RESEARCH ARTICLE / Araştırma Makalesi

Tryptase and Chymase Expression Differences of Mast Cells in Prostatic Adencarcinomas

Prostatik Adenkarsinomlarda Mast Hücrelerinin Triptaz ve Kimaz Ekspresyon Farklılıkları

Ali Aslan[1], Havva Erdem[2], Hilal Balta[3], Nilüfer Kadıoğlu[4], Feyza Başar[5], Handan Ankaralı[6]

[1] Department of Physiology, Ordu University of Medical Faculty, Ordu, Turkey
[2] Department of Pathology, Ordu University of Medical Faculty, Ordu, Turkey
[3] Department of Pathology, Training Hospital, Erzurum, Turkey
[4] Department of Pathology, Bozüyük State Hospital, Bilecik, Turkey
[5] Department of Pathology, Atatürk State Hospital, Düzce, Turkey
[6] Department of Biostatistics and Medical Informatics, Faculty of Medicine, Medeniyet University, Istanbul, Turkey

Yazışma Adresi / Correspondence:
Ali Aslan
Ordu University, Faculty of Medicine, Department of Physiology, Ordu, Turkey
T: **+90 505 486 82 14** E-mail: **draslan@yahoo.com**

Geliş Tarihi / Received : **24.01.2018** Kabul Tarihi / Accepted : **03.05.2018**

Abstract

Objective Mast cells (MCs) have been shown to have various roles in many tumors. MCs play roles in tumor progression via increasing angiogenesis and lymphangiogenesis. According to the presence of tryptase and chymase granules, mast cells are two varieties containing tryptase-chymase and containing tryptase. The aim of this study was to investigate the presence and distribution of these two mast cell types in PCa in terms of their tryptase and chymase content.
(Sakarya Med J, 2018, 8(2):229-234)

Materials and Methods In this study, immunohistochemical analysis of tryptase and chymase expression was performed retrospectively in 134 patients who have diagnosed PCa histopathologically, at the Department of Pathology of Faculty of Medicine, **** University Educational Research Hospital, between 2012 and 2014. Immunohistochemisrty experiment was done on the appropriate paraffin blocks by using mast cell tryptase and chymase antibodies. The research findings were evaluated statistically and accepted as P <0.05 level.

Results It has been observed that Mast Cell Chymase (MCTC) accumulates in intratumoral areas more than peritumoral areas (respectively; mean: 14,28 ± 14,06, mean: 12,12 ± 12,56). Mast Cell Tryptase (MCT) accumulates more than MCTC in intratumoral and peritumoral areas (P<0,001). MCT was observed to be more frequent in peritumoral areas than in intratumoral areas (respectively; mean: 33,94 ± 20,09, mean:33,50 ± 18,65). MCT and MCTC compared with regard to intratumoral and peritumoral areas. There wasn't a significant difference with MCT (p=0.723). However, MCTC was found the intratumoral area more than the peritumoral area (p=0.007).

Conclusion Mast cells numbers were increased around prostatic adenocarcinoma microenvironment. MCTC was tended to locate intratumoral whereas MCT was tended to the located peritumoral area.

Keywords Tryptase; chymase; prostatic adenocarcinomas; mast cell

Note: This article was presented as a poster at the 27th European Congress of Pathology 5 to 9 September 2015 in Belgrade, Serbia.

Öz

Amaç Mast hücrelerinin (MCs) birçok tümörde çeşitli rolleri olduğu gösterilmiştir. MCs, artan anjiyogenez ve lenfogenez yoluyla tümör progresyonunda rol oynamaktadır. Triptaz ve kimaz granüllerinin varlığına göre mast hücreleri; triptaz- kimaz içeren ve triptaz içeren olmak üzere iki çeşittir. Bu çalışmanın amacı, içeriklerine göre farklı bu iki mast hücre tipinin prostatik adenokarsinomalardaki (PCa) varlığı ve dağılımını araştırmaktır.**(Sakarya Tıp Dergisi, 2018, 8(2):229-234).**

Gereç ve Yöntem Bu çalışmada, 2012-2014 yılları arasında ***** Üniversitesi Tıp Fakültesi Eğitim Araştırma Hastanesi Patoloji Bölümü'nde histopatolojik olarak PCa tanısı alan 134 vakaya ait parafin bloklar kullanıldı. Uygun parafin bloklar seçilerek immunohistokimyasal olarak mast cell triptaz ve mast cell kimaz antikorları ile boyandı. Işık mikroskobunda pozitif boyanan mast hücreleri sayılarak değerlendirildi. İstatistiksel olarak değerlendirilen araştırma bulguları P<0.05 düzeyinde anlamlı kabul edilmiştir.

Bulgular Mast Hücre Kimazının (MCTC), intratümöral bölgelerde peritümöral bölgelere göre daha çok biriktiği gözlenmiştir (sırasıyla ortalama: 14,28 ± 14,06, ortalama: 12,12 ± 12,56). Mast Hücre Triptazı (MCT), intratümöral ve peritümöral bölgelerde MCTC'den daha fazla birikmiştir (P<0,001). MCT'nin peritümöral bölgelerde, intratümöral bölgelere göre, daha fazla olduğu gözlenmiştir (sırasıyla; 33.94 ± 20.09, ortalama: 33,50 ± 18,65). MCT ve MCTC'nin intratümöral alan ve peritümöral alanla ilişkisi karşılaştırıldı. MCT'de anlamlı fark yoktu (p=0.723).

Sonuç Mast hücreleri prostatik adenokarsinom mikroçevresinde sayısal artış göstermektedir. MCTC intratümöral yerleşme eğiliminde iken MCT peritümöral yerleşim eğilimi göstermiştir.

Anahtar Kelimeler Triptaz; kimaz; prostatik adenokarsinomlar; mast hücresi

Introduction

Mast cells (MCs), which are granulated cells, derived from bone marrow, migrate to the other organs and tissues.[1,2] One of the important roles of mast cells is releasing bioactive components. They are distributed in connective tissues neighboring to blood vessels and nerves, and also underneath the epithelial surfaces, and their role on the inflammation is well known.[2-5] MCs contributes to these processes by producing and releasing bioactive agents.

MCs increase angiogenesis, lymphangiogenesis, degradation of extracellular matrix components and mitosis, thus being effective in tumorigenesis.[6,10]

However, recent studies have shown that MCs activate collagen synthesis by activating the fibroblastic process. [8-11]

Tryptase and chymase are stored at different ratios in mast cell-specific granules as proteases.[5-12] The effects of mast cells that degrade extracellular matrix (ECM), enhance tumor spread and metastasis can be generated by these proteases. MCs have affinity to malignant tumors such as cutaneous malignancies, breast cancer and melanoma.[7-14]

On the other hand, connective tissue mast cells and mucosal mast cells, which contain tryptase, are called tryptase mast cells and are localized in the alveoli and small intestine mucosa, while those containing tryptase and chymase are called tryptase-chymase mast cells and located to skin and small intestine submucosa.[15]

Chymase is known to contribute to the stimulation of angiogenesis by providing ECM degradation. Chymase activates latent matrix metalloproteinases (MMPs) to degrade components of epithelial basement membranes and ECM, therefore, it is essential for tumor invasion and metastasis. A similar increase in the number of MCT, a potent proangiogenic factor has been documented in various malignancies including oral cancers.[11-14]

The present study was designed to determine the utility of MCT and MCTC in evaluating malignant prostate lesions (intratumoral area and peritumoral area).

Material and Method

The study protocol was reviewed and approved by the Ethics Committee of ****University with the approval number 2015/8. 134 biopsies were collected from patients with prostate pathology, including 134 cases of prostate cancer. Specimens were fixed in buffer formalin and paraffin embedded. Three-micrometer thick step sections were performed for each case.

Immunohistochemistry included primary antibodies as MCT and MCTC. We performed heat-induced epitope retrieval with pH 6.0 citrate solution (Novocastra, Newcastle upon Tyne, UK) for 30 minutes. Endogenous peroxidase blockage was performed with 3% hydrogen peroxide for 5 minutes. This step was followed by 30 minutes incubation with primary antibodies as MCT (Dako Glostrup Denmark, dilution 1:300, clone AA1) and MCTC (NeoMarkers Fremont, CA, ready to use, clone CC1). Bond Polymer Refine Detection System (Leica Biosystems, Newcastle upon Tyne, UK) was used and 3,3 diamino-benzidine dihydrochloride was applied as chromogen followed by

hematoxylin counterstaining. The individual MCT and MCTC were counted at 200x magnification (Ten peri and intratumoral areas were counted systematically).[16] MCs counting was manually done by calculating the average number for the two selected fields of the intratumoral and peritumoral (benign tissue without tumor) areas. The distribution of positive staining was manually evaluated independently by two experienced observers. All scores provided by the two observers were recorded. The images were made with the optical microscope Nikon camera.

Statistical Analysis

Wilcoxon Signed Ranks Test was used for determining the difference between the MCT and MCTC or intratumoral and peritumoral areas. The findings was shown as the frequency (n), mean, standard deviation (SD), median, IQR (interquartile range), minimum and maximum values. The results were accepted as significant at the level of P<0.05. All the statistical calculations were made with the SPSS 22.0 statistical program.

Results

The following table shows descriptive statistics included number of the MCT and the MCTC in 134 samples (intratumoral and peritumoral areas) (Figure 1-4). There was no significant difference in intratumoral and peritumoral areas of the MCT (P=0.723). However, the average of MCTC in the intratumoral area was higher than in the peritumoral area (P=0.007). Moreover, it was determined that total MCT area (intratumoral area + peritumoral area) was higher than total MCTC area (P<0,001) (Table 1).

Table 1. Intratumoral and peritumoral distribution rates of MC_T and MC_{TC}

	n	Mean	SE	Median	IQR	Minimum	Maximum	P
*MC_T								
intratumoral	134	33,50	1,61	30,00	24,00	0	98	0.723
peritumoral	134	33,94	1,74	29,50	23,00	0	98	
**MC_{TC}								
intratumoral	134	14,28	1,21	9,00	19,25	0	60	0.007
peritumoral	134	12,12	1,09	9,00	17,00	0	66	
Total expression								
MC_T	134	67,44	2,93	65,00	46,25	0	186	<0.001
MC_{TC}	134	26,40	2,12	18,00	31,00	0	115	

*MC_T: Mast Cell Tryptase
**MC_{TC}: Mast Cell Chymase

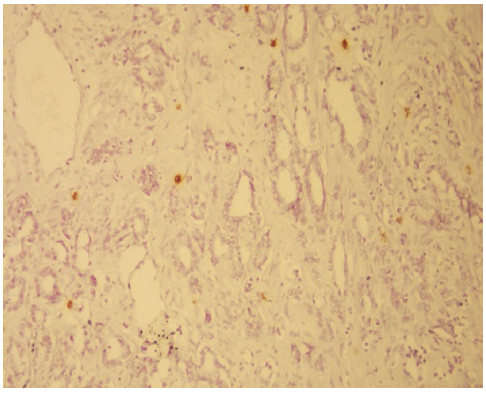

Figure 1. Mast Cell Chymase (MC_{TC}) ekspression in intratumoral area (x200)

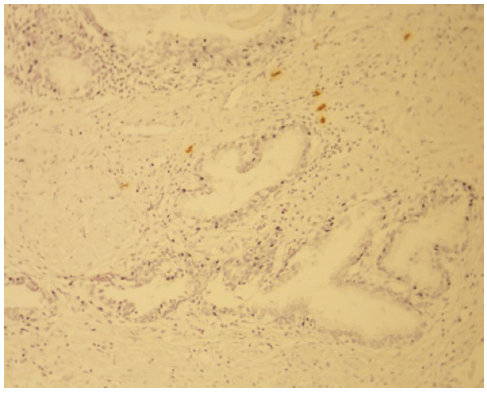

Figure 2. Mast Cell Chymase (MC_{TC}) ekspression in peritumoral area (x200)

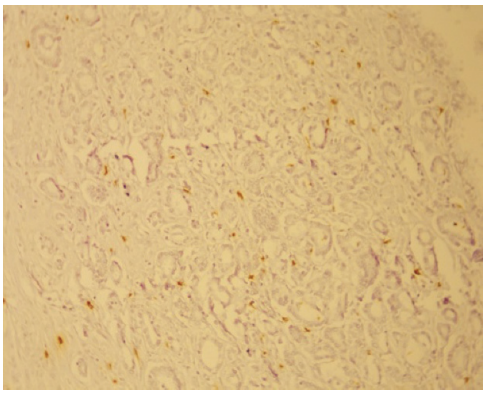

Figure 3. Mast Cell Tryptase (MC_T) ekspression in intratumoral area (x200)

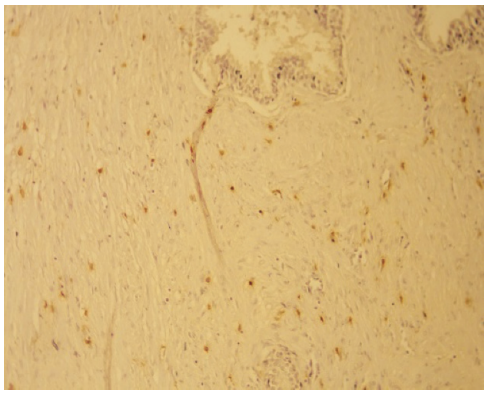

Figure 4. Mast Cell Tryptase (MC_T) ekspression in peritumoral area (x200)

Discussion

Prostate cancer is the second leading cause of cancer deaths in men. Changes in the prostate stroma may play a role in malignant progression as well as in benign prostatic hyperplasia.[14,17,18,19]

In a small number of studies on the role of prostate stromal cells in malignant and benign pathologies of the prostate. It is believed that the effect of stromal microenvironment and oxidative stress is an important factor in the development and progression of adenocarcinoma.[20, 21]

Several studies have shown that MCs may play a role in the pathogenesis of various tumors, such as melanocytic skin lesions or squamous cell carcinomas.[22, 23] It is also showed the relationship between MCs and tumors due to their cytotoxic action on tumor cells or their ability to deliver products with an anti-tumor effect.[24-26] It is not known that MCs are designed to stimulate the spread of tumor cells or inhibit of them.[27,28] However, several conflicting results have been reported about the correlation between the accumulation of MCs and survival in various cancers.[27-31] Some authors have considered mast cells as a target for cancer therapy. In addition, these researchers assessed the possible clinical relevance of mast cell degranulation in some malignant tumors.[29-32] Strouch et al. reported that MCT accumulating in tumor tissue may be a useful marker for distinguishing malignancies from benign tumors.[33] Previous studies that have revealed increased mast cell density in oral squamous cell carcinoma (OSSC) tissue, level of MCT was increased in the serum in OSCC patients' sera, but there was no significant difference between this level in the patients.[33,34] De Souza et al. presented that their results revealed changes in the expression of some mast cell-specific chymases, tryptase and carboxypeptidase A during tumor progression. After all, they have suggested that the role of mast cells during tumor progression can be directly related to their granule content.[35] Also, increased mast cell density has been shown in oral and esophageal squamous cell carcinoma (SCC).3[6,37] The positive correlation has been shown between mast cell density and poor prognosis of OSCC, even though there are controversies around this issue.[36-39] Globa et al. reported that tryptase-positive MCs density decreased in the intratumoral versus peritumoral areas. They reported that the significant correlation between tryptase and chymase in peritumoral areas of malignant lesions suggests a new mast cell phenotype, tryptase+chymase+CD117+. In addition, they reported that chymase involvement in malignant transformation is supported by the existence of partial correlations with Gleason score and chymase-positive MCs located peritumorally. They also reported that tryptase-positive MCs density decreased in the intratumoral versus peritumoral areas. Intratumor MCs were distributed among isolated tumor cells. They noted that in 13 cases (20.3%) intratumoral density of tryptase-positive MCs was higher than in the peritumoral areas.[21]

In this study, contrary to others, chymase increased intratumoral area but thryptase increased peritumoral area. The rise of mast cells around tumor microenvironment was obvious by a general evaluation. The accumulation of MCT was more than MCTC in intratumoral and peritumoral area. MCTC positive mast cells contain tryptase, therefore MCTC positive mast cells were MCT positive, too.

Carlini et al. reported that microvascular dansity was significantly higher in the peritumoral area than in the intratumoral area for total MCs and for tryptase-chymase phenotypes.[40] Erdem et al. showed that increased mast cell number but not its relation to prognostic parameters in prostate adenocarcinoma.16 Yadav et al. reported that subepithelial and deep distribution of tryptase and chymase positive mast cells play an important role in the pathogenesis of malignant transformation.[41] Cabanillas-Saez et al. have shown an increased number of MCT type mast cells in invasive uterine cervix carcinoma when compared with normal tissue. In addition, significantly increased numbers of mast cells were detected also in the close vicinity to the epithelium in cervical intraepithelial neoplasia and invasive carcinoma but not in the stroma.[42]

Mast cells stimulate angiogenesis in tumorigenesis but they have also other roles in these processes. For example, mast cell mediators such as tryptase and histamine cause tumor progression, not these mediators also affect immune response, therefore, supports tumorigenesis. Today, it is believed that the concentration and location of mast cells mediators and cofactors showed anticancer or procancer properties.[27] Studies conducted on mast cells containing chymase have led to the conclusion that chymase may be associated with tumor cell proliferation and metastasis.43 In this study, mast cell distribution containing chymase was found to be mostly intratumoral (P=0.007). It is thought to be associated with tumor progression.

In conclusion, recent studies appear to suggest that MCT and MCTC may represent a promising target in cancer treatment due to its proangiogenic activity.[43-45] However, the character of MCT and MCTC expression in cancer types needs to be understood better. In our study, MCT expression in prostate carcinoma (intratumoral areas and peritumoral areas) were observed to be higher. However MCTC expression in intratumoral areas were observed to be higher than peritumoral areas. MCTC might more important than MCT in prostate carcinomas formation.

1. Galli SJ, Nakae S, Tsai M. Mast cells in the development of adaptive immune responses. Nat Immunol 2005;6:135-42.
2. Sayed BA, Christy A, Quirion MR, Brown MA. The master switch: The role of mast cells in autoimmunity and tolerance. Annu Rev Immunol 2008;6:705-39
3. Slatter A, Smallman LA, Drake-Lee AB. Increasein epithelial mast cell numbers in the nasal mucosa of patientswith perennial allergic rhinitis. J Laryngol Otol 1996;110:929-933.
4. Yakanaka K, Fujisawa M, Tanaka H, Okada H, Arakawa S, Kamidono S. Significance of human testicularmast cells and their subtypes in male infertility. Human Reproduction 2000;15:1543-1547.
5. Gupta RK. Mast cell variations in prostate and urinary bladder. Arch Pathol 1970;89:302-305.
6. Ribatti D, Crivellato E. Mast cells, angiogenesis and cancer. Adv Exp Med Biol. 2011; 716: 270-88.
7. Ching S, Wallis RA, Yuan L, Davis PF, Tan ST. Mast cells and cutaneous malignancies. Mod Pathol 2006;19:149-159.
8. Yong LC. The mast cell: origin, morphology, distribution, and function. Exp Toxicol Pathol 1997;49:409-424.
9. Ribatti D, Crivellato E. The controversial role of mast cells in tumor growth. Int Rev Cell Mol Biol 2009;275:89-131.
10. Artuc M, Steckelings M, Henz BM. Mast cell–fibroblast interactions: human mast cells as source and inducer of fibroblast and epithelial growth factors. J Invest Dermatol 2002;118(3):391-5.
11. Fukushima H, Ohsawa M, Ikura Y, Naruko T, Sugama Y, Suekane T, et al. Mast cells in diffuse large B-cell lymphoma; their role in fibrosis. Histopathology 2006;49:498-505.
12. Caughey GH. Mast cell tryptases and chymases in inflammation and host defense. Immunological reviews. 2007;217:141-154.
13. Desai RS, Mamatha GS, Khatri MJ, Shetty SJ. Immunohistochemical expression of CD34 for characterization and quantification of mucosal vasculature and its probable role in malignant transformation of atrophic epithelium in oral submucous fibrosis. Oral Oncol 2010;46:553-558.
14. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57-70.
15. Irani AA, Schwartz LB. Neutral protease as indicators of human mast cell heterogeneity. In: Schwartz LB, ed. Neutral proteases of mast cells. Basel Karger: Monogr Allergy 1990;146-162.
16. Erdem H, Kayikci MA, Oktay M, Uzunlar AK, Tekin A, Sener E, et al. Mast cells numbers and peritumoral microvessel density of the prostatic adenocarcinomas and correlation with prognostic parameters. Med Glas (Zenica) 2013;10(2):293-297.
17. Liotta LA, Kohn EC. The microenvironment of the tumour-host interface. Nature 2001;411:375-379.
18. Matrisian LM, Cunha GR, Mohla S. Epithelial-stromal interactions and tumor progression: meeting summary and future directions. Cancer Research 2001;61:3844-3846.
19. Park CC, Bissell MJ, Barcellos-Hoff MH. The influence of the microenvironment on the malignant phenotype. Mol Med Today 2000;6:324-329.
20. Tuxhorn JA, Ayala GE, Rowley DR. Reactive stroma in prostate cancer progression. J Urol 2001;166:2472-2483.
21. Globa T, Şaptefr i L, Ceauşu RA, Gaje P, Cimpean AM, Raica M. Mast cell phenotype in benign and malignant tumors of the prostate. Pol J Pathol 2014;65(2):147-153.
22. Orhan N,Özcan ME, Memişoğulları R, Uçgun T, Kayıkçı MA, Demirin H.Prostat Kanserli Hastalarda Oksidatif Stres ve Paraksonaz Aktivite Azalması. Konuralp Tıp Dergisi 2015;(7)2:113-117.
23. Dyduch G, Oko K, Pescarini E. Mast cells in melanocytic skin lesions. An immunohistochemical and quantitative study. Pol J Pathol 2011;62:139-144.
24. Pyziak L, Stasikowska-Kanicka O, Danilewicz M, W growska-Danilewicz M. Immunohistochemical analysis of mast cell infiltrates and microvessel density in oral squamous cell carcinoma. Pol J Pathol 2013;64:276-280.
25. Farram E, Nelson DS. Mouse mast cells as anti-tumor effector cells. Cellular Immunology 1980; 55:294-301.
26. Ghiara P, Boraschi D, Villa L, Scapigliati G, Taddei C, Tagliabue A. In vitro generated mast cells express natural cytotoxicity against tumor cells. Immunology 1985;55:317-324.
27. Dyduch G, Kaczmarczyk K, Oko K. Mast cells and cancer: enemies or allies? Pol J Pathol 2012;63:1-7.
28. Dimitriadou V, Koutsilieris M. Mast cell-tumor cell interactions: for or against tumor growth and metastasis? Anticancer Res 1997;17:1541-1549.
29. Henderson WR, Chi EY, Jong EC, Klebanoff SJ. Mast cell-mediated tumor cell cytotoxicity. Role of the peroxidase system. JEM 1981;153:520-533.
30. Masaki T, Matsuzaki Y, Onitsuka T. Correlation between mast cells and survival rates in patients with pulmonary adenocarcinoma. Lung Cancer 1999;26:103-108.
31. Nechushtan H. The complexity of the complicity of mast cells in cancer. Int J Biochem Cell Biol 2010;42:551-4.
32. Samoszuk M, Corwin MA. Mast cell inhibitor cromolyn increases blood clotting and hypoxia in murine breast cancer. Int J Cancer 2003;107:159-63.
33. Strouch MJ, Cheon EC, Salabat MR, Krantz SB, Gounaris E, Melstrom LG, et al. Crosstalk between mast cells and pancreatic cancer cells contributes to pancreatic tumor progression. Clin Cancer Res 2010;16:2257-65.
34. Iamaroon A, Surawut P, Sumana J. Increase of mast cell and tumor angiogenesis in oral squamous cell carcinoma. J Oral Pathol Med 2003;32:195-9.
35. De Souza DA Jr, Toso VD, Campos MR, Lara VS, Oliver C, Jamur MC.Expression of mast cell proteases correlates with mast cell maturation and angiogenesis during tumor progression. PLoS One 2012;7(7):e40790.
36. Elpek G, Gelen T. The prognostic relevance of angiogenesis and mast cells in SCC of the esophagus. J Clin Pathol 2001;54:940-4.
37. Rojas IG, Spencer ML, Martínez A, Maurelia MA, Rudolph MI. Characterization of mast cell subpopulation in lip cancer. J Oral Path Med 2005;34:268-273.
38. Jaafari-Ashkavandi Z, Moshref M, Mashhadi-Abbas F, Sargolzaie S, Taghavi N. Evaluation of CD31 expression and mast cell count in dysplastic lesions and squamous cell carcinoma of the oral cavity. Iran Red Crescent Med J 2010;12:272-6.
39. Tinge B, Molin D, Bergqvist M, Ekman S, Bergström S. Mast cells in squamous cell esophageal carcinoma and clinical parameters. Cancer Genomics Proteomics 2010;7:25-9.
40. Carlini MJ, Dalurzo MC, Lastiri JM, Smith DE, Vasallo BC, Puricelli LI, Lauría de Cidre LS. Mast cell phenotypes and microvessels in non-small cell lung cancer and its prognostic significance.Hum Pathol 2010;41(5):697-705.
41. Yadav A, Desai RS, Bhuta BA, Singh JS, Mehta R, Nehete AP. Altered immunohistochemical expression of mast cell tryptase and chymase in the pathogenesis of oral submucous fibrosis and malignant transformation of the overlying epithelium. PLoS One 2014;9(5):e98719.
42. Cabanillas-Saez A, Schalper JA, Nicovani SM, Rudolph MI. Characterization of mast cells according to their content oftryptase and chymase in normal and neoplastic human uterinecervix. Int J Gynecol Cancer 2002;12:92-98.
43. Jiang Y, Wu Y, Hardie WJ, Zhou X. Mast cell chymase affects the proliferation and metastasis of lung carcinoma cells in vitro. Oncol Lett 2017 Sep; 14(3): 3193–3198.
44. Ammendola M, Leporini C, Marech I, Gadaleta, CD, Scognamillo G, Sacco R et al. Targeting Mast Cells Tryptase in Tumor Microenvironment: A Potential Antiangiogenetic Strategy. BioMed Research International 2014;2014:154702.
45. De Souza Junior DA, Santana AC, da Silva EZM, Oliver C, Jamur MC. The Role of Mast Cell Specific Chymases and Tryptases in Tumor Angiogenesis. BioMed Research International 2015;2015:142359.