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ORIGINAL ARTICLE

Comparison of Substance P Immunolocalization among Oral, Breast, Colorectal and Endometrial Carcinoma to Evaluate its Prognostic and Therapeutic Significance

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ABSTRACT

Present study was conducted to evaluate the expression and immunolocalization of Substance P (SP) in different grades of Oral Squamous Cell Carcinoma (OSCC), Breast Cancer (BC), Colorectal Cancer (CRC) and Endometrial Cancer (EC).

Patients and Methods: 40 OSCC, 22 BC, 30 CRC and 53 EC biopsies were immunohistochemically analyzed with SP antibody. 14 cases (35%) were well differentiated (WD), 14 cases (35%) moderately differentiated (MD) and 12 cases (30%) poorly differentiated (PD) OSCC. 2 cases (9%) were WD, 4 (18%) were MD and 16 (73%) were PD. There were 8 cases (27%) of PD, 14 (46%) MD and 8 cases (27%) of WD- CRC included in this study. In EC, there were 53 cases, 12 (22.69%) WD, 37 (67.8%) MD, 4 (75.4%) PD cases.

Results: 65% of the OSCC, 55% of BC, all cases of CRC and EC were positive for SP. 8% of WD, 93% of MD, 100% PD were SP-positive in OSCC; 50% WD, 75% MD, 50% PD cases were SP-positive in BC; all cases of CRC and EC were SP-positive. Regarding the intensity of SP stain, SP expression was highest (+3) in PD-OSCC, PD-BC, all CRC and PD-EC and vice versa. Expression increased with an increasing grade of tumor in all except CRC.

Conclusions: An association between grade of tumor and SP expression was observed in OSCC, BC and EC while no difference was observed in CRC. SP expression was in the increasing order from well to moderately differentiated cases in OSCC, BC and EC while it was reverse in CRC. We also suggest SP /NK-1R system as a potential therapeutic strategy to inhibit and manage OSCC, BC and EC.

Key words: Breast carcinoma, Oral squamous cell carcinoma, Colorectal carcinoma, Substance P, neurokinin 1 receptor

INTRODUCTION

Cancer is a heterogeneous group of diseases which represents abnormal and unwanted cellular growth with a tendency for local invasion and distant metastasis [1]. About 14.1 million new cases of cancer were diagnosed and 8.2 million deaths were related to cancer in a single year according to international statistics [2].

Oral cancer is the 6th common cancer globally [3]. With respect to oral cancer, tongue cancer incidence rate is increasing very rapidly. Tongue cancer is more common in young generation as compare to older. Major common factors of oral and neck cancer are smoking and alcohol use [4]. In South-Asian countries it it the second most common cancer [5]. Breast cancer (BC) is the leading cause of mortality among women globally [6]. More than 2 million women were diagnosed with BC inn 2018 [7]. BC is the 5th leading cause of death worldwide [8]. About 268,600 new cases of BCs were reported in 2019 [9].

In 2020, the third most common deadly cancer was colorectal cancer (CRC). Estimated 0.9 million individuals were diagnosed with CRC[10]. the death rate due to CRC in 2020 was 850,000. it may be a metastatic disease [11]. Sixth most common cancer is endometrial cancer (EC) in women. Recently there is no specific established screening method to diagnose EC. Endometrial hyperplasia diagnoses by proliferation [12] or lesion of endometrial gland which leads toward EC. It is more common in women with atypical hyperplasia [13]. Mainly it is known as cancer of women reproductive system [14][15]. Excessive use of contraceptive pills can also cause lesion of endometrial gland [16].

Substance P (SP), an 11 aminoacid neuropeptide and neurohormone is a famous member of the Tachykinin family of neuropeptides, encoded by TAC 1 gene [1]. Apart from its nociceptive role, pain and psychiatric disorders [1], it has also been found to be an important player in inflammation, obesity, gastrointestinal diseases [2] as well as cancers [17]. All of these studies were performed on cell lines, not on tissues. SP is released from the fifth cranial nerve, the trigeminal [18]. Three branches of trigeminal, gives innervation to the orofacial [20], mastication muscles [20] and facial muscles.

In a study conducted on mice, xenografter with human gall bladder cancer cells were analyzed to evaluate the SP/NK-1R involvement *in vivo.* SP/NK-1R was highly expressed in gall bladder cancer cells. SP was found to be contributor in cell proliferation, invasion, colonization and increased cellular growth. These effects were reversed with NK-1R antagonists. Furthermore, NF-κB p65 and cancer associated cytokines were increased and Akt inhibitor reversed this effect [21].

To date, SP and NK-1R have been observed to have contribution in underlying pathologies of different diseases and its role in cancer progression is under investigation [22]. Munoz and Covenas suggested that SP and its receptor Neurokinin-1 (NK-1R) are involved in the development and progression of cancer [23]. They reported that BC cells overexpress NK-1R which is associated to the viability of cancer cells and the proliferation is then caused by SP**.** SP-NK-1R is also involved in neoangiogenesis, invasion and metastasis in BC cells [24]. SP is also responsible for neovascularization and hence increased blood flow and tumor progression [25].

NK-1R antagonists may serve as treatment strategy for various carcinomas e.g lung [26]. According to our understanding, SP/NK-1 expression in linked to tumor grade through clinicopathological features and routine histological markers is still indefinite.

MATERIALS AND METHODS

This study was carried out in the Department of Pathology, Fatima Memorial Hospital, Lahore and The University of Lahore Teaching Hospital, University of Lahore, Lahore, Pakistan. 40 cases of OSCC, 22 cases of BC, 30 cases of CC and 53 cases of EC were taken after taking their clinicopathological data comprising of medical and personal history such as any drug of use, tumor site, clinical presentation, size, metastasis, tumor stage.

For staging of cancer American Joint Committee was followed for the purpose of data collection and differentiation of tumor. Grossing, Fixation, Dehydration and clearing (auto processing), Embedding, Sectioning, Staining, Mounting were the steps performed. Same methodology was adopted as done in our previous study [27]. Nuclear staining of SP protein as well as cytoplasmic staining of tumor cells for SP was reported as in our previous study [28].

RESULTS

Mean age was 53.5, 46.65, 45.74 and 45.74 years in OSCC, BC, CC and EC respectively (Table 1). Localization of SP was detected to be cytoplasmic. (Figure 1A: table 1). In MD cases little morphology of cells had been disrupted but so far they could be recognized. In OSCC, 13/14 cases (92.85%) were MD with +2 intensity of SP expression (Figure 1B: Table 1). In PD cases (12 cases, 100%) maximum intensity (+3) of SP was observed. The morphology of cells was extremely distorted and cells couldn't be simply distinguished (Figure 1C: Table 1).

SP expression intensity was maximum (+3) in PD and MD cases (Figure 1D,E,F: Table 1). In CRC, 100% of the cases were SP positive. SP cell stain intensity was high (+3) in WD as compared to MD and PD cases (Figure 1G,H,I: Table 1). Brain tissue was taken as positive control and showed SP positivity with maximum intensity +3(Figure 2). Mean age of EC patients was 45.74 years. 9 cases expressed weak SP positive intensity of +1 grade, 3 should mild or moderate intensity of +2 grade and none of them showed strong intensity. Among the MD cases,, 12 cases expressed +1 intensity, 21 had +2 and 4 had +3 intensity of SP stain. All the PD cases had +3 intensity of SP (Figure 1J,K,L: Table 1).

Table 1: Expression of Substance P in Oral, breast and colorectal carcinoma WD-Well differentiated, MD-Moderately differentiated, PD-Poorly differentiated

SP EXPRESSION								
OSCC (40 cases)			BC (22 cases)		CC (30 cases)		EC (53 cases)	
Mean age (yrs)	53.5		46.65		45.74		45.74	
Positive SP	26	65 %	12	55%	30	100%	53	100
Negative SP	14	38	10	45.4%	0	0	0	0
WD	14 (35%)		2 (9%)		8 (27%)		12 (22.69%)	
Positive	1	7.1	1	50%	8	100%	12	100
Negative	13	92.85	1	50%	0	0	0	0
+1	1	7.1	1	50%	0	0	9	75
+2	0	0	0	0	0	0	3	25
+3	0	0	0	0	8	100%	0	0
MD	14 (35%)		4 (18%)		14(46.6%)		37 (45.3%)	
Positive	13	92.85	3	75%	14	100%	37	100
Negative	1	7.1	1	25%	0	0	0	0
+1	1	7.1	1	25%	0	0	12	32.4
+2	12	85.71	1	25%	9	64.2%	21	56.7
+3	0	0	1	25%	5	35.7%	4	10.8
PD	12 (30%)		16 (73%)		8 (27%)		4 (7.54%)	
Positive	12	100	8	50%	8	100%	4	100
Negative	0	0	8	50%	0	0	0	0
+1	2	16.66	1	6.25%	6	75%	0	0
+2	4	33.33	3	18.75%	2	25%	0	0
+3	6	50	4	25%	0	0	4	100
SP EXPRESSION								
OSCC (40 cases)			BC (22 cases)		CC (30 cases)		EC (53 cases)	
Mean age (yrs)	53.5		46.65		45.74		45.74	
Positive SP	26	65 %	12	55%	30	100%	53	100
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WD	14 (35%)		2 (9%)		8 (27%)		12 (22.69%)	

Positive	1	7.1	1	50%	8	100%	12	100
Negative	13	92.85	1	50%	0	0	0	0
+1	1	7.1	1	50%	0	0	9	75
+2	0	0	0	0	0	0	3	25
+3	0	0	0	0	8	100%	0	0
MD	14 (35%)		4 (18%)		14(46.6%)		37 (45.3%)	
Positive	13	92.85	3	75%	14	100%	37	100
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+2	12	85.71	1	25%	9	64.2%	21	56.7
+3	0	0	1	25%	5	35.7%	4	10.8
PD	12 (30%)		16 (73%)		8 (27%)		4 (7.54%)	
Positive	12	100	8	50%	8	100%	4	100
Negative	0	0	8	50%	0	0	0	0
+1	2	16.66	1	6.25%	6	75%	0	0
+2	4	33.33	3	18.75%	2	25%	0	0
+3	6	50	4	25%	0	0	4	100

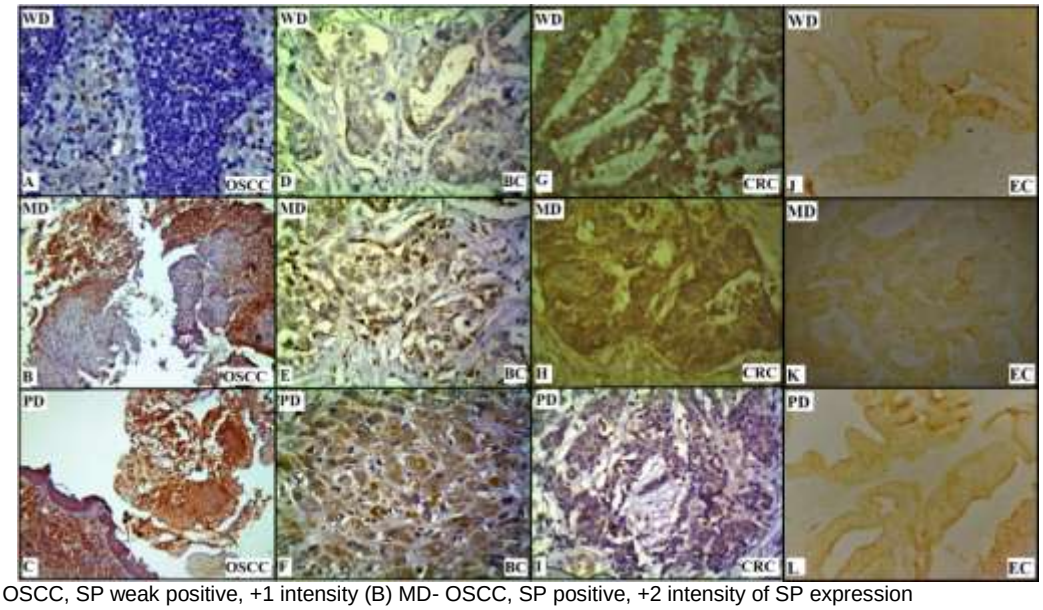

Fig. 1 (A)WD- OSCC, SP weak positive, +1 intensity (B) MD- OSCC, SP positive, +2 intensity of SP expression (C) PD- OSCC, SP strongly positive, intensity +3 (D) WD- BC, SP weak positive, +1 intensity .(E) MD- BC, SP positive, +2 intensity of SP expression (F) PD-BC, SP strongly positive, +3 intensity (G) WD- CRC, SP strongly positive, intensity +3 (H) MD- CRC, SP strong positive, +3 intensity (I) PD-CRC, SP weakly positive (J)WD-EC, SP weak positive, +1 intensity (K) MD- EC, SP positive, +2 intensity of SP expression (L) PD-EC, SP strongly positive, +3 intensity

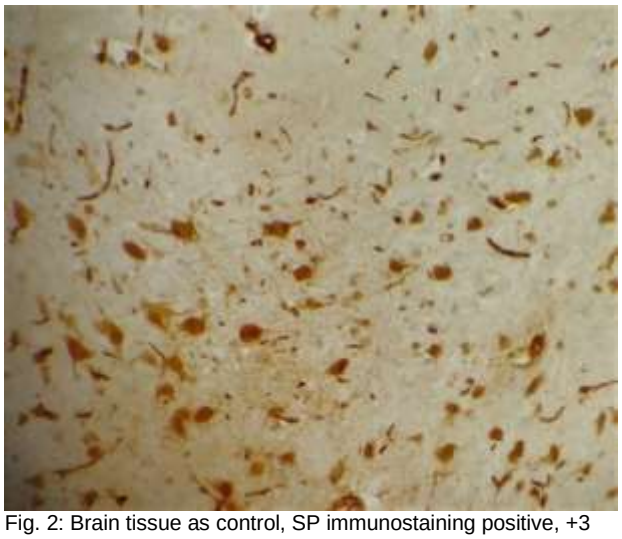

Fig. 2: Brain tissue as control, SP immunostaining positive, +3

DISCUSSION

Formerly, very few studies have been described about appearance of SP various cancers such as oral squamous cell carcinoma, lung cancer, prostate cancer, colorectal cancer etc. But there is insufficient information available about the role of SP in these cancers. Tachykinins may contribute to carcinogenesis by promoting angiogenesis, cell division, proliferation, invasion and regulating cell cycle control. SP is an important member of TK family and its role is mediated by 7-transmembrane receptor NK-1R. Both act as key players for the progression of cancer by initiating the signaling pathways. As a result, translation mechanism is triggered and effector proteins are synthesized that modulate the genetic expression [29][30].

It is suggested that SP activates the mast cells via NK-1R to initiate its nociceptive role [31]. This is actually the underlying pathological mechanism and the signalling cascade of SP. The other mediators involved in this pathway needs to be explored as well. In one of our

previous studies, we tried to explore the other contributors through computational analysis. Nerve growth factor beta polypeptide **(**NGFB), VIP, Neurokinin 1 Receptor (NK-1R), Neurokinin 2 Receptor (NK-2R), Neurokinin 3 Receptor (NK-3R), Tachykinin 3 (TAC3), Neuropeptide S (NPS), Kininogen 1 (KNG1), Neurotensin (NTS), Cholecystokinin (CCK), Gastrin releasing peptide (GRP) and Pro-thyrotropin-releasing hormone (TRH) were also found to be interacting with SP but it needs to be investigated by experimental studies [32].

A case-control study was conducted in Iran to evaluate the level of serum SP and distribution of NK-1R in breast tissue. There were 41 cases and 34 controls. It was found that SP initiates cancer progression, increased vascularizaion, metastasis through NK-1R. Cases showed significant serum SP levels and there was no association with tumor grade. Regarding the tissue distribution of NK-1R, an increased expression and intensity was observed in tumor tissues and it increased with increasing grade. NK-1R expression was found to be cytoplasmic [33].

In our previous study, we have reported a decreased expression of SP in trigeminal nerves of brainstems of SIDS victims, while an amplified expression in sudden intrauterine death victims. It is an obvious indicator of an important respiratory role of SP in brainstems [34]. In alternative study, an increased SP and NK-1R expression was observed in pre-cancerous oral epithelium and a role of SP during the initial stages of carcinogenesis was suggested [35] but in our study we observed an increased expression in later stage of OSCC, BC and EC although it was expressed in all the grades. Hence, SP overexpression with poor prognosis was proposed.

A few studies reported on Substance P, NK-1R and its association with cancer progression but they give only limited information, mostly done on cell lines of mouse model. In one of these studies, 3 miR-206 target sites were identified in 3'-untranslated region of NK-1R gene through *in silico* analysis. RT-PCR was performed for quantification and confirmation of expression of miR-206 and the NK-1R expression was done through immunohistochemistry in 82 BC tissues. miR-206 gene was identified by dual-luciferase reporter assay, RT-PCR and western blotting. Transwell migration and invasion, colony formation and proliferation assays were done to evaluate the effects of miR-206 expression on different aspects of BC cell behaviour *in vitro.* miR-206 was observed to be over-expressed in BC cell lines and tissues but it was inversely correlated with TNM staging and lymph node metastasis [36].

In this study we revealed the SP expression in all grades of breast, oral and colorectal cancer. Expression was commonly positive in breast and oral cancer and the intensity increased with growing grade. Here, SP expression was found to be associated with the poor prognosis and progression of disease. Intensity of expression increased with the growing grade. So, it may be suggested as not only a prognostic but a diagnostic marker as well in these cancers. However, further studies are recommended to confirm the fact by increasing the sample size. Earlier studies on BC cell lines have also shown SP overexpression. As a result of nociceptive stimuli, SP is released from the cancer cells resulting in cellular proliferation [37], metastasis and neovascularization [38]. These functions are carried out by autocrine role while inflammation is triggered by paracrine. Therefore, resulting in increased absorptivity of blood brain barrier [39].

A surge in SP, decreases the apoptosis [40] by regulating the immune mediators IL4, IL6 and IL10 [41] consequently, an uncontrolled cell division, proliferation and thus metastasis. SP binds to its receptor NK-1R as a result of these cascades after phosphorylating the antiapoptotic protein kinase, AKT [42]. But these finding were not studies so far in cancers and need to be explored further.

CONCLUSION

SP can be used as a prognostic marker in OSCC, BC and EC and should be explored for its therapeutic potential along with NK-1R. This biomarker should also be investigated in other carcinomas to evaluate its association with decrease progression.

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