ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Which Material Should Be Used for Mast Cell Evaluation in Gastric Cancer: Endoscopic Material or Resection Material?

Mide Kanserinde Mast Hücresi Değerlendirmesinde Hangi Material Kullanılmalı: Endoskopik Materyal mi, Rezeksiyon Materyali mi?

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ABSTRACT

Aim: *Histopathological examination has an important place in the evaluation of parameters that are important in the prognosis of gastric tumors. In addition to the prognostic data included in the guidelines, other observed findings that may be important for the tumor behavior are also evaluated in the histopathological examination. Mast cells, which are among the elements of the immune system, are among these findings. In this study, it is aimed to compare the endoscopic biopsy materials and resection materials, which have the potential to be used for the evaluation of mast cells.*

Material and Method: *Nineteen gastric tumor cases with endoscopic biopsy and resection material belonging to the same patient were included in the study. Toludine blue histochemistry was applied to the sections obtained from the paraffin blocks of the preparations representing the tumor. In the light microscopic evaluation, the area with the highest concentration of mast cells was selected at 100× magnification, and then 100 cells were counted inside and around the tumor at 400× magnification. Mast cells staining positively with toludine blue were noted in these 100 cells. Mann-Whitney-U was used in the analysis of the significance of mast cell number between groups, and Pearson's test was used in the correlation between groups.*

Results: *In the endoscopic biopsy material, the mean number of mast cells inside the tumor (MCIT) was 1.32±2.65, the mean number of mast cells around the tumor (MCAT) was 1.0±1.76; in the resection materials, the average number of MCIT was calculated as 4.84±4.86, and the average number of MCAT was calculated as 5.63±6.99. A statistically significant difference was observed between the number of MCIT (p=0.001) and the number of MCAT (p=0.000) between endoscopic biopsies and resection materials in the analyzes. When all the materials were included in analysis, it was determined that the number of MCIT and the number of MCAT showed a positive correlation. However, when endoscopic biopsies and resection materials were compared, it was noted that there was no correlation in terms of MCIT or MCAT.*

Conclusion: *Mast cells, which are an important element of the immune response, are evaluated with different aspects in gastric cancers as in various tumors. Considering the importance of tumor and tumor microenvironment analysis as well as the results of the presented study, it is thought that mast cells, which have the potential to be an important marker in gastric tumors in the future, should be evaluated in the resection material, and endoscopic material evaluations do not reflect the real picture.*

Key words: *gastric cancer; mast cell; endoscopic biopsy; resection material*

ÖZET

Amaç: *Mide tümörlerinin prognozunda önemli olan parametrelerin değerlendirilmesinde histopatolojik inceleme önemli bir yer tutmaktadır. Histopatolojik incelemede kılavuzlarda yer alan prognostik verilerin dışında gözlenen tümör davranışı için önemli olabilecek diğer veriler de değerlendirilmektedir. İmmun sistem elemanları arasında yer alan mast hücreleri bu veriler arasında yer almaktadır. Bu çalışmada mast hücrelerinin değerlendirilmesi için kullanılabilme potansiyeli olan endoskopik biyopsi materyalleri ile rezeksiyon materyallerinin karşılaştırılması amaçlanmaktadır.*

Materyal ve Metot: *Çalışmaya aynı hastaya ait endoskopik biyopsi ve rezeksiyon materyali bulunan 19 mide tümörü olgusu dâhil edilmiştir. Tümörü temsil eden preparatlara ait parafin bloklardan elde edilen kesitlere toludin blue histokimyası uygulanmıştır. Işık mikroskopik değerlendirmede mast hücrelerinin en yoğun olduğu alan 100× büyütmede seçilmiş ve sonrasında 400× büyütmede tümör içinde ve çevresinde 100 hücre sayılmıştır. Bu 100 hücrenin içinde yer alan toludin blue ile pozitif boyanan mast hücreleri not edilmiştir. Mast hücresi sayısının gruplar arası anlamlılığı analizlerinde Mann-Whitney U, gruplar arası korelasyonda Pearson testi kullanılmıştır.*

Bulgular: *Endoskopik biyopsi materyalinde tümör içinde yer alan mast hücre sayısı (TİMH) ortalama 1,32±2,65, tümör çevresi mast hücresi sayısı (TÇMH) ortalama 1,0±1,76; rezeksiyon materyallerinde TİMH sayısı ortalama 4,84±4,86, TÇMH sayısı ortalama 5,63±6,99 olarak hesaplanmıştır. Analizlerde endoskopik biyopsiler ve rezeksiyon materyalleri arasında TİMH sayısı (p=0,001) ve TÇMH sayısı (p=0,000) arasında istatistiksel anlamlı farklılık izlenmiştir. Tüm olgular incelendiğinde TİMH sayısı ile TÇMH sayısının pozitif korelasyon gösterdiği saptanmıştır. Ancak endoskopik biyopsiler ile rezeksiyon materyalleri kıyaslandığında TİMH veya TÇMH açısından herhangi bir korelsayon olmadığı dikkati çekmiştir.*

Sonuç: *İmmun yanıtın önemli bir unsuru olan mast hücreleri çeşitli tümörlerde olduğu gibi mide kanserlerinde de farklı yönleri ile değerlendirilmektedir. Sunulan çalışma sonuçları yanısıra tümör ve tümör mikroçevre incelemesinin önemi göz önünde bulundurulduğunda gelecekte mide tümörlerinde önemli bir belirteç olma potansiyeli bulunan mast hücrelerinin rezeksiyon materyalinde değerlendirilmesi gerektiği, endoskopik materyal değerlendirmelerinin gerçek tabloyu yansıtmadığı düşünülmektedir.*

Anahtar kelimeler: *mide kanseri; mast hücresi; endoskopik biyopsi; rezeksiyon materyali*

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Introduction

Gastric cancer is the fifth most commonly diagnosed cancer worldwide in both gender, responsible for more than 1,000,000 new cases and an estimated 783,000 deaths in 2018[1]. Although survival rates have increased in the last few decades due to current treatment protocols, managing the disease still poses significant challenges[2]. As the stage of stomach cancer increases, the survival rate decreases significantly. Although the 5-year survival rate of non-metastatic gastric cancer cases is over 50%, the 5-year survival rate decreases to 30% because most of the diagnoses are made in advanced stages[3,4].

One of the most important risk factors for gastric cancer is *Helicobacter pylori* infection among the modifiable factors. Other modifiable risk factors include tobacco history, socioeconomic status, and salty meat consumption, and non-modifiable risk factors include age, gender, race, and genetics[5,6].

Histopathological examination has an important place in the evaluation of parameters that are important in the prognosis of gastric tumors. Histopathologically, tumor localization, histological type, histological grade, tumor depth (pT stage), condition of surgical margins, treatment effect in the presence of neoadjuvant therapy, lymphovascular and perineural invasion, regional lymph node metastases (pN stage), presence of distant metastases (pM stage), presence of intestinal metaplasia, presence of low/high grade dysplasia, presence of *Helicobacter pylori gastritis*, presence of autoimmune chronic atrophic gastritis, presence of polyps are evaluated[7].

The immune system is vital in controlling tumor growth and progression[8]. Gastric cancer cells have the ability to modulate the immune system and evade detection[9]. Mast cells are a group of innate immune cells that have immunomodulatory effects on tumor progression, such as angiogenesis, tumor microenvironment reconstruction, and interaction with other immune cells[10–13]. However, many unexplained areas remain regarding the phenotype, functional regulation and clinical correlation of mast cells in the human gastric cancer microenvironment. Studies investigating the effect of mast cells on prognosis in gastric cancer have shown that the presence and amount of mast cells, tryptase activity, and various parameters of the tumor and survival are related[14–17]. This raises the question of "how to evaluate mast cells, which can be used as a biomarker?" in gastric cancer. In this context, the current study evaluated the correlation between the number of mast cells observed inside and/or around the tumor, and between endoscopic biopsies and resection materials.

Materials and Methods

The study is approved by the Kafkas University Faculty of Medicine Ethical Committee (11.03.2021-02). 19 gastric tumor cases with endoscopic biopsy and resection material belonging to the same patient diagnosed in Kafkas University Health Research and Application Center between 01.06.2014 and 01.06.2016 were included in the study. Cases without pathology archive material were not included in the study. Hematoxylin&eosin stained slides of the cases were obtained from the pathology archive and the diagnosis was confirmed. Then, the slides representing the tumor were selected, sections of 4 micron thickness were taken from the blocks of these slides, and histochemical staining was performed with toludine blue (Sigma-Aldrich, St Louis, USA) in accordance with the kit's instruction manual.

Toludine blue stained slides were evaluated by a light microscope (Olympus BX46, Japan). During the evaluation, the area with the highest concentration of mast cells was selected at 100× magnification, and then 100 cells were counted in and around the tumor at 400× magnification. Mast cells staining positively with toludine blue were noted in these 100 cells.

Statistical Package for Social Sciences (SPSS) program version 15.0 package program (Released 2006. SPSS program for Windows, Version 15.0, SPSS Inc. Chicago, USA) was used for statistical analysis. Mann Whitney-U test was used to analyze the significance of mast cell number between groups, and the Pearson test was used for intergroup correlation. Values with $p<0.05$ were considered statistically significant in the 95% confidence interval analyses.

Results

In the evaluation of endoscopic biopsy material of 19 gastric tumor cases, the mean number of mast cells in the tumor was 1.32 ± 2.65 (minimum 0, maximum 10), and the mean number of mast cells around the tumor was 1.0 ± 1.76 (minimum 0, maximum 7). When the resection materials were evaluated, the mean number of mast cells in the tumor was 4.84 ± 4.86 (minimum

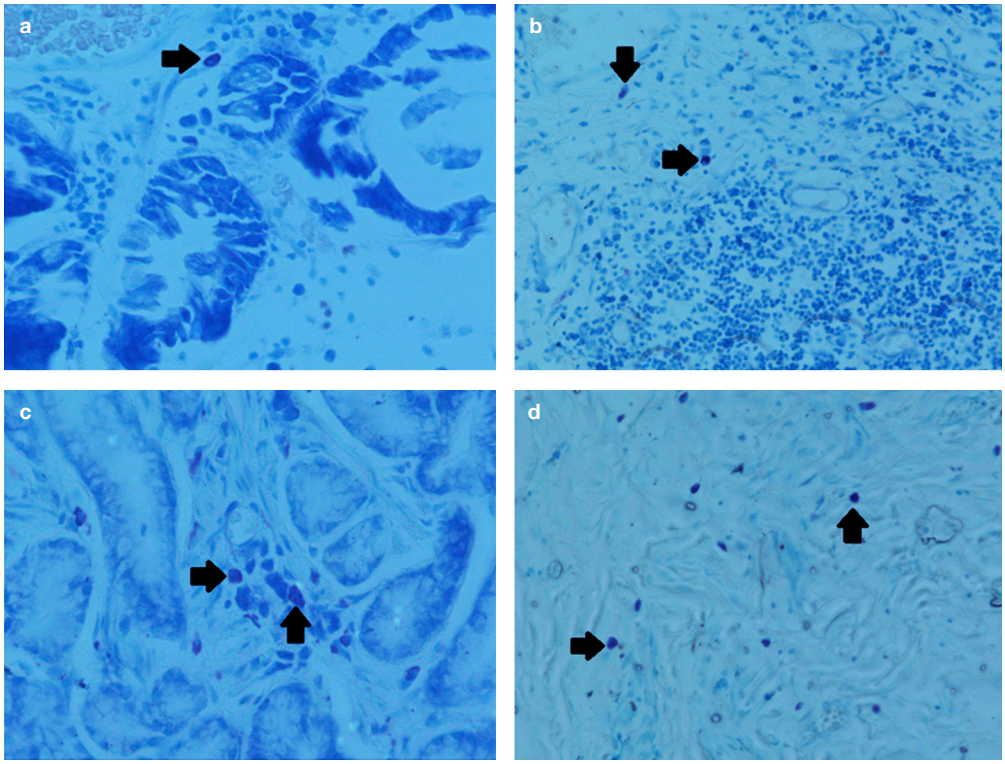

Figure 1. *a–d. Intra-tumoral mast cells (black arrow) in endoscopic biopsy material, Toludine blue, 400× (a); peri-tumoral mast cells (black arrow) in endoscopic biopsy material, Toludine blue, 200× (b); intra-tumoral mast cells (black arrow) in resection material, Toludine blue, 400× (c); peri-tumoral mast cells (black arrow) in resection material, Toludine blue, 200× (d).*

0, maximum 17), and the mean number of mast cells around the tumor was 5.63±6.99 (min 0, max 30). Intratumoral and peritumoral mast cells of endoscopic biopsy and resection materials were demonstrated in Fig. 1.

In the statistical analyzes performed, a statistically significant difference was observed between the endoscopic biopsies and resection materials in the number of mast cells within the tumor (p=0.001) and the number of mast cells around the tumor (p=0.000). When all cases were examined, it was found that the number of mast cells in the tumor and the number of mast cells around the tumor showed a positive correlation with the Pearson test (p=0.000, correlation coefficient 0.704). Similarly, when endoscopic cases and resection materials were examined separately, it was observed that the number of intra-tumor mast cells in the endoscopic material (p=0.003, correlation coefficient 0.643) and the resection material were positively correlated with the number of mast cells around the tumor (p=0.002, correlation coefficient 0.660). However, when endoscopic biopsies and resection materials were compared, it was noted that there was no correlation in terms of intra-tumoral mast cells (p=0.074, correlation coefficient -0.419). In addition, no correlation was observed between the endoscopic biopsies and the resection material when the mast cells around the tumor were compared (p=0.325, correlation coefficient -0.239).

Discussion

Gastric cancer is among the leading causes of death from cancer in the world[1]. In gastric cancer, in which the incidence and mortality rates increase with age 18, histopathological examination remains at a key point. A significant portion of the data proven to be of prognostic importance on the tumor is obtained through histopathological examination. These data are presented in standard pathology reports according to current guidelines[7]. However, apart from the known prognostic important data, observationally different findings form the basis for studies that will affect the behavior of the tumor, and therefore, the treatment and prognosis. Inflammatory response to tumors and tumor immunology are frequently encountered as one of these issues.

The immune system is critical in tumorigenesis and the control of tumor growth and progression[8]. In a healthy immune system, under normal conditions, tumor cells with abnormal genetic structure and behavior are detected and eliminated before they turn into detectable malignancies. However, as in many cancer types, malignant cells in gastric cancer can modulate the immune system and evade detection[9].

Mast cells exert their immunomodulatory effects on tumor progression mainly through angiogenesis, tumor microenvironment reconstruction, and interaction with other immune cells[10–12]. This system, which plays a critical role in maintaining normal microenvironment tissue homeostasis, is an obstacle to tumorigenesis. However, an abnormal microenvironment alters homeostasis and creates the necessary environment for tumorigenesis. The inflammatory microenvironment containing mast cells, macrophages, lymphocytes, neutrophils, and natural killer cells may form the basis for tumorigenesis[19]. This raises the question of whether mast cells are a factor or an inhibitor in tumor development. Mast cells are long-lived cells and have the potential to respond quickly to changes in their microenvironment. By degranulation, they can release large amounts of immunomodulatory compounds and cause a massive proinflammatory response in and around the tumor, which may be detrimental to cell survival[20]. However, as tumor proliferation with critical granules containing trophic or mitogenic factors, the medium can also lead to the formation of an enriching microenvironment[21].

A study evaluating the relationship between gastric cancer and mast cells reported that the percentage of mast cells increased significantly in the advanced stages of the tumor, and mast cells might induce tumor progression. In the same study, it is suggested that the increase in the percentage of mast cells is positively correlated with the overall survival of gastric cancer patients[22]. Similarly, another study stated that there is a significant positive correlation between the number of infiltrating mast cells in gastric cancers and clinical features such as tumor size. In addition, it has been reported that the overall survival rate of the patient is lower independently in patients with increased intratumoral mast cell count, and disease-free survival is inversely correlated with intratumoral mast cell levels[21]. Tryptase is considered an indicator of mast cell activity. It has been reported that tryptase-positive mast cells are correlated with new vessel formation in gastric cancers[22]. In a study published in 2017, it was reported that tryptase expression is an independent predictor of overall survival and recurrence-free survival in gastric cancer cases; It has been suggested that the combination of tryptase expression and tumor-node-metastasis (TNM) stage has higher prognostic power than each of these markers alone in predicting survival[16]. A study that included both gastric and colorectal cancers found that tryptase-positive mast cells in the primary tumor tissue showed a positive correlation with the number of metastatic lymph nodes, regardless of tumor staging or location[17].

As seen in the studies, mast cells infiltrating the tumor, whether they have positive or negative effects on tumorigenesis, have the potential to be used as a useful clinical prognostic marker in the future. Future targeted treatment protocols may include blocking the protumorigenic effects of tumor-infiltrating mast cells and/or increasing their proinflammatory activity. In this context, the detection of the mast cell gains importance. In our technical study on which pathological material would be more appropriate to detect the mast cell, the mean number of intra-tumoral mast cells in all materials was 3.08±4.25, and the mean number of mast cells around the tumor was 3.32±5.55. When endoscopic biopsy and resection materials were evaluated, it was noted that the mean of intra-tumoral ($p=0.001$) and peri-tumoral ($p=0.000$) mast cells in endoscopic materials was statistically significantly lower than that of resection material. Endoscopic biopsies are thought to contain a more limited area for selection, although the same amount and quality of areas are selected.

When the number of intra-tumoral mast cells and the number of peri-tumoral mast cells were evaluated individually in endoscopic cases ($p=0.003$, correlation coefficient 0.643) and resection materials ($p=0.002$, correlation coefficient 0.660), it was observed that there was a statistical correlation in both material types. This indicates that the tumor microenvironment shows a similar distribution of mast cells in the tumor area. However, no correlation was found when the endoscopic and resection materials were compared with intra-tumoral (Pearson correlation coefficient -0.419, $p=0.074$) and peri-tumoral (Pearson correlation coefficient -0.239, $p=0.325$) mast cells. This indicates that intra- and peri-tumor mast cells in endoscopic materials are not representative of those in resection materials. The statistically significant difference in the mean of mast cell numbers also confirms this proposition.

As a result of our study, the necessity of using resection material that better represents the tumor and tumor microenvironment has been demonstrated in the evaluation of mast cells, which can potentially be an important marker in gastric tumors in the future. In addition, it is thought that endoscopic biopsy materials should not be used in the evaluation of mast cells because they cannot adequately express the tumor and its microenvironment, and the adequacy of endoscopic biopsies should be questioned in studies to be conducted in terms of other possible different markers.

Statement of Ethics

The study is approved by the Kafkas University Faculty of Medicine Ethical Committee (11.03.2021-02).

Conflict of Interest Statement

All the authors declare no conflict of interest.

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