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Histochemical Use Of Giemsa In Detecting Tumor Associated Tissue Eosinophilia In Histopathological Biopsies Of Squamous Cell Carcinoma.

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ABSTRACT:

BACKGROUND:

Tumor associated tissue eosinophilia has been the center of attention of histopathologists because this might be a diagnostic and a prognostic indicator in carcinomas. Various researches have been done regarding the relationship of tissue eosinophilia with the tumor using various stains. The aim of this study was to identify the role of a special stain, giemsa, in detecting tumor associated tissue eosinophilia in tumor microenvironment of histopathological samples of squamous cell carcinoma.

MATERIAL AND METHODS:

In this study, we evaluated 61 paraffin embedded blocks of moderately differentiated squamous carcinoma and 17 of poorly differentiated squamous cell carcinoma. Each block was processed and was stained with special stain, giemsa. Each slide was viewed under microscope and tumor associated tissue eosinophils were counted in 10 high power fields by two observers.

RESULTS:

The tumor associated tissue eosinophilia was higher in cases of moderately differentiated squamous cell carcinoma than poorly differentiated squamous cell carcinoma. However, no significant relationship was found between the macroscopic features of the tumor and the number of eos/10HPF.

CONCLUSION:

It is concluded that there is increased number of tumor associated tissue eosinophils in MDSCC than PDSCC.

KEYWORDS:

Giemsa. Tumor associated tissue eosinophilia. Moderately differentiated squamous cell carcinoma (MDSCC). Poorly differentiated squamous cell carcinoma (PDSCC)

INTRODUCTION:

The initiation of the cancer is the result of combat between the accumulation of driver gene mutations and epigenetic transformations (Tomasetti et al, 2017; Dawson, 2017). The immune system continuously eliminates the mutant cells that are generated during the process of cell division. Cancer cells resistant to immunity may slip through this defense mechanism and develop tumors (Zitvogel et al, 2013).

Typical SCC presents as nests of pleomorphic malignant squamous epithelial cells arising from the epidermis and extending into the dermis. The malignant cells are often large with abundant eosinophilic cytoplasm and vesicular nucleus with variable keratinization. According to WHO grading system, moderately differentiated tumors (MDSCC) exhibit more nuclear pleomorphism, an increased number of mitoses (including abnormal mitoses), and few keratin pearls. Poorly differentiated tumors (PDSCC) are characterized by a lack of squamous differentiation, a high mitotic rate (including abnormal mitoses), and absence of keratin pearls (Streider et al, 2017).

Peripheral blood eosinophils were first identified by Paul Ehrlich. They are derived from their pluripotent precursor cells present in the bone marrow. There, they mature and enter the circulation (Kay, 2015; Johnston et al, 2017). When eosinophils are activated, they release biological mediators which can have positive or negative effects on target organs (Varricchi et al, 2016). They have an important ability to initiate biochemical program of inflammation and repair (Rosenberg et al, 2013; Furuta et al, 2014). Other functions of eosinophils include the induction of protective response against viruses, microbes and helminthes (Samarasinghe et al, 2017).

Normal microenvironment maintains cellular homeostasis by providing a barrier to tumor development. Incorrect signals change tissue environment and cause either initiation or promotion of tumor growth. Smouldering inflammation is the emblem of cancer development. Eosinophils produce a number of mediators for example cationic proteins i.e. ECP, MBP, EPX,

EDN, chemokines and cytokines. They have the ability to leave the blood and migrate to the site of inflammation as well as into the tumor microenvironment. . IL-5 is the most important activator of eosinophils. Migration is mediated by the interaction between integrins that are expressed on endothelial cells and activated eosinophils (Bochner, 2015).

Eosinophils have been found in the tumor microenvironment of many experimental models. Tumor associated tissue eosinophilia is defined as peri or intra-tumor eosinophilic infiltrate not associated with tumor necrosis or ulceration (Dorta et al, 2002). The precise mechanism underlying the eosinophilic infiltration of the tumor remains unknown. It is thought that carcinomas such as lung carcinoma produces IL-5 that activates and promotes eosinophilic infiltration. Eosinophilic infiltration in cancers can also be mediated by chemokines produced by tumor associated immune cells. In this regard, macrophages and mast cells are the most important in eosinophil recruitment (Granata et al, 2010). Several methods are employed to identify eosinophils e.g. cationic proteins and different special stains, there is still a need to identify a special stain which is most effective to identify them under microscopy. A special stain for determining tissue eosinophils is required for early diagnosis. This will provide a basis for further research in evaluating squamous cell carcinoma.

MATERIALS AND METHOD:

This study was conducted on 61 paraffin embedded blocks of moderately differentiated squamous cell carcinoma and 17 blocks of poorly differentiated squamous cell carcinoma that were processed from biopsies from different regions of body. The samples were selected through convenient sampling from the biopsies handed over to the Histopathology department from year 2018 to 2020. The inclusion criteria was biopsies from both genders that were presented in the Department of Histopathology. The exclusion criteria included metastatic disease and patients with more than one malignancy. The data was collected from the hospital record and the pre-operative tests of the patients. 4 to 5 micrometer thick sections were cut in microtomy (Joshi and Keijkar, 2013). 1 ml giemsa solution (ready to use) and 9 ml distilled water were mixed to form a working solution. The slide was stained with giemsa for 15-20 minutes. The slide was washed and air dried (Bancroft and Gamble, 2008). The slides were air dried, mounted with DPX and used for observation. All these slides were evaluated with 400X magnification. 10 non overlapping fields were selected and eosinophil count was recorded. The number of eosinophils per 10HPF (eos/10HPF) were counted in both categories of MDSCC and PDSCC using student's t test. The

clinical presentation (growth/ulcer) of the tumor was also compared to the eos/10HPF. $P < 0.01$ was used to determine the statistical significance.

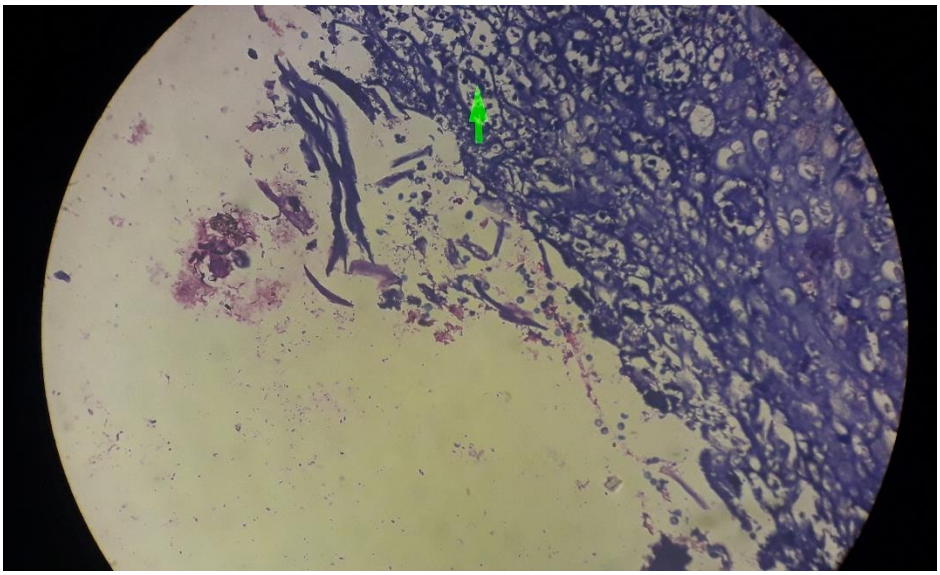

Figure 1: photo micrograph of giemsa stained MDSCC

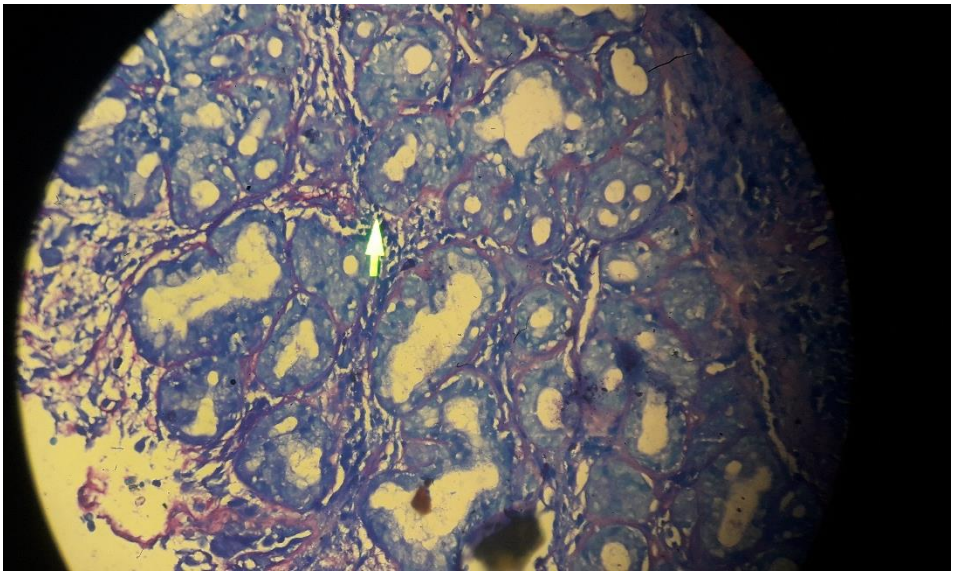

Figure 2: photo micrograph of giemsa stained PDSCC

STATISTICAL ANALYSIS:

The cases included 25 biopsies from female (67.9%) and 53 from male patients (32.1%). The cases include 47 oropharyngeal SCC (60.3%), 5 genitourinary SCC (6.4%), 10 cutaneous (12.8%) and 16 gastrointestinal SCC (20.5%).

Table 1: Frequency table for age

age

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	30-39	7	9.0	9.0	9.0
	40-49	17	21.8	21.8	30.8
	50-59	17	21.8	21.8	52.6
	60-69	15	19.2	19.2	71.8
	70-79	15	19.2	19.2	91.0
	80-89	5	6.4	6.4	97.4
	Above 90	2	2.6	2.6	100.0
	Total	78	100.0	100.0	

Table 2: Frequency table for gender

gender

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	f	25	32.1	32.1	32.1
	m	53	67.9	67.9	100.0
	Total	78	100.0	100.0	

Table3: Frequency table for category of SCC

category

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Oropharyngeal SCC	47	60.3	60.3	60.3
	Gastrointestinal SCC	16	20.5	20.5	80.8
	Cutaneous	10	12.8	12.8	93.6
	Genitourinary SCC	5	6.4	6.4	100.0
	Total	78	100.0	100.0	

Table 4: T-test for clinical presentation to eos/10HPF

T-Test (Growth/Ulcer to eos/10HPF giemsa)

Group Statistics

	Growth/Ulcer	N	Mean	Std. Deviation	Std. Error Mean
eos/10HPF giemsa	growth	72	25.86	14.703	1.733
	ulcer	6	24.83	12.254	5.003

Independent Samples Test

Table 5: T-test for macroscopic features of SCC to eso/10HPF

		Levene's Test for Equality of Variances		t-test for Equality of Means						
								Std. Error	95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Difference	Lower	Upper
eos/10HPF giemsa	Equal variances assumed	1.508	.223	.166	76	.868	1.028	6.184	-11.290	13.345
	Equal variances not assumed			.194	6.265	.852	1.028	5.294	-11.795	13.851

The mean number of eos/10HPF in SCC presenting as growth is 25.86 and the mean in ulcer is 24.83. The t-test reveals the results to be insignificant and thus, no relationship was found between macroscopic features of SCC to number of eos/10HPF. The mean number of eos/10HPF in MDSCC is 31.95 which is significantly higher than that in PDSCC (3.65). The t-test showed that the number of eos/10HPF were significantly higher in cases of MDSCC than in PDSCC.

Group Statistics

	type of scc	N	Mean	Std. Deviation	Std. Error Mean
eos/10HPF giemsa	MDSCC	61	31.95	9.507	1.217
	PDSCC	17	3.65	1.412	.342

T-Test

(Type of SCC to eos/10HPF giemsa)

Group Statistics

	type of scc	N	Mean	Std. Deviation	Std. Error Mean
eos/10HPF giemsa	MDSCC	61	31.95	9.507	1.217
	PDSCC	17	3.65	1.412	.342

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
eos/10HPF giemsa	Equal variances assumed	27.478	.000	12.182	76	.000	28.304	2.323	23.676	32.931
	Equal variances not assumed			22.384	68.267	.000	28.304	1.264	25.781	30.827

Table 6: T-test for type of SCC to eos/10HPF

DISCUSSION:

Tumor associated tissue eosinophilia (TATE) is characteristically defined by the presence of eosinophils as a component of peri- or intra-tumoral inflammatory infiltrate which is not associated with any necrosis. Over the past years, many researches are being conducted to

identify the role of immune cells in tumor microenvironment; the mast cells and eosinophils are the most important ones under consideration. However, research has not led to a final decisive conclusion regarding the role of eosinophils in cancer. Tumor associated tissue eosinophilia has been linked to a prognosis varying from good, bad to null effect.

It has been suggested that tissue eosinophils associated with squamous cell carcinoma can predict the invasive nature of the tumor (Jain et al., 2014). Tissue eosinophilia is also associated to regional recurrence of squamous cell carcinoma (Rakesh et al., 2015). The infiltration of the lesion site by eosinophils has also been related to progression of cervical cancer (Xie et al., 2015). The tissue eosinophils are also markedly increased in invasive head and neck carcinomas as compared to non-invasive carcinomas (Alrawi et al., 2005). Another study reveals that the intense tumor associated tissue eosinophilia can predict occult lymph node metastasis in patients with early oral squamous cell carcinoma (Oliveira et al., 2012).

All the above mentioned studies give a contrasting opinion about the role of eosinophils in tumor microenvironment. These studies suggest that eosinophils can mediate tumor growth or rejection by indirectly affecting other cells through the secretion of a number of mediators.

Many stains have been used to identify eosinophils in peripheral blood as well as tissue sections. Immunostains, Hematoxylin & eosin and congo red have been used to identify eosinophils in tissue sections.

Giemsa stain has been used over the past decades to stain blood cells in peripheral blood smears. In human blood samples, Wright Giemsa stain is particularly used to identify eosinophils specifically (Caballero et al., 2020). It stains the bi-lobed nuclei of eosinophils as red which makes eosinophils easy to recognize. A more effective combination stain is also recognized to identify cells in blood samples known as Leishman-Giemsa stain (Akhlaghi and Ahmadi-Hamedani, 2019). To date, the major role of giemsa stain has been to identify Helicobacter Pylori in histopathological samples and it is quite effective in that (Alkhamiss, 2020). This study is in accordance with various other studies that suggest an increase in number of eosinophils in MDSCC than in PDSCC (Bankur, 2016).

CONCLUSION:

Giemsa can be used as a stain to identify tumor associated tissue eosinophilia in histopathological samples of squamous cell carcinoma. There is increased number of tumor

associated tissue eosinophils in MDSCC than in PDSCC of various regions of body which is consistent with many previous researches.

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