ORIGINAL RESEARCH

Assessment of serum cortisol levels in patients with oral squamous cell carcinoma

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ABSTRACT
One of the important factor in the host response to tumor is the lymphocytic infiltration, along the tumor front which provide an immunologic barrier to spread of tumor. This barrier can be affected by the release of cytokine factor released by the tumor which lead to increased cortisol levels in the serum. Patient's clinical parameters were recorded which include age, sex, tumor location, tobacco and alcohol use and medical history. Through clinical examination in which the site of the lesion and the extent of the lesion were assessed. The clinical staging was assessed by AJCC TNM staging.Tumor size was divided into T1, T2, T3 & T4 stage and mean serum cortisol level is calculated in each group. There were no patients in T1 stage. In T2 stage mean serum cortisol level was 9.15 with SD +_ 1.94. In T3 stage mean cortisol level was 15.58 with SD +_ 2.75 and in T4 stage mean serum cortisol was 20.65 with SD ±1.07. Cortisol levels was compared with clinical tumor size using kruskalwallis ANOVA test. Significant change in cortisol levels were found with the tumor size since p=0.0001i.e.p<0.05. Pair wise comparison by Man-Whitney 'U' test was done within the group which showed a significant change in cortisol levels with tumor size. Since p=0.0062 i.e., p<0.05.

Key words: Serum cortisol levels, oral squamous cell carcinoma, TNM staging

INTRODUCTION

Oral cancer represents the third most common form of malignancy in the developing countries[1]. Oral squamous cell carcinoma is the most frequent malignancy in the mouth accounting to 95% of all oral malignant lesions[2]. Cancer progression has now been established to be a multifactorial phenomenon.

The main factor which affects tumor progression can be divided into two categories. One is tumor related factor and another is host response to tumor. One of the important factor in the host response to tumor is the lymphocytic infiltration, along the tumor front which provide an immunologic barrier to spread of tumor. This barrier can be affected by the release of cytokine factor released by the tumor which lead to increased cortisol levels in the serum. One of the most important hormone involved in physiological regulation is cortisol, a Glucocorticoid produced by the cortex of the adrenal gland. Cortisol secretion occurs in response to physical and psychological stress and its level in the blood are modulated by feedback inhibition mechanism. There are studies which have shown that in tumors of breast, ovary, kidney, lung and colon cancer high cortisol levels have been associated with an advanced clinical stage and they may include immunosupression and facilitate tumor progression [3].However this hormonal dysregulation has not yet been investigated extensively in patients with oral cancer. Therefore in this study, we evaluated serum cortisol levels in patients with oral squamous cell carcinoma and correlate it with clinical staging (TNM staging), HPR grading (BRYNE'S grading) and nodal metastasis. [4]

METHODOLOGY

This is a prospective study involving 25 biopsy proven cases of squamous cell carcinoma of oral cavity. This study was approved by institutional review board ethics committee.

Selection criteria

Inclusion criteria

- Patients with biopsy proven case of squamous cell carcinoma.

Exclusion criteria

- Patient withrecurrence of oral cancer.
- Patient with previous radiotherapy.
- Patients with previous history of endocrine disorders.
- Patients who have received steroid therapy in the last 5yrs.

METHOD

Patient's clinical parameters were recorded which include age, sex, tumor location, tobacco and alcohol use and medical history. Through clinical examination in which the site of the lesion and the extent of the lesion were assessed. The clinical staging was assessed by AJCC TNM staging.

Plasma sample

All the patients were fasting in the morning before blood collection, in order to prevent the changes in hormonal levels due to oral intake. Blood samples were collected from the patients in the early morning along with blood for routine blood investigation. Patients' blood was drawn in a plain syringe and sent to department of biochemistry, SDM College of medical sciences and hospital to assess the serum cortisol levels. Then serum cortisol level estimation was done using ADIVA centaur CP assay which is a competitive immunoassay using direct chemiluminescent technology.

The obtained serum cortisol levels was correlated withTNM staging which was done prior to the surgery and then correlated with histopathological grading of the excised tumor using BRYNE'S invasive tumor front grading system and nodal metastasis which was confirmed with histopathology of the neck specimen.

RESULTS

Out of 25 patient, 20 patients (80%) were males and 5 patients (20%) were Females.

25 patients were divided into 3 age groups. Group 1 from 21-40yr which included 7 patients (28%), Group 2 from 41-60yrs which included 14 patients (56%) and Group 3 above 61yrs which included 4 patients (16%). The mean age of patients was 48.24yrs with SD +_ 12.40 The mean age of males was 46.95yrs with SD +_ 13.14 and mean age of females was 53.4yrs with SD +_ 7.77.

These patients were biopsy proven case of squamous cell carcinoma of oral cavity involving Buccal mucosa(BM) 18 patients (72%), tongue 4 patients (16%), Floor of the mouth(FOM) 1 patient (4%), Retro mandibular trigone(RMT) & BM 1 patient (4%), lip & BM 1 patient (4%).

Kruskal Wallis ANOVA test was used to compare the cortisol levels with clinical TNM stage. TNM stage was divided separately according to tumor size (T), regional nodal involvement (N) and distant metastasis (M) and was compared with cortisol levels.

Tumor size was divided into T1, T2, T3 & T4 stage and mean serum cortisol level is calculated in each group. There were no patients in T1 stage. In T2 stage mean serum cortisol level was 9.15 with SD +_ 1.94. In T3 stage mean cortisol level was 15.58 with SD +_ 2.75 and in T4 stage mean serum cortisol was 20.65 with SD ±1.07. Cortisol levels was compared with clinical tumor size using kruskalwallis ANOVA test. Significant change in cortisol levels were found with the tumor size since p=0.0001i.e., p<0.05. Pair wise comparison by Man-Whitney 'U' test was done within the group which showed a significant change in cortisol levels with tumor size. Since p=0.0062 i.e., p<0.05.

Regional lymph node was divided into N0, N1, N2 & N3 stages and mean cortisol level was calculated in each group. In N0 mean cortisol level was 9.26 with SD +_ 2.39. In N1 mean cortisol level was 14.25 with SD +_ 4.90. In N2 stage mean cortisol level was 20.93 with SD +_ 0.95 and there were no patients in N3 stage.

Cortisol level was compared with clinical nodal stage using kruskalwallis ANOVA test. Significant changes in cortisol levels were found with nodal stage since p=0.0138i.e., p<0.05.

Pair wise comparison by Man Whitney 'U' test was done within the group which showed a significant change in cortisol level in between N0 v/s N1 & N0 v/s N2stage since p=0.033i.e., p<0.05. But between stage N1 v/s N2 no much significance was since p=0.0807. In this study there was no incidence of distant metastasis.

Comparison of overall clinical TNM stage with cortisol levels was done by kruskalwallis ANOVA test which showed a significant change in cortisol levels. Since p=0.0001 i.e., p<0.05.Pair wise comparison by Man Whitney 'u' test was done within the group which showed a significant change in cortisol levels. Since p=0.025i.e., p<0.05. But betweenT2N1M0 v/s T2N0MO and T4N1M0 v/s T4N2M0 there was no much significance. Since p=0.999.

Tumor mean score (TNM) group wise distribution according to BRYNE'S histopathological grading system is divide into 3 groups. Group1-2 patients (8%), group 2-11 patients (44%) and group-3 12 patients (48%).

Comparison of TMS score with cortisol levels was done using Kruskal Wallis ANOVA test. In good group mean cortisol level was 14.95 with SD +_ 9.55. In moderate group mean cortisol level was 10.96 with SD +_ 4.35 and in poor group mean cortisol level was 14.53 with SD +_4.96. There was no significance change in cortisol levels when compared with TMS score since p=0.22.

Table 1: Comparison of clinicalT stages with Cortisol levels (µg/dl) by Kruskal WallisANOVA

T stages	Means	Std.Dev.	Sum of ranks
T2	9.15	1.94	107.00
T3	15.58	2.75	103.00
T4	20.65	1.07	115.00
Total	12.99	5.13	
H-value	18.5785		
P-value	0.0001*		
Pair wise comparison by Mann-Whitney U test			
T2 vs T3	P=0.0010*		
T2 vs T4	P=0.0012*		
T3 vs T4	P=0.0062*		

*p<0.05

Table 2: Comparison of clinical N stages with Cortisol levels (µg/dl) by Kruskal Wallis ANOVA

N stages	Means	Std.Dev.	Sum of ranks
N0	9.26	2.39	69.50
N1	14.25	4.90	209.50
N2	20.93	0.95	46.00
Total	12.99	5.13	
H-value	8.5610		
P-value	0.0138*		
Pair wise comparison by Mann-Whitney U test			
N0 vs N1	P=0.0153*		
N0 vs N2	P=0.0339*		
N1 vs N2	P=0.0807		

*p<0.05

Table 3: Comparison of TNM stages with Cortisol levels (µg/dl) by Kruskal Wallis ANOVA

TNM stages	Means	Std.Dev.	Sum of ranks
T2N0M0	9.26	2.39	69.5
T2N1M0	8.94	0.89	37.5
T3N1M0	15.58	2.75	103.0
T4N1M0	20.47	1.31	69.0
T4N2M0	20.93	0.95	46.0
Total	12.99	5.13	
H-value	18.5815		
P-value	0.00001*		
	Pair wise comparison by Mann-Whitney U test		
T2N0M0 vs T3N1M0	p=0.0032*		
T2N1M0 vs T2 N0M0	p=0.9999		
T2N1M0 vs T3N1M0	p=0.0062*		
T2 N0M0 vs T4N2M0	p=0.0339*		
T2N1M0 vs T4N1M0	p=0.0254*		
T2N0M0 vs T4N1M0	p=0.0126*		
T2N1M0 vs T4N2M0	p=0.0528		
T3N1M0 vs T4N1M0	p=0.0201*		
T3N1M0 vs T4N2M0	p=0.0455*		
T4N1M0 vs T4N2M0	p=0.9999		

*p<0.05

Table 4: TMS{tumour mean score} groups wise distribution

TMS	No of patients	% of patients
Good	2	8.00
Moderate	11	44.00
Poor	12	48.00
Total	25	100.00

Table 5: Comparison of groups of TMS scores with Cortisol levels (µg/dl) by Kruskal WallisANOVA

TMS	Mean	Std.Dev.	Sum of ranks
Good	14.95	9.55	30.50
Moderate	10.96	4.35	111.50
Poor	14.53	4.96	183.00
Total	12.99	5.13	
H-value		2.9749	
p-value		0.2260	

DISCUSSION

Beside the known effects of the immune system, studies have shown that neurohormonaldysregulation may influence the behavior of certain types of cancer. Here the influence on tumor microenvironment, viral oncogenesis and immune system is emphasized. There is an evidence linking stress, concomitant behavioral response patterns and resultant neurohormonal and neurotransmitter changes to cancer development and progression. Stress can also influence the expression of viral oncogenes and replication of tumorgenic viruses. Glucocorticoids regulates a wide variety of cellular processes through Glucocorticoids receptor mediated activation or repression of target genes. Glucocorticoids can also activate oncogenic viruses and inhibit antitumor and antiviral cellular immune responses. Glucocortcoids such as cortisol might function in a synergistic fashion with catecholamine's to facilitate cancer growth. In lung carcinoma cells, cortisol increases beta AR density {beta adregenicreceptor} and potentiates the isoproterenol induced increase in cAMP accumulation so it is possible that stressful situations characterized by both increased catecholamine's and cortisol concentrations might have greatest impact on cancer related process.[5]
Cancer progression has now been established to be multifactorial phenomenon. The main factors which affects this can be divided into 2 categories

1. Tumor related factor.
2. Host response to the tumor.

One of the most important factor in the host response to tumor is the lymphocytic infiltration along the tumor front which provides an immunologic barrier to spread of the tumor. This barrier can be affected by the release of cytokine factor released by the tumor which lead to increased cortisol levels in serum. [6]
One of the most important hormone involved in physiological regulation is cortisol, a Glucocortcoids produced by cortex of the adrenal gland in response to ACTH, cortisol secretion occurs in response to physical and psychological stress and its levels in blood are modulated by feedback inhibition.
In some type of tumors, high cortisol levels have been associated with an advanced clinical stage and they may induce immunosupression and facilitates tumor progression[17]. Increased cortisol levels have been related to worsen disease prognosis and poorer response to treatment in patients with breast, ovary, kidney, lung and colon cancer.

A study was conducted in 31 patients with metastatic breast cancer to know the hormonal levels of HPA system. 15 healthy women were included in the study as control group. The results showed that breast cancer patients had significant elevation in the basal cortisol levels compared to controls. Metastatic breast cancer patients had higher cortisol levels than early-stage breast cancer patients. This study concludes that breast cancer is associated with hyperactive adrenal gland, which may be due to physiological stress associated with presence of metastasis in the circulation.[7]
112 patients were examined for the association between IL-6, diurnal cortisol rhythms and feets of depression in the epithelial ovarian cancer patients. Salivary samples were collected 3 days pre surgery and pre surgical blood sample IL-6 was measured by ELISA and cortisol by a chemiluminicence immunoassay. Advanced stage ovarian cancer patients demonstrated elevations in cortisol levels. In vegetative and affective depressive symptoms, plasma IL-6 and cortisol when compared with patients of low malignant potential tumors. These results demonstrate significant relationship between IL-6, cortisol and vegetative depression and may have implications for treatment of depression in ovarian cancer patients. [8]

CONCLUSION

From this study we conclude thatcortisol estimation can be used as a biomarker associated with disease's clinical status.

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