Benha Journal of Applied Sciences (BJAS) **print: ISSN 2356–9751**
Vol. (7) Issue (9) (2022), (47-53) **online: ISSN 2356–976x**
http://bjas.journals.ekb.eg

Relation between Serum and Fecal Calprotectin and Atopic Dermatitis

[1]A.A.Ali, [1]A.M.El-refaei, [1]S.H.Ahmed and [2]N.F.alhusseini
[1]Dermatology, Venereology and Andrology, Dept., Faculty of Medicine, Benha Univ., Benha, Egypt
[2]Biochemistry and Molecular Biology, Dept., Faculty of Medicine, Benha Univ., Benha, Egypt
E-mail: dr.ahmedabdallah90@yahoo.com

Abstract

Atopic dermatitis (AD), often called atopic eczema, is one of the most common inflammatory skin conditions that affects both children and adults and may result in chronic, pruritic, and recurrent dermatitis. The objectives of this research were to determine the clinical relevance of serum and faecal calprotectin in children with AD over a range of ages, to analyse the relationship between the two, and to clarify the relationship between the two and disease severity. For this case-control research, participants were chosen from the general population whereas AD patients were drawn from the outpatient clinic of the Dermatology, Venereology, and Andrology Department at Benha University Hospitals. The findings also showed that xerosis was present in every instance of atopy, as well as pit alba in 40%, k pilaris in 38%, oral in 22%, and ocular in 22%. The levels of serum and faecal calprotectin in the AD group were considerably greater than those in the control group. We discovered no significant variations in AD group serum or faecal calprotectin concentration by age, gender, or family history. There was a positive connection between serum and faecal calprotectin and AD severity, but not with age at onset, disease duration, or disease stage. Higher concentrations of serum and faecal calprotectin were thought to be an indicator of vulnerability to and severity of AD.

Key words: Serum Calprotectin, Fecal Calprotectin, Atopic Dermatitis.

1. Introduction

As one of the most common inflammatory skin conditions, atopic dermatitis (AD) also goes by the name "atopic eczema." This condition may result in chronic, pruritic, and recurrent dermatitis. The physical and physiological symptoms of atopic dermatitis severely diminish the quality of life for those who suffer from it. The cost of treating AD, which includes medication, inpatient care, and follow-up care, has been rising rapidly in low- and middle-income nations. Additionally, children with this condition have social disadvantages, such as frequent absences from school and difficulties participating in regular activities [1].

AD is a chronic inflammatory pruritic skin condition that may afflict anybody of any age. AD is more common in youngsters, where its prevalence is estimated at 20%, than in adults, when it is only 10%. Africa and the Middle East are two of the emerging areas where the frequency of AD is on the rise [2].

Calprotectin is a calcium-zinc binding protein secreted by monocytes and neutrophils that has been implicated in a wide range of physiological and pathological processes, such as the control of immune response, the suppression of cell proliferation, and the control of the growth of pathogenic microorganisms [3].

Activated human neutrophils, monocytes, and macrophages exhibit high levels of calprotectin, also known as MRP8/14, a heterodimer composed of two intracellular calcium-binding proteins, S100A8 (MRP8) and S100A9 (MRP14). In reaction to stress, phagocytes actively release calprotectin. More than 20 years ago, researchers discovered a link between it and inflammation. Calprotectin was discovered not only as a receptor for advanced glycation end products (RAGE), but also as an endogenous activator of Toll-like receptor 4. [4]. Several chronic inflammatory disorders, such as rheumatoid arthritis, allograft rejection, inflammatory bowel disease, cancer, and lung diseases, have been linked to increased plasma levels of calprotectin [5].

Previous research has shown a favourable link between serum calprotectin and inflammatory skin conditions such psorias and between faecal calprotectin and children with atopic dermatitis. However, the role of serum calprotectin as an inflammatory and immunological marker in children with atopic dermatitis has not been investigated before.

The objectives of this research were to determine the clinical relevance of serum and faecal calprotectin in children with AD over a range of ages, to analyse the relationship between the two, and to clarify the relationship between the two and disease severity.

2. Patients and Methods

Type of the study

This study was conducted as a case - control study.

Study population

The study was conducted on fifty children suffering from AD in addition to ten apparently healthy individuals of matched age and sex as a control group, with age ranged from 6 months to 15 years old. Fifty AD children was tested for serum calprotectin and ten of them tested for fecal calprotectin in addition to ten apparently healthy individuals tested for serum calprotectin and five of them tested for fecal calprotectin**.**

Patients were recruited from outpatient clinic of Dermatology, Venereology and Andrology Department of Benha University Hospitals during the period from January 2021 to July 2021.

Inclusion criteria

Patients with AD, the diagnosis were based on clinical findings, pattern of British association according to Hanifin and Rajka criteria [6]. Those patients with different degrees of severity according to score of AD**,** with age group from 1 year up to 16 years old.

2.4Exclusion criteria

Any patient with any of the following condition was excluded from this study:

- Patient with congenital heart disease or bronchial asthma at time of the sample
- un controlled diabetes mellitus or renal failure
- those using topical treatment for less than two weeks or systemic treatment less than one month prior to the study.

Ethical consideration

This study was approved by the Research Ethical Committee of Benha Faculty of Medicine. Before taking blood samples, a written informed consent was taken from each patient and normal volunteer in the control group.

Methods

All patients were subjected to the following

Full history taking

- Personal history: Name, age, sex, occupation, residence and smoking or special habit of medical importance.
- Present history: Onset, course and duration of AD.
- Past history: History of medications (type and duration), associated systemic diseases, endocrinal problems and previous surgery.
- History of previous treatment of AD (type, dose and duration).
- Family history of AD.

Clinical Examination

All patients were subjected to the following:

- Complete general and dermatological clinical examination in order to clinically assess AD lesions, determine the distribution, the extent of AD and to exclude other diseases and deformities.
- Clinical details of all patients were recorded.

Assessment of atopic dermatitis severity: scoring atopic dermatitis (SCORAD)

This Scoring Atopic Dermatitis (SCORAD) calculator evaluates the severity of the atopic lesions based on affected body area and intensity of plaque characteristics.

Affected area (role of nine): a possible maximum of (100%).

- Head and neck (up to 9%).
- Upper extremities (up to 9% each).
- Anterior trunk (up to 18%).
- Back (up to 18%).
- Lower extremities (up to 18% each).
- Genitals (up to 1%).

Intensity: a representive area of eczema is selected. In this area, the intensity of each following signs is assessed as none (0), mild (1), moderate (2) or severe (3) with a maximum of (18).

- Redness.
- Swelling.
- Oozing.
- Scratch marks.
- Skin thickening.
- Dryness.

Subjective symptoms: on a scale from 0 (none) to 10 (maximum severity).

Itch and sleeplessness each is scored by patient or relative (0) is no itch or sleeplessness and (10) is the worst imaginable itch or sleeplessness with maximum (20).

The SCORAD formula is: A/5+7B/2+C. (A) stands for affected area, (B) stands for intensity, (C) stands for subjective symptoms. The result range is between 0 and 103 overall score of 0–24 was categorised as mild, 25–49 as moderate and 50–103 as severe [7].

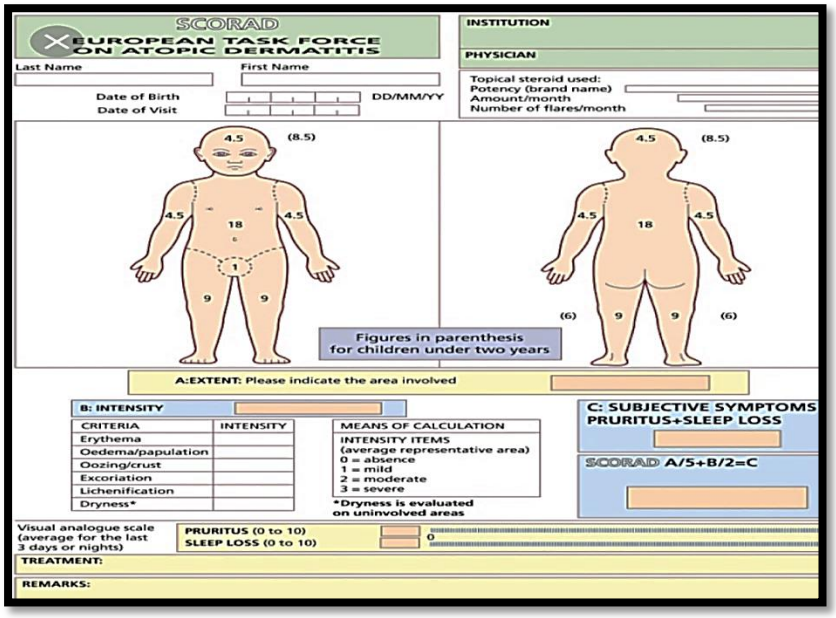

Fig. (1) Assessment severity of AD by score AD [8]

Laboratory investigations:

All participants were tested for determination of serum and fecal levels of calprotectin.

Measurement of serum calprotectin level by using enzyme-linked immunosorbent assay (ELISA) technique.

- **Sampling**

Three ml venous blood was collected from each subject by clean venipuncture using disposable plastic syringe and placed on plain tube (without anticoagulant) for serum separation. The tube was left at room temperature for 30 minutes till coagulation, and centrifuged (at 1500 rpm for 15 minutes).The resultant serum was aliquoted and stored at -20°C for further testing.

- **Analysis**

Serum calprotectin was measured using "Human calprotectin **(**CALPRO) ELISA Kit" provided by Shanghai Sunred Biological Technology Co., Ltd - human elisa kit, Website: www. srbooo.com, E-mail address: shanghai@srbooo.com, Phone number: 862166162175.

Statistical Analysis

The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science **(IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.).** Data were presented and suitable analysis was done according to the type of data obtained for each parameter.

- **Normality of data**
- **Descriptive statistics:**
  - Median and range for non-parametric numerical data.
  - Frequency and percentage of non-numerical data.
- **Analytical statistics:**
  - **Mann Whitney Test (U test)**
  - **Chi-Square test**
  - **Fisher's exact test**
  - **Correlation analysis**
  - **The ROC Curve (receiver operating characteristic)** provides a useful way to evaluate the sensitivity and specificity for quantitative diagnostic measures that categorize cases into one of two groups. The optimum cut off point was defined as that which maximized the AUC value. AUC is that a test with an area greater than 0.9 has high accuracy, while 0.7–0.9 indicates moderate accuracy, 0.5–0.7, low accuracy and 0.5 a chance result**.**
  - **Regression analysis**
- **Probability of results**

A p value is considered significant if <0.05 at confidence interval 95%.

3. Results

Table (1) Clinical features in all studied AD cases

	AD N=50
Age of onset (years)	Median (range) 1.5(0.2-7)
Duration (years)	4.8(0.5-12)
Xerosis	N (%) 50(100%)
Pit alba	20(40%)
K pilaris	19(38%)
Oral	11(22%)
Eye	11(22%)

Median age of onset was 1.5 years, ranged from 2 months to 7 years; median disease duration was 4.8 years, ranged from 6 months to 12 years.

Other manifestations of atopy included xerosis in all cases, pit alba in 40%, k pilaris in 38%, oral in 22% and eye in 22% table (1).

Table (2) Comparison of serum and fecal calprotectin levels among cases and control groups

	Control N=10		**AD N=50**		**P**
	median	**range**	**median**	**range**	
Serum Calprotectin (µg/ml)	3.5	2.7-5.1	7.4	3.9-23.2	<0.001
Fecal Calprotectin (µg/g)	3.8	2.6-8.5	6.4	2.2-12.2	0.002

Man Whitney test was used for comparison of numerical parameters.

AD group showed significantly higher serum calprotectin level when compared to control group (median=7.4 versus 3.5, p<0.001).

In addition, AD group showed significantly higher fecal calprotectin level when compared to control group (median=6.4 versus 3.8, p=0.002) table (2).

Table (3) Comparison of serum Calprotectin level according to other parameters in AD group

		Serum calprotectin level (µg/ml)				p
		N	median	minimum	maximum	
Gender	Males	24	7.7	5.2	22.0	0.153
	Females	26	7.05	3.9	23.2	
Family history	Negative	30	7.2	3.9	22.0	0.513
	Positive	20	7.7	4.2	23.2	
pit alba	Negative	30	7.45	4.2	22.0	0.367
	Positive	20	6.55	3.9	23.2	
k pilaris	Negative	31	6.9	3.9	23.2	0.149
	Positive	19	7.9	5.7	22.0	
oral	Negative	39	7.5	3.9	23.2	0.214
	Positive	11	6.9	4.2	17.0	
eye	Negative	39	7.5	3.9	23.2	0.214
	Positive	11	6.9	4.2	17.0	

Man Whitney test was used for comparison of numerical parameters.

No significant associations ($p>0.05$) were found regarding serum Calprotectin level according to other parameters in AD group table (3)

Table (4) Comparison of fecal Calprotectin level according to other parameters in AD group

		Fecal calprotectin level (µg/g)				p
		N	median	minimum	maximum	
Gender	Males	24	6.3	2.2	12.2	0.998
	Females	26	6.35	3.0	10.2	
Family history	Negative	30	6.35	2.2	12.2	0.797
	Positive	20	6.55	2.4	10.6	
pit alba	Negative	30	6.55	2.2	12.2	0.874
	Positive	20	6.05	3.0	10.6	
k pilaris	Negative	31	6.1	2.2	12.2	0.757
	Positive	19	6.6	2.4	8.8	
oral	Negative	39	6.4	2.4	12.2	0.861
	Positive	11	5.8	2.2	9.1	
eye	Negative	39	6.4	2.4	12.2	0.861
	Positive	11	5.8	2.2	9.1	

Man Whitney test was used for comparison of numerical parameters.

No significant associations ($p>0.05$) were found regarding fecal Calprotectin level according to other parameters in AD group table (4).

Table (5) Correlations of serum calprotectin level with age, onset, duration, severity in AD group

	Serum Calprotectin level	
	rs	p
Age	0.016	0.911
Age of onset	0.146	0.311
Duration	-0.022	0.880
Scorad	0.944	<0.001

rs, Spearman's correlation coefficient.

This table shows that serum calprotectin level showed significant positive correlation only with SCORAD scores ($p<0.001$) table (5).

Table (6) Correlations of fecal calprotectin level with age, onset, duration, severity in AD group

	Fecal Calprotectin level	
	rs	p
Age	0.074	0.611
Age of onset	0.103	0.477
Duration	0.054	0.710
Scorad	0.515	<0.001

rs, Spearman's correlation coefficient.

This table shows that fecal calprotectin level showed significant positive correlation only with SCORAD scores ($p<0.001$) table (6).

4. Discussion

Four in ten of the patients we looked at had a favourable family history. Corresponded with the findings of Dold et al. [9], who reported that 40% of patients with atopic dermatitis had a history of atopic illness in their siblings. In addition, Bohme et al. [10] discovered that 43% had a history of atopy in one or both parents.

The median age of start in this research was 1.5 years, with a range of 2 months to 7 years, and the median duration of illness was 4.8 years, with a range of 6 months to 12 years. Our findings are quite comparable to those of Camargo et al. [11], who found a median age of AD start of 3 months, with a range of 2 months to 1 year; the discrepancy might be attributable to differences in weather or sample size.

Other atopic signs, such as xerosis in all instances, pityriasis alba in 40%, keratosis pilaris in 38%, and oral and/or ocular manifestations in 22%, were seen in AD patients in the present research.

In contrast to these findings, Wahab et al. [12] found that xerosis was present in 43.8% of AD patients, infra-orbital folds in 39.5%, keratosis pilaris in 14.8%, pityriasis alba in 14.3%, facial erythema in 1.9%, and cheilitis in 10.5%. Additional research by Stefaniak et al. [13] found that xerosis was present in 38% and pruritus was present in 72%. The mismatch can result from differences in the studies' sample sizes or the weather.

Numerous researchers have linked allergic illness development to alterations in gut microbiota and intestinal inflammation [14]. Epithelial damage from an altered gut microbiota leads to increased intestinal inflammation, altered gut permeability, and immunological balance, all of which influence the progression of allergic disorders and the eventual onset of AD [15].

Serum and faecal calprotectin levels were found to be considerably higher in the AD group compared to the control group in the present study.

The faecal calprotectin levels of patients with IBD were also shown to be greater than those of the non-IBD group, according to research by Mahmoud et al. [16]. Fecal calprotectin levels are increased in inflammatory bowel disease by anything that stimulates neutrophil activity or migration into the gut lumen. Increased amounts of faecal calprotectin have been seen in individuals with rheumatological illness, necrotizing colitis, infectious colitis, and colorectal cancer [17].

Among the S100 family of proteins is calprotectin (S100A8/A9) [18]. Therefore, Jin et al[19] .'s findings that S100A8/A9 levels were likewise increased in the skin lesions and serum of AD patients corroborated our findings. They demonstrated the pro-inflammatory features of S100A8 and S100A9, which have earned them the name "damage associated molecular pattern molecules" (DAMPs).

In this investigation, we found that serum and faecal calprotectin levels were substantially linked with SCORAD score. Disease severity is associated with S100A8/A9 levels, according to Jin et al. [19]. One possible route of DAMP-mediated inflammation in AD is reflected in the overexpression of S100A8 and S100A9 expressions triggered by IL-17A and house-dust mites (HDMs), as well as the increase of IL-33 production by S100A9-treated keratinocytes.

In addition, Kim et al. [20] showed that sensitization with Oxazalon caused AD mice dramatically increased faecal calprotectin levels after 1 week and for up to 6 months. Interestingly, probiotic-treated mice showed a significant reduction in the elevated level of calprotectin seen in Oxazalon-induced AD animals. These results suggest that probiotics may mitigate the elevation of calprotectin seen in Oxazalon-induced AD.

As Bjarnason [21] elucidated, there is a connection between gut microbiota and faecal calprotectin, which functions as an inflammatory marker, and this elevation of faecal calprotectin plays a pathogenic role in the gut inflammatory process. They also shed light on the idea that probiotics or living microorganisms may impact the host by giving healthy gut bacteria and providing health advantages; this is supported by a reduction in faecal calprotectin following administration of probiotics for 4 weeks.

Our data demonstrated that the serum calprotectin level was not significantly correlated with the duration of the condition. However, Purchiaroni et al. [22] showed that chronic inflammatory responses cause calprotectin levels to rise, thus it's possible that the conflicting findings are because to weather or sample size differences. By contrast, Johnson and Ownby [23] reported that elevated calprotectin levels are common in chronic IBD and allergy disorders.

With a focus on skin-derived anti-microbial peptides and AD-related cytokines and chemokines, Chieosilapatham et al. [24] proposed a connection between the innate immunological capabilities of keratinocytes and the pathogenesis of AD.

Our findings are supported by those of Seo et al. [25], who found that children with elevated faecal calprotectin levels also had higher SCORAD index scores for AD. The average faecal calprotectin level in those with severe AD was substantially greater than those with mild to moderate AD. There was a statistically significant relationship between the faecal calprotectin level and the SCORAD index ($r=0.303$, $p=0.014$), but no other parameters. However, it is yet to be determined whether or not faecal calprotectin has a direct role in the pathogenetic pathways in infants with AD.

Pathirana et al. [26] reported that faecal calprotectin concentrations seem to be age-dependent in infancy, whereas the present investigation found no significant correlation between faecal calprotectin and age.

Also, Orivuori et al. [27] found that children with faecal calprotectin levels above the 90th percentile were more likely to develop AD by the age of 6 compared to

those with faecal calprotectin levels below the 90th percentile.

These findings indicate that severe intestinal inflammation during childhood is associated with an increased risk of developing allergy disorders as an adult. Intestinal inflammation seems to be regulated in part by the microbiota, which appears to be constantly colonising the gut [28].

At 2 months of age, the immune system is young, the gut is permeable, and the typical flora has not yet been formed, yet Orivuori et al. [27] found that there was a considerable drop in faecal calprotectin early in life. We know that faecal calprotectin levels are greater in infants than they are in older children. The median faecal calprotectin level was greatest (589.5 g/g) in the first 30 days of life, as documented by Günaydn et al. [29] who studied this phenomenon. It was hypothesised that elevated amounts of faecal calprotectin seen in the first month of life were connected to the continuous process of gastrointestinal development. Bacterial colonisation of the intestines in the first few months of life results in the production of chemoattractants that encourage granulocyte transepithelial migration and raise calprotectin levels in the faeces. Subclinical physiological inflammation in the gastrointestinal system, leading to transepithelial migration of granulocytes, is another possible explanation for the elevated levels of faecal calprotectin seen in the postpartum period. Calprotectin has been shown to have a variety of biological activities, such as antibacterial and antifungal activities and immunomodulatory properties, and can have a positive effect on host defences, such as the gastrointestinal system, in healthy infants under physiological conditions during the first weeks of life.

Neither the serum nor the faecal calprotectin levels were significantly correlated with any other parameters in the AD group ($p>0.05$ for both).

5. Conclusion

Higher concentrations of serum and faecal calprotectin were associated with increased risk for developing AD and a more severe form of the disease.

References

[1]. L. Kolb, SJ. Ferrer-Bruker, Atopic Dermatitis. [Updated 2022 May 23]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK448071/

[2]. MJ. Cork, SG. Danby, GS. Ogg, Atopic dermatitis epidemiology and unmet need in the United Kingdom. Journal of Dermatological Treatment, 2020 Nov,vol.16.pp.31-801.

[3]. D. Carroll, A. Corfield, R. Spicer and P. Cairns, Faecal calprotectin concentrations and diagnosis of necrotizing enterocolitis. Lancet,vol.361.pp. 310-311,2003.

[4]. E. Leclerc, G. Fritz, SW. Vetter and CW. Heizmann, Binding of S100 proteins to RAGE: an update. Biochim Biophys Acta,vol.1793.pp.993–1007,2009.

[5]. I. Bjarnason and R. Sherwood, Fecal calprotectin: a significant step in the noninvasive assessment of intestinal inflammation. J Pediatr Gastroenterol Nutr,vol.33.pp.11–13,2001.

[6]. J. Hanifin, G. Rajka, "Diagnostic features of Atopic Dermatitis," Acta Dermato-Venereologica, supplement,vol.92.pp.44–47,1980.

[7]. Oranje AP, Glazenburg EJ, Wolkerstorfer A, de Waard-van der Spek FB. Practical issues on interpretation of scoring atopic dermatitis: the SCORAD index, objective SCORAD and the three-item severity score. Br J Dermatol. 2007 Oct,vol.157.pp.645-8.

[8]. C. Charman, H. Williams, Outcome measures of disease severity in atopic eczema. Archives of Dermatology,2000 Jun,vol.1.pp.136-763.

[9]. S. Dold, M. Wjst, E. Von Mutius, P. Reitmeir, E. Stiepel, Genetic risk for asthma, allergic rhinitis, and atopic dermatitis. Archives of disease in childhood. 1992 Aug 1;67(8):1018-22.

[10]. Böhme M, Wickman M, Lennart Nordvall S, Svartengren M, Wahlgren CF. Family history and risk of atopic dermatitis in children up to 4 years. Clinical & Experimental Allergy. 2003 Sep,vol.33.pp.1226-31.

[11]. CA. Camargo, D. Ganmaa, R. Sidbury, K. Erdenedelger, N. Radnaakhand, B Khandsuren, Randomized trial of vitamin D supplementation for winter-related atopic dermatitis in children. Journal of Allergy and Clinical Immunology. 2014 Oct,vol.1.pp.134-831.

[12]. S. Wahab, J. Ahmed, K. Sharif-ul-Hasan, Pars plana surgical capsulotomy for posterior capsular opacification (PCO). J Pak Med Assoc. 2011 Jan;61(1):14-7. PMID: 22368895.

[13]. AA. Stefaniak, A. Zubkiewicz-Kucharska, Ł. Matusiak, A. Noczyńska, JC. Szepietowski, Itch in Children with Type 1 Diabetes: A Cross-Sectional Study. Dermatol Ther (Heidelb). 2020 Aug,vol.10.pp.745-756.

[14]. T. Zuo, SC. Ng, The gut microbiota in the pathogenesis and therapeutics of inflammatory bowel disease. Frontiers in microbiology,vol.9.pp.22-47,2018.

[15]. D. Zheng, T. Liwinski, & E. Elinav, Interaction between microbiota and immunity in health and disease. *Cell Res* 30, https://doi.org/10.1038/s41422-020-0332-7,vol.8.pp.492–506,2020.

[16]. HS. Mahmoud, HM. Fayed, M. El Dosoky, M.Tag-Adeen, Assessment of Fecal Calprotectin level in Inflammatory, Infectious and Malignant Gastrointestinal Diseases. SVU-International Journal of Medical Sciences. 2021 Aug,vol.1.pp.14-24.

[17]. H. Hovstadius, D. Lundgren, P.Karling, Elevated Faecal Calprotectin in Patients with a Normal Colonoscopy: Does It Matter in Clinical Practice?

A Retrospective Observational Study. Inflamm Intest Dis. 2021 May,vol.6.pp.101-108.

[18]. B. Kim, M. Siracusa, S. Saenz, TSLP elicits IL-33- independent innate lymphoid cell responses to promote skin inflammation. Science Translational Medicine,vol.5.pp.170-180, 2013.

[19]. S. Jin, CO. Park, JU. Shin, JY. Noh, YS. Lee, NR. Lee, HR. Kim, S. Noh, Y. Lee, JH. Lee, KH. Lee, DAMP molecules S100A9 and S100A8 activated by IL-17A and house-dust mites are increased in atopic dermatitis. Exp Dermatol. 2014 Dec,vol.23.pp.938-41.

[20]. MJ. Kim, JY. Kim, M. Kang, MH. Won, SH. Hong, Y. Her, Reduced fecal calprotectin and inflammation in a murine model of atopic dermatitis following probiotic treatment. International journal of molecular sciences. 2020 May,vol.31.pp.39-68.

[21]. I. Bjarnason, The Use of Fecal Calprotectin in Inflammatory Bowel Disease. Gastroenterol Hepatol (N YPMID): 28420947; PMCID: PMC5390326.),2017 Jan,vol.13.pp.53-56.

[22]. F. Purchiaroni, A. Tortora, M. Gabrielli, F. Bertucci, G. Gigante, G. Ianiro, V.Ojetti, E.Scarpellini, A. Gasbarrini, The role of intestinal microbiota and the immune system. Eur. Rev. Med. Pharm. Sci,vol.17.pp.323–333,2013.

[23]. C.C.Johnson, D.R. Ownby, The infant gut bacterial microbiota and risk of pediatric asthma and allergic diseases. Transl. Res,vol.179.pp.60–70,2017.

[24]. P. Chieosilapatham, C. Kiatsurayanon, Y. Umehara, JV. Trujillo-Paez, G. Peng, H. Yue, LT. Nguyen, F. Niyonsaba, Keratinocytes: Innate immune cells in atopic dermatitis. Clinical & Experimental Immunology. 2021 Jun,vol.204.pp.296-309.

[25]. S.C. Seo, S.H. Ahn, S. Ri, Y.Yoon, J.H. Byeon, S.H. Kim, W. Yoon, Y. Yoo, Elevated fecal calprotectin levels are associated with severity of atopic dermatitis in children. Asian Pac. J. Allergy Immunol,vol.36.pp.82–87,2018.

[26]. WGW. Pathirana, SP. Chubb, MJ. Gillett, SD. Vasikaran, Faecal Calprotectin. Clin Biochem Rev. PMID: 30828114; PMCID: PMC6370282,2018 Aug,vol.39.pp.77-90.

[27]. Laura Orivuori, & Kirsi Mustonen, & Marcus Goffau, & Saara Hakala, & Monika Paasela, & Caroline Roduit, & Jean Dalphin, & Jon Genuneit, & Roger Lauener, & Josef Riedler, & Juliane Weber, & Erika Mutius, & Juha Pekkanen, & Hermie Harmsen, & Outi Vaarala, & Petra Pfefferle, High level of fecal calprotectin at age 2 months as a marker of intestinal inflammation predicts atopic dermatitis and asthma by age 6. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 45. 10.1111/cea.12522,vol.8.pp.12-30, 2015.

[28]. SJ. Jeong, The role of fecal calprotectin in pediatric disease. Korean J Pediatr. 2019 Aug,vol.62.pp.287-291.

[29]. BS. Günaydın Şahin, G. Keskindemirci, TA. Özden, Ö. Durmaz, G. Gökçay, Faecal calprotectin levels during the first year of life in healthy children. Journal of Paediatrics and Child Health. 2020 Nov,vol.56.pp.1806-11.