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Beyond Inflammation: Decoding the Bacterial Landscape of Granulomatous Mastitis

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ABSTRACT

Background: Idiopathic granulomatous mastitis (IGM) is a rare, chronic inflammatory breast disease with an unclear etiology. This study aimed to investigate the potential microbial involvement in IGM by detecting bacterial DNA in biopsy samples.

Methods: This cross-sectional study included 22 patients with histopathologically confirmed IGM, selected through convenience sampling from Besat Hospital, Sanandaj, Iran, in 2019. DNA was extracted from biopsy samples, and the 16S rRNA gene was amplified using universal primers. The amplified products were sequenced, and bacterial species were identified using NCBI BLAST.

Results: The mean age of the patients was 35.23 years. DNA analysis revealed *Escherichia coli* in 21 of 22 samples (95.5%) and *Staphylococcus lugdunensis* in 1 sample (4.5%). The most common inflammatory symptom was *erythema*, observed in 8 patients (36.4%), while *deep collections* were the most frequent tissue abnormality, found in 10 patients (45.5%).

Conclusion: The detection of *E.coli* in most samples suggests a potential bacterial role in IGM pathogenesis. Further research, including control samples from normal breast tissue, is needed to validate these findings and evaluate the potential benefits of molecular testing in clinical practice.

INTRODUCTION

Idiopathic granulomatous mastitis (IGM), first reported by Kessler and Wolloch in 1972, presents a significant treatment challenge. IGM is an uncommon, recurrent inflammatory breast disease with a benign prognosis and an elusive pathogenesis.[1,2] Although prevalence data for this rare condition in women remains scarce, it has been reported as 0.37%, equivalent to 37 cases per 10,000 women in the USA and 0.24%, or 24 cases per 10,000 women, in Europe.[3,4] The disease typically peaks around age 30 and is more common in women with a history of breastfeeding and pregnancy. Interestingly, non-breastfeeding cases have been linked to antidepressant use, leading to hyperprolactinemia [5] Clinically, IGM often mimics breast carcinoma, complicating diagnosis and occasionally resulting in incorrect treatment. Recent findings suggest a racial association, with most reported cases originating from the Middle East and Mediterranean regions.[3,5]

The etiology of IGM remains unclear due to its rarity and complex presentation. Various hypotheses have been proposed, including autoimmune reactions, infectious agents—particularly bacterial species—and hormonal dysregulation.[6] While IGM

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predominantly affects women around the age of 30, the precise mechanisms underlying its development continue to be explored. Some studies suggest an association with bacterial infections, including *Corynebacterium kroppenstedtii*.[7] Bacterial involvement appears to differ by region, with studies highlighting the prevalence of specific bacterial strains in different populations.[8] Additionally, prolonged breastfeeding duration and hyperprolactinemia remain significant hormonal factors associated with disease onset.[9] While antibiotic therapy has been explored as a potential treatment for IGM, its effectiveness remains inconsistent. Recent studies indicate that certain antibiotics may be effective, particularly in cases with an identified bacterial component, but results vary based on the pathogen involved and the patient's immune response.[10–12] In this study, we focused on identifying the etiologic agent and detecting bacterial genes in clinical samples.

METHODS

Demographic data of patients

In this cross-sectional study conducted at Besat Hospital in Sanandaj, Iran, in 2019, 22 patients with histopathologically confirmed IGM were included using a convenience sampling method from individuals presenting to the hospital during the study period. Inclusion criteria were histopathologically confirmed IGM cases, while exclusion criteria were patients with known infectious or autoimmune conditions. Patients were selected via convenience sampling from those presenting to Besat Hospital in 2019. Demographic and histopathologic characteristics were collected and analyzed to describe the features of this condition. Descriptive statistics were applied to summarize the data. Continuous variables, such as age and breastfeeding duration, were described using means and standard deviations. Categorical variables, including marital status, contraceptive use, and affected breast, were presented as frequencies with percentages.

Microbiological assay

Stocked biopsies collected from patients were used for culture and DNA extraction. About 100µL of homogenized samples were streaked on sheep blood agar and incubated for 48 hours at 37°C. DNA was extracted from biopsy samples using a kit (Favorgen, Taiwan). PCR was performed using the following universal primers: 27F: AGRGTTYGATYMTGGCTCAG and 1492R: RGYTACCTTGTTACGACTT under standard Polymerase chain reaction (PCR) conditions to find the 16S rRNA gene of any bacterial strain.[13] For PCR, we used a master mix (ParsToos, Iran) for 50µL, including 50ng DNA and 0.4µM of each primer. PCR products were visualized in a 1% gel electrophoresis. The PCR products were sequenced by the Sanger method, and the results were analyzed using NCBI BLAST.

RESULTS

A comprehensive analysis was conducted which provides valuable insights into the demographic, reproductive, and clinical characteristics of IGM patients. The demographic data underscores the role of reproductive history, particularly pregnancy and breastfeeding, while the presence of *Escherichia coli* in 21 out of 22 cases (95.5%) supports the hypothesis of bacterial involvement. Additionally, hormonal factors, such as prolonged breastfeeding and contraceptive use, may influence disease susceptibility. Clinically, the predominance of deep collections and erythema aligns with inflammatory processes commonly associated with bacterial infections. Collectively, these findings highlight the significance of the presented data in understanding IGM's complex etiology, addressing concerns about its relevance.

Demographic data of patients

The demographic characteristics of the 22 patients with IGM are presented in Table 1. The mean age of diagnosis was 35.23 years (SD, 5.53), with the youngest patient being 23 years old and the oldest 44 years old. The average height was 162.5 cm (SD, 7.48), ranging from 150 cm to 178 cm, while the mean weight was 76.82 kg (SD, 10.72), with values ranging from 50 kg to 97 kg, with considerable variability, potentially highlighting the role of metabolic factors in disease development. All 22 patients (100%) were married. Regarding reproductive history, the mean number of pregnancies was 2.14 (SD, 1.08), with 9 patients (40.9%) reporting only one pregnancy, and 5 patients (22.7%) reporting three or more pregnancies. The mean number of abortions was 0.32 (SD, 0.57), with 17 patients (77.3%) having no history of abortion and 5 patients (22.7%) having experienced at least one abortion. The mean number of children was 1.77 (SD, 0.87), with 11 patients (50.0%) having 1 child and 4 patients (18.2%) having 3 or more children. Bacterial DNA analysis revealed the presence of *E. coli* in 21 out of 22 cases (95.5%), while *S. lugdunensis* was detected in 1 case (4.5%), suggesting a potential bacterial etiology in IGM pathogenesis.

The reproductive and hormonal factors associated with IGM are outlined in Table 2. The mean breastfeeding duration among the patients was 39.0 months (SD, 19.73), with 7 patients (31.8%)

Table 1. Characterization of demographic factors in idiopathic granulomatous mastitis patients.

No	Bacteria	Marital status	Age at diagnosis	Height	Weight	Number of pregnancies	Number of abortions	Number of children
HM1	*E. coli*	Married	38	162	78	1	0	1
HM2	*E. coli*	Married	40	170	85	3	1	2
HM3	*E. coli*	Married	41	156	68	1	0	1
HM4	*E. coli*	Married	33	158	78	3	0	2
HM5	*E. coli*	Married	35	157	56	1	0	1
HM6	*E. coli*	Married	37	161	86	2	0	2
HM7	*E. coli*	Married	26	170	80	1	0	1
HM8	*E. coli*	Married	30	165	85	1	0	1
HM9	*E. coli*	Married	40	165	78	3	0	3
HM10	*E. coli*	Married	38	150	71	3	1	2
HM11	*E. coli*	Married	44	155	75	3	1	2
HM12	*E. coli*	Married	32	157	5	3	0	3
HM13	*E. coli*	Married	27	178	78	1	0	1
HM14	*E. coli*	Married	44	150	90	4	0	4
HM15	*E. coli*	Married	31	170	85	2	0	2
HM16	*E. coli*	Married	23	163	76	1	0	1
HM17	*E. coli*	Married	38	154	67	2	0	2
HM18	*E. coli*	Married	38	173	97	3	2	1
HM19	*E. coli*	Married	36	161	77	3	1	2
HM20	*E. coli*	Married	32	169	70	1	0	1
HM21	*E. coli*	Married	36	163	73	1	0	1
HM22	*S. lugdunensis*	Married	36	168	87	4	1	3

breastfeeding for exactly 24 months and 5 patients (22.7%) breastfeeding for 48 months or longer. The mean age at first pregnancy was 23.23 years (SD, 5.53), with 3 patients (13.6%) reporting their first pregnancy at age 18, and 4 patients (18.2%) becoming pregnant for the first time at age 30 or older. The mean menarche age was 13.45 years (SD, 1.22). Among the patients, 15 individuals (68.2%) experienced menarche at 13 or 14 years, while 2 individuals (9.1%) reported menarche before the age of 12.

Table 2. Conditions associated with IGM patients

No	Breastfeeding period by month	Age of first pregnancy	Menarche age	Taking contraceptives or hormone therapy	Family history of the disease	Breast size	Affected breast
HM1	24	24	13	No	No	Medium	Right
HM2	39	20	13	Yes	No	Medium	Bilateral
HM3	24	27	12	No	No	Small	Bilateral
HM4	48	19	14	No	No	Small	Right
HM5	18	30	15	No	No	Medium	Bilateral
HM6	48	19	14	No	No	Medium	Left
HM7	18	21	13	No	No	Medium	Right
HM8	24	26	16	No	No	Medium	Bilateral
HM9	72	20	13	No	No	Large	Bilateral
HM10	48	17	11	Yes	No	Medium	Left
HM11	48	36	14	No	No	Medium	Right
HM12	72	18	12	No	No	Large	Bilateral
HM13	24	21	15	Yes	No	Medium	Bilateral
HM14	72	25	14	Yes	No	Large	Left
HM15	48	18	12	No	No	Large	Right
HM16	24	19	12	No	No	Medium	Bilateral
HM17	27	28	14	No	No	Medium	Right
HM18	24	35	14	Yes	No	Large	Right
HM19	48	22	15	No	No	Medium	Bilateral
HM20	12	27	13	No	No	Medium	Left
HM21	24	16	13	Yes	No	Medium	Right
HM22	72	23	14	No	No	Medium	Bilateral

In terms of contraceptive use, 6 patients (27.3%) had a history of contraceptive or hormone therapy use, whereas 16 patients (72.7%) reported no such history. Breast size analysis indicated that 15 patients (68.2%) had medium-sized breasts, 5 patients (22.7%) had large breasts, and 2 patients (9.1%) had small breasts. Regarding laterality, 10 patients (45.5%) presented with bilateral involvement, 8 patients (36.4%) with right-sided involvement, and 4 patients (18.2%) with left-sided involvement. These observations suggest that prolonged breastfeeding and hormonal influences, including contraceptive use, may contribute to the development of IGM.

The clinical findings of the patients with IGM are summarized in Table 3. Among inflammatory symptoms, erythema was the most commonly observed feature, present in 8 patients (36.4%). *Pain* was reported by 5 patients (22.7%), *redness* by 2 patients (9.1%), and peau d'orange by 1 patient (4.5%). Notably, 6 patients (27.3%) reported no inflammatory symptoms. Tissue-related symptoms predominantly involved *deep collections*, which were detected in 10 patients (45.5%), while *mass formation* was identified in 6 patients (27.3%). The remaining 6 patients (27.3%) exhibited no tissue abnormalities. Regarding skin destruction, *superficial collections* were found in 4 patients (18.2%), *fistula formation* occurred in 2 patients (9.1%), and 16 patients (72.7%) showed no signs of skin destruction. These findings illustrate the significant inflammatory involvement of deeper tissues in IGM and the relatively lower frequency of superficial skin damage.

Table 3. Distributions of clinical findings of IGM patients

	Inflammatory symptoms				Tissue symptoms		Skin destruction	
No	Pain	Redness	Erythema	Peau d'orange	Deep collections	mass	superficial collection	fistula
HM1	No	No	No	No	No	No	No	No
HM2	No	No	No	No	No	No	No	No
HM3	No	No	No	No	Yes	No	No	No
HM4	No	No	No	No	No	Yes	No	No
HM5	No	No	No	No	No	No	Yes	No
HM6	No	No	No	No	Yes	No	No	No
HM7	No	No	No	No	No	Yes	Yes	No
HM8	No	No	Yes	No	Yes	No	No	No
HM9	Yes	No	Yes	No	No	No	No	Yes
HM10	No	No	Yes	No	No	No	No	No
HM11	Yes	No	Yes	No	No	Yes	Yes	No
HM12	Yes	No	Yes	No	Yes	No	No	No
HM13	No	No	No	No	No	Yes	No	No
HM14	Yes	No	No	No	Yes	No	No	No
HM15	No	No	No	No	Yes	No	No	No
HM16	Yes	Yes	Yes	No	Yes	No	No	No
HM17	No	No	Yes	Yes	No	No	Yes	No
HM18	No	No	No	No	No	No	No	Yes
HM19	No	No	No	No	Yes	No	No	No
HM20	No	No	Yes	No	No	Yes	No	No
HM21	No	Yes	No	No	Yes	No	No	No
HM22	No	No	No	No	Yes	Yes	No	No
Positive (%)	22.7	9.1	36.4	4.5	45.5	27.3	18.2	9.1

Microbiological assay

Culture results for all samples were negative. Sequencing results indicated that all samples had bacteria. Except for one case in which *S. lugdunensis* was detected, in all other cases, *E. coli* was present.

DISCUSSION

Our study provides new insights into the role of bacterial involvement in IGM by highlighting the predominance of *E. coli* in biopsy samples. These findings challenge previous assumptions that bacterial involvement in IGM is rare or predominantly associated with *C. kroppenstedtii*.[6,14] While *E. coli* has historically been recognized as a cause of mastitis in bovine populations, its consistent detection in human IGM tissues, as observed in our study, suggests a potential pathogenetic role that warrants further investigation. This discovery may have therapeutic implications, particularly for antibiotic selection and resistance patterns.[15]

The demographic patterns observed in our cohort further support the existing literature on IGM. The mean age of 35.23 years aligns with previous reports that identify IGM as a condition typically affecting

women of reproductive age.[16] Our findings also emphasize the role of hormonal factors, as evidenced by the mean breastfeeding duration of 39 months and the fact that 27.3% of patients reported contraceptive use. This reinforces the hypothesis that prolonged exposure to hormonal stimuli, including hyperprolactinemia, may contribute to the disease process.[17] This aligns with observations from Metanat *et al.*, who reported similar hormonal patterns in their cohort.[3] Additionally, the high frequency of bilateral breast involvement in nearly half of the cases (45.5%) aligns with reports indicating the symmetrical nature of IGM in hormonally active women.[6]

The predominance of inflammatory symptoms, particularly erythema (observed in 36.4% of patients), underscores the inflammatory nature of the disease. The prevalence of deep collections (45.5%) and superficial skin destruction (18.2%) aligns with findings by Co *et al.*, who also reported significant tissue involvement in chronic mastitis cases.[18] The co-occurrence of these symptoms with bacterial DNA presence suggests an inflammatory process potentially driven by bacterial dysbiosis rather than sterile granulomatous inflammation alone.[19] This insight challenges traditional perspectives that consider IGM a primarily autoimmune condition.[20] Furthermore, recent studies suggest that inflammatory processes may also be exacerbated by genetic predispositions, such as polymorphisms affecting immune response genes.[21]

Our findings also raise questions about potential regional and environmental factors influencing bacterial profiles in IGM. The near-exclusive detection of *E. coli* may reflect specific environmental exposures or population-specific microbiota patterns, as suggested by Krawczyk *et al.*, who reported regional variations in bacterial profiles of granulomatous mastitis.[22] Further research incorporating multi-center data could elucidate whether such bacterial patterns are unique to our study region or part of a broader epidemiological trend. Additionally, the regional distribution of IGM cases, with a higher prevalence in the Middle East, further supports the need for region-specific diagnostic and therapeutic protocols.[3,8]

A key limitation of our study is the absence of control tissue samples from healthy breast tissue. Recent research has highlighted the presence of a normal breast microbiome, which may influence inflammatory processes.[23] Without comparative data from normal tissue, distinguishing pathogenic bacterial involvement from commensal microbiota is challenging. Future studies should incorporate control samples to accurately assess bacterial contributions to idiopathic granulomatous mastitis and distinguish between microbial colonization and true infection.

CONCLUSION

Although current evidence does not conclusively establish the link between *E. coli* and IGM, this hypothesis can pave the way for further research. Detailed investigations and clinical studies can help clarify the role of this bacterium in the onset and exacerbation of IGM. While this study demonstrates the presence of *E. coli* in the majority of IGM biopsy samples, further research is necessary to investigate the potential role of bacterial involvement in the disease's pathogenesis and its implications for future therapeutic approaches.

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CONFLICTS OF INTEREST

None to declare.

ETHICAL CONSIDERATIONS

The study was approved by the Ethics Committee of Kurdistan University of Medical Sciences.

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DATA AVAILABILITY

Data related to this study are presented in the article.

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