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Original Article

Significance of platelet parameters in squamous cell carcinoma of oral cavity – A case-control study

ABSTRACT

Introduction: Tumor microenvironment plays an important role in cancer progression. Platelets are one of the components of the tumor environment shown to have a role in cancer survival and progression.

Materials and Methods: Ninety-six cases of squamous cell carcinoma (SCC) cases of the oral cavity and 96 age/sex-matched healthy controls were considered for the study. Data regarding platelet count, platelet distribution width (PDW), mean platelet volume (MPV), Platelet-Large Cell Ratio (P-LCR), Plateletcrit (PCT), platelet/neutrophil ratio (PNR), platelet/lymphocyte ratio (PLR), and Platelet/Monocyte Ratio (PNR) from automated hematology analyzer records and clinicopathological data from the Department of Pathology were captured. These data were compared between cases and controls and also with tumor size, tumor grade, lymph node status, and tumour node metastasis (TNM) stage of cases.

Results: Mean ± standard deviation for platelet count, PDW, MPV, P-LCR, PCT, PNR, PLR and PMR among cases were 315.03 ± 98.26, 10.94 ± 1.66, 9.91 ± 0.77, 23.52 ± 5.64, 0.31 ± 0.086, 62.55 ± 31.51, 149.34 ± 61.32, and 498.67 ± 194.91, respectively, and among controls were 287.88 ± 74.11, 10.84 ± 1.18, 9.89 ± 0.72, 23.45 ± 4.55, 0.29 ± 0.061, 60.27 ± 21.02, 138.71 ± 49.28, and 497.64 ± 172.28, respectively. The association between means of platelet count, PDW, P-LCR, and PCT among cases and controls were statistically significant ($P = 0.020$, 0.006, 0.030, and 0.000, respectively). No statistically significant association was found between means of platelet count, PDW, MPV, P-LCR, PCT, PNR, PLR, and PMR versus tumor size, lymph node status, and tumor grades. The association between the means of PCT/PMR and TNM Stages I and II were statistically significant ($P = 0.029$ and 0.016, respectively).

Conclusions: Platelet count, morphology, and functions are altered in oral SCC. Platelet activation plays an important role in oral cancer. PCT and PMR can be used to predict the progress of oral SCC as a cost-effective inflammatory marker.

KEY WORDS: Oral cavity cancer, platelet count, platelet Indices, platelet/lymphocyte ratio, platelet/monocyte ratio, platelet/neutrophil ratio, squamous cell carcinoma

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INTRODUCTION

Worldwide incidence of oral cancer is 4/100,000 population. In India, oral cancer is the second-most common cancer in both genders constituting about 10.3% of all cancer cases.[1] Oral cancer contributes to 29.6% of all cancers in a study at Kolar, southern part of India.[2]

Recent studies suggest the role of platelets in tumor biology. Platelet interacts with the tumor and promotes tumor growth, invasion, immune protection, and angiogenesis by secreting various growth factors and cytokines.[3] Thrombocytosis is reported as poor prognostic factor in cancers such as breast, lung, gastric and gynecological cancers.[4-7]

Platelet indices are considered biomarkers of platelet activation and are related to the morphology and proliferation kinetics of the platelets. Mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), and platelet-large cell ratio (P-LCR) are common constituent of platelet indices obtained in automated hematology analyzers.[8]

Various ratios such as Neutrophil/Lymphocytes ratio and platelet/lymphocytes ratio (PLR) are

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Submitted: 15-May-2021
Accepted in revised form: 16-Jul-2021
Published: 22-Sep-2022

Access this article online
Website: www.cancerjournal.net
DOI: 10.4103/jcrt.jcrt_786_21
Quick Response Code:

Cite this article as: Anand A, Raju K, Azeem Mohiyuddin SM. Significance of platelet parameters in squamous cell carcinoma of oral cavity – A case-control study. J Can Res Ther 2022;18:1036-41.

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indicators of the role of inflammatory cells toward cancer. Inflammatory cells are an important constituent of the tumor microenvironment.[9]

Our aim is to study various platelet parameters such as platelet count, platelet indices (MPV, PDW, PCT, P-LCR), and ratios like platelet/neutrophil ratio (PNR), PLR, and Platelet/Monocyte Ratio (PMR) in squamous cell carcinoma (SCC) of oral cavity cases and age/sex-matched controls.

MATERIALS AND METHODS

Ethical clearance from the institutional ethics committee was obtained. A case-control study was conducted in the Department of Pathology. Ninety-six cases of SCC of the oral cavity from January 2019 to December 2020 were retrospectively captured for the study. Recurrent cancer, postchemotherapy, postradiotherapy cases, and cases with metastatic deposits to the oral cavity were excluded from the study.

Clinical details of the patient such as duration of lesion, site of biopsy, histological grading, tumor size, lymph node status, and TNM stage of carcinoma were retrieved from department records and hospital record section. TNM classification was done on the basis of the eighth edition of the American Joint Committee on cancer recommendations.[10] Radiology findings (magnetic resonance imaging/ultrasonography) regarding the size of the lesion, lymph node status, and stage of disease were captured from the medical record department. Data such as platelet count, MPV, PDW, PCT, and P-LCR were captured from departmental records (hematology section). PNR, PLR, and PMR were calculated by dividing platelet count by absolute neutrophil count, absolute lymphocyte count and absolute monocyte count, respectively, obtained in hematology analyzer.

Ninety-six age/sex matched controls having no SCC of oral cavity were captured from department records. Data regarding age, sex, platelet count, PCT, PDW, MPV, P-LCR, PLR, PNR, and PMR were retrieved from the hematology section. PNR, PLR, and PMR were calculated as in cases. The association of platelet parameters between cases and controls and also with size, grade, nodal metastasis, and TNM staging of tumor in cases were assessed.

Data were entered in Microsoft excel sheet and statistical analysis was performed using SPSS Version 22.0 software (IBM SPSS Statistics, Somers NY, USA). Mean ± standard deviation, range was calculated for each variable. Independent *t*-test was done for calculating equality of means. ANOVA test was applied to find the association of means among the TNM stages and grades of tumor. *Post-hoc* test was done after ANOVA to derive statistical significant association of means between more than two study groups. $P < 0.05$ was considered significant.

RESULTS

The mean age of cases was 53.23 ± 10.07 years. The mean age of controls was 51.9 ± 10.03 years. 63 (65.63%) out of 96 cases were female and 33 (34.37%) were male. For better representation 63 age-matched female controls and 33 age-matched male controls were randomly captured. Out of 96 cases, 66 cases (68.75%) had tumor of size ≤4 cm and 30 cases (31.25%) had tumor of >4 cm size. Out of 96 cases, 46 cases (47.92%) had positive lymph nodes and 50 cases (52.08%) had negative lymph nodes. Out of 96 cases, 60 cases (62.5%) showed features of well-differentiated SCC, 27 cases (28.12%) showed features of moderately differentiated SCC and 09 cases (9.37%) showed features of poorly differentiated SCC. Among 96 cases of oral squamous cell cancer, 23 cases (23.95%) had TNM Stage I disease, 25 (26.04%) had TNM Stage II disease, 31 (32.29%) had TNM Stage III disease, and 17 (17.70%) had TNM Stage IV disease.

The mean platelet count in cases was 315.03 ± 98.26 thousands/cubic mm and in controls was 287.88 ± 74.11 thousands/cubic mm. The association of the means of platelet count between cases and controls was statistically significant with $P = 0.020$. In cases mean PDW was 10.94 ± 1.66% and in controls was 10.84 ± 1.18%. The association of the means of PDW between cases and controls was statistically significant with $P = 0.006$. In cases mean MPV was 9.92 ± 0.77 fL and in controls was 9.89 ± 0.72 fL. There was no statistically significant association in mean MPV between cases and controls ($P = 0.22$). Mean P-LCR in cases was 23.53 ± 5.64% and in controls was 23.45 ± 4.55%. The association of mean P-LCR between cases and controls was statistically significance with $P = 0.030$. In cases mean PCT was 0.31 ± 0.086% and in controls was 0.29 ± 0.061%. The $P = 0.000$ which showed statistical significant association in the mean of PCT between cases and controls [Table 1].

In cases mean PNR was 62.55 ± 31.51 and in controls was 60.27 ± 21.01. There was no statistical significant association in mean PNR between cases and controls ($P = 0.069$). Mean PLR in cases was 149.34 ± 61.32 and in controls was 138.71 ± 49.28. The association of the mean PLR between cases and controls was statistically insignificant with $P = 0.114$. Mean PMR in cases was 498.67 ± 194.91 and in controls was 497.64 ± 172.28. The association of the mean

Table 1: Comparison of platelet parameters with cases and controls

Parameters	Mean±SD		*t*	*P*
	Case group	Control group		
Platelet count (×10^9/L)	315.03±98.26	287.88±74.11	2.162	0.020
PDW (%)	10.94±1.66	10.84±1.18	0.476	0.006
P-LCR (%)	23.53±5.64	23.45±4.55	0.104	0.030
PCT (%)	0.31±0.086	0.29±0.061	1.919	0.000

SD=Standard deviation, PDW=Platelet distribution width, P-LCR=Platelet-large cell ratio, PCT=Plateletcrit

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PMR between cases and controls was statistically insignificant with $P = 0.192$.

Tumor size were classified into two groups; group 1 with tumor size ≤4 cm and group 2 >4 cm. Mean for platelet count, PDW, MPV, P-LCR, PCT, PNR, PLR, and PMR in cases having tumor size ≤4 cm were 312.70 ± 97.53 thousands/cubic mm, 10.99 ± 1.66%, 9.94 ± 0.79 fL, 23.62 ± 5.64%, 0.30 ± 0.085%, 62.33 ± 27.68, 146.77 ± 62.05, and 501.61 ± 199.15, respectively, and in cases having tumor size >4 cm were 320.17 ± 101.35 thousands/cubic mm, 10.83 ± 1.68%, 9.87 ± 0.75 fL, 23.31 ± 5.71%, 0.31 ± 0.090%, 63.03 ± 39.19, 154.99 ± 60.34, and 492.20 ± 188.39, respectively. There was no statistical association between means of platelet count, PDW, MPV, P-LCR, PCT, PNR, PLR, and PMR between cases having tumor size of ≤4 cm and tumor size of >4 cm with $P = 0.281$, 0.826,0.579, 0.867, 0.270, 0.127 0.366, and 0.354, respectively [Table 2].

Lymph node involvement in cases was classified into two groups; Group 1 as positive lymph nodes and Group 2 as negative lymph nodes. Mean for platelet count, PDW, MPV, P-LCR, PCT, PNR, PLR and PMR in cases having positive nodal status were 309.20 ± 94.28 thousands/cubic mm, 10.74 ± 1.65%, 9.82 ± 0.72 fL, 23.01 ± 5.17%, 0.30 ± 0.086%, 61.48 ± 34.30, 151.11 ± 70.65, and 480.83 ± 181.20, respectively, and for cases having negative nodal status were 320.40 ± 102.45 thousands/cubic mm, 11.12 ± 1.66%, 10 ± 0.82 fL, 23.99 ± 6.04%, 0.32 ± 0.087%, 63.53 ± 29.02, 147.72 ± 51.96, and 515.08 ± 207.19, respectively. There was no statistical association of means of platelet count, PDW, MPV, P-LCR, PCT, PNR, PLR, and PMR between cases having positive nodal status and negative nodal status with $P = 0.831$, 0.835, 0.068, 0.169, 0.691, 0.464, 0.283, and 0.182, respectively [Table 2].

There was no statistically significant association of means of platelet count, PDW, MPV, P-LCR, PCT, PNR, PLR, and PMR between tumor grades (well differentiated, moderately differentiated, and poorly differentiated) showing $P = 0.915$, 1.438, 2.584, 0.649, 0.098, 2.479, 0.312, and 0.133, respectively [Table 2].

The association of mean PCT among different TNM stages was found to be statistically significant with $P = 0.029$. *Post-hoc* analysis showed statistically significant association of mean PCT between TNM Stage I and TNM Stage II only ($P = 0.017$). The association of mean PMR among different TNM stages was found to be statistically significant with $P = 0.016$. *Post-hoc* analysis showed statistically significant association of mean PMR between TNM Stage I and TNM Stage II only ($P = 0.020$). No statistical significant association was found between means of platelet count, PDW, MPV, P-LCR, PNR, and PLR among TNM stages with $P = 0.107$, 0.502, 0.753, 0.649, 0.793, 0.861, and 0.126, respectively [Table 2].

DISCUSSION

Worldwide incidence of oral cancer is 4/1,00,000 population. Oral cancer is the second-most common cause of cancer in India constituting about 10.3% of all cancer cases in both genders.[1] Consumption of tobacco, exposure to tobacco smoke, betel quid chewing, improper oral hygiene, exposure to the Human Papilloma Virus are the major risk factor for oral cancer.[11] Oral cancer is a public health problem in India because of its higher incidence and mortality. Majority of cases present with late stage which contributes to the higher mortality. Early diagnosis has a better prognosis.[12] There is a need of finding of a cost-effective marker which can help in the early diagnosis of cancer as well as which can predict prognosis in the early stages.

Table 2: Comparison of platelet parameters with tumor characteristics

Tumor characteristics	Number of cases	Mean±SD							
		Platelet count (thousand/cubic mm)	PDW	MPV	P-LCR	PCT	PNR	PLR	PMR
Tumor size (cm)									
≤4	66	312.70±97.53	10.99±1.66	9.94±0.79	23.62±5.64	0.30±0.085	62.33±27.68	146.77±62.05	501.60±199.15
>4	30	320.17±101.35	10.83±1.68	9.87±0.75	23.31±5.71	0.31±0.090	63.03±39.19	154±60.33	492.20±188.39
Lymph nodes									
Present	46	309.20±94.28	10.74±1.65	9.82±0.72	23.01±5.17	0.30±0.086	61.48±34.30	151.11±70.65	480.83±181.20
Absent	50	320.40±102.45	11.12±1.66	10±0.82	23.99±6.04	0.32±0.087	63.53±29.02	147.72±51.96	515.08±207.19
Grades									
Well differentiated	60	314±98.83	10.78±1.45	9.83±0.69	23.43±5.54	0.31±0.088	63.35±31.03	148±53.56	505.87±199.99
Moderately differentiated	27	312.93±98.37	11.39±1.98	10.19±0.95	24.30±6.29	0.32±0.089	54.63±27.27	155.22±80.32	490.94±196.26
Poorly differentiated	09	328.22±104.69	10.62±1.83	9.68±0.48	21.87±4.16	0.31±0.081	80.93±40.92	136.82±47.24	473.86±172.33
TNM stage									
Stage I	23	352.30±124.42	10.92±1.74	9.99±0.88	23.56±6.34	0.35±0.099	66.46±36.69	152.67±56.45	665.10±210.00
Stage II	25	282.84±74.77	10.85±1.40	9.87±0.70	23.08±5.13	0.28±0.065	61.89±20.40	144.02±75.79	481.83±161.50
Stage III	31	311.55±98.73	11.26±1.74	9.98±0.69	24.29±5.49	0.31±0.092	62.84±39.26	197.45±40.74	553.33±118.46
Stage IV	17	318.29±75.70	10.51±1.78	9.76±0.88	22.74±5.92	0.30±0.066	57.70±21.96	163.44±89.22	638.65±258.80

PCT and PMR showed statistical significant associations between different TNM stages (P=0.029 and 0.016, respectively). SD=Standard deviation, PDW=Platelet distribution width, P-LCR=Platelet-large cell ratio, PCT=Plateletcrit, PMR=Platelet/monocyte ratio, TNM=Tumor, nodes, and metastases, MPV=Mean platelet volume, PNR=Platelet/neutrophil ratio, PLR=Platelet/lymphocyte ratio

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Literature shows the role of platelets in tumor growth and prognosis. Platelet count and platelet indices are part of routine automated hematology analyzer. They are cost-effective and readily available markers.[13]

In the present study, mean platelet count was 315.03 thousands/cubic mm in cases and 287.88 thousands/cubic mm in controls. The association between the mean platelet count between cases and controls was statistically significance with $P = 0.020$. Thrombocytosis was seen among cases. This finding is consistent with the findings of Lu *et al*. where the mean platelet count in oral SCC cases ($n = 253$) was found to be significantly higher than that of controls (267.2 ± 79.1 thousands//cubic mm vs. 253.3 ± 59.6 thousands/cubic mm).[14] Our findings are comparable with the findings of Kannar *et al*. where the mean platelet count was 336.82 ± 100.66 thousands/cubic mm in cases ($n = 107$) and 314.25 ± 44.47 thousands/cubic mm in controls ($n = 68$). Increase mean platelet count was seen among cases. However, the difference in mean platelet count between cases and controls was statistically insignificant ($P = 0.083$) in the study done by Kannar *et al*.[15]

In the present study, there was no significant association between platelet count and tumor size, lymph node status, tumor grades, and TNM stages. However, Lu *et al*. ($n = 253$) reported that platelet counts were significantly higher in the larger tumor, presence of lymph node metastasis, late-stage malignancy, presence of distant metastasis, and tumor recurrence.[14]

In the present study, mean PDW was high among oral SCC cases as compared to healthy controls (10.94% ± 1.66% vs. 10.84% ± 1.18%) and it was statistically significant. PDW is indicator of volume variability in platelet size. This finding is comparable with the findings of Kannar *et al*. where mean PDW was 12.35 ± 2.97% in cases and 11.67% ± 1.42% in controls but was not statistically significant.[15] Zhang *et al*. showed a statistically significant increase in mean PDW among gallbladder carcinoma cases compared to normal controls (16.3 ± 2.1% vs. 15.0 ± 2.2%, $P < 0.01$).[16] In the present study, there was no significant association between PDW and tumor size, lymph node status, tumor grades, and TNM stages. High PDW was reported as unfavorable prognostic factor in laryngeal cancer and esophageal SCC.[16-18] However, conflicting findings do exist in literature. Low PDW was reported as an unfavorable predictive factor for survival in non-SCC of lungs.[19]

MPV represents the average volume of platelets and is considered as the index of platelet activation. In the present study, there was no statistical association was seen between the mean MPV of cases (9.91 ± 0.77 fL) and of controls (9.89 ± 0.71 fL). Eryilmaz *et al*. showed higher MPV value in the head-and-neck cancer patients compared with controls (10.2 fL vs. 9.7 fL, $P = 0.02$).[20] MPV shows different values in different types of cancers. Kannar *et al*. showed significantly decrease in MPV in oral cancer cases compared to controls. Cases of oral cancer showed mean MPV of 7.89 ± 0.92 fL while controls showed 7.61 ± 0.26 fL ($P = 0.014$).[15] Shen *et al*. showed significantly decrease in MPV in cervical cancer cases compared to controls. Cases of cervical cancer showed mean MPV of 8.6 ± 1.3fL while controls showed 9.2 ± 0.9 fL ($P < 0.001$).[21] Variation in results of MPV might be because of differences in methodologies, effect of ethylenediaminetetraacetic acid (EDTA), and time of analysis. Platelets show time-dependent swelling with EDTA. The recommended measuring time of MPV is within 120 min after venipuncture.[15,22]

P-LCR is defined as the percentage of platelets that exceed the normal platelet volume (12fL). Platelet size indicates platelet activation. The present study showed statistical significant association of mean P-LCR between cases (23.53 ± 5.64%) and controls (23.45 ± 4.55%). Increase P-LCR value was seen in cases as compared to controls. However, studies done by Inagaki *et al*. and Yang *et al*. showed statistically significantly lesser P-LCR value in nonsmall-cell lung carcinoma and cervical cancer cases, respectively, as compared to respective controls. This difference in the finding maybe because of the different study population or tumor heterogeneity.[23,24] No statistical significant association was seen in the mean of P-LCR value with tumor size, tumor grade, and TNM stage.

PCT indicate volume occupied by platelets in the blood. In the present study, mean for PCT was 0.31 ± 0.086% for cases and 0.29 ± 0.061% for controls. The association of mean of PCT between cases and controls was statistical significance ($P = 0.000$). The higher value of PCT was seen in cases as compared to control. This finding is comparable with the findings of Zhu *et al*., Ma *et al*. and Kannar *et al*. in colorectal cancer, epithelial ovarian cancer, and oral SCC, respectively.[15,25,26] Association of mean of PCT between cases and controls was found to be statistically significant in the study done by Zhu *et al*.[25] However, conflicting results do exist in the literature regarding the value of PCT in different cancers. Oncel *et al*. found low PCT values in lung carcinoma patients as compared to control.[27]

Statistical significant association was found in PCT values between Stage I and II oral cancer in the present study. This finding is comparable to the finding of Zhu *et al*. in colorectal carcinoma ($n = 783$). Statistical significant association was found in the mean PCT values among various stages of colorectal carcinoma.[25] No statistical significance was found in mean PCT value and tumor size, tumor grades, and lymph node status in cases in the present study.

The present study did not find a significant association in mean values of PNR, PLR, and PMR between cases and controls. In the present study, mean PNR was 62.55 ± 31.51 for cases and 60.27 ± 21.01 for controls. The association of the mean

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PNR between cases and controls was found to be statistically insignificant ($P = 0.590$). No statistically significant association was found in mean PNR with tumor size, nodal status, and TNM stages in the present study. There is a scarcity of data regarding PNR and cancer.

PLR is the most commonly studied parameter in different cancers. In the present study, mean PLR was 149 ± 61.32 for cases and 138.67 ± 49.18 for controls. The association of the mean PLR between cases and controls was found to be statistically insignificant ($P = 0.114$). No statistical significant association was found in mean PLR with tumor size, nodal status, and TNM stages. However, Jonska-Gmyrek *et al*. showed prognostic significance of PLR for overall survival in cervical adenocarcinoma patients.[28] Chen *et al*. showed that PLR can independently predict disease-free survival and overall survival in oral SCC patients ($n = 306$) who underwent surgery.[29] This difference in findings may be because of heterogeneity of tumors or because of the different study population.

There was statistically significant association in the mean PMR value between Stage I and II of oral cancer in the present study. There is the scarcity of data regarding PMR and cancer. However recent studies showed the role of monocytes in tumor biology. Monocytes are reported to show heterogeneity as monocytes may show protumor or antitumor functions. Release of tumoricidal mediators and phagocytosis of tumor cells leads to antitumor effect. Protumor effects include promotion of angiogenesis, remodeling of extracellular matrix, recruitment of regulatory T-cells, and differentiation into tumor-associated macrophages.[30]

The limitation of the present study is, this is a retrospective, unicentric study with a limited sample size. However the strength of the study is, there was a statistical significant association of platelet count, PDW, P-LCR, and PCT between cases and controls, where the values were higher in cases compared to controls. This indicates alteration in platelet count, morphology and hence function in oral cancer patients. In addition, PCT and PMR showed statistical significant association between Stage I and II in cases, where the values were higher in Stage I compared to Stage II. This indicates the role of platelets in the progress of the disease. Hence, similar studies can be done in the larger population as multicentric studies by which platelets can be used as a cost-effective marker in oral SCC.

CONCLUSIONS

Platelet count, morphology, and functions are altered in oral SCC. Platelet activation plays an important role in oral cancer. PCT and PMR can be used to predict the progress of oral SCC in early stage as a cost-effective inflammatory marker.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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