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Original Research Article

Evaluation of hematological parameters in oral cancer and oral pre-cancer

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ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) is the most common public health issue in Indian population. Quite a large number of OSCC cases are preceded by potentially malignant disorders of oral cavity. The need for simple diagnostic marker for early diagnosis and thus better therapeutic outcome is imperative. The current study aims to evaluate hematological parameters such as hemoglobin, bleeding time, clotting time, total leucocyte count (TLC) and differential leucocyte count (DLC) in OSCC and oral potentially malignant disorder cases along with normal healthy controls.
Methods: A total of 150 subjects; 50 in each group were taken and 2.5 ml of blood were withdrawn from each subject and TLC, DLC and hemoglobin assessment was done using autoanalyzer while bleeding time and clotting time was recorded through Duke's method and modified Dale's method respectively.
Results: All the data were tabulated and recorded as mean±standard deviation and comparison was done using one-way ANOVA test ($p<0.05$). TLC count, neutrophil count and lymphocyte count showed statistically significant difference amongst three groups while other parameters such as hemoglobin percentage, bleeding time, clotting time, eosinophil count, monocyte count and basophil count were statistically non-significant.
Conclusions: This study showed TLC count, neutrophil count and lymphocyte count might prove as useful determinant factor in oral squamous cell carcinoma and oral potentially malignant disorders. However, further study with larger sample size is required to establish their role as diagnostic, prognostic marker or predictor of malignant transformation.

Keywords: Bleeding time, Clotting time, Haemoglobin, Hematological, Differential leucocyte count, Oral potentially malignant disorders of oral cavity, Oral squamous cell carcinoma, Total leucocyte count

INTRODUCTION

Oral cancer reports for approximately 3% of all malignancies and affects 2,70,000 patients annually worldwide.[1] It is noteworthy that majority of the oral cancer derived from oral precancerous lesions such as leukoplakia, oral submucous fibrosis, amongst others.[1] Oral pre-cancer lesions have quite high malignant transformation rate.[2] Numerous biomarkers are have been studied in order to provide early diagnosis and treatment, thus better survival rate of the patients.[3] Many attempts have been done on blood biochemistry and hematology to explore the etiology of cancers and to establish this as tumor markers. Quantitative alteration in the serum during tumor initiation and progression takes place. Thus, authors aim to study the haematological

profile in oral squamous cell carcinoma and oral precancer cases.

METHODS

The study took place in the hematology unit of Department of Oral Pathology and Microbiology, King George's Medical University from the period of January 2019 till December 2019. Institutional ethical clearance and informed consent from the patients were duly obtained. A total of 150 subjects participated in the study. The subjects were divided into three groups.

Group I involve 50 subjects with clinical and histopathologically confirmed cases of oral cancer. Group II consists 50 subjects with clinical and histopathologically confirmed cases of oral pre-cancer (includes oral leukoplakia, erythroplakia, oral lichen planus and oral submucous fibrosis) and Group III includes 50 subjects of normal healthy control.

Inclusion criteria

Subjects within age range of 20-70 years, only clinically and histopathologically confirmed cases of oral cancers cases, only clinically confirmed cases of oral leukoplakia, erythroplakia, oral submucous fibrosis, oral lichen planus and histopathologically confirmed having oral epithelial dysplasia were included in the study.

Exclusion criteria

Patients suffering with chronic diseases such as diabetes, hypertension, renal, cardiac or hepatic disease, subjects with periodontitis, subjects with history of bleeding or clotting disorders such as hemophilia etc., subjects with previous history of malignancy or has undergone any cancer treatment, patients having malignancy at any other site other than oral cancer were excluded from the study.

A total 2.5 ml of blood was drawn from the patient in a vial and hematological parameters were assessed using Erba-Transasia B7256 autoanalyzer. The bleeding time was carried out by the Duke's method wherein a standard deep cut was made on the finger pulp with a lancet of 3 mm depth, the first drop of blood was wiped out subsequently the blood was blotted on a filter paper every 30 sec until the blood stopped oozing. The normal bleeding time by this method is 1-5 minutes.[4] The whole blood clotting time was carried out by modified Dale's method wherein the similar prick is made into the finger pulp and blood is taken into a standard glass capillary tube by the capillary action. Subsequent to this the end of capillary tube is broken every 30 sec until the clot is formed and the end of capillary tube starts hanging. The normal clotting time by this method is 5-11 minutes.[4]

All the data were tabulated and mean value with standard deviation were calculated (Table 1). The data were analysed by using SPSS (statistical package for software solutions) version- 18 software (IBM Inc. NY, USA) The comparison between the three groups was calculated using one-way ANOVA test ($p \leq 0.05$) (Table 2).

RESULTS

The mean age in oral cancer group were 50.92±15.192 years; in potentially malignant disorders of oral cavity were 45.08±14.241 years while in the control group were 38.00±14.842 years (Table 1). There were 76% males and 24% females in group 1; 74 % males and 26% females in group 2 and there were 50% males and 50% females in the control group (Figure1).

Table 1: Mean data along with standard deviations of all the variables.

Parameters	Group 1 (n=50)		Group 2 (n=50)		Group 3 (n=50)		Total (n=150)		P value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Age in years	50.92	15.192	45.08	14.241	38.00	14.842	44.37	15.601	0.000
Hb (%)	12.9531	1.67445	13.0348	2.31935	12.8855	1.62895	12.9555	1.88223	0.922
BT (min)	2.2622	0.14344	2.3536	0.26664	2.2745	0.23938	2.2963	0.22597	0.088
CT (min)	4.3990	0.26838	4.4856	0.32033	4.3936	0.21127	4.4252	0.27015	0.157
TLC (10^9/l)	8087.755	2412.435	8164.000	2586.523	6903.636	1650.248	7689.610	2296.947	0.006*
N (cells/mm^3)	63.9184	10.43998	62.9600	8.87317	58.5091	9.36280	61.6753	9.80322	0.009*
L (cells/µl)	24.6122	9.72072	26.2000	7.57008	30.3818	7.98956	27.1883	8.75429	0.002*
E (cells/µl)	7.7755	4.26842	7.5800	2.46684	7.4727	3.19058	7.6039	3.35760	0.900
M (cells/µl)	3.6122	2.02912	3.3400	1.30321	3.6000	1.62845	3.5195	1.66949	0.654
B (cells/µl)	0.0000	0.00000	0.0000	0.00000	0.0000	0.00000	0.0000	0.00000	0.00000

Hb- Haemoglobin, BT- Bleeding time, CT- Clotting time, TLC- Total leucocyte count, N- Neutrophils, L- Lymphocytes, E- Eosinophils, M- Monocyte, B- Basophils.

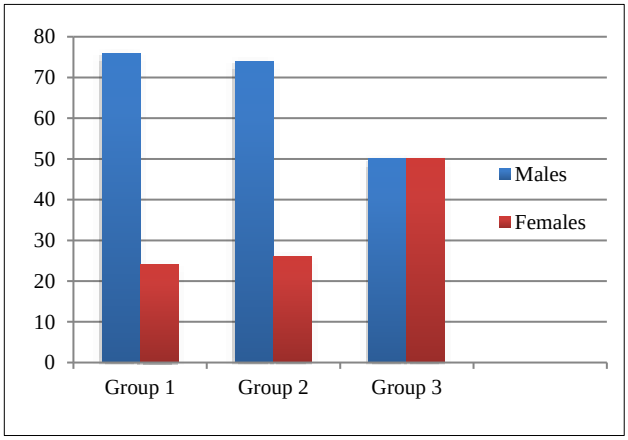

Figure 1: Gender distribution among three groups.

The mean hemoglobin in group 1 was 12.9531±1.67445; in group 2 was 13.0348±2.31935 and group 3 was 12.8855±1.62895. The mean bleeding time in oral cancer subjects was 2.2622±0.14344 in oral pre-cancer subjects was 2.3536±0.26664 and in control subjects was 2.2745±0.23938. The mean clotting time in group I was 4.3990±0.26838; in group 2 was 4.4856±0.32033 and in group 3 was 4.3936±0.211. The means TLC count in group 1, 8087.75±2412.43; in group 2 was 8164±2585.52 while in control group is 6903.63±1650.24843. The mean neutrophil count in group 1 was 63.918±10.439, in group 2 was 62.960±8.8731 while in group 3 was 58.509±9.3628. The mean lymphocyte count in group 1 was 24.612±9.7207; in group 2 was 26.200±7.5700 and in group 3 was 30.381±7.98956. The mean eosinophil count in cancer subject was 7.7755±4.26842; in oral pre-cancer subject was 7.5800±2.4668 and in normal healthy control 7.6039±3.3576. The mean monocyte count was 3.6122±2.0291 in oral cancer subjects; 3.3400±1.3032 in oral precancer subjects and 3.600±1.6284 in normal healthy controls. The mean basophil count in all the three groups of oral cancer, oral pre-cancer and normal healthy control was 0.000±0.000 (Table 1).

The mean TLC count, neutrophil count and the lymphocyte count between the three groups were statistically significant. The other parameters when compared were found to be statistically non-significant. (Table 1 and 2).

Table 2: Comparison of different parameters between groups using one-way ANOVA test.

Variables		Sum of squares	df	Mean square	F	P value
Age in years	Between groups	4314.157	2	2157.078	9.901	0.000
	Within groups	32681.347	150	217.876		
	Total	36995.503	152			
Hb (%)	Between groups	0.585	2	0.292	0.082	0.922
	Within groups	541.461	151	3.586		
	Total	542.045	153			
BT (min)	Between groups	0.247	2	0.124	2.465	0.088
	Within groups	7.566	151	0.050		
	Total	7.813	153			
CT (min)	Between groups	0.271	2	0.135	1.877	0.157
	Within groups	10.896	151	0.072		
	Total	11.166	153			
TLC (10^9/l)	Between groups	5.300E7	2	2.650E7	5.305	0.006
	Within groups	7.542E8	151	4994881.628		
	Total	8.072E8	153			
N (cells/mm^3)	Between groups	880.427	2	440.214	4.809	0.009
	Within groups	13823.339	151	91.545		
	Total	14703.766	153			
L (cells/µl)	Between groups	934.924	2	467.462	6.541	0.002
	Within groups	10790.614	151	71.461		
	Total	11725.539	153			
E (cells/µl)	Between groups	2.418	2	1.209	0.106	0.900
	Within groups	1722.420	151	11.407		
	Total	1724.838	153			
M (cells/µl)	Between groups	2.389	2	1.194	0.425	0.654
	Within groups	424.053	151	2.808		
	Total	426.442	153			
B (cells/µl)	Between groups	0.000	2	0.000		
	Within groups	0.000	151	0.000		
	Total	0.000	153			

Hb- Haemoglobin, BT- Bleeding time, CT- Clotting time, TLC- Total leucocyte count, N- Neutrophils, L- Lymphocytes, E- Eosinophils, M- Monocyte, B- Basophils.

DISCUSSION

The prevalence of oral cancer is highest in India as per the World Oral Health Report 2003. Oral cancer ranks number one amongst men and number three amongst women in India. Oral cancer constitutes 12% of all cancers in men and 8% of all cancers among women.[5] The presence of epithelial dysplasia is considered to play a pivotal role in malignant transformation.[6] The quest for a definite biomarker in oral cancer and oral pre-cancerous lesions for early diagnosis and thus better therapeutic outcome in different biological media including blood, saliva and urine are is still on.[7-9] The current study aim to compare and evaluate total leucocyte count, differential leucocyte count, bleeding time and clotting time in oral precancerous and oral cancer lesions.

Inflammation plays a critical role in tumorigenesis.[10] It plays an elemental role in tumor initiation, progression and metastasis and also in conversion from potentially malignant lesion to full blown malignancy.[11] Systemic Inflammatory response are being evaluated as one of key biomarker in oral cancer. It has been suggested that Systemic inflammatory response may be the effect of the tumor hypoxia or necrosis or the consequence of the local tissue injury.[12] The most used biomarkers that reflect a systemic inflammatory response are white blood cell subtypes.[13] White blood cell count is highly variable because it is responsive to diverse acute and chronic stimuli. It is increased in infection, stress and smoking.[13] The stromal tissue of tumors has high WBC count and inflammatory cells and their cytokine production seems to co-relate with tumour severity.[14]

Tsai et al in his study on oral cavity cancer, showed that the peripheral total white blood cell (WBC) count, monocyte, and neutrophil counts and neutrophil lymphocyte ratio increased with the stage T4 and poor tumour differentiation.[15] Kuss L et al, found altered lymphocyte homeostasis in head and neck squamous cell carcinoma cases, which persisted for months, or years after curative therapies.[16] Grim et al reported that WBC count was associated with risk of cancer death.[17] Erlinger et al postulated WBC count with cancer mortality.[18] Shankar et al also found an association between high WBC count and cancer mortality.[19] The evidence seems to be increasing that, cellular proliferation in an environment rich in, inflammatory cells, growth factors and activated stroma is associated with DNA damage that can potentiate the growth of cancer cells.[20]

Despite the evident link in coagulation cascade and cancer in the literature, this study showed no significant difference in bleeding time and clotting time between oral pre-cancer, oral cancer and normal controls.[21] Still, further studies with more specific parameters are needed to conclude to the relationship between hemostatsis, coagulation cascade and oral cancer and oral pre-cancer lesions.

The correlation between hemoglobin status and head and neck cancer or potentially malignant lesion of oral cavity is still unclear. Studies suggested that pre-chemo-radiation hemoglobin level to be an important determinant of outcome in carcinoma esophagus.[22] For other risk factors, such as p53 mutation, loss of heterozygosity (LOH), HPV, etc.[23] Claudia Cordilla in the study revealed hemoglobin status is associated with lymph node metastasis in squamous cell carcinoma of oral cavity but not with initial T stage.[24] Also, contrary to this study finding, Bhattacharjee et al showed statistically significant difference in hemoglobin value between squamous cell carcinoma of oral cavity and epithelial precursor lesion with the control.[22]

CONCLUSION

This study showed statistically significant difference between total leucocyte count, neutrophil count and lymphocyte count but other parameters such as hemoglobin status, basophil count, monocyte count, eosinophil count, bleeding time and clotting time showed no such difference. There are various confounding factors, which also influence the variation in hematological parameters. Still further studies with larger sample size and with more precise study design is needed.

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