SUMMARY

Peripheral blood Leukocyte subsets

In Leukoplakia patients, significantly reduced mean percentage of Lymphocytes and increased Neutrophils to Lymphocytes ratio was observed as compared to healthy controls.

In Oral Squamous Cell Carcinoma (OSCC) patients, significantly increased mean percentage of Neutrophils and Monocytes, decreased mean percentage of Lymphocytes with enhanced Neutrophils to Lymphocytes ratio was observed as compared to healthy controls. Further, OSCC patients had significantly reduced percentage of Lymphocytes as compared to Leukoplakia patients.

In relation to clinicopathological parameters, high mean percentage of Neutrophils was observed in patients with buccal mucosa patients as compared to other anatomical sites. Also, high mean percentage of monocytes was found in patients whose mucosal margins were involved by tumor as compared to patients without margin involvement.

With respect to disease status, a trend of high incidence of disease relapse with reduced disease free survival (DFS) was seen in patients with high mean percentage of Neutrophils and elevated Neutrophils to Lymphocytes ratio as compared to their respective counterparts.

Peripheral blood T cell subsets

In Leukoplakia patients, increased mean percentage of Effector T cells (CD8^+CD45RA⁻) and Memory T cells (CD4^+CD45RO^+) and decreased mean percentage of Naive T cells
(CD4+CD45RA⁻), CD161⁺CD56⁻ and CD161⁺CD56⁺ NK T subpopulations was observed as compared to healthy controls.

In OSCC patients, significantly increased mean percentage of Helper T cells with increased Helper to Cytotoxic T cell ratio, increased mean percentage of Regulatory T cells and decreased mean percentage of CD161⁺CD56⁺ NK T subpopulation was observed as compared to healthy controls. A trend of decreased mean percentage of CD161⁺CD56⁻ NK T subpopulation was also observed in OSCC patients as compared to healthy controls.

Further, OSCC patients showed significantly decreased mean percentage of Effector T cells (CD8⁺CD45RA⁻) and increased mean percentage of Naive T cells (CD4⁺CD45RA⁺) and Regulatory T cells as compared to Leukoplakia patients.

In relation to clinicopathological parameters, significantly reduced mean percentage of total T cells was found in male than female and in patients without vascular permeation as compared to patients with vascular permeation. Moreover, a trend of low total T cells was found in patients with habit of tobacco and alcohol as compared to patients without habit.

Regarding αβ T cells, a trend of low percentage of αβ T cells was seen in male than female and in patients with T3 tumor size as compared to other tumor sizes. γδ T cells did not show significant correlation with any clinicopathological parameters in the present study.
SUMMARY AND CONCLUSION

Regarding Cytotoxic T cells and their subsets, a trend of low mean percentage of Cytotoxic T cells was seen in histological grade II and III tumors as compared grade I tumor. Further, a significantly decreased mean percentage of Naive T cells (CD8^+CD45RA^+) and increased mean percentage of Effector T cells (CD8^+CD45RA^-) was observed in patients with lip lesion as compared to other anatomic sites.

Regarding Helper T cells and their subsets, significantly decreased mean percentage of Helper T cells was found in patients with habit of tobacco and alcohol as compared to patients without habit. Also, patients with T_2, T_3 and T_4 tumor size had significantly low mean percentage of Helper T cells as compared to T_1 tumor size. In contrast, low mean percentage of Helper T cells was found in N_0 patients as compared to N_1 and N_2 patients. Further, significantly high mean percentage of Naive T cells (CD4^+CD45RA^+) was observed in patients with > 45 years of age and involvement of margin by tumor as compared to their respective counter parts. Moreover, a trend of high percentage of Memory T cells was observed in patients with N_2 nodal status than N_0 and N_1 nodal status. Further, a trend of reduced Naive (CD4^+CD45RA^+) and increased Memory T cells (CD4^+CD45RO^+) was found in N_1 and N_2 patients than N_0 patients.

Regarding Regulatory T cells, a trend of high mean percentage of Regulatory cells was observed in patients with lip lesion as compared to other anatomic sites.

Regarding NK T cell subpopulations, high mean percentage of CD161^+CD56^- NK T subpopulation was noted in females than males. Further, a trend of increased mean percentage of CD161^-CD56^- NK T cell subpopulation was found in patients without habit than patients with habit. Also, a trend of increased mean percentage of CD161^+
CD56⁺ NK T cell subpopulation was observed in smokers than tobacco chewers, chewers and smokers and alcohol users.

With respect to disease status, significantly high incidence of disease relapse with reduced DFS was seen in patients with low mean percentage of γδ T cells and CD161⁺CD56⁻ NK T cell subpopulation as compared to their high mean percentage in blood. Moreover, a trend of high incidence of death with reduced overall survival (OS) was observed in patients with low mean percentage of αβ T cells as compared to its counterpart.

In multivariate survival analysis of peripheral blood Leukocytes and T cell subsets along with clinicopathological parameters, presence of vascular permeation emerged as independent prognostic factor at step 1, followed by low percentage of γδ T cells in peripheral blood at step 2 for predicting reduced DFS and high percentage of Regulatory T cells in blood was found as independent prognostic factor for predicting reduced OS.

**Tumor infiltrating T cell subsets**

High number of tumor infiltrating T cells was observed in tumor stroma, followed by tumor margin and tumor nest.

The ratio of Regulatory T cells to Cytotoxic and Helper T cells was found elevated in tumor nest as compared to tumor stroma and tumor margin.
In relation to clinicopathological parameters, regarding Cytotoxic T cells in tumor nest a trend of low number of Cytotoxic T cells in tumor nest was seen in patients with vascular permeation as compared to patients without vascular permeation.

Regarding Cytotoxic T cells in tumor stroma, low number of Cytotoxic T cells in tumor stroma was observed in patients with ≤ 45 years of age and in males as compared to their respective counter parts. Further, with respect to disease stage, significantly low number of Cytotoxic T cells in tumor stroma was found in stage III disease as compared to stage I, stage II and stage IV disease.

Similarly, low number of Cytotoxic T cells in tumor margin was observed in patients with ≤ 45 years of age and in males as compared to their respective counter parts. Further, low number of Cytotoxic T cells in tumor margin was seen in patients with habit of tobacco and alcohol as compared to patients without habit. A trend of low number of Cytotoxic T cells at tumor margin was observed in patients without lymphatic permeation as compared to patients with lymphatic permeation.

Regarding Helper T cells in tumor nest, a trend of low number of Helper T cells in tumor nest was seen in patients with vascular permeation as compared to patients without vascular permeation. Further, regarding Helper T cells in tumor stroma and tumor margin, low number of Helper T cells was seen in patients with neural invasion as compared to patients without neural invasion.

Regarding Regulatory T cells, a trend of high number of Regulatory T cells in tumor nest was observed in histological grade III tumors as compared to grade I and II. In contrast,
low number of Regulatory T cells in tumor stroma and tumor margin was seen in histological grade III tumors than grade I and II.

Further, regarding Regulatory to Cytotoxic T cells ratio, significantly increased ratio was observed in tumor margin of male patients than female. Whereas, Regulatory to Helper T cells ratio was not found to alter with any of clinicopathological parameters.

With respect to disease status, a trend of high incidence of disease relapse along with reduced DFS was seen in patients with high number of Regulatory T cells than low number of Regulatory T cells in tumor nest. Similarly, increased Regulatory to Cytotoxic T cell ratio in tumor nest and tumor stroma correlated with high incidence of disease relapse. Further, with respect to OS, a trend of high incidence of disease death along with reduced OS was seen in patients with high number of Regulatory T cells than low number of Regulatory cells in tumor nest. Similarly, increased Regulatory to Cytotoxic T cell ratio in tumor nest was correlated with high incidence of death. Moreover, a trend of high incidence of death with reduced overall survival was observed in patients with low number of Helper T cells as compared to high number in tumor stroma.

In multivariate survival analysis of tumor infiltrating T cells along with clinicopathological parameters, tumor size emerged as independent prognostic factor entered at step 1, followed by presence of vascular permeation at step 2 and increased Regulatory to Cytotoxic T cell ratio in tumor stroma at step 3 for predicting DFS. Further, tumor size entered at step 1, followed by low number of Helper T cells in tumor nest at step 2 for predicting OS.
**T cell subsets in Hyperplasia and Dysplasia**

Cytotoxic and Regulatory T cell subsets were found to be heterogeneously distributed in epithelial and lamina propria layer in Hyperplasia and Dysplasia patients. High number of T cells was found to be present in lamina propria as compared to proliferative or dysplastic epithelium.

Comparing Hyperplasia and Dysplasia patients, a trend of increased number of Cytotoxic as well as Regulatory T cells were observed in epithelial layer of Dysplasia patients as compared to Hyperplasia patients.

Further, comparing Dysplasia and OSCC patients, significantly increased number of Cytotoxic T cells and Regulatory T cells in tumor nest and tumor stroma were seen as compared to epithelial and lamina propria layer of Dysplasia patients.

Further, increased ratio of Regulatory to Cytotoxic T cells was observed in patients who developed Carcinoma from Dysplasia.

**Microvessels density (MVD) in Hyperplasia, Dysplasia and OSCC**

Further, the significant increase in number of MVD was found in OSCC patients as compared to Dysplasia patients. However, no significant difference in number of MVD was found between Hyperplasia and Dysplasia patients.

Further, no significant correlation of MVD with clinicopathological parameters was observed in present study.
In relation with circulating T cell subsets, significantly increased incidence of high MVD was observed in patients with low circulating Regulatory T cells as compared to high Regulatory T cells. Further, in correlation with tumor infiltrating T cell subsets, a trend of increased incidence of high MVD was observed in patients with high Helper T cells as compared to low Helper T cells in tumor nest.

In Multivariate survival analysis of peripheral blood Leukocyte and T cell subsets, tumor infiltrating Lymphocytes along with clinicopathological parameters, an increased ratio of Regulatory to Cytotoxic T cell in tumor stroma emerged as independent prognosticator at step 1, presence of vascular permeation at step 2, increased percentage of Regulatory T cell in blood at step 3, reduced percentage of CD161^+CD56^+ NK T subpopulation in blood at step 4 for predicting reduced DFS. Further, low number of Helper T cells in tumor stroma was found to be an independent prognostic factor for predicting poor OS.
Figure 40: Circulating Leukocyte and T cell subsets in Leukoplakia and OSCC patients
Figure 41: T cell subsets in Hyperplasia, Dysplasia and OSCC patients

CONCLUSION

An altered systemic immune response was observed in patients with Oral Squamous Cell Carcinoma (OSCC). Among Leukocyte subsets Neutrophils and Monocytes found increased with a decrease in Lymphocytes. In addition increase in Regulatory T cells and decrease in NK T cells in circulation indicate severe systemic immune suppression in OSCC patients. Tobacco and alcohol induced suppression of circulating Helper T cells suggest the potential influence of carcinogen on T cells. Also, with increasing
tumor size and with advancement of histological grade reduction in circulating Helper T cells and Cytotoxic T cells explained immunosuppressive mechanism of tumor. Higher proportion of Effector T cells and lower Naive T cells in circulation of Leukoplakia suggests effective systemic immune response. However, noticeable lymphopenia and defective proliferation and activation of circulating T cells in OSSC in comparison with Leukoplakia suggest altered systemic immune response during carcinogenesis. Further, Microvessels determination revealed migration of Regulatory T cells from blood to the tumor site as well as high Microvessel density in Carcinoma in comparison to Dysplasia correlated high angiogenesis with immune cell infiltration at tumor site. An elevated Regulatory to Cytotoxic and Helper T cell ratio in tumor nest implied a strong immune suppression within tumor microenvironment. Further, reduced Cytotoxic T cells infiltration in advanced stage patients and reduced Helper T cells in patients with neural invasion revealed the deprived function of immune system during tumor invasion. Thus, suppression of systemic immune response, deprivation of local immune response was also observed at tumor site. Further, a distinct infiltration of T cells was observed in tissue, as lesion became malignant; the infiltration of T lymphocytes increased in step wise manner from Hyperplasia to Dysplasia to Carcinoma. Moreover, an increased Regulatory to Cytotoxic T cells ratio in patients who developed Carcinoma from Dysplasia suggested crucial role of Regulatory T cells during tumor progression. Moreover, elevated Neutrophils to Lymphocytes ratio along with low γδ T cells and NK T cells with high Regulatory T cells in circulation and high Regulatory to Cytotoxic T cells ratio in tumor stroma were found to be indicators of poor prognosis of OSCC patients.
Hence, analysis of circulating \( \gamma \delta \) T cells, NK T cells and Regulatory T cells and tumor infiltrating Cytotoxic and Regulatory T cell subsets helps in scrutinizing immunosuppressed patients who are at high risk for relapse. Such patients may potentially benefit from the implementation of immunotherapy along with conventional treatments to stimulate T cell immune response.