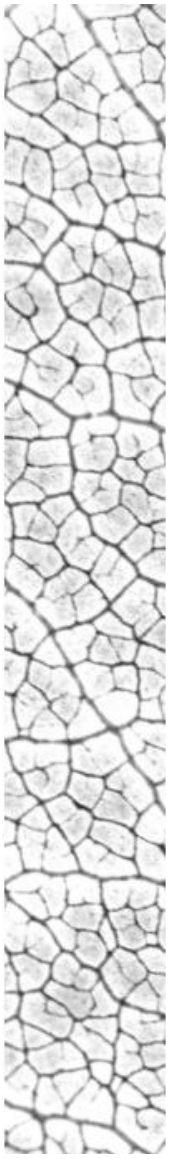

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Seborrheic dermatitis in children caused by *Malassezia restricta* and *Malassezia furfur* detected by polymerase chain reaction

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Abstract
Seborrheic dermatitis is a recurrent skin disease with a predilection in areas having many sebaceous glands. The etiology of seborrheic dermatitis is still unknown. Several factors are involved in its etiopathogenesis, such as Malassezia, activity of sebaceous disorders, and individualized education. The diagnosis of seborrheic dermatitis is based on the clinical morphology of the scales and erythema, which is typical in sebum-rich areas. If necessary, the diagnosis requires histopathological examination and species identification through culture or polymerase chain reaction. The goal of the management of seborrheic dermatitis is to release and eliminate scales and crust, change fungal colonization, control infections, and regulate erythema and itching. Therapies, such as topical antifungals and corticosteroids, can be given. This literature presents a case of a baby diagnosed with seborrheic dermatitis established through history, physical examination, and Polymerase chain reaction (PCR) to identify the causative microorganism. The results of the examination carried out showed the involvement of *Malassezia furfur and Malassezia restricta*. We then provided topical corticosteroid and antifungal treatments which led to repair of the lesion within 2 weeks.

Keywords: *Malassezia furfur, Malassezia restricta*, Polymerase chain reaction**,** Seborrheic dermatitis in children

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INTRODUCTION

Seborrheic dermatitis is a chronic recurring skin disease with a predilection for areas having a lot of sebaceous glands. All ages, from infants to adults, can suffer from seborrheic dermatitis. Although the pathogenesis of the disease is not fully understood, seborrheic dermatitis has been associated with excess sebum production and the presence of *Malassezia* (Terroe, Kapantow, & Kandou, 2015. Azhari et al. 2017). *Malassezia furfur* (formerly known as *Pityrosporum ovale*) is thought to cause immune abnormalities and vulnerabilities and also influence androgen glands that produce an increase in the number and activity of the sebaceous glands (Paller, Mancini, 2011). Other literature mentioned that 13 species of *Malassezia* are known to date (*Malassezia furfur*, *Malassezia obtusa*, *Malassezia globosa*, *Malassezia slooffiae*, *Malassezia sympodialis*, *Malassezia pachydermatis, Malasssezia restricta, Malasssezia yamatoensis, Malassezia nana, Malassezia japonica, Malassezia equine, Malassezia caprae, Malassezia dermatis*) (Dessinioti, Katsambas, 2013). *Malassezia* is a normal flora that inhabits the human skin and is suggested to play an important role in the development of seborrheic dermatitis based on the evidences that the common lesions of seborrheic dermatitis are related to the distribution of sebaceous glands where *Malassezia* preferentially colonizes; however, not all of these *Malassezia* species were found in seborrheic dermatitis, implying that there may exist specific strains capable of causing the disease (Hajar, 2015. Collins, Hivnor, 2012).

The incidence of seborrheic dermatitis generally occurs at all ages but often presents in the first 3 months of life up to 70% and the fourth to seventh decade of life, whereas the incidence in infants is associated with the size and activity of sebaceous glands at their age. Newborns have large sebaceous gland with high sebum secretion nearly as adults. Adult sebaceous glands are no longer associated with seborrheic dermatitis, because the activity of sebaceous glands will reach its peak at the beginning of puberty; however, new

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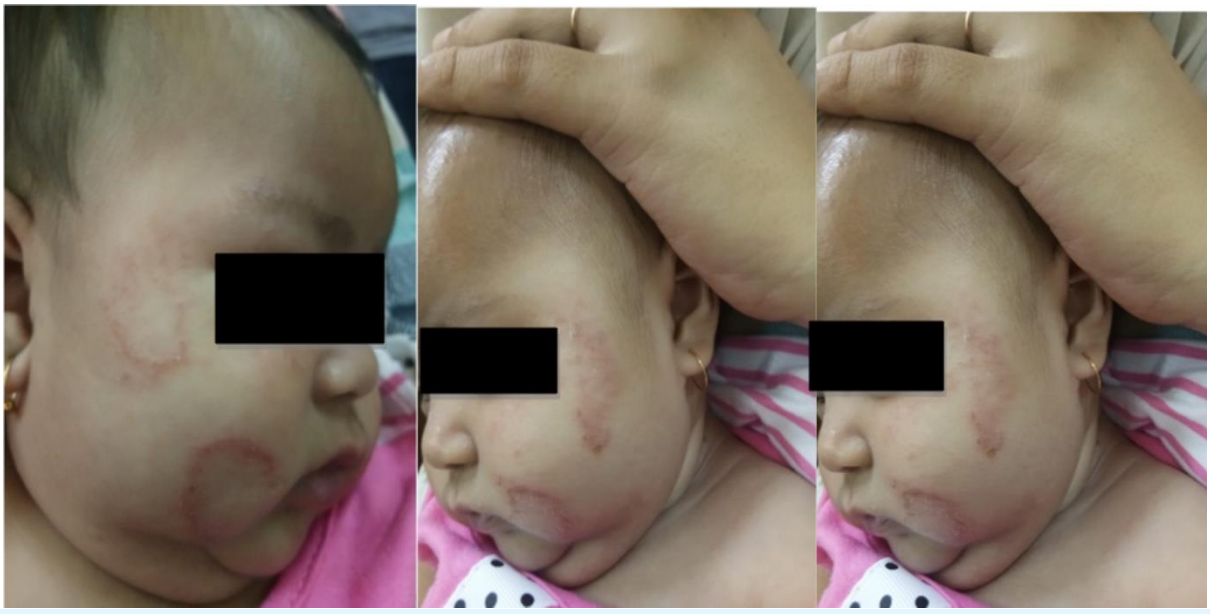

Fig. 1. The first day of treatment showed nummular erythema macules with active lesion margins accompanied by scaling and excoriation of the facial area

abnormalities usually appear a few decades later (Hajar, 2015).

Seborrheic dermatitis in infants is commonly known as infantile seborrheic dermatitis. Its predilection is on vertex part of the scalp (cradle cap) in the form of erythematous plaques with tawny inherent scaling and will then spread to all parts of the scalp. In addition, crust also exists. Lesions can be found on the face (nasolabial folds, eyebrows, upper eyelid, and forehead) and neck and spread at the back and extremities, manifesting as plaque inflammation and intertrigo in the area of the axilla and groin. Lesions can also be found in the diaper area (Collins, Hivnor, 2012. Widaty, Marina, 2016). The symptoms of seborrheic dermatitis are mainly chronic, persistent, and recurrent. The severity of seborrheic dermatitis varies from mild erythema and pruritus to severe, oily, thick scale with a burning or tingling sensation. Characteristically, seborrheic dermatitis in infants has a relatively different feature in contrast with seborrheic dermatitis in older ages, such as non-pruritic skin eruption with dry, thick, adherent, and flaking scale, and may be accompanied by erythematous rash on intertriginous folds of the trunk and extremities. The diagnosis can be confirmed based on the location of the affected skin and scaling properties, such as dry or greasy scaling, smooth, or rough, layer or layered, and color. On the other hand, in difficult cases where diagnosis is difficult to distinguish from one another, histopathological examination is necessary (Collins, Hivnor, 2012).

This paper reports a case of seborrheic dermatitis caused by *Malassezia restricta* and *Malassezia furfur* detected by Polymerase chain reaction (PCR) in children aged 3 months, who were treated with corticosteroids and topical antifungals, providing clinically meaningful changes.

MATERIAL AND METHODS

A 3-month-old baby boy patient was consulted from the pediatric surgery department with a diagnosis of anorectal malformation disorder in high post-colostomy positions and with skin complaints in the form of red spots on the face since 1 week ago. The spots are initially small in size and then spread. Children seem to scratch often, especially when sweating or crying. There is no fever. Treatment history is only in the form of a baby cream application.

Physical examination showed the general condition of the patient who was in a state of moderate pain. There was good patient awareness, vital signs are within normal limits, and there was no malnutrition. Dermatological examination showed nummular erythematous macules with active lesion margins accompanied by scaling and excoriation of the facial area (**Fig. 1**). From history and physical examination, it can be concluded that the patient has seborrheic dermatitis.

The treatment given to the patient was an antifungal miconazole cream, which was applied to the lesion every morning, afternoon, and night. In addition, the patient was also given hydrocortisone corticosteroid cream, which was applied every morning. At the follow-up 1 week later, the redness of the lesion had diminished, and the patient rarely scratched, but there were still erythema macules on the face (**Fig. 2**).

PCR was used to identify the microorganisms that caused seborrheic dermatitis. Skin scrapings obtained will undergo a DNA extraction process using the

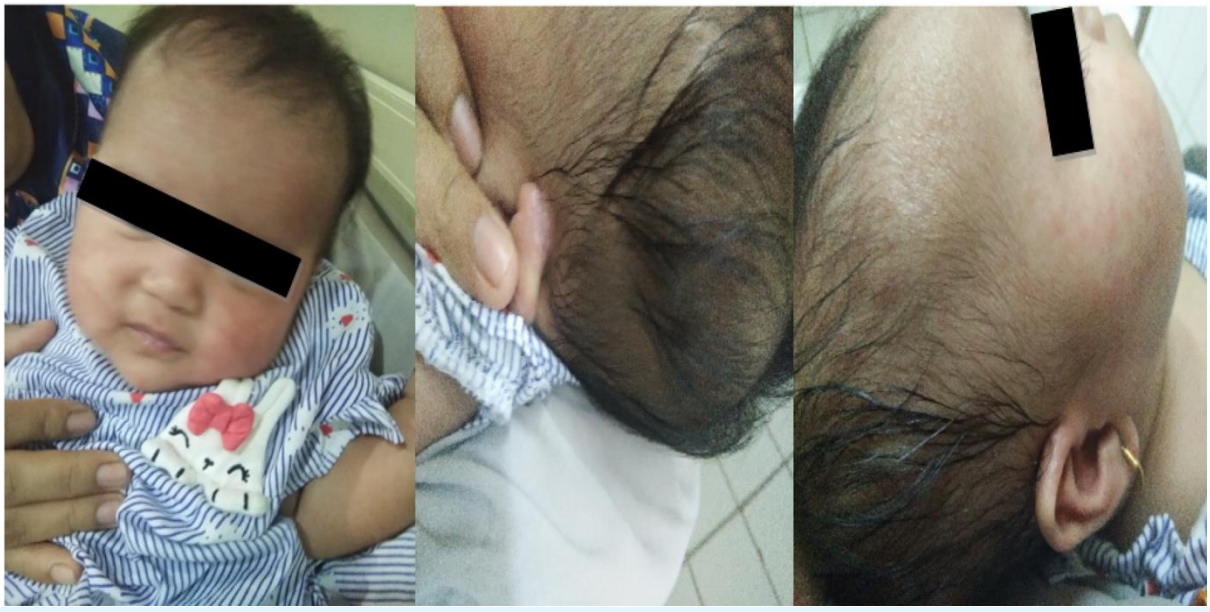

Fig. 2. Follow-up 1 week after treatment showed that there was still a macular erythema on the face

Geneaid method. This is done by adding 200 µl of GST buffer and 20 µl of proteinase K, followed by vortexing for 10 s and incubating at 60°C overnight to lyse all the scales. The sample is then centrifuged to obtain the supernatant layer. The supernatant layer obtained will then be mixed with 200 µl of GSB buffer solution and 200 µl of absolute ethanol and will be transferred to the GD column for another centrifugation. A 400 µl of buffer W1 was added to the GD column, followed by a centrifuge, and the liquid was discharged. After the liquid is discharged, the process continues with the addition of 600 µl of buffer wash and 100 ml of ethanol. After centrifuging, the GD column was transferred into a 1.5-ml Eppendorf tube and was mixed with 100-µl buffer elution that had been previously heated and allowed to stand for 3 min. The sample will be centrifuged again. The GD column will be removed, and the liquid contained in the Eppendorf tube is a DNA product from the extracted sample, which is ready to undergo PCR.

After sample collection and extraction, PCR was carried out twice for three species of *Malassezia* at once and each species. In the first PCR, primers with forward sequences 5'-GGATCATTAGTGATTGCCTTTATA-3' and reverse 5'-TCCTCCGCTTATTGATATG-3' were selected to allow amplification of target DNA for three species of *Malassezia* (*Malassezia furfur*, *Malassezia globosa*, and *Malassezia restricta*). DNA amplification of *Malassezia* species aims to detect the presence or absence of *Malassezia* in general in the examined sample. PCR amplification was carried out in a final volume of 25 µl. Each reaction contains 5 µl of DNA template, 12.5 µl of Green Master Mix, 0.5 µl of forward primer, 0.5 µl of reverse primer, and 6.5 µl of nuclease-free water. Initial denaturation was carried out at 94ºC for 5 min and continued with 30 denaturation cycles at the same temperature for 30 s, annealing at 57°C for 1 min, and extension at 72ºC for 50 s, with the final extension at 72°C for 10 min.

A second PCR was carried out for each *Malassezia* species, where the primers chosen to enable the amplification of target DNA for each Malassezia species were as follows: forward 5'-CTACTCGCGTACAACGTCTCTG-3' and reverse 5'-TTCGCT.GCGTTCTTCATCGA-3' for *Malassezia furfur*; forward 5'-CAATAAGTGTGTCTCTGCGG-3' and reverse 5'-TTCGCTGCGTTCTTCATCGA-3' for *Malassezia globosa*; and forward 5'-CTTGGTTGGACCGTCACTG-3' and reverse 5'-AGGCGGATGCAAAGTGTCTC-3' for *Malassezia restricta*. This second PCR will identify *Malassezia* species specifically. PCR amplification was performed in a final volume of 25 µl. Each reaction contained 5 µl of the template DNA, 12.5 µl of Green Master Mix, 0.5 µl of forward primer *Malassezia furfur*, 0.5 reverse primer *Malassezia furfur*, 0.5 µl of forward primer *Malassezia globosa*, 0.5 reverse primer *Malassezia globosa*, 0.5 µl of forward primer *Malassezia restricta*, 0.5 reverse primer *Malassezia restricta*, and 4.5 µl of nuclease-free water. Initial denaturation was carried out at 94°C for 5 min and continued with 30 denaturation cycles at 94°C for 30 s, annealing at 62°C for 1 min, and extension at 72°C for 40 s, with a final extension at 72°C for 10 min. Amplified products were visualized by 1.5% (w/v) agarose gel electrophoresis in TBE buffer, stained with ethidium bromide (0.5 µg/ml), and photographed under UV transillumination.

RESULT

PCR results showed a band with a size of 320 bp for *Malassezia restricta*-specific primers and 230 bp for

Fig. 3. Follow-up 1 week after treatment the patient. We performed a result PCR using a specific primer for DNA amplification of *Malassezia* species and found a band of 320 bp for *Malassezia restricta* and 230 bp for *Malassezia furfur*

Fig. 4. Controls in the second week showed macular erythema on the face had disappeared and returned to normal skin colour

Malassezia furfur-specific primers; thus, it can be concluded that DNA of *Malassezia restricta* and *Malassezia furfur* was positive in patient samples (**Fig. 3**).

Based on history, physical examination, and PCR of the patient's skin sample, we made the diagnosis of seborrheic dermatitis caused by *Malassezia restricta* and *Malassezia furfur*. The treatments given are 10 g of hydrocortisone cream, applied every morning, and 5 g of miconazole cream, applied every morning, afternoon, and night on the patient's skin lesions. In the follow-up period 1 week later, the reddish lesions had disappeared, and the erythematous macules on the face had returned to normal skin color (**Fig. 4**). Medication is continued with the application of miconazole cream every morning and night.

DISCUSSION

The diagnosis of seborrheic dermatitis is based on clinical morphology of typical scaling and erythema in sebum-rich areas. If necessary, the diagnosis requires histopathological examination and identification of species of fungi through culture or PCR (Jusuf, Nasution, Ullyana, 2018. Kibar, Aktan, Bilgin, 2015). Examination of dermatology status showed the presence of macular erythematous nummular shape, edge active lesions, scaling, and excoriation in the facial region. Literature study mentioned that the clinical manifestation of the disease is characterized by scaly, greasy and slightly yellowed with limits less strict (Terroe, Kapantow, & Kandou, 2015).In infants, the skin disorder is usually white or yellowish, oily, focal, or diffuse scale, and crusts can form on the scalp (Clark, Pope, Jaboori, 2015). Lesions may thicken and form the cradle cap. The cradle cap is usually the initial lesion and the only manifestation of seborrheic dermatitis in infants. In the face, external ear, folds postauricular, weight, and body folds areas, there are infrequently erythematous patches that are demarcated with white until yellow color scales appearance. In the diaper area, seborrheic dermatitis appears as erosion of demarcated, erythematous, greasy, and scaly fused to form large confluent lesions. In the infantile seborrheic dermatitis, a baby usually does not cry and does not feel itchy. Dermatitis in infants usually resolves itself within a few weeks to a few months (Leung, Barankin, 2015. Pandita, et al. 2015). Within the first week of treatment for our patient, reddish spots have disappeared, but erythema macules still appear on the face. In the following week, the erythema macules have disappeared and returned to the patient's normal skin color.

Seborrheic dermatitis is a chronic recurring skin disease with a predilection for areas having a lot of sebaceous glands (Terroe, Kapantow, & Kandou, 2015· Azhari, et al. 2017).The prevalence in adults is estimated between 1% and 3%, with more incidence in men than in women (Borda,Wikramanayake, 2015).The etiology of seborrheic dermatitis is unknown. Several factors are involved in the etiopathogenesis of the disease, such as *Malassezia*, sebaceous gland activity, and individual susceptibility. The role of *Malassezia* evidenced by the improvement of the lesions suffered due to the administration of antifungal therapy. The dimorphic fungus *Malassezia* is lipophilic and is naturally a part of the normal flora of the skin and can be isolated by scraping the skin with sebum-rich areas of the body, such as the chest, back, and head areas. In 90% of healthy skin, this fungus can change the role from commensal to pathogenic due to several predisposing factors, such as changes in the microflora of the skin and/or changes in host defense (Azhari, et al. 2017)

PCR test results using specific primers detecting *Malassezia* found a band with a size of 320 bp indicating

positive DNA *Malassezia restricta* and 230bp which was positive DNA *Malassezia furfur*. PCR was an enzymatic method for DNA amplification in vitro and was used to identify viruses, bacteria, parasites, and fungi molecularly (Ehtisham, et al. 2016). In this case, PCR was used to identify *Malassezia* spp., which is thought to be one of the causes of seborrheic dermatitis. Research carried out in Iran identified that the dominant *Malassezia* spp. causing seborrheic dermatitis using PCR were *Malassezia furfur* and *Malassezia restricta* (Mahmoudabadi, Zarrin, Azish, 2014).

The goal of therapeutic management of seborrheic dermatitis is to remove and eliminate scaling and crusting, inhibit colonization of fungi to control secondary infection, and reduce erythema and itching.[1] Treatment of seborrheic dermatitis in infants is mainly composed of emollients that help loosen scales. The scales can then be removed by rubbing with a cloth or a baby comb. On the face, therapies, such as topical antifungals corticosteroids and calcineurin inhibitors, can be given (Clark, Pope, Jaboori, 2015).

This patient was given topical antifungal miconazole cream, applied in the morning, afternoon, and evening. In addition, the patient was given topical corticosteroid hydrocortisone cream, applied in the morning. When choosing a topical antifungal agent of choice for acute and long-term treatment of seborrheic dermatitis of the face and body, one must consider its effectiveness, low side effect profile, and cost (lark, Pope, Jaboori, 2015). Miconazole cream is available in packs of 2% gel and shampoo. This agent has a good penetration ability in the stratum corneum after topical application on the skin (Chowdhry, Gupta, 2017). A low potency topical corticosteroid or medium has been successful in reducing the symptoms of seborrheic dermatitis and just as effective as other antifungal and anti-inflammatory agents. Corticosteroids are a second-line agent for long-term use associated with the thinning of the skin and the formation of telangiectasia.[10] Hydrocortisone is a mild topical corticosteroid agent used to reduce inflammation and itching from seborrheic dermatitis. A study showed that administration of hydrocortisone 1% gives the same effect as the provision of sertaconazole 2% during the 4 weeks of treatment (Balighi, et al. 2017). Several studies have shown that 1–2% ketoconazole can significantly decrease the severity of seborrheic dermatitis versus placebo, giving a remission rate equivalent to corticosteroids, with almost 44% fewer side effects. In a single study evaluating the efficacy of clotrimazole and miconazole, these agents were almost as effective against seborrheic dermatitis as corticosteroids. Hydrocortisone 1% tends to be a therapeutic choice but must be used with caution due to its side effects (Collins, Hivnor, 2012).

Seborrheic dermatitis in infants usually resolves spontaneously within a few weeks to a few months. Most cases settle at the age of 12 months. In adolescent and adult patients, the disease can progress from chronic to severe. However, in some cases of seborrheic dermatitis in infants, the disease can result to complications when there are accompanying *Candida* infection. Post-inflammatory pigment changes may occur, especially in pigmented individuals. Blepharoconjunctivitis can also occur (Leung, Barankin, 2015). In patients who are refractory to topical treatment or for those with significant secondary bacterial infection, bacterial culture and systemic antibiotics may be necessary (Paller, Mancini, 2011).

CONCLUSION

In conclusion, various factors, such as immunity, heredity, and sebaceous glands, play an important role in the development of seborrheic dermatitis. The role of sebaceous glands in the pathogenesis of seborrheic dermatitis must certainly be considered due to the distinctive distribution of lesions. However, the role of microorganisms especially *Malassezia* species is essential because it causing seborrheic dermatitis. Various modalities are available in detecting the involvement of microorganisms in the process of seborrheic dermatitis development. Nonetheless, further research is still needed to map the significance of the role of various species of *Malassezia* and other microorganisms to trigger the occurrence of this disease.

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