Cross-sectional Study

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Investigation of multi-infections and breast disease comorbidities in granulomatous mastitis

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Introduction: Granulomatous mastitis (GM) is an inflammatory breast disease typically caused by infection, posing diagnostic challenges. It can coexist with other breast disorders or multiple infections, which have been vaguely discussed. This study investigates the incidence of multi-infection and breast disease comorbidities in GM.

Method: The study enroled 63 females who had a confirmed diagnosis of GM. Laboratory investigations and bacterial cultures had been conducted for all the cases. The patients had undergone ultrasonography examination utilizing the LOGIQ E9 system. Core needle biopsy had been done to procure tissue samples for histopathological examination. Thorough scrutiny and assessment of patients' records were performed. The variables encompassed age at presentation, breastfeeding data, parity, smoking status, seasonal affliction, hair-washing agents, exposure to radiation, comorbidities, and clinical, ultrasound and histopathological findings.

Results: The patients' ages ranged from 24 to 50. Breastfeeding history was positive in nearly all cases (97%). The majority of cases exhibited multiparity (81%). In total, 63.5% were passive smokers. Multi-infections were detected in six cases (9.5%). Among them, *B. cepacia complex* and *Toxoplasma gondii* were identified in two cases (3.16%). Other multi-infections involved *Staphylococcus epidermidis* and *Toxoplasma gondii*, *Burkholderia cepacia* and *S. kloosii* and *Toxoplasma gondii*, *Staphylococcus epidermis* and *Brucella* spp., *Candida* spp. and *Brucella* spp. Histopathological analysis revealed GM comorbidities with other breast diseases in 35% of the cases.

Conclusion: Multi-infections and breast disease comorbidities may further complicate diagnosis and management of GM. The findings of this study may raise additional questions about the nature of the disease or potential complications associated with it.

Keywords: granulomatous mastitis, infection, comorbidities, inflammatory disease.

Introduction

Granulomatous mastitis (GM) is a chronic inflammatory breast disorder that often presents a diagnostic challenge due to its clinical and radiological resemblance to a commoner and more crucial disease, breast cancer (BC)[1,2]. The aetiology of GM remains enigmatic, with suggested associations with infections, hormonal imbalances, autoimmune responses, and genetic predisposition[1,3,4]. GM predominantly affects women of childbearing age, especially postpartum and lactating women. Hormonal imbalances, particularly hyperprolactinemia, are considered one of the potential risk factors due to their association with breastfeeding and hormonal fluctuations[2,5,6]. Despite affecting the patient's quality of life and psychological well-being, the disease is also associated with a financial burden, particularly in low-income populations[7,8]. The symptoms of GM are often nonspecific, and patients may present with breast pain, erythema, skin changes, and palpable masses. These clinical manifestations closely mimic those of BC, necessitating a comprehensive evaluation[2,6]. The disease masquerades as various breast disorders, including sarcoidosis, and autoimmune diseases[1,5,9]. Furthermore, GM can coexist with other breast pathologies like lobular carcinoma in situ (LCIS) and ductal carcinoma in situ (DCIS), highlighting diagnostic complexities[10,11]. Multi-infection in GM, albeit rare, introduces an intriguing facet to its complexity in diagnosis and management[5]. Multi-infection not only influences disease progression but also impacts the choice of therapeutic interventions[12,13].

The current study investigates the incidence of multi-infection and breast disease comorbidities (BDC) in GM patients. The study has been prepared regarding the STROCSS guideline[14].

HIGHLIGHTS

- Granulomatous mastitis is a chronic inflammatory breast disorder that presents a diagnostic challenge.
- The disease masquerades as various breast disorders or can coexist with other breast pathologies.
- Multi-infection in granulomatous mastitis, albeit rare, introduces an intriguing facet to its complexity in diagnosis and management.

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Method

Study design

This was a single-centre cross-sectional study spanned from June 2020 to June 2023. The study enroled 63 female participants who had a confirmed histopathological diagnosis of GM.

Eligibility criteria

The study included patients who had confirmed histopathological diagnoses of GM. Exclusion criteria encompassed cases with indeterminate GM diagnoses and individuals with a documented history of breast disease or pre-existing chronic conditions prior to the onset of GM.

Laboratory investigation

The DYNEX DSX full-automated ELISA (DYNEX Technologies) had been utilized for the detection of Brucella IgG and *Candida albicans*. Infection by *Toxoplasma gondii* had been diagnosed using CHORUS Trio (DIESSE Diagnostica Senese).

Bacterial culturing

For all patients, tissue specimens had been aspired through needle aspiration and subsequently dispatched to the microbiology centre. The culturing process encompassed the utilization of four distinct agars: blood agar base, chocolate agar (610005, Liofilchem), MacConkey agar (610028, Liofilchem), and Mueller Hinton agar (NCM0036A, NEOGEN). Employing the streak plate method, culturing was conducted, followed by overnight incubation (Heratherm IGS60, Thermo Fisher Scientific). The identity of bacterial species was determined through BD Phoenix M50 (Becton, Dickinson, and Company).

Ultrasound examination

The patients had undergone ultrasonography utilizing the LOGIQ E9 system (GE Healthcare) equipped with an ML6–15 transducer operating at a frequency range of 5–15 MHz. With the patients in a supine position, two primary ultrasound scanning methods were employed. The radial approach provided a comprehensive view of breast anatomical structures, particularly ducts and lobules. Conversely, the anti-radial technique enabled cross-sectional imaging of ductal and lobular tissues, furnishing insights into potentially suspicious areas. Additionally, an axillary evaluation had been performed.

Tissue sampling and histopathological evaluation

The patients had undergone core needle biopsies to procure tissue samples for histopathological examination. The specimens had been meticulously labelled and immersed in neutral buffered formalin (10%) for ~10 h, maintained at a temperature of 25°C. A tissue processor (Histo-Tek@ VP1- Sakura) had been employed to facilitate tissue dehydration and fixation for 18–22 h. Paraffin wax was used to embed the processed tissues, ultimately forming blocks (Histo Core Arcadia H, Leica company). Subsequent staining with hematoxylin and eosin (H&E) was done. The screening had been carried out by a specialized histopathologist employing a light microscope.

Data collection and statistical analysis

Patient information, encompassing medical records and clinical data, was obtained through face-to-face interviews or via a review of the centre's database. Thorough scrutiny and assessment of records were performed. The variables encompassed age at presentation, breastfeeding data, parity, smoking status, seasonal affliction, exposure to radiation, co-infections, and clinical, ultrasound and histopathological findings. Microsoft Excel (2019) was utilized for data organization and encoding, while Statistical Package for the Social Sciences (SPSS) Version 25 facilitated data analysis.

Results

The study included 63 female patients diagnosed with GM (Figs. 1 and 2), spanning ages from 24 to 50. Breastfeeding history was positive in nearly all cases (97%). The mean breastfeeding duration was 25.93 months. In terms of parity, the majority of cases exhibited multiparity (81%). In total, 63.5% were passive smokers. The prevalence of aggressive symptoms was observed to be higher during the summer months. All patients used benzene-containing shampoo, and 20.63% also used oxygen alongside it. More than once radiation exposure was reported in 79.36% of cases (Table 1). Multi-infections were detected in six cases (9.5%). Among them, *B. cepacia complex* and *T. gondii* were identified in two cases (3.16%). Other multi-infections involved *S. epidermidis* and *T. gondii*, *B. cepacia* and *S. kloosii* and *T. gondii*, *S. epidermis* and *Brucella* spp., *Candida* spp. and *Brucella* spp. (Table 2). Histopathological analysis revealed GM comorbidities with other breast diseases in 35% of the cases. The most prevalent accompanying condition was ductal ectasia (DE) (23.9%) (Fig. 3), followed by papilloma (8%), chronic mastitis (6.3%), and fat necrosis (5%) (Table 3). Furthermore, both multi-infection and BDC demonstrated complex appearance due to weak compatibility between clinical and histopathological diagnosis and incompatibility between ultrasound and histopathology (Tables 4 and 5).

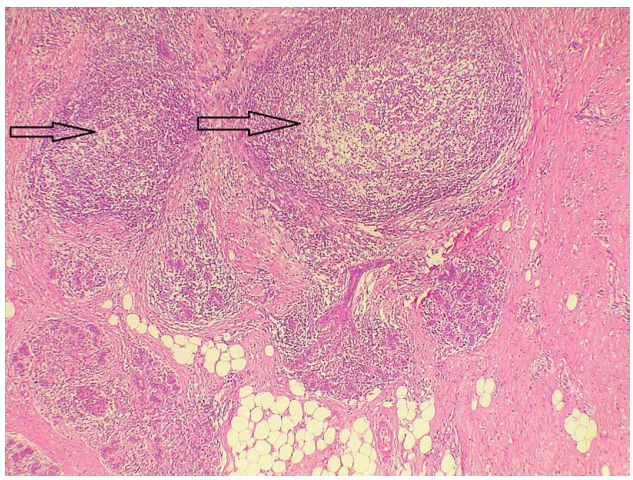

Figure 1. The section shows lobular granulomatous mastitis, (dark arrows), with the inflammation centred on the breast lobules.

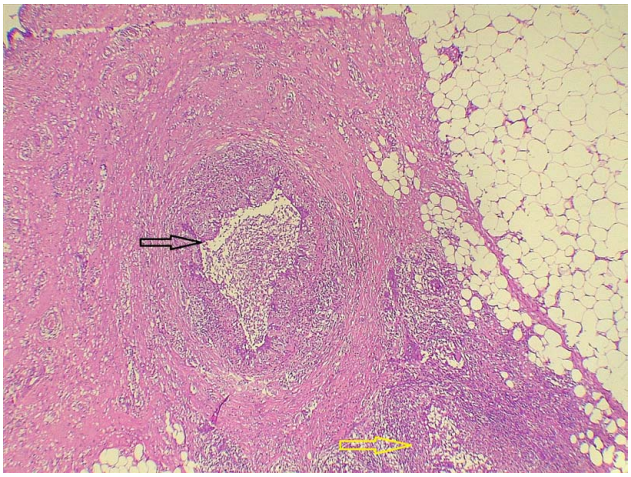

Figure 2. The section shows dilated ducts (dark arrow) with the inflammation and granulomatous inflammation below it (yellow arrow).

Discussion

GM refers to an inflammatory breast condition frequently observed in women of childbearing age, particularly within three years postpartum[15]. It was first described in 1972 by Kessler and Wolloch[16]. The condition involves the emergence of non-necrotizing chronic granulomatous inflammation in the breast lobules. However, both its cause and prevalence remain enigmatic[4]. In a study conducted by Baslaim *et al.*[17], involving 1106 cases with benign breast diseases, only 20 cases (1.8%) were identified as GM. GM typically manifests in women aged 30–45, presenting as a soft mass that may be accompanied by characteristics like erythema, peau d'orange texture changes, and nipple inversion. These features can pose challenges in distinguishing GM from BC. In severe or chronic instances, complications such as abscess, ulceration, and fistula or sinus formation are frequently observed[3]. The vast majority of affected individuals are parous, and GM in nulliparous females is an exceptionally

Table 1

Demographic features of GM patients

Variables	Frequency/percentages
Age range (mean ± SD)	24–50 years. (35.7 ±1.56)
Breast-fed time (mean)	25.93 months
Parity, *n* (%)	
Monoparous	9 (14.3)
Multiparous	51 (81.0)
Nulliparous	3 (4.7)
Clinical presentations, *n* (%)	
Breast pain	50 (79.4)
Fever or chill	29 (46.0)
Breast erythema/firmness	26 (41.3)
Breast oedema	19 (30.2)
Sweating at night	2 (3.2)
Smoking status	
Non-smoking	22 (34.92)
Former smoker	1 (1.58)
Passive smoker	40 (63.49)
Seasonal affliction	
Winter	23 (36.50)
Summer	40 (63.49)
Using benzene-contained Shampoo	
With oxygen	13 (20.63)
Without oxygen	50 (79.3)
Exposure to radiation (X-ray, ultrasound)	
Once	10 (15.87)
More than once	50 (79.36)
Unknown	3 (4.76)
Breast-fed history	
Yes	61 (96.8)
No	2 (3.17)
Breastfeeding type	
Pure	45 (73.8)
Mixed	16 (26.2)

GM, granulomatous mastitis.

Table 2

Multi-infections in patients with GM

Isolated	Frequency/percentage, *n* (%)
B. cepacia complex + *Toxoplasma gondii*	2 (3.16)
Staphylococcus epidermidis + *Toxoplasma gondii*	1 (1.58)
Burkholderia cepacian + *S. kloosii*, + *Toxoplasma gondii*	1 (1.58)
Staphylococcus epidermis + *Brucella* spp.	1 (1.58)
Candida spp. + *Brucella* spp.	1 (1.58)
Total	6 (9.5)

GM, granulomatous mastitis.

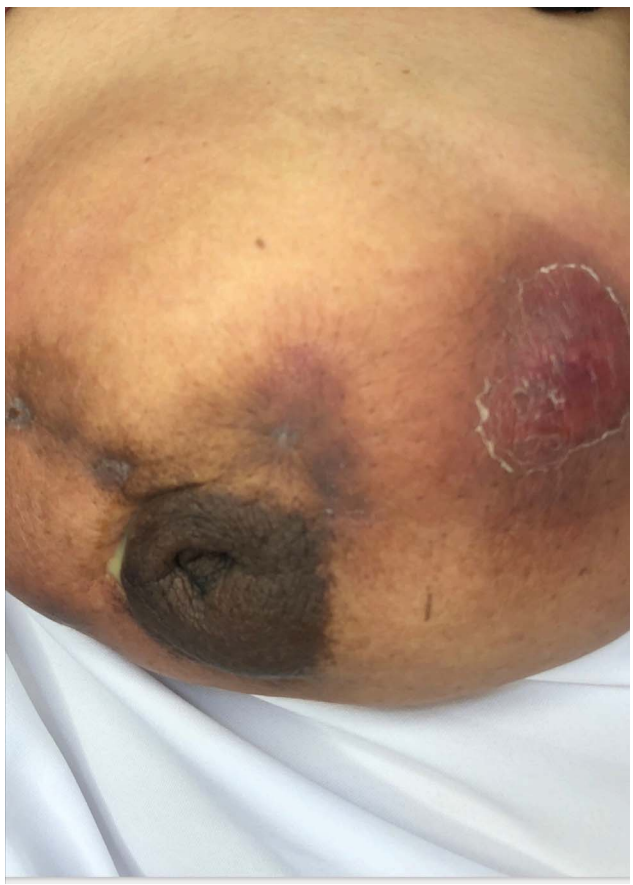

Figure 3. Typical clinical presentation of granulomatous mastitis -associated ductal ectasia including ulceration abscesses and fistulae.

Table 3

Breast disease comorbidities with GM regarding the clinical findings, ultrasonography, and histopathology

Comorbidities + GM	Clinical, *n* (%)	Ultrasonography, *n* (%)	Histopathology, *n* (%)
GM + DE	8 (12.7)	21(33.3)	10 (15.9)
GM + DE + papilloma	0	0	5 (8)
GM + chronic mastitis	0	2 (3.2)	4 (6.3)
GM + fat necrosis	0	0	3 (5)

GM, granulomatous mastitis.

Table 5

Harmonization between histopathology and sonography

	Ultrasonography				
Histopathology	GM, *n* (%)	Non-GM, *n* (%)	*Kappa*	(95% CI)	*P*
Granulomatous mastitis	12 (19)	51 (81)	−0.025	0.566	0.257
Non-granulomatous mastitis	0	0			

GM, granulomatous mastitis.

rare occurrence[6,18]. Kutsuna and colleagues documented two cases of GM induced by *C. kroppenstedtii* infection in nulliparous young women. Additionally, there are two other reported cases of nulliparous women with the same diagnosis. Both had been under long-term phenothiazine regimens, which are known to elevate serum prolactin levels. It has been suggested that hormonal changes associated with drug-induced hyperprolactinemia could heighten the vulnerability to GM following *C. kroppenstedtii* infection[18]. Consistent with existing literature, the majority of cases in this study involved young women of reproductive age, with a mean age of 35.7. Most of the cases were parous, and their mean duration of breastfeeding was 25.93 months. Among the cases, there were three nulliparous women, aligning with the well-documented scarcity of the disease in this particular group. When combining our cases with previously reported ones, the total count of documented GM cases in nulliparous women remains merely seven. None of the three nulliparous cases in our study exhibited signs of bacterial infection, and there was no history of phenothiazine use or other medication intake among them. Consequently, the precise origin of the disease in these specific cases remains elusive. Predominantly, the cases in our study presented with breast pain (79.4%), followed by fever/chills (46%), and erythema or breast firmness (41.3%).

GM can arise from either infectious or non-infectious origins, and recognizing this condition is crucial due to its potential to mimic BC, both in clinical presentation and radiographic appearance. Infections linked to GM encompass tuberculosis, actinomycosis, syphilis, and *Corynebacterium*, or fungal infections. These agents are believed to activate the immune system, resulting in the formation of granulomas. On the other hand, non-infectious contributors involve hormonal fluctuations, smoking, and autoimmune disorders such as granulomatosis with polyangiitis, giant cell arteritis, and sarcoidosis. The challenge lies in the fact that distinguishing between these entities through clinical and radiographic means is intricate[12,19]. In the current study, bacterial, protozoan, and fungal infections were identified, whereas instances of tuberculosis were not detected. Moreover, none of the participants exhibited any of the autoimmune diseases previously mentioned. It is noteworthy that ~63.5% of the cases had a history of exposure to cigarette smoke, and 79.36% of the cases had been exposed to radiation on multiple occasions. Additionally, 95.3% of the subjects experienced parturition at least once. The aetiological factors potentially contributing to the development of GM in our study population appear to encompass a complex interplay of passive cigarette smoke inhalation, infectious agents, and hormonal fluctuations.

GM can occasionally occur in unique cases of breast abscesses caused by different types of infections, mostly involving gram-positive bacteria like *Staphylococcus aureus* and *Corynebacterium*[1,3,19] However, Alasket *et al.*[1] reported an exceptionally rare instance of GM associated with *Salmonella*. There is also some evidence suggesting that *Corynebacterium* may play a role in the development of GM[12,18,19]. Taylor's study, for instance, discovered *Corynebacterium* in the breast tissue of 55% of 64 GM patients, particularly among those who had fever or an abscess[18]. Additionally, it revealed that 9 out of 12 women with *Corynebacterium* infections showed pathological signs of GM[18]. Various *Corynebacterium* species, including nonlipophilic ones such as *Corynebacterium accolens*, *Corynebacterium striatum*, *Corynebacterium amycolatum*, *Corynebacterium minutissimum*, and the more recently identified lipophilic species *Corynebacterium kroppenstedtii*, have been associated with breast infections[13]. Mirini-Alado *et al.* also reported an unusual occurrence where GM coexisted with two fungal infections of *Histoplasma* sp. and *Paracoccidioides* sp. In both cases, infection resulted from the inhalation of fungal spores, typically originating from mould, and usually presents with no noticeable symptoms, often resolving on its own. It is well-documented that subclinical hematogenous dissemination to other organs can occur following a benign primary lung infection in such cases[12].

In 1987, Adams and colleagues mentioned the first case of concurrent GM and Erythema nodosum (EN)[20]. While polyarthritis and EN are infrequent, they represent the most common systemic manifestations of GM. It is worth noting that occurrences of GM complicated solely by EN are exceptionally rare[9]. Akin *et al.* reported the presence of both GM and EN in a series of 11 cases, while Hida and colleagues highlighted the first case of

Table 4

Harmonization between histopathology and clinical diagnosis

	Clinical diagnosis				
Histopathology	GM, *n* (%)	Non-GM, *n* (%)	*Kappa*	(95% CI)	*P*
Granulomatous mastitis	42 (66.6)	21 (33.4)	0.036	(−0.101, 0.162)	0.566
Non -granulomatous mastitis	0	0			

GM, granulomatous mastitis.

EN associated with GM attributed to *Corynebacterium* infection[9,21]. Furthermore, another study outlined a case where a patient presented simultaneously with both GM and Sjögren's syndrome[22]. The concurrence of GM and BC has rarely been mentioned in the literature[5,23]. Only a few cases have been reported, and in three of these cases, both conditions were observed within the same breast. Among these cases, two were associated with invasive ductal carcinoma (IDC)[24,25], while in one instance, the coexistence was with both IDC and DCIS, occurring simultaneously[5]. In another case, the conditions manifested in separate breasts but simultaneously; one breast exhibited GM, while the other had IDC[26]. Additionally, Oddo *et al*.[27] reported a case of concurrent GM with *Coryneform* bacteria and DCIS within the same breast. Lastly, Tavakol *et al*.[10] discussed and presented the first recorded case of simultaneous LCIS and idiopathic GM in a patient. The coexistence of GM and carcinoma rekindles the classic and extensively discussed debate concerning the interplay among mastitis, infection, and BC. This phenomenon gives rise to the hypothesis that inflammation and chronic infection might indeed have a connection to cancer development[27]. Yoshida and colleagues reported an exceedingly rare case involving the simultaneous presence of non-IDC and GM. While it is plausible that BC and GM coincided incidentally in this case, several factors warrant consideration. These include the patient's young age, the absence of any family history of such conditions, and the noteworthy observation of an intertwined distribution of BC and GM in the histopathological images. These elements suggest that a potential relationship between these two conditions cannot be dismissed outright[11]. The objectives of the present study were twofold: first, to investigate the prevalence of multi-infections in GM, an aspect that has been rarely examined; and second, to explore BDC with GM. In our study, no multi-infections were observed among bacteria (of the same species) and fungi as previously mentioned. However, within our cases, we identified a multi-infection rate of 9.5%. Two cases had co-infections with the *B. cepacia complex* and *T. gondii*. In the remaining cases, co-infections were found per case, including *S. epidermidis* with *T. gondii*, *B. cepacian* with *S. kloosii*, and *T. gondii*, *S. epidermis* and *Brucella* spp., and *Candida* spp. with *Brucella* spp. To the best of our knowledge, multi-infections involving various microorganisms such as bacteria, protozoa, and fungi alongside GM have not been previously well addressed. Regarding the coexistence of GM with other breast diseases, our findings revealed that DE occurred independently in 15.9% of cases, while DE combined with papilloma was present in 8% of cases. Chronic mastitis was observed in 6.3% of cases, and fat necrosis was identified in 5% of cases. Altogether, the total rate of BDC with GM reached 35.2%. It is noteworthy that all concurrent conditions were of a benign nature, and no instances of BC were detected. This underscores the rarity of GM co-occurring with BC, consistent with existing literature.

In GM cases, breast ultrasound has proven to be diagnostically valuable in nearly 80% of cases[3]. Following the recognition of GM as a distinct pathological entity, it became evident that this condition could mimic invasive BC both in terms of clinical presentation and imaging characteristics. Consequently, breast biopsy, particularly core needle biopsy, emerged as the pivotal and indispensable method for establishing accurate diagnoses[27]. In our study, we observed complex manifestations of both multi-infections and BDC. These complexities arise from the limited compatibility between clinical and histopathological diagnoses, as well as the complete incompatibility between ultrasound and histopathology. These findings reinforce the notion that histopathology is the ideal standard diagnostic modality. In our study, all cases underwent core needle biopsy, and the diagnoses were subsequently validated through histopathological examination. This study could shed light on the multi-infection aspect of GM, potentially providing groundbreaking insights for future research aiming to statistically establish correlations between these two medical conditions. However, the limitations of this study can be summarized as a small sample size, a lack of a control group, and a short-term study.

Conclusion

Although GM poses challenges in diagnosis and is prone to misdiagnosis due to its resemblance to BC and an unknown aetiology, additional factors such as co-infections and concurrent diseases may further complicate both diagnosis and management. The findings of this study may raise additional questions about the nature of the disease or potential complications associated with it.

Ethical approval

The ethical approval for the study was provided by the ethical committee of Sulaimani Polytechnic University Ethical approval No. CHOO22.

Consent

Written informed consent was obtained from the patient for publication and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

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The research has got no funding.

Author contribution

N.K.E.: participated in preparing and drafting the manuscript, participated in data collection. performed the data analysis. A.M.S.: major contribution of the idea, designed the study. Authors contributed equally to the manuscript and read and approved the final version of the manuscript.

Conflicts of interest disclosure

There are no conflicts of interest.

Research registration unique identifying number (UIN)

The study has been registered in the research registry with number of researchregistry 9570 https://www.researchregistry.com/registernow#home/registrationdetails/651960bce7586c00290f7b69/.

Guarantor

Abdulwahid Mohammed Salih.

Data availability statement

Not applicable.

Provenance and peer review

Not commissioned, externally peer-reviewed.

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