and after treatment using the Pearson correlation test. This score indicates a positive linear relationship that is satisfactory.

4. DISCUSSION

The research's prior findings all point to the importance of focusing on the difficult problem of identifying and treating intraoral premalignant lesions. Additionally, by utilizing the least toxic medicines available, the numerous advantages of chemopreventive therapy that have been researched over the years, along with their involvement in regression and prevention of malignant lesion transformation, have been explored. Photographs obtained before and after treatment and reviewed by more than two examiners in this investigation demonstrated a definite improvement on the clinical level. Patients who participated in this study also saw improvements, as evidenced by changes in the size and texture of the lesions, and they were quite happy with the outcomes. On a histological level, the specimen analyzed following carotenoid therapy (A-Vitpon) showed a marked shift in the levels of epithelial dysplasia along with cellular and nuclear pleomorphism and a decrease in the expression of p53, compared to the pre-treatment specimens.

These findings corroborated a prior finding in a study conducted with Lippman et al. (19) that p53 expression in oral lesions with the potential to be malignant can exhibit varied expression when treated with carotenoids, particularly beta-carotene [19]. According to their findings, p53 accumulation was greater in lesions with higher dysplasia grades, and this accumulation was negatively correlated with the carotenoid therapy's degree of response. An evident response to chemopreventive medication was observed in another study that examined RAR-β, another biomarker [20]. This data supports the theory that using beta-carotene increased RAR-β expression and served as a helpful marker of the protein's receptiveness to treatment [21].

The findings of this investigation were also compared to another study conducted by Papadimitrakopoulou et al., (22), where they employed a comparable chemotherapeutic protocol. In that study, 26 patients with mild, moderate, and severe dysplasia affecting the larynx, oral cavity, and oropharyngeal area were enrolled. The patients suffered from premalignant lesions of different degrees. It has been demonstrated that, during the 6-month assessment, lesions with low levels of p53 expression demonstrated a higher rate of response to the therapy than lesions with high levels of p53 expression [22].

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There was a small discrepancy between the findings of this study and the prior research in that the moderate dysplastic lesions with higher levels of p53 expression prior to therapy were more responsive and expressed lower levels of p53 following therapy. The majority of dysplastic cells are repressed during the S phase and hurried towards the apoptotic phase, which helps to explain this observation [23].

In contrast, patients with lung cancer (n = 39) who received betacarotene 50 mg on alternate days did not appear to have lower positive up-regulation of some biological markers, such as p53, cyclin D1, PCNA, and RAR-β, when compared to those (n = 20) who received a placebo, according to a study by Liu et al. (24), which evaluated the long-term use of beta carotene therapy in patients with lung carcinogenesis and its effect on some biological markers [24].

Retinoids, which include betacarotene and vitamin A and provitamin A, are involved in the control of cell growth, development, differentiation, and morphogenesis [25]. Additionally, they have been shown to stop premalignant lesions that affect the skin, cervix, and oral cavity from turning into cancer. They have also been shown to reduce the risk of primary head and neck tumors metastasizing [26, 27].

Overexpression of p53 has generally been shown to be considerable and reasonably high in premalignant lesions that have progressed to malignancy. This was connected to the observation that stem cells exhibiting p53 mutations were the source of dysplastic fields in every case [28]. There have been conjectures regarding the potential involvement of chemotherapypreventive treatment in the downregulation of these mutant doer molecules [29]. Future research on a variety of biomarkers, particularly at the baseline, is necessary to validate and identify the mechanism via which chemopreventive therapy can stop potentially malignant illnesses from turning malignant.

5. CONCLUSION

In recent years, the efficacy of chemopreventive medicines has been assessed using biomarkers such as p53, with encouraging outcomes. In this study, the use of p53 as a marker has proven to be very beneficial, particularly in light of the encouraging outcomes of using AViton as the preferred therapy agent and occasionally the removal of varied premalignant lesions. In some of the difficult illnesses that dentists typically treat in the dental office, more research into alternative chemopreventive therapies can be quite helpful. This study also demonstrated that, after the therapy was expressed, premalignant lesions with a higher degree of dysplasia had a significant drop in p53 expression.

FUNDING
NIL

CONFLICT OF INTEREST
NIL

ACKNOWLEDGEMENT
NIL

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Journal homepage: https://ijcdr.apdch.edu.in
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