

Expression of human chorionic gonadotropin- β in tissue specimens, saliva and urine of oral squamous cell carcinoma patients

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Abstract

Background: Oral squamous cell carcinoma (OSCC) remains as one of the leading causes of death in many of the developing countries including India. Early detection helps in improving the prognosis and survival rates. Over the years, tumor markers continue to play an important role in diagnosing and monitoring cancer progression. The ectopic production human chorionic gonadotropin- β (hCG- β) is one such marker that is seen in various nontrophoblastic cancers and serves as a marker for tumor prognosis. Few immunohistochemical studies have shown the presence of hCG- β in oral cancers too. The present study investigated the immunohistochemical expression, levels of hCG- β in saliva and urine of various grades of OSCC patients and correlated it with their histopathological grading.

Materials and Methods: Tissue sections of 50 histologically confirmed OSCC were subjected to immunohistochemical staining by using hCG- β antibody (well differentiated – 21, moderately differentiated – 21 and poorly differentiated – 8). The levels of hCG- β in saliva and urine were estimated in these individuals, by using Beckman Coulter Access 2 automated immunoassay system and comparisons drawn.

Results: hCG- β immunopositivity was seen in 8 (38%) of 21 well-differentiated, 11 (52%) of 21 moderately differentiated and 6 (75%) of 8 poorly differentiated OSCC specimens. The levels of hCG- β in both saliva and urine were increased in poorly differentiated (0.40 and 1.19 mIU/ml) than moderately (0.3 and 0.76 mIU/ml) and well-differentiated (0.36 and 0.48 mIU/ml) OSCC patients.

Conclusion: Immunohistochemical expression, salivary and urine levels of hCG- β could serve as an independent prognostic indicator in OSCC patients.

Keywords: Human chorionic gonadotropin- β , oral cancer, oral squamous cell carcinoma, saliva, urine

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INTRODUCTION

Oral cancer, a malignant neoplasm of the oral cavity, is among the top three types of cancers in India.^[1] More than

90% of oral cancers are histopathologically diagnosed as squamous cell carcinomas.^[2] Severe alcoholism, use of tobacco, betel nut chewing and human papillomavirus are

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the most common risk factors for oral cancer.^[3] It has one of the lowest survival rates and remains unaffected despite recent therapeutic advances.^[4] However, early diagnosis has helped in improving the prognosis and survival rates. Tumor markers secreted from cancer cells play an important role in early diagnosis, prognosis of cancer and monitoring treatment response as well as to check for cancer recurrence.^[5,6]

Human chorionic gonadotropin- β (hCG- β) is one such marker normally produced by the placental syncytiotrophoblasts in healthy pregnant women from 6 to 8 days after conception and so also is expressed in trophoblastic malignancies.^[7,8] Studies have shown that the ectopic production of hCG- β in serum is expressed in various nontrophoblastic malignant tumors.^[9-21] It has been revealed that a positive immunohistochemical staining of hCG- β and its increased levels in serum are indicative of, the aggressiveness of the tumors, their metastatic potential and a poor prognosis.^[6,9,16-18] A study by Meda *et al.*^[22] on salivary gland tumors also showed similar results.

Literature reveals that there are only two studies regarding hCG- β immunoexpression in oral cancer tissues,^[18,23] one study on levels of hCG- β in serum^[6] and none on saliva and urine. Hence, the present study aimed to investigate the immunohistochemical expression, levels of hCG- β in saliva and urine of various grades of oral squamous cell carcinoma (OSCC) patients.

MATERIALS AND METHODS

The study included 50 tissue samples from patients presenting with squamous cell carcinoma of the oral cavity admitted to the various hospitals in the city between 2016 and 2018. Tissue sample, saliva and urine were obtained from each patient and used for various assays.

Evaluation of human chorionic gonadotropin- β immunostaining

Formalin-fixed and paraffin-embedded 50 biopsy specimens of clinically diagnosed and microscopically confirmed OSCC were used. They included 21 well-differentiated, 21 moderately differentiated and 8 poorly differentiated grades of OSCC.

Paraffin blocks of all cases were sectioned onto polylysine-coated slides. The avidin-biotin-peroxidase method was performed using the primary monoclonal antibodies (BioGenex, India) against hCG- β . Due protocol for immunohistochemistry was followed as per the manufacturer's instruction at controlled room temperature.

Linking antibody and horseradish peroxidase (HRP) complex (BioGenex super sensitive polymer - HRP detection kit) were added, and peroxidase activity was visualized with diaminobenzidine. Placental tissue sections were used as positive controls [Figure 1] and lack of primary antibody as negative control.

The immunoassay sections were compared with the corresponding hematoxylin and eosin-stained sections to establish topographic relationship between the positive stained areas and histopathological diagnoses. The entire tumor section was assessed for the immunohistochemical evaluation, and separate immune scores were assigned for the tumor components. Scoring was done by two independent examiners.

The degree of positive staining was evaluated by semi-quantitative scoring on a scale of 0–3 for intensity (I) such as negative, weak, moderate and strong and for percentage of positive cells (P) on a 0–4 scale as negative, <25%, 26%–50%, 51%–75% and >75%. For each section, a final score was obtained by multiplying the percentage of positive cells (P) by the staining intensity scores (I), and staining expression was represented as weak (0–3), moderate (4–5) and strong (6–7).

Evaluation of human chorionic gonadotropin- β in saliva and urine

Unstimulated salivary samples (spit technique) and urine samples were collected from patients in a sterile graduated plastic container under standardized conditions and centrifuged and transferred to vials to be maintained at -20°C . Samples were then assayed for hCG- β levels by using Beckman Coulter Access 2 automated immunoassay system [Figure 2].

RESULTS

Immunohistochemical tissue expression of human chorionic gonadotropin- β

hCG- β immune reactive cells were found in 25 out of 50 specimens of OSCC (50%). hCG- β was positive in 8 (38%) of 21 well-differentiated, 11 (52%) of 21 moderately differentiated and 6 (75%) of 8 poorly differentiated OSCC specimens [Table 1].

The staining intensity ranged from weak to high and involved only focal areas. Poorly differentiated OSCC specimens showed moderate to high staining intensity with increased percentage of cell involvement, whereas moderate and well-differentiated OSCC specimens showed weak to moderate intensity with involvement of few cells [Figures 3-5]. The immunostaining was localized in the

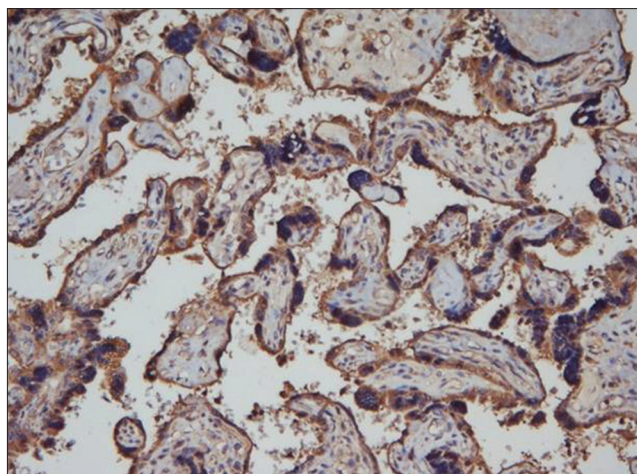


Figure 1: Immunohistochemical staining of human chorionic gonadotropin- β in syncytiotrophoblasts and cytotrophoblasts of placental villi (x20)

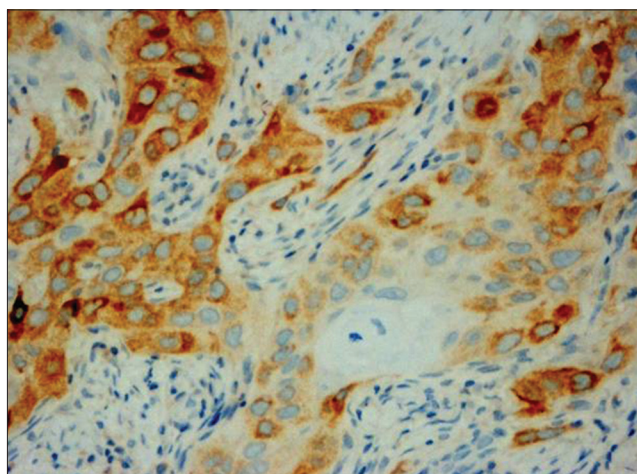


Figure 3: Immunohistochemical staining of human chorionic gonadotropin- β in cytoplasmic tumor cells of well-differentiated oral squamous cell carcinoma (x40)

cytoplasm and cell membrane of cancer cells. The nuclei showed consistently negative staining.

Human chorionic gonadotropin- β levels in saliva and urine samples

Poorly differentiated OSCC patients showed increased levels of hCG- β in both saliva and urine (0.40 and 1.19 mIU/ml) than moderately (0.3 and 0.76 mIU/ml) and well-differentiated (0.36 and 0.48 mIU/ml) OSCC patients. On comparison, the urine levels of hCG- β were higher than saliva of all the three grades [Table 2].

Saliva, urine levels and histological expression of human chorionic gonadotropin- β

Positive hCG- β immunoexpression of moderately differentiated OSCC cases showed higher levels in saliva and urine than negative cases. In well-differentiated OSCC,



Figure 2: Evaluation of human chorionic gonadotropin- β in saliva and urine. (a) saliva and urine containers, (b) vials, (c1 and c2) Access human chorionic gonadotropin- β kit, (d) Beckman Coulter Access 2 immunoassay system

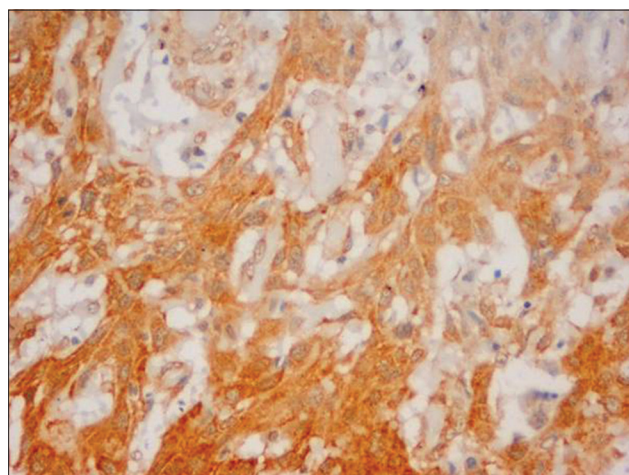


Figure 4: Immunohistochemical staining of human chorionic gonadotropin- β in cytoplasmic tumor cells of moderately differentiated oral squamous cell carcinoma (x40)

positive cases showed higher levels in saliva and lower levels in urine when compared with negative cases. In poorly differentiated OSCC, positive cases showed higher levels in urine and lower levels in saliva when compared with negative cases [Table 3].

DISCUSSION

Diagnostic delay has been shown to be a significant factor in oral cancer progression. Early diagnosis and referral

Table 1: Human chorionic gonadotropin β immunohistochemical expression in different grades of oral squamous cell carcinoma cases

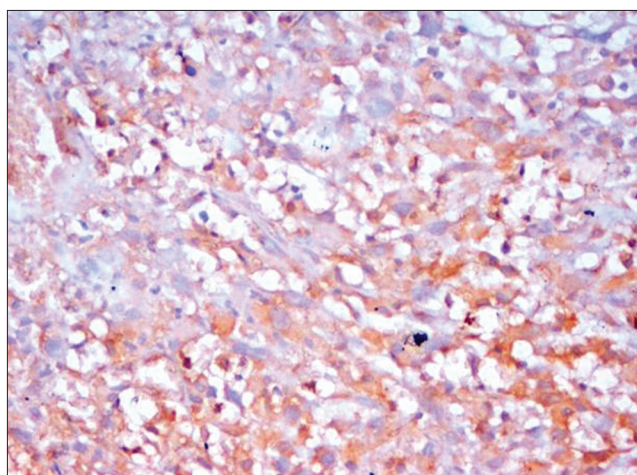
Grade of OSCC	Number of cases	Positive cases (%)	Staining intensity	Percentage of positive cells
Well differentiated	21	8 (38)	Weak to moderate	<25
Moderately differentiated	21	11 (52)	Moderate	26-50
Poorly differentiated	8	6 (75)	Moderate	26-50

OSCC: Oral squamous cell carcinoma

Table 2: Human chorionic gonadotropin β levels in saliva and urine of different grades of oral squamous cell carcinoma cases

Grade of OSCC	Mean levels of hCG- β in saliva (mIU/ml)	Mean levels of hCG- β in urine (mIU/ml)
Well differentiated	0.36	0.48
Moderately differentiated	0.3	0.76
Poorly differentiated	0.4	1.19

OSCC: Oral squamous cell carcinoma, hCG: Human chorionic gonadotropin

**Figure 5:** Immunohistochemical staining of human chorionic gonadotropin- β in cytoplasmic tumor cells of poorly differentiated

are thus a cornerstone to improve survival rates and to reduce high mortality. In recent years, tumor markers played an important role in early detection and assessment of prognosis.^[4-6]

hCG- β a type of tumor maker was studied extensively in trophoblastic and nontrophoblastic malignancies.^[7-21] Studies revealed that excessive production of hCG- β in serum, urine and immunohistochemical expression in these malignancies could serve as marker for early detection and prognostic indicator.^[6,9,16-18,22]

Over the years, several studies have been performed to establish methods for diagnosis or prognosis of various oral diseases including cancer with saliva, because of its easy accessibility, noninvasive collection, low cost, requires minimal training and also as a mass screening tool.^[24]

There are very limited studies of hCG- β immunohistochemical expression on OSCC and no

studies on saliva and urine, and hence, the present study was undertaken to investigate the immunohistochemical expression, levels of hCG- β in saliva and urine of various grades of OSCC patients.

In the present study, hCG- β immunoreactive cells were found in 50% of the OSCC specimens. Studies done by Bhalang *et al.*^[18] and Singh *et al.*^[23] on OSCC specimen's revealed similar results, wherein a 64% and 43.3% of positivity were reported, respectively. Other nontrophoblastic malignancies showed a variation in the frequency of hCG- β expression in tumor specimens [Table 4].

Previous studies revealed that poorly differentiated or anaplastic or dedifferentiated cells exhibit more positive immunorexpression of hCG- β than well-differentiated cells, thus indicating that hCG- β immunoreactive tumor cells are more aggressive and have higher metastatic potential.^[9,18,27] Similar results were found in the current study also, wherein poorly differentiated OSCC specimens showed more positivity (75%) than well (38%) and moderately differentiated (52%) ones. Although the exact mechanism of hCG- β production in nontrophoblastic tumors including OSCC is still not known, findings in our study also support the differentiation theory wherein hCG- β production could be an event in the course of dedifferentiation or arrested differentiation of the malignant cells.^[18]

It was believed that hCG- β also appears to increase the growth of tumor cells in culture by preventing apoptosis.^[23] The results from present and previous studies suggest that hCG- β is an aggressive tumor marker and its expression could be attributed to decreased survival rate and poor prognosis of the disease.

Studies done by Bhalang *et al.*^[18] and Singh *et al.*^[23] revealed that hCG- β appears to be a tumor-associated marker because it was not expressed by normal or uninvolved oral epithelium. Thus, hCG- β could be useful in cytologic studies for the identification of OSCC cells. hCG- β may also help in subclassifying OSCC based on the frequency of the positivity.

A study done by Hedström *et al.*^[6] on OSCC patients and a case report by Turner *et al.*^[28] in head-and-neck squamous

Table 3: Comparison of human chorionic gonadotropin β immunoexpression, levels in saliva and urine of different grades of oral squamous cell carcinoma cases

Grade of OSCC	Mean levels of hCG- β in saliva (mIU/ml)		Mean levels of hCG- β in urine (mIU/ml)	
	Immunonegative	Immunopositive	Immunonegative	Immunopositive
Well differentiated	0.33	0.41	0.57	0.34
Moderately differentiated	0.32	0.36	0.75	0.85
Poorly differentiated	0.52	0.36	0.37	1.47

OSCC: Oral squamous cell carcinoma, hCG: Human chorionic gonadotropin

Table 4: Summary of immunohistochemical studies localizing Human chorionic gonadotropin- β in different malignancies

Source (year)	n	Tumor type	Percentage positive staining
Buckley and Fox (1979) ^[17]	60	Colorectal	26
Wachner <i>et al.</i> (1984) ^[12]	129	Breast	16
Sheaff <i>et al.</i> (1996) ^[14]	80	prostate	12
Syrgos <i>et al.</i> (1998) ^[9]	36	pancreatic	15
Bhalang <i>et al.</i> (1999) ^[18]	45	OSCC	64
Lundin <i>et al.</i> (200) 1 ^[11]	239	Colorectal	52
Hotakainen <i>et al.</i> (2003) ^[16]	229	Renal cell	35
Nowak-Markwitz <i>et al.</i> (2004) ^[13]	8	Ovarian	8
Li <i>et al.</i> (2005) ^[25]	42	Esophageal	71.4
Murhekar <i>et al.</i> (2009) ^[20]	150	Gastric	25
Venyo <i>et al.</i> (2010) ^[26]	86	Urothelial	38
Masrouha <i>et al.</i> (2012) ^[21]	32	Osteosarcoma	5
Meda <i>et al.</i> (2018) ^[22]	21	Salivary gland	12.5
Singh <i>et al.</i> (2019) ^[23]	30	OSCC	43.3

OSCC: Oral squamous cell carcinoma

cell carcinoma revealed that increased levels of hCG- β in serum showed higher recurrence and poor survival rate. Based on this, hCG- β levels were also evaluated in saliva and urine of the same patients.

Poorly differentiated OSCC patients showed increased levels of hCG- β in both saliva and urine (0.40 and 1.19 mIU/ml) than moderately (0.3 and 0.76 mIU/ml) and well-differentiated (0.36 and 0.48 mIU/ml) OSCC patients. On comparison, the urine levels of hCG- β were higher than saliva of all three grades. To the best of our knowledge, this was the first study to estimate hCG- β levels in saliva and urine of OSCC patients.

However, when the same was compared with that of the immunoexpressivity of hCG- β in tissue samples, higher levels of hCG- β were found in salivary samples of moderate and well-differentiated OSCC than that of poorly differentiated OSCC. While the same was not true with the urinary levels, which showed higher levels in moderately and poorly differentiated OSCC when compared to well-differentiated OSCC (positive immunoexpression). While these results are indicative of a relationship between the immunoexpressivity of hCG- β in tissue samples to that of the salivary and urine samples a certain amount of clarity with regard to the association needs to be drawn, to provide a conclusive evidence

that hCG- β in saliva and urine can indeed be used as a tumor/prognostic marker.

CONCLUSION

The results of our study suggest that hCG- β immunoexpression could serve as prognostic indicator for OSCC patients. Although the salivary and urine levels were high in poorly differentiated OSCC patients, it showed mixed results on comparison with hCG- β immunoreactivity. Hence, it requires more studies to draw final conclusions.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Elango JK, Gangadharan P, Sumithra S, Kuriakose MA. Trends of head and neck cancers in urban and rural India. *Asian Pac J Cancer Prev* 2006;7:108-12.
2. Salahshourifar I, Chog VK, Thomas G, Zain RB. Geometric DNA copy number of alterations from precursor of oral lesions to oral squamous cell carcinoma. *Oral oncol* 2014;50:404-12
3. Petti S. Lifestyle risk factors for oral cancer. *Oral Oncol* 2009;45:340-50.
4. Islam M, Datta J, Lang JC, Teknos TN. Down regulation of RhoC by microRNA-138 results in de-activation of FAK, Src and Erk1/2 signaling pathway in head and neck squamous cell carcinoma. *Oral Oncol* 2014;50:448-56.
5. Malati T. Tumour markers: An overview. *Indian J Clin Biochem* 2007;22:17-31.
6. Hedström J, Grenman R, Ramsay H, Finne P, Lundin J, Haglund C, *et al.* Concentration of free hCG beta subunit in serum as a prognostic marker for squamous cell carcinoma of the oral cavity and oropharynx. *Int J Cancer* 1999;84:525-8.
7. Stenman UH, Tiitinen A, Alfthan H, Valmu L. The classification, functions and clinical use of different isoforms of HCG. *Hum Reprod Update* 2006;12:769-84.
8. Cole LA. Biological functions of hCG and hCG-related molecules. *Reprod Biol Endocrinol* 2010;8:102.
9. Syrgos KN, Fyssas I, Konstandoulakis MM, Harrington KJ, Papadopoulos S, Milingos N, *et al.* Beta human chorionic gonadotropin concentrations in serum of patients with pancreatic adenocarcinoma. *Gut* 1998;42:88-91.

10. Tashjian AH Jr, Weintraub BD, Barowsky NJ, Rabson AS, Rosen SW. Subunits of human chorionic gonadotropin: unbalanced synthesis and secretion by clonal cell strains derived from a bronchogenic carcinoma. *Proc Natl Acad Sci U S A* 1973;70:1419-22.
11. Lundin M, Nordling S, Lundin J, Alfthan H, Stenman UH, Haglund C. Tissue expression of human chorionic gonadotropin beta predicts outcome in colorectal cancer: A comparison with serum expression. *Int J Cancer* 2001;95:18-22.
12. Wachner R, Wittekind C, von Kleist S. Immunohistological localization of beta-HCG in breast carcinomas. *Eur J Cancer Clin Oncol* 1984;20:679-84.
13. Nowak-Markwitz E, Jankowska A, Szczerba A, Andrusiewicz M, Warchol JB. Localization of human chorionic gonadotropin beta subunit transcripts in ovarian cancer tissue. *Folia Histochem Cytobiol* 2004;42:123-6.
14. Sheaff MT, Martin JE, Badenoch DF, Baithun SI. beta hCG as a prognostic marker in adenocarcinoma of the prostate. *J Clin Pathol* 1996;49:329-32.
15. Rubin MR, Bilezikian JP, Birken S, Silverberg SJ. Human chorionic gonadotropin measurements in parathyroid carcinoma. *Eur J Endocrinol* 2008;159:469-74.
16. Hotakainen K, Ljungberg B, Haglund C, Nordling S, Paju A, Stenman UH. Expression of the free beta-subunit of human chorionic gonadotropin in renal cell carcinoma: Prognostic study on tissue and serum. *Int J Cancer* 2003;104:631-5.
17. Buckley CH, Fox H. An immunohistochemical study of the significance of HCG secretion by large bowel adenocarcinoma. *J Clin Pathol* 1979;32:368-72.
18. Bhalang K, Kafrawy AH, Miles DA. Immunohistochemical study of the expression of human chorionic gonadotropin- β in oral squamous cell carcinoma. *Cancer* 1999;85:757-62.
19. Butler SA, Ikram MS, Mathieu S, Iles RK. The increase in bladder carcinoma cell population induced by the free beta subunit of human chorionic gonadotrophin is a result of an anti-apoptosis effect and not cell proliferation. *Br J Cancer* 2000;82:1553-6.
20. Murhekar KM, Anuratha JN, Majhi U, Rajkumar T. Expression of human chorionic gonadotropin beta in gastric carcinoma: A retrospective immunohistochemical study. *Indian. J Med Paediatr Oncol* 2009;30:99-102.
21. Masrouha KZ, Khattab R, Tawil A, Abdallah A, Saghie S, Haidar R, *et al.* A preliminary investigation of Beta-hCG expression in patients with osteosarcoma. *J Bone Joint Surg Br* 2012;94:419-24.
22. Meda S, Reginald BA, Reddy BS. Immunohistochemical study of the expression of human chorionic gonadotropin- β in salivary gland tumors. *J Can Res Ther* 2018;14:952-6.
23. Singh J, Swaminathan U, Sharada P, Alur JB, Chowdhury P, Mrinal U. Estimation of expression of beta-human chorionic gonadotropin levels through progression of disease from normal to epithelial dysplasia to malignancy. *J Oral Maxillofac Pathol* 2019;23:108-13.
24. Markopoulos AK, Michailidou EZ, Tzimagiorgis G. Salivary markers for oral cancer detection. *Open Dent J* 2010;4:172-8.
25. Li DM, Li SS, Zhang YH, Zhang HJ, Gao DL, Wang YX. Expression of human chorionic gonadotropin, CD44v6 and CD44v4/5 in esophageal squamous cell carcinoma. *World J Gastroenterol* 2005;11:7401-4.
26. Venyo AK, Herring D, Greenwood H, Maloney DJ. The expression of beta human chorionic gonadotrophin (β HCG) in human urothelial carcinoma. *Pan Afr Med J* 2010;7:20.
27. Iles RK, Purkis PE, Whitehead PC, Oliver RT, Leigh I, Chard T. Expression of beta human chorionic gonadotrophin by nontrophoblastic nonendocrine 'normal' and malignant epithelial cells. *Br J Cancer* 1990;61:663-6.
28. Turner JH, Ross H, Richmon J. Secretion of beta-HCG from squamous cell carcinomas of the head and neck. *Otolaryngol Head Neck Surg* 2010;143:169-70.