Estimation of expression of beta-human chorionic gonadotropin levels through progression of disease from normal to epithelial dysplasia to malignancy

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Abstract

Background: Gonadotropins have been extensively studied in trophoblastic and nontrophoblastic tumors of breast, gastric, bladder, parathyroid, renal cell and cervical carcinomas, with a significant increase in tissue expressions. Serum levels of beta-human chorionic gonadotropin (β-hCG) and its tissue expression were found more in oral squamous cell carcinoma (OSCC) patients with a significant diagnostic and prognostic value. No such study has been done on oral epithelial dysplasia (OED).

Aims and Objective: To evaluate the expression of β-hCG in OED and the feasibility of using this marker for early diagnosis and to see its progression from normal to dysplasia to malignancy.

Materials and Methods: The study population consisted of thirty histologically confirmed cases of OED and thirty cases of OSCC. Fifteen normal tissues were also included in the study. All the tissue samples were subjected to immunohistochemical (IHC) staining using antimouse β-hCG antibody.

Results: The IHC expression of β-hCG was completely negative in normal cases (Group 1 [n = 15]), whereas 13 (43.3%) cases of OED (Group 2 [n = 30]) and 13 cases (43.3%) of OSCC (Group 3 [n = 30]) showed diffuse cytoplasmic staining in dysplastic surface epithelium and epithelial islands of OSCC. This difference was statistically significant with P = 0.007.

Conclusion: We conclude that the expression of β-hCG increased from normal mucosa to dysplasia to OSCC, suggesting that it is involved in the early stage of carcinogenesis and progression of the disease.

Keywords: Beta-human chorionic gonadotropin, immunohistochemical, oral squamous cell carcinoma

INTRODUCTION

The concept of a step-wise progress of cancer in the oral mucosa, i.e., the initial presence of a precursor (premalignant/precancerous) lesion subsequently developing into cancer, is well established. The term dysplasia, principally encountered in epithelium, was introduced by Reagon in the year 1958 which means atypical, anomalous proliferation.

In 1876, a Hungarian dermatologist detected leukoplakia. Schwimmer dotted that 80% of leukoplakic lesions are...
premalignant, and the likelihood of malignancy increases by 3% per year in adults older than 35 years. The following keratotic lesions have high potential malignant and dysplastic changes: lichen planus, smokeless tobacco, alveolar keratosis and other similar leukoplakic lesions. The likelihood of dysplastic changes is higher in thicker and more granular lesions.

Head-and-neck squamous cell carcinomas (HNSCCs) are among the most destructive of tumors, with oral squamous cell carcinoma (OSCC) representing the vast majority. More than 11 million people are diagnosed with cancer every year. It is estimated that there will be 16 million new cases every year by 2020. The tendency for local and regional metastases owing to the close proximity and uninhibited infiltration of local lymph nodes is high, and this is thought to be the greatest contributor to the morbidity and mortality associated with OSCC. Five-year survival rates are reportedly as low as 9% for some parts of the oral cavity, largely due to late-stage diagnosis when tumor, node, metastasis Stage IV has occurred.[3]

In India alone, 2.5 lakh new patients are diagnosed with HNSCC, of whom about three-fourths are in an advanced stage. Among the HNSCCs, carcinoma of the oral cavity and oropharynx predominates in the Indian population. The prognosis of these patients depends on various factors such as age of patient, size of tumor, site of tumor, thickness of tumor, degree of differentiation and spread into regional lymph nodes. The spectrum of HNSCC varies from place to place within the country.[2]

Survival significantly increases between 66% and 85% when OSCC is detected and treated before lymph node infiltration. Early detection also improves morbidity accompanying the treatment of OSCC, with late-stage diagnosis associated with poorer prognosis. Although it has not been previously reported, it follows that diagnosis and management at the “precancerous” stage would further improve survival rates.[1]

OSCC is commonly preceded by a range of tissue and cellular alterations consistent with carcinoma, yet restricted to the surface epithelial layer, termed oral epithelial dysplasia (OED). These changes often manifest in a clinical mucosal lesion.

Tumor markers are biochemical substances elaborated by tumor cells either due to the cause or effect of malignant process. A tumor marker produced by the tumor and when present in significant amounts indicates the presence of cancer. Tumor markers may be present as intracellular substances in tissues or may be released into the circulation and appear in serum. Continuing search for suitable tumor markers in serum tissue and body fluids during neoplastic process is of clinical value in the management of patients with various malignancies.[3]

Beta-human chorionic gonadotropin (β-hCG) is normally produced in significant amounts only during pregnancy. It is also ectopically produced by trophoblastic as well as non-trophoblastic (colon, prostate, bladder, breast and lung) carcinomas. β-hCG has, therefore, been proposed as a cancer marker of broad utility.

In general, β-hCG and/or subunit synthesis is seen in poorly differentiated tumors. β-hCG expression is prominent in metastatic cancers and is associated with a poor prognosis. Survival time of patients with β-hCG expressing cervical, pancreatic and colorectal cancers has been reported to be statistically shorter than those with β-hCG-negative neoplasms. In bladder cancer as well, the serum level of β-hCG is considered a prognostic indicator of disease. The presence of β-hCG has been linked to chemoresistance and radio-resistance and the molecule has been shown to increase invasiveness.[3,4]

**Objectives of the study**

1. To identify and evaluate the immunohistochemical (IHC) expression of β-hCG in oral mucosal biopsy of healthy volunteers (Group I, n = 15)
2. To identify and evaluate the IHC expression of β-hCG in oral mucosal biopsy of OED (Group II, n = 30)
3. To identify and evaluate the IHC expression of β-hCG in oral mucosal biopsy of OSCC. (Group III, n = 30)
4. To compare the IHC expression of β-hCG in normal oral mucosa, OED and OSCC.

**MATERIALS AND METHODS**

The study population consisted of thirty histologically confirmed cases of OED and thirty cases of OSCC. Fifteen normal tissues were also included in the study. All the tissue samples were subjected to IHC staining using antimouse β-hCG antibody.

**RESULTS**

The control tissue (placenta) showing positive expression of β-human chorionic gonadotropin. The IHC expression of β-hCG was completely negative in tissue sections of healthy volunteers (Group I [n = 15]) as indicated in, Graph 1 and Table 1, whereas
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13 (43.3%) cases of OED (Group 2 [n = 30]) as indicated in Graph 1 and Table 1 and 13 (43.3%) cases of OSCC (Group 3 [n = 30]) as indicated in Graph 1 and Table 1 showed diffuse cytoplasmic staining in dysplastic surface epithelium and epithelial islands of OSCC. This difference was statistically significant with \( P = 0.007 \).

When the expression of β-hCG between normal oral mucosa and various stages of OED using Chi-square test was compared, it showed complete negativity in normal oral mucosa cases, with 50% positivity in mild epithelial dysplasia and 40% positivity in both moderate and severe epithelial dysplasia as indicated in Figure 1a-c, Graph 2 and Table 2.

This difference was statistically significant with \( P = 0.02 \).

When the expression of β-hCG between normal oral mucosa and various stages of OSCC using Chi-square test was compared, it showed complete negativity in normal oral mucosa cases, with 40% positivity in well-differentiated OSCC, 40% positivity in moderately differentiated OSCC, and 50% positivity in poorly differentiated OSCC, as indicated in Figure 1d-f, Graph 3 and Table 3.

### Table 1: Comparison of expression of β-human chorionic gonadotropin between different study groups using Chi-square test

| Groups             | Absent, n (%) | Present, n (%) | \( \chi^2 \) | \( P \) |
|--------------------|---------------|----------------|------------|-------|
| Normal mucosa      | 15 (100.0)    | 0 (0.0)        | 9.949      | 0.007*|
| Dysplasia          | 17 (56.7)     | 13 (43.3)      |            |       |
| SCC                | 17 (56.7)     | 13 (43.3)      |            |       |

*This difference was statistically significant with \( P = 0.007 \). SCC: Squamous cell carcinoma

### Table 2: Comparison of expression of β-human chorionic gonadotropin between normal mucosa and various stages of epithelial dysplasia using Chi-square test

| Groups             | Absent, n (%) | Present, n (%) | \( \chi^2 \) | \( P \) |
|--------------------|---------------|----------------|------------|-------|
| Normal mucosa      | 15 (100)      | 0 (0.0)        | 9.465      | 0.02* |
| Mild dysplasia     | 5 (50)        | 5 (50)         |            |       |
| Moderate dysplasia | 6 (60)        | 4 (40)         |            |       |
| Severe dysplasia   | 6 (60)        | 4 (40)         |            |       |

*This difference was statistically significant with \( P = 0.02 \)

### Table 3: Comparison of expression of β-human chorionic gonadotropin between normal mucosa and various stages of squamous cell carcinoma using Chi-square test

| Groups         | Absent, n (%) | Present, n (%) | \( \chi^2 \) | \( P \) |
|----------------|---------------|----------------|------------|-------|
| Normal mucosa  | 15 (100)      | 0 (0.0)        | 9.465      | 0.02* |
| WD SCC         | 6 (60)        | 4 (40)         |            |       |
| MD SCC         | 6 (60)        | 4 (40)         |            |       |
| PD SCC         | 5 (50)        | 5 (50)         |            |       |

*This difference was statistically significant with \( P = 0.02 \). SCC: Squamous cell carcinoma, WD: Well differentiated, MD: Moderately differentiated, PD: Poorly differentiated
This difference was statistically significant with $P = 0.02$.

**DISCUSSION**

Cancer results from the outgrowth of a clonal population of cells from tissue. Oral carcinogenesis is a molecular and histological process featuring genetic and phenotypic markers for each stage, which involves enhanced function of several oncogenes and/or the deactivation of tumor suppressor genes, resulting in the loss of cell cycle checkpoints.[5] The development of cancer, referred to as carcinogenesis, can be modeled and characterized in a number of ways. One way to describe this process is to illustrate the essential features of both cancer cells and tumors: the “hallmarks” of cancer. Cancer development requires the acquisition of the following six fundamental properties: self-sufficient proliferation, insensitivity to antiproliferative signals and evasion of apoptosis, unlimited replicative potential, the maintenance of vascularization and tissue invasion and metastasis. Cancer can also be considered with regard to a step-wise development functionally grouped into the following three phases: initiation, promotion and progression. Initiation is characterized by genomic changes within the “cancer cell,” such as point mutations, gene deletion and amplification and chromosomal rearrangements, leading to irreversible cellular changes. Tumor development is promoted by the survival and clonal expansion of these “initiated” cells. Progression encompasses a substantial growth in tumor size and either growth-related or mutually exclusive metastasis.[6]

According to the histological model of oral carcinogenesis, cells chronically exposed to environmental carcinogens progress through the stages of reactive hyperkeratosis, epithelial hyperplasia, degrees of dysplasia and intraepithelial carcinoma, leading to invasive carcinoma.[8] The degree of dysplasia is the best guide to the potential progression of oral lesions. Severe epithelial dysplasia has an overall malignant transformation rate of about 16%, but studies show a wide range of 7%–50%. Moderate dysplasia has a malignant transformation potential of 3%–15%, whereas mild epithelial dysplasia shows a very low risk (<5%). It is always assumed, however, that there is a temporal progression of disease, analogous to multistage carcinogenesis and that mild dysplasia will progress to severe dysplasia and then to carcinoma.[7]

Oral cancers are mostly diagnosed between 50 and 79 years of age, 96.6% being over 40 years as studied by Omar.[8] The mean ages of OSCC without and with nodal involvement included in our study were 52.4 and 54.4 years, respectively, which was consistent with the study of Omar.[8] Despite the advances of therapeutic approaches, percentages of morbidity and mortality of OSCC have not improved significantly during the last 30 years. Percentages of morbidity and mortality in males are 6.6/100,000 and 3.1/100,000, respectively, while in females, the corresponding percentages are 2.9/100,000 and 1.4/100,000.[9]

Studies of Prabhu et al.[10] have shown that the incidence and prognosis of OSCC differs based on the site of origin of the lesion. They found that, in India, buccal mucosal OSCCs are most commonly followed by tongue lesions and the least common cases of OSCC arise from the floor of the mouth (0.2–0.6 per 10,000 OSCC cases).[10] They also inferred that 25% of lip lesions, 75% of labial mucosal lesions, 78% of buccal mucosal lesions and 67% of tongue lesions showed lymph node metastasis.[10]
HCG is a glycoprotein hormone that biochemically consists of two polypeptide subunits (alpha and beta chains) with attached carbohydrate side chains. The alpha-subunit is shared by other glycoprotein hormones such as luteinizing hormone, follicle-stimulating hormone and thyroid-stimulating hormone and the beta-subunits are unique for each hormone. This hormone is also known to be produced by neoplastic cells in the tumors of trophoblastic origin. Further, the hormone is also widely used for the diagnosis of pregnancy, pregnancy-associated disorders and trophoblastic disease such as gestational trophoblastic disease in which the serum and urine levels of beta-hCG are almost always elevated. Similar findings were also reported by Guo et al.[12] in trophoblastic malignancies.

However, it is well established that production of beta-hCG is not restricted to these tumors alone, and this hormone is secreted by several nontrophoblastic neoplasms as reported by a study conducted by Marcillac et al.[13] They found that nontrophoblastic cancers demonstrated elevated levels of serum beta-hCG in 30%–72% of tumors of pancreas, 9% of stomach cancers, 11% of tumor of liver and 35%–47% of bladder cancers. Findings of the study of Marcillac et al.[13] on tumors of pancreas and bladder cancer were consistent with those of studies of Alfhah et al.[14] and Iles et al.[15] who also demonstrated similar findings in tumors of pancreas and bladder cancer, respectively. Yet, other studies have demonstrated the prognostic significance of beta-hCG in urothelial and ovarian cancers.

The present study also indicated a statistically significant difference in IHC expression of beta-hCG between oral mucosa of healthy volunteers and OSCC cases. These findings were inconsistent with those of a study conducted by Bhalang et al.[16] who demonstrated similar findings in oral fibromas and OSCC.

The beta-hCG expression in OSCC patients was also consistent with that of Crawford et al.[17] (who demonstrated similar findings in cervical carcinomas). The studies of Murhekar et al.[18] Li et al.[19] and Venyo et al.[20] demonstrated similar findings in gastric carcinomas, esophageal carcinomas and urothelial carcinomas, respectively, and the results of their study were also in favor of our study on OSCC patients.

The expression of beta-hCG is mainly cytoplasmic which was also evident in the present study, and its production by tumor cells is explained by differentiation theory, according to which the retrodifferentiation of tumor cells into an invasive and highly proliferative tissue type resulted in the transformation of moderately differentiated primary tumor into poorly differentiated lesion. Beta-hCG also appears to enhance the growth of tumor cells in culture by preventing apoptosis. All these findings suggested that beta-hCG is an aggressive tumor marker and its expression could be attributed to decreased survival rate and poor prognosis of the disease. The results obtained from the present study also support the differentiation theory and further strengthen the fact that the presence of beta-hCG in tumor cells indicates a higher grade of malignancy and defines the aggressiveness of the tumor.

The present study showed an increased expression of beta-hCG in tissue sections of OED cases and OSCC cases with 43.3% positivity in the above mentioned groups respectively. It showed diffuse cytoplasmic staining in dysplastic surface epithelium of the oral epithelial dysplasia cases and in epithelial islands of OSCC cases.

The present study demonstrated a statistically significant increase in beta-hCG levels in tissue sections of patients with OED and OSCC when compared to healthy volunteers, suggesting that beta-hCG could be considered as a tumor-associated marker for OED and OSCC.

CONCLUSION

The present study was undertaken to evaluate the IHC expression of beta-hCG in normal oral mucosa, OED and OSCC.

The following observations were made from the present study:

- The expression of beta-hCG was significantly more in OED and OSCC compared to that of normal oral mucosa
- The expression of beta-hCG increased from well-differentiated to moderately differentiated to poorly differentiated squamous cell carcinoma
- Thus, we conclude that the expression of beta-hCG increased from normal oral mucosa to OED to OSCC, suggesting that it is involved in the early stage of carcinogenesis and progression of the disease.

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Conflicts of interest
There are no conflicts of interest.

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