

# Cytokines the Etiology of Idiopathic Granulomatous Mastitis

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## ABSTRACT

**Objective:** To investigate the roles of cytokines in the etiopathogenesis of idiopathic granulomatous mastitis (IGM).

**Design:** Case-control study.

**Place and Duration of Study:** Istanbul Training and Research Hospital, Istanbul, Turkey, from September 2020 to January 2021.

**Methodology:** Idiopathic Granulomatous Mastitis patients in active or remission who were admitted to the breast diseases outpatient clinic and healthy volunteers were included prospectively in the present study. The IL-1 $\beta$ , IFN- $\alpha$ 2, IFN- $\gamma$ , TNF- $\alpha$ , MCP-1, IL-6, IL-8, IL-10, IL-12p (p70), IL-17A, IL-18, IL-23 and IL-33 values were measured with Flow Cytometry. The blood samples were taken before the treatment in the active IGM group. The ages, physical examination findings, menopausal and smoking conditions, and treatment methods were also evaluated.

**Results:** A total of 32 patients including 19 patients with active and 13 in remission, and 18 controls, were inducted making up a total of 50 subjects. The mean age was 37.18 $\pm$ 7.15. The IL-1 $\beta$ , TNF- $\alpha$ , IL-10, and IL-18 values were lower in patients with IGM than in the control group. Granulomatous Mastitis patients smoked more than the healthy participants. When the active patients, remission patients, and control group were evaluated together, no significant differences were detected in cytokine levels.

**Conclusion:** The autoimmune and granulomatous reactions may not play a role in the etiopathogenesis of IGM because of the low levels of Th1 and Th17-related cytokines. However, some to baseline reference ranges were established.

**Key Words:** Idiopathic granulomatous mastitis, Cytokine, Autoimmunity, Smoking.

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## INTRODUCTION

Idiopathic granulomatous mastitis (IGM) is a rare chronic and inflammatory disease of the breast. It was identified by Kessler and Wolloch in 1972 for the first time.<sup>1</sup> It is observed more frequently in the premenopausal period, especially in women at reproductive age with a history of breastfeeding.<sup>2</sup>

Granulomas occurs when a great number of inflammatory cells are organized, especially mononuclear phagocytes. An infectious agent or foreign body triggers the formation of granulomas in general.<sup>3</sup> Among granulomatous reactions with known etiology, there are infections such as tuberculosis, sarcoidosis, fungal infections, and autoimmune diseases, such as giant-cell arteritis.

Although granulomatous mastitis is an inflammatory disease resulting in the formation of granulomas, its etiology has not yet been elucidated.<sup>4</sup> However, no significant etiological factors were distinguished in the blood-related parameters of autoimmunity in previous studies.<sup>5</sup>

Cytokines are small proteins, which regulate the growth, differentiation, maturation, and response of certain cell types. There is generally an increase in IL-4, IL-5, and IL-13 in allergic diseases; IL-17, and IL-23 in autoimmune diseases; IL2, Interferon Gamma (IFN- $\gamma$ ) levels in sarcoidosis and granulomatous diseases.<sup>6</sup>

The aim of this study was to evaluate blood cytokines levels with a large cytokine panel, which is important in inflammation to evaluate the role of these cytokines in IGM development and possible etiologies.

## METHODOLOGY

Patients attending the Breast Diseases outpatients Clinic, Istanbul Training and Research Hospital, Istanbul, Turkey, diagnosed with IGM on histopathological examination; patients who were previously diagnosed with IGM and patients who were in remission between September 2020 and January 2021; and

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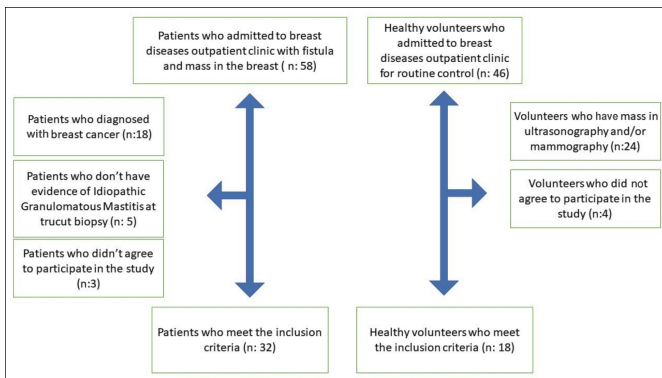
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healthy volunteers who did not have any breast diseases complaints or findings in breast ultrasonography and/or mammography, were included. The study was performed after receiving the approval of the Local Human Ethics Committee. Patients were informed about the study, and informed consent were obtained from all the patients. The study was registered with a public trials system after ethics committee approval (NCT04540237).

Inclusion criteria of the study were admitting with pain, fistula and mass complaint in the breast, detecting inflammatory lesion in ultrasonography, evaluation of trucut biopsy as IGM, and negative tuberculosis tests for active granulomatous mastitis patients; for patients with remission, having IGM diagnosed histopathologically in history, lack of active discharge, redness, and mass complaints, and if treatment was applied for IGM previously, having finished the treatment at least three months ago; and for healthy volunteers, no mass in ultrasonography and/or mammography, no lesions with fistula in the breast, and no mastitis history in the past. Exclusion criteria of the study were pregnancy, having any type of cancer, and suspected malignancy in trucut biopsy (Figure 1).



**Figure 1: Flow diagram of the patients and healthy volunteers.**

Five cc blood was taken into tubes with jelly from the volunteers. The serum was stored after the necessary labeling was made at  $-80^{\circ}\text{C}$  freezer. After all of the samples were collected, serum samples were dissolved and prepared according to user guidelines of the cytokine measurements kit which is Human Inflammation Panel 1 (13-plex) with Filter Plate Kit (LEGENDplex™, Biolegend, USA, Cat. no: 740808). Cytokine measurement performed on the same day in flow cytometry (FACSLyric™, BD, USA) for IL-1 $\beta$ , IFN- $\alpha$ 2, IFN- $\gamma$ , TNF- $\alpha$ , MCP-1, IL-6, IL-8, IL-10, IL-12p (p70), IL-17A, IL-18, IL-23 and IL-33. The ages, physical examination findings, menopausal status, smoking status, blood cytokine levels, and treatment methods of the volunteers were recorded and compared among groups.

Statistical analyses were performed with SPSS version 17.0 Program. The suitability of the variables to normal distribution was checked with histogram graphs and Kolmogorov-Smirnov Test. Mean, standard deviation for normally distributed variables, and median (IQR) values for non-normally distributed results were used in presenting descriptive analyses. Categorical variables were compared with the Pearson Chi-square test.

Those with normal distribution were evaluated with Student T-test and groups that did not show normal distribution were evaluated with Mann Whitney U-test. Comparison of the four groups was performed with one-way ANOVA in those with normal distribution and with Kruskal Wallis test in those who did not show normal distribution. When the p-value was below 0.05, it was evaluated as statistically significant.

## RESULTS

A total of 50 patients, including 32 patients and 18 healthy individuals, were included in the study. The mean age was  $37.18 \pm 7.15$  years, and all were premenopausal. A total of 19 patients were in the active group, and 13 were in the remission group. According to the physical examination findings of active IGM patients mass, redness, and pain were universally present (100% each), with fistula (78.9%), and abscess (84.2%) formation in the majority.

In the evaluation among patients and control group, IL-1 $\beta$ , TNF- $\alpha$ , IL-10, and IL-18 values were significantly higher in the control group than in patients' group (Table I). When smoking rates were compared, it was observed that the patients smoked significantly more than the healthy volunteers (62.5% vs. 33.3%, respectively,  $p:0.048$ ).

When control, active, and remission groups were compared, no significant differences were detected in the parameters among the groups other than smoking rates (Table II). When smoking rates were compared among the three groups, it was found that the active group smoking rate was significantly higher than that in the remission and control groups (78.9%, 38.5%, and 33.3% respectively,  $p:0.011$ ).

No significant differences were detected in any parameters except smoking in parameters evaluated in active and remission groups. It was found that active patients smoked more than those in remission (78.9% vs. 38.5%, respectively,  $p = 0.020$ ).

When active IGM group and control group were compared, IL-1 $\beta$ , TNF- $\alpha$ , IL-10, IL-18 values were statistically higher in control group ( $p = 0.030$ ,  $p = 0.040$ ,  $p = 0.045$ , and  $p = 0.033$ , respectively). The smoking rate in active IGM patients was significantly higher compared to the Control Group (78.9%, and 33.3%, respectively,  $p = 0.005$ ). There were no statistically significant differences between the remission and control groups in all the parameters.

Systemic steroids were used for six patients in the active and seven patients in remission group, local steroids were used for ten patients in the active and four patients for in remission group as the treatment method. No treatment-spontaneous regression was used for the rest of the IGM patients.

## DISCUSSION

The etiology of IGM has not yet been clearly elucidated, and clinical symptoms can be confused with different diseases. Many different treatment methods are tried to explain dark spots in the diagnosis and etiology.

**Table I: Distribution of the cytokine levels of the patient and healthy volunteers (SD: standard derivation).**

	Patients Mean ± SD	Control Mean ± SD	p-value
Age (year)	35.94±6.16	39.39±8.36	0.102
IL-1β (pg/ml)	5.03±4.32	9.93±9.72	0.027
IFN-α2 (pg/ml)	2.96±0.12	2.96±0.14	0.841
IFN-γ (pg/ml)	4.30±3.55	7.31±8.75	0.485
TNF-α (pg/ml)	8.01±8.41	15.23±15.73	0.033
MCP-1 (pg/ml)	93.805±56.11	139.75±110.99	0.208
IL-6 (pg/ml)	18.75±39.44	19.94±22.95	0.269
IL-8 (pg/ml)	43.51±85.07	28.25±30.62	0.299
IL-10 (pg/ml)	4.01±2.55	6.38±5.09	0.034
IL-12p70 (pg/ml)	4.27±0.59	4.78±1.53	0.186
IL-17A(pg/ml)	4.36±0.26	4.35±0.28	0.986
IL-18 (pg/ml)	91.96±66.96	121.56±50.17	0.029
IL-23 (pg/ml)	6.79±5.64	12.02±12.67	0.430
IL-33(pg/ml)	4.27±1.06	4.86±2.23	0.560

**Table II: Evaluation of the cytokine levels examined among all the groups (SD: standard derivation).**

	Active Mean ± SD	Remission Mean ± SD	Control Mean ± SD	p-value
Age (year)	34.68 ±6.41	37.77±5.51	39.39±8.36	0.127
IL-1β (pg/ml)	4.65±2.07	6.25±6.34	9.93±9.72	0.081
IFN-α2 (pg/ml)	2.98±0.15	2.92±0.01	2.96±0.14	0.398
IFN-γ (pg/ml)	3.73±0.73	5.14±0.52	7.31±8.75	0.784
TNF-α (pg/ml)	6.74±3.12	9.84±12.73	15.23±15.73	0.097
MCP-1 (pg/ml)	100.99±58.10	81.45±53.16	139.75±110.99	0.287
IL-6 (pg/ml)	22.48±49.78	13.60±15.46	19.94±22.95	0.539
IL-8 (pg/ml)	55.36±98.99	26.21±58.73	28.25±30.62	0.538
IL-10 (pg/ml)	3.70±1.15	4.45±3.28	6.39±5.09	0.104
IL-12p70 (pg/ml)	4.15±0.26	4.44±0.86	4.78±1.53	0.186
IL-17A (pg/ml)	4.36±0.26	4.36±0.27	4.35±0.28	1.000
IL-18 (pg/ml)	87.94±59.04	97.83±79.33	121.56±50.17	0.090
IL-23 (pg/ml)	5.91±1.92	8.06±8.59	12.02±12.67	0.438
IL-33 (pg/ml)	4.02±0.35	4.63±1.58	4.86±2.23	0.697

It is stated in some studies that there is a relation between IGM and some hormonal diseases, oral contraceptives, autoimmunity, various microorganisms, smoking, and alpha-1 antitrypsin deficiency.<sup>7</sup>

One of these most likely reasons emphasized is autoimmunity.<sup>8</sup> The evidence reported as a cause for this is using steroids and immunosuppressive in treatment, the togetherness with non-breast findings, such as erythema nodosum and arthritis, and immunohistochemically T lymphocyte dominance.<sup>7,9</sup> The markers which are related to autoimmune diseases such as serum RF, complement, ANCA, c- ANCA, interleukin 2 receptor, and angiotensin-converting enzyme, were not different in IGM patients in many previous studies.<sup>5</sup>

Another marker related to autoimmunity is cytokines. A very small number of studies in the literature evaluate the relations between IGM and IL-17, IL-23, and IL-33.<sup>10-12</sup> It was found that the IL-33 levels were significantly higher in IGM patients compared to control group in the study limited to only about IL-33.<sup>12</sup> In this study, IL-33 levels did not have any differences in active nor in remission patients compared to the control group. According to referred study in the literature, which evaluated the relationship between IGM and IL-23 with 26 IGM patients, which is another cytokine associated with autoimmunity, it was determined that IGM patients had significantly higher IL-23 values compared to 15 healthy

volunteers.<sup>8</sup> In this study, however, no significant differences were detected in 32 IGM patients compared to 18 healthy volunteers.

Basically, T helper 17 (Th17) and IL-17A play a significant role in the early response to many extracellular pathogens and in the chronic inflammatory response to chronic infection, allergy, and autoimmunity.<sup>13</sup> There are only two studies in the literature evaluating the relation between IL-17 and IGM. No significant differences were detected between IGM patients and the control group in one of them; however, in the other study, IGM patients had significantly high levels of IL-17.<sup>8,14</sup> In this study, no significant difference was detected between the groups in terms of IL-17A levels.

Chronic Granulomatous Disease (CGD) develops after defect in NADPH oxidase complex and proceeds with dysregulated inflammation and granuloma formations. NADPH regulates IL-8 mRNA negatively.<sup>15</sup> IL-8 levels are elevated because of this in chronic granulomatous patients. Elevated IL-8 levels were detected in both active and remission IGM patients compared to the control group in the only study in the literature evaluating IL-8 levels in IGM patients.<sup>14</sup> Although the results were not statistically significant in our study, IL-8 levels were high in IGM patients compared to the control group and IL-8 levels were higher in active IGM patients than in other groups.

IL-10, which plays an anti-inflammatory role, was high in IGM patients compared to the control group in the said study in the literature evaluating the relation between IGM and IL-10. When active, remission, and control groups were compared, it was found that this difference continued.<sup>14</sup> IL-10 levels were lower than the control group statistically significant in IGM patients in this study. However, this significance disappeared when active, remission, and control groups were compared. This result supports immune dysregulation occurring in IGM patients.

In a previously reported case, granulomatous mastitis developed as a result of anti-TNF treatment in a patient who was diagnosed with psoriasis.<sup>16</sup> In another study, it was found that TNF- $\alpha$  values were lower in IGM patients than in the control group, although not at significant levels.<sup>14</sup> TNF- $\alpha$  value was significantly lower in IGM patients compared to the control group in this study. In the subgroup analyses, a significant decrease was detected in TNF- $\alpha$  values in active IGM patients compared to healthy volunteers.

There are no studies evaluating the relation between IL-1 $\beta$ , IFN- $\gamma$  and IGM patients according to the literature review. In this study, it was found that IFN- $\gamma$  levels, were lower in IGM patients than in the control group, however, these differences were not significant. When we evaluated the relation between IL-1 $\beta$ , IL-18, IL-12p70, and IGM, it was found that IL-1 $\beta$  and IL-18 values were significantly higher in the control group than in IGM patients, however, IL-12p70 was not different in IGM patients than in the control group. IL-1 $\beta$  maturation is controlled by a multiprotein complex called the inflammasome (NLRP3). This Inflammasome contains an enzyme called caspase 1, which is required for the activation of both IL-1 $\beta$  and IL-18.<sup>17</sup> IL12 and IL 18 also play a significant role on T helper 1 response and releasing IFN- $\gamma$ , TNF- $\alpha$ .<sup>18</sup> The authors believe that the low levels of TNF- $\alpha$ , IL-18, and IFN- $\gamma$  in IGM patients show that Inflammasome Th1, which plays role in the release of these cytokines, may not play roles in IGM etiopathogenesis.

One of the limitations of this study is the small sample size. The other limitation is lack of immunophenotyping of the patients. The reference values of cytokines for these patients could not be determined because of the very small number of studies conducted on IGM patients and the low number of patients. The reason for different results from literature data might be that the cytokine measurement method was bead-based immunoassay on Flow Cytometry instead of ELISA. More studies are needed with these two methods for the optimization of conclusions.

## CONCLUSION

Cytokines, which play roles in known autoimmune and Granulomatous reactions, especially cytokines related to Th1 and Th17, might not play a role in the etiopathogenesis in GM patients. Different mechanisms may also be effective.

## ETHICAL APPROVAL:

The study was performed after receiving the approval of the Istanbul Training and Research Hospital Clinical Research Ethics Committee.

## PATIENTS' CONSENT:

Informed consent was obtained from all the patients included in the study to publish the data.

## COMPETING INTEREST:

The authors declared no competing interest.

## AUTHORS' CONTRIBUTION:

CC, UOI: Designed the study.

CC, AEN, EF, MG: Collected the data.

CC, AEN, EF, MG, UOI: Analysed the data and wrote the manuscript.

UOI: Critically reviewed the paper.

All authors approved the final version of the manuscript to be published.

## REFERENCES

1. Kessler E, Wolloch Y. Granulomatous mastitis: A lesion clinically simulating carcinoma. *AM J Clin Pathol* 1972; **58(6)**:642-6. doi: 10.1093/ajcp/58.6.642.
2. Wang J, Xu H, Li Z, Li F, Yang Y, Yu X, et al. Pathogens in patients with granulomatous lobular mastitis. *Int J Infect Dis* 2019; **81**:123-7. doi: 10.1016/j.ijid.2019.01.034.
3. Lacambra M, Thai TA, Lam CC, Yu AM, Pham HT, Tran PV, et al. Granulomatous mastitis: The histological differentials. *J Clin Pathol* 2011; **64(5)**:405-11. doi: 10.1136/jcp.2011.089565.
4. Diesing D, Axt-Flidner R, Hornung D, Weiss JM, Diedrich K, Friedrich M. Granulomatous mastitis. *Arch Gynecol Obstet* 2004; **269(4)**:233-6. doi:10.1007/s00404-003-0561-2. Doi: 10.1007/s00404-003-0561-2.
5. Illman JE, Terra SB, Clapp AJ, Hunt KN, Fazzio RT, Shah SS, et al. Granulomatous diseases of the breast and axilla. Radiological findings with pathological correlation. *Insights Imaging* 2018; **9**:59-71. doi: 10.1007/s13244-017-0587-9.
6. Zhou F, Yu LX, Ma ZB, Yu ZG. Granulomatous lobular mastitis. *Chronic Dis Transl Med* 2016; **2**: 17-21. doi: 10.1016/j.cdtm.2016.02.004.
7. Borish LC, Steinke JW. 2. Cytokines and chemokines. *J Allergy Clin Immunol* 2003; **111(2 Suppl)**:S460-75. doi: 10.1067/mai.2003.108.
8. Saydam M, Yilmaz KB, Sahin M, Yanik H, Akinci M, Yilmaz I, et al. New findings on autoimmune etiology of idiopathic granulomatous mastitis: Serum IL-17, IL-22 and IL-23 levels of patients. *J Invest Surg* 2020; **11**:1-5. doi: 10.1080/08941939.2020.1725190.
9. Gumus M, Akkurt ZM, Gumus H. Is erythema nodosum coexisting with lesions of the breast a suggestive sign for idiopathic granulomatous mastitis? *Turk J Surg* 2018; **34**: 71-3. doi: 10.5152/turkjsurg.2017.3161.
10. Grant CR, Liberal R, Holder BS, Cardone J, Ma Y, Robson SC, et al. Dysfunctional CD39(POS) regulatory T cells and aberrant control of T-helper type 17 cells in autoimmune

- hepatitis. *Hepatol* 2014; **59(3)**:1007-15. doi: 10.1002/hep.26583.
11. Zhao J, Zhang Z, Luan Y, Zou Z, Sun Y, Li Y, et al. Pathological functions of interleukin-22 in chronic liver inflammation and fibrosis with hepatitis B virus infection by promoting T helper 17 cell recruitment. *Hepatol* 2014; **59(4)**:1331-42. doi: 10.1002/hep.26916.
  12. Yigitbasi MR, Guntas G, Atak T, Sonmez C, Yalman H, Uzun H. The role of interleukin-33 as an inflammatory marker in differential diagnosis of idiopathic granulomatous mastitis and breast cancer. *J Invest Surg* 2017; **30(4)**:272-6. doi: 10.1080/08941939.2016.1240270.
  13. Crome SQ, Wang AY, Levings MK. Translational mini-review series on Th17 cells: Function and regulation of human T helper 17 cells in health and disease. *Clin Exp Immunol* 2010; **159**:109. doi: 10.1111/j.1365-2249.2009.04037.x.
  14. Koksai H, Vatansev H, Artac H, Kadoglou N. The clinical value of interleukins-8, -10, and -17 in idiopathic granulomatous mastitis. *Clin Rheumatol* 2020; **39(5)**: 1671-7. doi: 10.1007/s10067-020-04925-8.
  15. Lekstrom-Himes JA, Kuhns DB, Alvord WG, Gallin JI. Inhibition of human neutrophil IL-8 production by hydrogen peroxide and dysregulation in chronic granulomatous disease. *J Immunol* 2005; **174(1)**:411-7. doi: 10.4049/jimmunol.174.1.411.
  16. Garraud T, Barbarot S, Berthelot JM, Le Goff B. Occurrence of a granulomatous mastitis and aseptic osteitis after anti-TNF therapy in a patient with pustular psoriasis. *Joint Bone Spine* 2016; **83(1)**:107-8. doi: 10.1016/j.jbspin.2015.04.007.
  17. Guarda G, Dostert C, Staehli F, Cabalzar K, Castillo R, Tardivel A, et al. T cells dampen innate immune responses through inhibition of NLRP1 and NLRP3 inflammasomes. *Nature* 2009; **460(7252)**:269-73. doi: 10.1038/nature08100.
  18. Shigehara K, Shijubo N, Ohmichi M, Takahashi R, Kon S, Okamura H, et al. IL-12 and IL-18 are increased and stimulate IFN-gamma production in sarcoid lungs. *J Immunol* 2001; **166(1)**:642-9. doi: 10.4049/jimmunol.166.1.642.

