# THE CORRELATION BETWEEN VITAMIN D LEVEL IN SERUM AND THE PERCENTAGE OF BASOPHILS IN CHILDREN WITH ATOPIC DERMATITIS

**FINAL ASSIGNMENT**

**To Meet the Requirements to Obtain a Medical Degree**


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## STATEMENT OF AUTHENTICITY

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State that this final assignment is truly the work of my own, not the takeover of the writings or thoughts of others. If later proven that this final assignment is plagiarized, I am willing to accept sanctions for my act.

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## ABSTRACT

Abdul Hakkeem, Jafrina Jasmin. 2019. **The correlation between the vitamin D level in serum and the percentage of Basophil in children with atopic dermatitis in Saiful Anwar Hospital.** Final Assignment, Medical Program, Faculty of Medicine, Brawijaya University. Supervisors (1) Dr. dr. Wisnu Barlianto, M.Si.Med, SpA(K), (2) Dr. Bayu Lestari M. Biomed

Vitamin D has the ability to repress inflammation and its immunomodulatory property allows it to be used as a treatment for Atopic Dermatitis. Patients with Atopic Dermatitis have high amounts of Basophils in their skin lesions. The aim of this study was to determine the correlation between the level of Vitamin D and the percentage of Basophils in children with Atopic Dermatitis in Saiful Anwar Hospital. In this crss-sectional study, the measurement of Vitamin D level in patients was conducted using the Enzyme-linked Immunosorbent Assays (ELISA) method. The values were measured in nmol/L. Meanwhile, Basophils were measured using a Hematolgy Analyzer. There were 12 patients in this study, five males and seven females, aged 6.33 ± 2.96 months. There were seven patients with Vitamin D deficiency (25 (OH) D < 20 ng/mL). Three patients had Vitamin D insufficiency (25 (OH) D 20-30 ng/mL) and only two patients had sufficient amount of Vitamin D (25 (OH) D > 30 ng/mL). The relationship between Vitamin D level and Basophil percentage were analysed using the one-way Anova test and Pearson correlation. A significant negative correlation was detected between Vitamin D levels and Basophil percentage ($p = 0.029$, $r = -0.626$). In conclusion, there is a correlation between Vitamin D levels and Basophil percentage in patients with Atopic Dermatitis. Whereby the lower the Vitamin D level, the higher the Basophil percentage.

Keywords: Atopic Dermatitis, Vitamin D, Basophils

## ABSTRAK

Abdul Hakkeem, Jafrina Jasmin. 2019. **Korelasi antara Vitamin D di serum dan presentase Basofil pada anak-anak dengan Dermatitis Atopik di Rumah Sakit Saiful Anwar.** Tugas Akhir, Program Studi Kedokteran, Fakultas Kedokteran Universitas Brawijaya. Pembimbing: (1) Dr. dr. Wisnu Barlianto, M.Si.Med, SpA(K), (2) Dr. Bayu Lestari M. Biomed

Vitamin D memiliki kemampuan untuk menekan peradangan dan properti imunomodulatornya memungkinkan ia untuk digunakan sebagai pengobatan untuk dermatitis atopik. Pasien dengan Dermatitis Atopik memiliki jumlah Basofil yang tinggi di dalam lesi kulit mereka. Tujuan dari penelitian ini adalah untuk mengetahui hubungan antara kadar Vitamin D dan persentase Basofil pada anak-anak dengan Dermatitis Atopik di Rumah Sakit Saiful Anwar. Desain penelitian ini adalah analitik observasional dengan pendekatan cross-sectional. Pengukuran kadar Vitamin D pada pasien dilakukan dengan menggunakan metode *Enzyme-linked Immunosorbent Assays (ELISA)*. Nilainya diukur dalam nmol / L. Sementara itu, Basofil diukur dengan *Hematology Analyzer* menggunakan sampel darah pasien. Ada 12 pasien dalam penelitian ini, lima laki-laki dan tujuh perempuan, berusia 6,33 ± 2,96 bulan. Ada tujuh pasien dengan defisiensi vitamin D (25 (OH) D <20 ng / ml). Tiga pasien memiliki kekurangan vitamin D (25 (OH) D 20-30 ng / ml) dan hanya dua pasien yang memiliki jumlah vitamin D (25 (OH) D> 30 ng / ml) yang cukup. Hubungan antara kadar Vitamin D dan jumlah Basophil dianalisis menggunakan uji *Anova* satu arah dan korelasi *Pearson*. Korelasi negatif yang signifikan terdeteksi antara kadar Vitamin D dan jumlah Basofil (p = 0,029, r = -0,626). Kesimpulannya, terdapat korelasi antara kadar vitamin D dan jumlah Basofil pada pasien dengan Dermatitis Atopik. Dimana semakin rendah kadar Vitamin D, semakin tinggi jumlah Basophil.

Kata kunci: Dermatitis Atopik, Vitamin D, Basofil

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## ABBREVIATIONS

AD: Atopic Dermatitis

CD: Cathelicidin D

ELISA: Enzyme-linked Immunosorbent Assays

IG-E: Immunoglobulin E

IL-33: Interleukin-33

LPS: Lipopolysaccharides

SPSS: Statistical Package for the Social Sciences

TLR: Toll-like receptor

TMB: Tetramethylbenzidine

TSLP: Thymic stromal lymphopoeitin

UVB: Ultraviolet B

VDBP: Vitamin D binding protein

VDR: Vitamin D receptor

# CHAPTER 1
# INTRODUCTION

## 1.1 Background

Atopy is the genetic susceptibility to develop allergic hypersensitivity. Dermatitis is a term that refers to the inflammation of the skin that can have various causes and develops in multiple forms. Atopic dermatitis (AD) is commonly known as eczema or atopic eczema, and is a chronic inflammatory skin disease (Kim *et al*., 2019).

Atopic dermatitis is a skin disease which usually begins in early infancy but is also known to affect a decent number of adults. The incidence of AD is estimated to be 15-20% in children and 1-3% in adults. Approximately 13% of children and 9.1% of adults suffering from AD were identified as Asians. In Indonesia, the prevalence of atopic dermatitis is approximately 1.1% in 13 to 14 year olds (Nutten, 2015).

The pathogenesis of atopic dermatitis is contributed by various factors such as genetic, mechanical, environmental, epidermal, pharmacologic and immunologic. In fact, a study in Hong Kong showed that atopic dermatitis in around 70% of patients is caused by genetic factors. Defective Fillagrin gene has known to cause moderate to severe AD in up to one third of people in East Asia (Boguniewicz and Leung, 2013). The primary reason for the development of atopic dermatitis is defective skin barrier which allows the entry of antigens and eventually causes the production of inflammatory cytokines. The main symptom of AD is said to be incessant pruritus or severe itching, which involves an

intermittent course of flares and remission that occur without known reasons. In children, some of the symptoms of AD include dry and scaly skin, lightened or darkened skin spots, rashes on the neck and face, especially around the eyes and thick skin also known as lichenification. When these children grow up, they may have discolouration and easily irritated skin (Baird, 2016).

In order to successfully treat AD, patients need a treatment that proves the skin with hydration and moisturization. The most important aspect of treating AD is the identification and elimination of triggers. Some of the medications that can be used for severe AD include systemic or topical steroids as well as antihistamines (Kim, 2019).

Basophils are a type of white blood cells or leukocytes that differentiate and mature in the bone marrow and circulate in the peripheral blood (Metz *et al*., 2008). Granulocytes are a group of leukocytes that are comprised of basophils, eosinophils and neutrophils. Basophils are mostly involved in the immune system where they mediate hypersensitivity reactions. In the event of an infection, basophils migrate to the site of infection and release various mediators such as chemokines, cytokines and proteases (Augustyn *et al*., 2016).

Studies have shown that patients with atopic dermatitis have an increased spontaneous release of basophils. Patients with more severe atopic dermatitis are known to have activated circulating basophils and an increased release of basophils (Redrup *et al*., 1998). Other than atopic dermatitis, basophils also invade skin lesions in skin disorders like chronic idiopathic urticaria and allergic contact dermatitis (Wu *et al*., 2002).

Vitamin D has the ability to suppress inflammatory responses and improve the integrity of the permeability barrier. As a result, it can be a possible medication for various skin diseases, including atopic dermatitis. The presence of Vitamin D in human cause a reduction in the amount of circulating basophils (Searing and Leung, 2010).

This observational study was conducted in order to determine the relationship between the amount of vitamin D in serum and the percentage of basophils in children who were diagnosed with atopic dermatitis in Saiful Anwar Hospital Malang.

## 1.2 Problem Statement

### 1.2.1 General Problem Statement

Is there a correlation between the level of vitamin D in serum and the percentage of basophils in children with atopic dermatitis in the paediatric department of Saiful Anwar Hospital?

### 1.2.2 Specific Problem Statements

1. How is the level of vitamin D in serum in children with atopic dermatitis in the paediatric department of Saiful Anwar Hospital?
2. Does the percentage of basophils in children with atopic dermatitis in the paediatric department of Saiful Anwar Hospital vary in different Vitamin D status?

3. Is there a correlation between the level of Vitamin D in serum and the percentage of basophils in children with atopic dermatitis in the paediatric department of Saiful Anwar Hospital?

## 1.3 Research Objectives

### 1.3.1 General Objective

The general objective of this study is to determine if there is a correlation between the vitamin D level in serum and the percentage of basophils in children with atopic dermatitis in the paediatric department of Saiful Anwar Hospital.

### 1.3.2 Specific Objectives

The specific objectives of this study are as follows:

1. To determine the level of vitamin D in serum in children with atopic dermatitis in the paediatric department of Saiful Anwar Hospital
2. To determine the percentage of basophils in children with atopic dermatitis in the paediatric department of Saiful Anwar Hospital in different Vitamin D status
3. To prove that there is a correlation between the Vitamin D level in serum and the percentage of basophils in children with atopic dermatitis in the paediatric department of Saiful Anwar Hospital

## 1.4 Benefits of Research

The benefits that can be derived from this study involve both the academic and clinical field.

Academic Benefits:

1. To contribute to the knowledge in regards to the correlation between the amount of Vitamin D in serum and the percentage of basophils in children with atopic dermatitis.
2. To serve as a basis for further research on atopic dermatitis and its therapy.

Clinical Benefits:

1. To contribute to the clinical information regarding vitamin D as a possible therapy for atopic dermatitis.
2. To serve as a basis for the development of the method used to evaluate the prognosis of atopic dermatitis

# CHAPTER 2
# LITERATURE REVIEW

## 2.1 Atopic Dermatitis

### 2.1.1 Definition and Epidemiology

Atopic dermatitis (AD) or commonly known as eczema is a chronic and pruritic inflammatory skin disease. The word *atopic* is derived from the Greek which translates to “strange” and the word *dermatitis* means inflammation of the skin. According to the Japanese Dermatological Association, the definition of atopic dermatitis is “a disease whose main lesion is itching eczema with recurrent remissions and exacerbations”. It is a chronically relapsing illness caused by multiple stimulative factors. AD usually begins in early infancy but is also found in a significant number of adults (Kim, 2019).

One of the most common diseases that affect children and infants is atopic dermatitis. The prevalence of AD as high as 20% in most countries and continues to increase. Approximately 30% of infants are afflicted with atopic dermatitis and in adults, the prevalence of AD ranges from 1 to 3%. Atopic dermatitis in adults is more common in Asia than in western countries. Females are found to be slightly more susceptible to AD with a ratio of female: male ratio of 1.3:1. In Indonesia, the prevalence of atopic dermatitis is 1.1% in 13 to 14 year olds (Nutten, 2015).

Some of the clinical manifestations of AD includes hyper pigmented skin especially around the eyes or dark spots on the skin, scaly and dry skin, cracked

skin that can sometimes cause pain or bleeding, skin creases, rashes and itchiness. The itchiness is usually worse at night and the rashes experienced may be filled with pus and develop on the face, wrists and forearms. Infants with AD may develop red, scaly and crusty areas on their cheeks, scalp and front of the arms and legs (Leung and Bieber, 2003).

### 2.1.2 Pathogenesis

The pathogenesis of atopic dermatitis has not been fully understood. This is because AD and its development is caused by a complex interaction between multiple factors. However, a defective epidermal barrier is the main factor that contributes to the clinical manifestations of AD (Pandaleke, 2014).

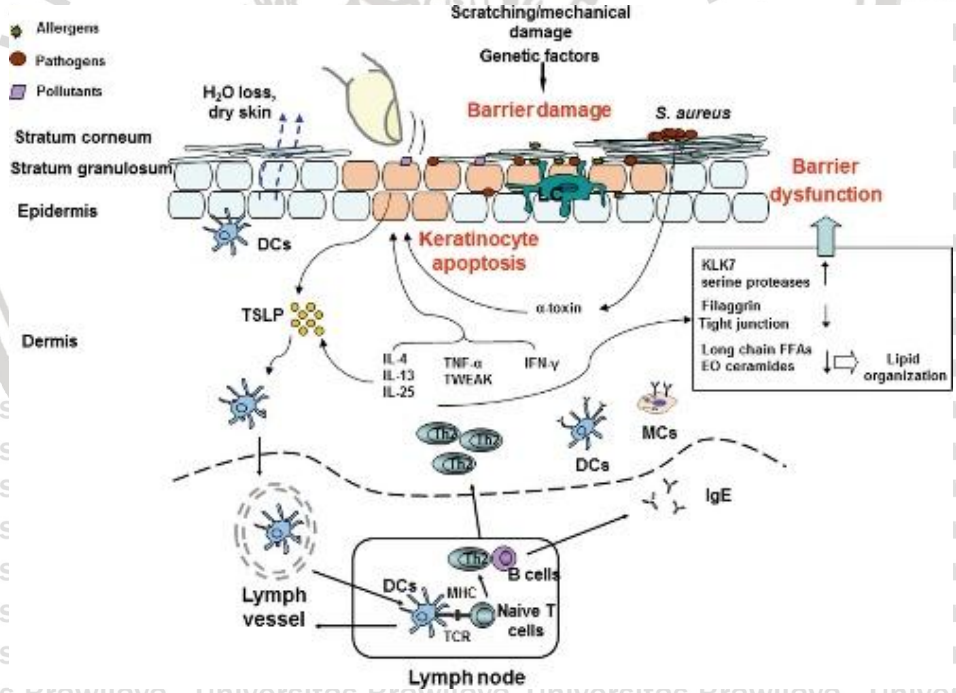


**Figure 2.1:** Pathogenesis of Atopic Dermatitis (Peng and Novak, 2015)

Genetic, immunologic and mechanical factors induce the damage of epidermal barrier, causing the antigen-presenting cells to easily come in contact with allergens, bacterial and viral antigens. Activated antigen-presenting cells migrate to lymph nodes and cause naïve T cells to become Th2 cells. Increased Th2 cytokines, TNF-α and IFN-γ damage the epidermal barrier function. This induces keratinocyte apoptosis and disrupt the function of tight junctions and increase Th2 responses by enhancing TSLP expression of epithelial cells. Furthermore, colonizing pathogens including *Staphylococcus aureus* damage barrier function due to the release of virulence factors which will induce keratinocyte death and eventually boost Th2-type inflammation. This

means that the pathogenesis of AD is contributed by both genetic and immunologic factors (Peng and Novak, 2015).

Patients with AD are known to have a mutation of the FLG (Filaggrin gene) which is responsible for the encoding of the protein (pro)-filaggrin, an important protein in the epidermis. Genetic defect in FLG disrupts the epidermis and results in contact between the immune cells in the dermis and antigens in the external environment. Scratching causes epidermal inflammation and is known as the 'itch scratch cycle'. This results in itchiness and scratching will lead to inflammation. (Pandaleke, 2014).

The damaged skin barrier causes migration of activated antigen-presenting cells into the lymph glands and migration from naive T cells to T helper 2 cells (Th2). Increased Th2 cytokines and Tumour Necrosis Factor α (TNF-α) along with IFN-γ is the reason for further damage of the skin barrier since it causes keratinocyte apoptosis induction. This impairs the tight junction function and increases the response of Th2 by raising the thymic stromal lymphopoetin (TLSP) expression from epithelial cells (Peng and Novak, 2015).

AD can also occur in patients with defective innate immunity that results in susceptibility to viral and bacterial infections. Initially, the T cell response will be dominated by Th2. Eventually, a shift in a Th1 dominant response will lead to the release of cytokines and pro-inflammatory chemokines known as interleukin (IL) 4, IL 5 and TNF which will stimulate the production of IgE and a systemic inflammatory response. This series of events will produce the clinical manifestations of AD such as pruritus (Pandaleke, 2014).

It is also worth noting that environmental and pharmacologic factors as well as microbial exposure could be considered responsible in the development of atopic dermatitis. For instance, dust mites and pollen are known to be able to

trigger an eczema flare up. AD is also a common occurrence in people who easily react towards chemicals or irritants such as cleaning products like soaps and detergents (Jones, 2018).

### 2.1.3 Diagnosis

Atopic dermatitis can be diagnosed with clinical manifestations presented by patients. While there are several factors that should be taken into consideration such as a family history of AD, environmental conditions and specific triggers (Kim *et al.*, 2016).

1. Patient must have three or more of the following major criteria:

   i) Pruritus

   ii) Dermatitis affecting flexural surfaces in adults and the face and extensors in infants

   iii) Chronic or relapsing dermatitis

   iv) Personal or family history of cutaneous or respiratory atopy

2. Patient must have at least three of the following minor criteria:

   i) Features of the so-called “atopic facies”: facial pallor or erythema, hypo pigmented patches, infraorbital darkening, infraorbital folds or wrinkles, cheilitis, recurrent conjunctivitis and anterior neck folds

   ii) Trigger of atopic dermatitis: food, emotional factor, environmental factors and skin irritants like wool, solvents and sweat

   iii) Complications of atopic dermatitis such as susceptibility to cutaneous viral and bacterial infections, impaired cell-mediated immunity,

immediate skin-test reactivity, raised serum IgE, keratoconus and anterior sub capsular cataracts.

iv) Others: early age of onset, dry skin, ichthyosis, hyper linear palms, keratosis pilaris (plugged hair follicles of proximal extremities), hand and foot dermatitis, nipple eczema, white dermatographism and perifollicular accentuation

**2.1.4 Assessment of AD Activity**

The best validated scoring system for Atopic Dermatitis is SCORAD. SCORAD (Score of Atopic Dermatitis) is a clinical tool used to assess the extent and severity of AD. It is used before and after treatment to test the effectiveness of the treatment. The interpretation of SCORAD is done based on three categories, which are the areas affected, the intensity of the signs and the subjective symptoms experienced (Chopra *et al.,* 2017).

The areas affected are assessed using the rule of 9 by calculating the percentages of different parts of the body. 9% for the head and neck, 9% and 18% for each upper and lower limb, 18% for the anterior trunk and back as well as 1% for the genitals. The total area is 'A' which has a possible maximum of 100%. The intensity of the signs are examined based on six criteria which are erythema (redness), papulation or oedema (swelling), oozing or crusting, excoriation (scratching), lichenification (leathery) and dryness (ichthyosis). The score ranges from 0-3 which translates to absent, mild, moderate or severe. The total scores are added to give 'B' a maximum of 18 points. The subjective symptoms such as pruritus or sleeplessness can be scored from 0 to 10 where 0

indicates absence of symptoms and 10 indicates the worst imaginable condition. The scores are added to give 'C' with a maximum of 20 points (Hanifin, 2018).

The SCORAD assessment for atopic dermatitis is divided into the following:

- Mild: <15 score
- Moderate: 15-40 score
- Severe: >40 score

**2.1.5 Management**

Non-pharmacologic measures for atopic dermatitis are just as critical as pharmacologic treatments. The most important of which is identifying and eliminating factors that cause atopic dermatitis. Some of the factors that trigger AD are allergens such as dust mites, animal dander and pollen, foods like milk, eggs, nuts and fish as well as environmental conditions including extreme temperatures, seasonal changes, humidity and smoke. Patients should steer clear of irritants which encompass soaps, detergents, creams and topical medications. Certain materials worn on the skin such as wool and synthetic fabrics can also provoke the outbreak of eczema. Other factors include hormonal changes in women, especially during pregnancy or menstruation and infections caused by bacteria and virus (Chen *et al*., 2007).

It is crucial for patients with atopic dermatitis to add elements of hydration and moisture to the skin. Hydrating the skin is needed to remove scales, crusts, irritants and allergens. Moisturizers on the other hand, are useful to stop xerosis

(dry skin) and trans-epidermal dehydration while reducing the severity of the disease. Patients with AD should shower or bath regularly and moisturise immediately. Steroid cream or ointment should be applied to red or itchy areas while moisturisers should be applied on other parts of the body. Wet compresses would be useful to control itching during the night (Shwartz, 2019).

Pharmacologic therapy for AD includes topical corticosteroids such as Hydrocortisone, Triamcinolone and Flurandrenolide. Systemic corticosteroids like Prednisone as well as immunomodulatory drugs like Tacrolimus and Pimecrolimus can be used too. Antihistamines such as Diphenhydramine, Hydroxyzine, Doxepin and Pramoxine may help relieve itching caused by AD. Patients may also be prescribed with antimicrobials like Mupirocin, Erythromycin, Oxacillin, Amoxicillin, Acyclovir and Ketoconazole to treat infections (Kim *et al*., 2016).

## 2.2 Basophils

### 2.2.1 Basophils and their functions

Basophils were discovered in 1897 by Paul Ehrlich. They are myeloid cells which are also the least common granulocytes that are found in the blood (Obata *et al*., 2019). Granulocytes are a group of white blood cells formed by basophils, eosinophils and neutrophils. Basophils have large, purplish black cytoplasmic granules that conceal the underlying double-lobed nucleus. They are formed in the bone marrow and are released into the peripheral blood where they circulate. From the circulation, basophils migrate to tissues to synthesize and store an inflammatory modulator called histamine (Borriello *et al*., 2014).

### 2.2.2 Basophils in Atopic Dermatitis

In the event of an allergic reaction, basophils infiltrate into the inflammatory sites. This is not only true in atopic dermatitis, but also in other conditions such as asthma and allergic rhinitis. The accumulation of basophils and mast cells can be seen in allergic reactions. Their migration, differentiation and activation are needed for inflammatory responses (Metz *et al*., 2008).

Basophils play a significant role in allergic diseases as they aid in the secretion of various mediators, such as cytokines, chemokines and proteases. The activation of basophils is responsible for the release of histamine, IL- and IL-13 (Marone *et al*., 2000). Studies show that skin lesions of patients who have been diagnosed with AD are enriched with basophils. On the other hand, no basophils were found in the healthy control group (Siracusa *et al*., 2013).

## 2.3 Vitamin D

### 2.3.1 Metabolism of Vitamin D

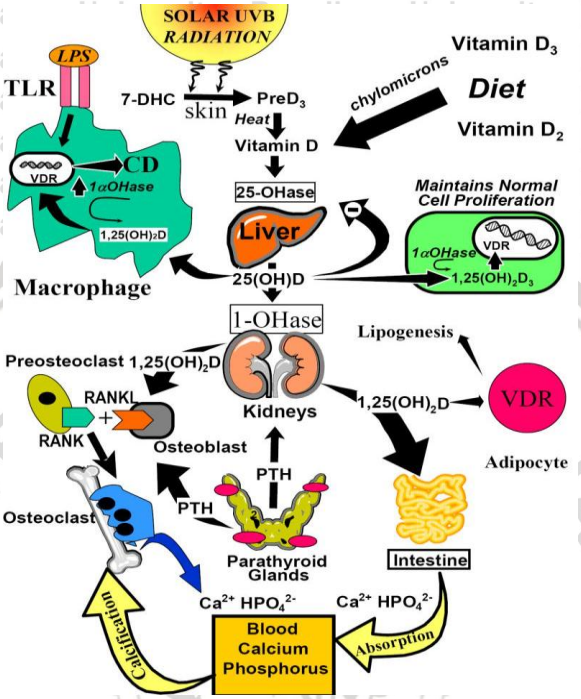


**Figure 2.2:** The metabolism of vitamin D (Holick, 2009)

When exposed to sunlight, 7-dihydrocholesterol (7-DHC) is photolyzed to previtamin D3 (preD3). PreD3 is converted to vitamin D3 by body heat. Vitamin D produced in the skin together with Vitamin D2 and Vitamin D3 enter the circulation and are either stored in the body's adipocytes or enter the liver to be converted to 25-hydroxyvitamin D [25 (OH) D]. For calcium metabolism, 25 (OH) D is converted in the kidneys to 1.25-dihydroxyvitamin D [1.25 (OH) 2 D] which interacts with its vitamin D receptor (VDR) in the small intestines and on osteoblasts for calcium regulation and phosphorus metabolism. 25 (OH) D is metabolized in various tissues and cells for the regulation of cell proliferation and differentiation. It also induces cathelicidin D (CD) in macrophages. 1.25 (OH) 2 D is induced in the macrophage and is controlled by the 2/1 toll-like receptors (TLR) and its interaction with lipopolysaccharide (LPS). Circulating concentrations of 1.25 (OH) 2 D helps to increase the production of insulin and decrease the production of renin as well as alter adipocyte lipogenesis (Holick, 2009).

The majority of Vitamin D for humans are sourced from animal-based food products such as fish, eggs, milk and beef liver as well as solar ultraviolet B. Vitamin D that is produced in the skin or ingested is hydroxylated in the liver to form 25-hydroxycholecalciferol or 25(OH) D, also known as calcifediol. This

reaction is catalysed by vitamin D 25-hydroxylase which is the product of the *CYP2R1* human gene. The product is released into the plasma where it is bound to vitamin D-binding protein. Calcifediol is transported to the kidneys and hydroxylated to form calcitriol (1.25 dihydroxycholecalciferol, 1.25 (OH) $_2$D). This process is catalysed by the enzyme 25-hydroxyvitamin $D_3$ 1-alpha-hydroxylase, which is the product of *CYP27B1* human gene. Parathyroid hormone and low levels of calcium or phosphate can increase the activity of *CYP27B1* (Holick, 2009).

After finally being converted in the kidney, calcitriol is released into the circulation, throughout the body including to target organs of intestine, kidney and bone by binding to vitamin D-binding protein. Calcitriol is responsible for mediating most of the physiological actions of vitamin D. It is also synthesized by other cells including monocyte-macrophages in the immune system where it acts locally as a cytokine to modulate body defences against microbes by stimulating the innate immune system (Holick, 2009).

### 2.3.2 Vitamin D Deficiency

Vitamin D deficiency is defined as 25 (OH) D <20 ng/mL while Vitamin D insufficiency is 25 (OH) D 20-30 ng/mL. Finally, Vitamin D sufficiency is confirmed when 25 (OH) D >30 ng/mL (Tangpricha, 2019).

Some of the factors that influence Vitamin D deficiency are sunlight exposure, dietary habits, old age, body weight and BMI as well as reduced production of Vitamin D in the skin. There are specific diseases that result in low Vitamin D levels such as renal diseases and gastrointestinal disorders (Harbolic, 2019).

## 2.4 Vitamin D and Atopic Dermatitis

Vitamin D plays a crucial role in both adaptive and innate immunity. Recently, vitamin D receptors (VDR) were discovered and detected in many tissues and cells in humans including almost all immune cells such as B cells, T cells, dendritic cells, neutrophils and macrophages. In innate immunity, Vitamin D induces antimicrobial peptide synthesis but inhibits the expression of TLR (Toll-like receptor) and the pro-inflammatory cytokine production. In adaptive immunity, Vitamin D causes the reduction of Th-1 cytokine production and inhibition of T cell proliferation (Hoxha, 2014).

Vitamin D is known to be able to suppress inflammatory responses and improve the integrity of the permeability barrier which allows it to be a possible therapeutic intervention for various skin diseases, including atopic dermatitis (Searing and Leung, 2010).

# CHAPTER 3

# CONCEPTUAL FRAMEWORK AND HYPOTHESIS

## 3.1 Conceptual Framework

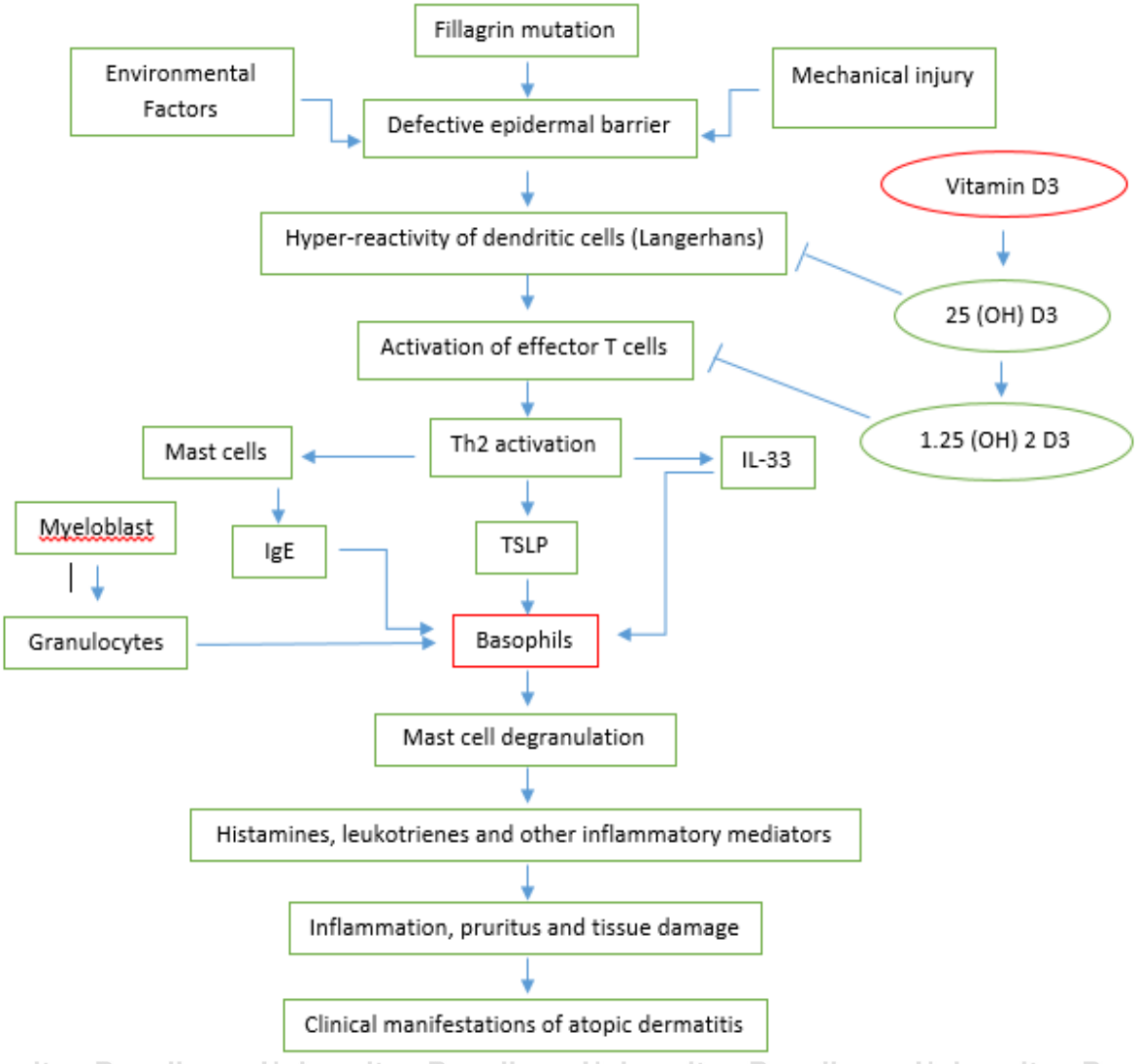


**Figure 3.1** Conceptual Framework

Explanation:

: Variable measured

: Induces

: Inhibits

Based on the conceptual framework, the pathogenesis of atopic dermatitis is initiated by a defective epidermal barrier caused by mechanical injuries, environmental factors or fillagrin mutation. This results in the hyper-reactivity of dendritic or Langerhans cells. Effector T cells are activated and subsequently Th2 activation occurs. With that, IgE formed by mast cells together with IL-33 and TSLP regulate basophils. Basophils cause degranulation of mast cells to produce histamine, leukotrienes and other inflammatory mediators. As a result, inflammation, pruritus and tissue damage may occur followed by other clinical manifestations of atopic dermatitis (Peng and Novak, 2015).

1.25 (OH) 2 D3 is an active metabolite of vitamin D which plays an important role in the immune system regulation. It has the ability to inhibit the hyper reactivity of dendritic cells and the activation of effector T cells. This affects the mast cells and granulocytes produced which suppresses basophils in the body. As a consequence, vitamin D reduces the clinical manifestations of atopic dermatitis as well as improving the quality of lives of patients with atopic dermatitis (Hoxha, 2014).

**3.2 Hypothesis**

1. The level of vitamin D in serum in children with atopic dermatitis in the paediatric department of Saiful Anwar Hospital is lower than the normal range
2. The percentage of basophils in children with atopic dermatitis in the paediatric department of Saiful Anwar Hospital is higher in patients with Vitamin D deficiency

3. There is a negative correlation between the Vitamin D level in serum and the percentage of basophils in children with atopic dermatitis in the paediatric department of Saiful Anwar Hospital

# CHAPTER 4

# RESEARCH METHOD

## 4.1 Research Design

The design of this research was analytic observational with a cross-sectional approach. The variable observation on the research subjects and the data retrieval were carried out simultaneously.

## 4.2 Research Population and Subjects

The population of this research was all children at the Polyclinic of Immunologic Allergy at the Paediatric department of Saiful Anwar Hospital in Malang. Whereas the subject of this research was children who were diagnosed with at the Polyclinic of Immunologic Allergy at the Paediatric department of Saiful Anwar Hospital in Malang. Research subjects were gathered using the consecutive sampling technique.

The inclusion criteria for the research subjects include:

- Children of the age of 0-10 years that have been diagnosed with AD by a paediatrician
- Must not have consumed vitamin D in the past 6 months
- Parents of patients must be willing to allow their children to participate in the research and sign the informed consent

The exclusion criteria for the research subjects include:

- Patients with Atopic Dermatitis and secondary infection
- Patients with severe infections, congenital heart diseases, autoimmune diseases and malignancy

### 4.3 Sample Size

For cross-sectional research with numeric variables, the formula for calculating the number of samples was as follows (Dahlan, 2011):

$$n = \frac{(Z\alpha)^2 \times p \times (1-p)}{d^2}$$

$$n = \frac{(1.96)^2 \times 0.03 \times 0.97}{0.01}$$

$$n = 11.17$$

Note,

n = number of minimal samples

α = significant values, the significant value used in this research was 0.05 and an interval confident of 95%, until Zα = 1.96

p = the proportion of dependent and independent variable in the previous research (0.03)

d = the acceptable degree of error (0.1)

Based on the formula, a minimum of 11 samples were required for each group.

## 4.4 Research Variables

### 4.4.1 Independent Variables

The independent variable in this research was the level of Vitamin D (25 (OH) D)

### 4.4.2 Dependent Variables

The dependent variable in this research was the percentage of basophils

## 4.5 Operational Definition

### 4.5.1 Atopic Dermatitis

Atopic dermatitis is a chronic inflammatory skin disease. It can be diagnosed based on several clinical criteria (Tada, 2002):

- Pruritus with scars of scratching
- With 3 or more of the following clinical manifestations:
    - Eczema on skin folds (cheeks, forehead and other parts of the body for children under the age of 4 years
    - Lesions on the folds of the elbow, knee, ankle and around the neck including the cheeks for children under the age of 10 years
    - A history of atopy such as asthma or hay fever
    - Dry skin that was widely spread
    - Affects children below the age of 2

### 4.5.2 Vitamin D Level

The level of vitamin D measured was 25 (OH) D, which is the active form of vitamin D and the most dominant component in serum. The measurement was

done using the Enzyme-linked Immunosorbent Assays (ELISA) method. A concentration of below 20 ng/mL indicates vitamin D deficiency (Harrington, 2018). Vitamin D insufficiency is when 25 (OH) D falls in the range of 20-30 ng/mL. Vitamin D sufficiency is defined as 25 (OH) D >30 ng/mL (Tangpricha, 2019).

In this research, the subjects were separated into three groups. There was one group with sufficient level of vitamin D, one with Vitamin D deficiency and the remaining group with vitamin D deficiency and insufficiency.

### 4.5.3 Percentage of Basophils

The normal percentage of basophils ranges from 0.5 to 1.0% (Nall, 2018). Though a low percentage of basophils do not provide any reason for concern, increased basophils indicate an active allergic response. Basophils were measured by automated calculation using Hematology Analyzer (Curry *et al*., 2019).

## 4.6 Research Tools and Materials

a. Patient identity data

b. Form of Atopic Dermatitis patient

c. Tools and materials for blood sampling:

- Vacutainer filled with EDTA
- Tourniquet
- 70% alcohol swab

- 5 cc syringe
- Ice pack
- Cool box
- Sterile gloves

d. Tools and materials for Vitamin D level measurement:

- Human ELISA kit 25(OH) D3
- ELISA reader
- Centrifuge
- Micropipette
- Blue tip
- Yellow tip
- PBS
- Aquadest

## 4.7 Research Procedure

### 4.7.1 Blood Sampling

The blood sampling was conducted in the laboratory of the Pathology Clinic of Saiful Anwar Hospital by the laboratory staff. The blood sample of 5 cc was obtained from the median cubital vein for each subject.

The procedure began with washing hands with soap and water and then confirming the patient identity. After applying the tourniquet, the vein was palpated and the tourniquet was released. Sterile gloves were used and the area was wiped with 70% alcohol swab. The tourniquet was reapplied 3 - 4 inches above the puncture site, the needle was inserted at a 30° angle. After the specimen was collected, the tourniquet and the needle were removed. Direct pressure was applied at puncture site to stop bleeding. The specimens were mixed by inverting the tube 4 - 8 times. The sample was labelled and sent for tests.

**4.7.2 Sample Management**

The collected blood sample was divided into two parts, 2 ml for complete blood count and 3 ml to be made into serum via centrifugation at 3000 rpm for 5 minutes and then stored at 4°C.

**4.7.3 Measurement of 25(OH) D3**

The measurement of 25 (OH) D3 was conducted using the Enzyme-linked Immunosorbent Assays (ELISA) method in ng/mL. In the first incubation step, the sample was added to the solid phase along with the calibrator and the control. In this mix, the vitamin D binding protein (VDBP) and the VDBP-antibody were also added. 25 (OH) D in the sample competed with the tracer which allowed the VDBP antibody to be bound to the vitamin binding protein. The increased concentrations of 25 (OH) D in the sample resulted the reduction of the amount of binding protein which was immobilized to the well via the tracer. Around this time, a washing step was employed to eliminate compounds that were unbound. Using incubation, the quantification of VDBP was achieved with a host specific

peroxidase labelled antibody which utilised a TMB in place of an enzyme substrate. To end this reaction, an acidic stopping solution was added and the colour turns yellow. There was an indirect proportion between intensity of the yellow colour and the 25 (OH) D concentration in the sample.

**4.7.4 Measurement of Basophil percentage**

The measurement of basophil percentage was done using the Hematology Analyzer. A capillary tube was filled with blood. The specimen was allowed to flow down the tube until it was the dry end, inserted vertically into the sealant and pushed to the bottom of the tray. The tube was twisted when removing from the sealant to prevent the sealing plug from being extracted. The sealed end of the tube was gently tapped on a flat surface and the capillary tube was wiped off. It was then placed carefully in the centrifuge tube holder with the sealant end down. The tube was not forced but slid into the tube holder. All the tube positions were numbered on the rotor and used to record the position of each specimen. With the tube holders and tubes in place, the lid was locked firmly and the specimen was centrifuged.

### 4.8 Research Flow

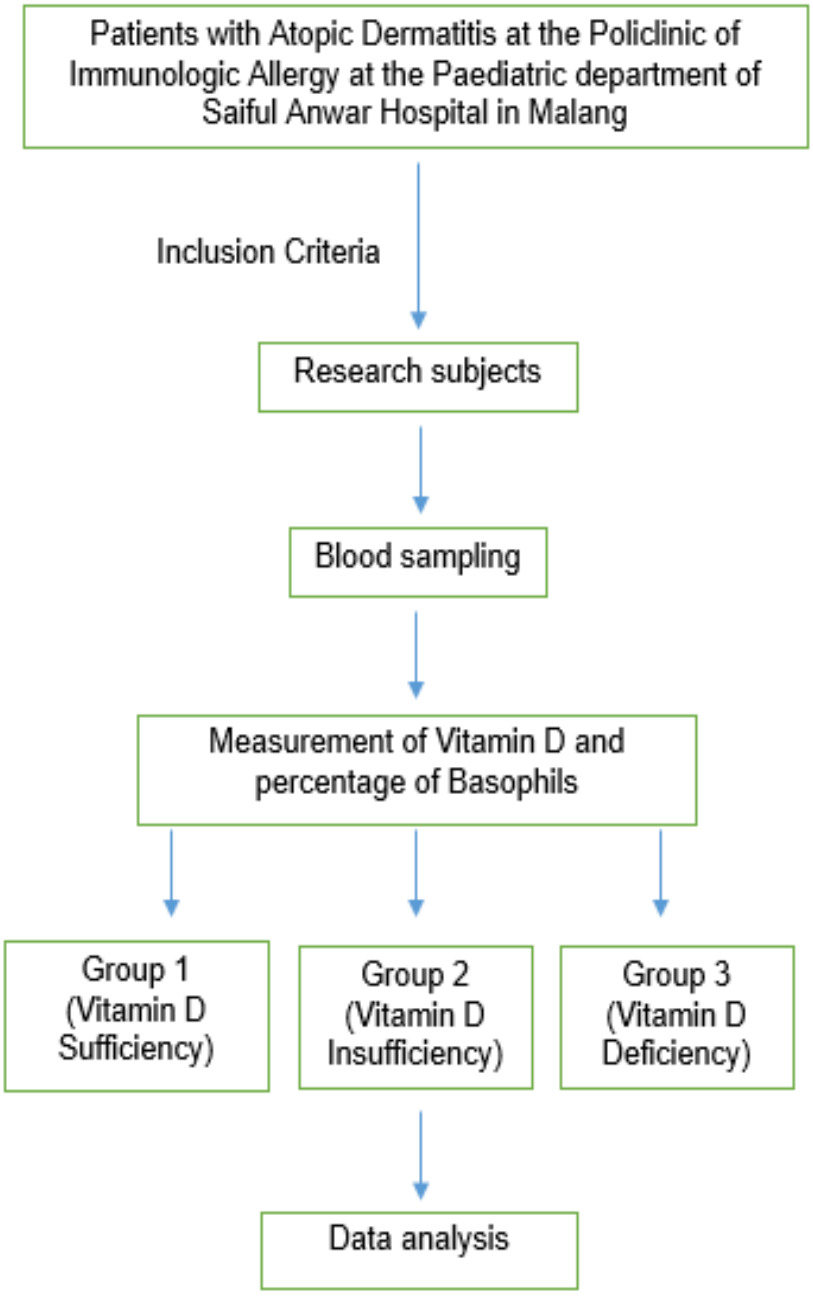


**Figure 4.1** Research Flow

### 4.9 Data Analysis

The data on subject characteristics will be displayed in tabulated form with percentage. The statistical analysis between the two groups was done with one-way Anova test when there was a normal data distribution. For data that is not normally distribution, the Mann-Whitney test should be conducted. For categorical data, a Chi-Square test should be done. In order to determine the relationship between variables, the Pearson correlation test was used when the data was normally distributed and the Spearman test when the data is not normally distributed. The statistical analysis was valid when $p \leq 0.05$. All calculations were done using the 23.0 version SPSS software for Windows.

# CHAPTER 5

# RESEARCH RESULTS AND DATA ANALYSIS

## 5.1 The Characteristics of the Research Subjects

The aim of this study to determine the correlation between Vitamin D levels and the percentage of Basophils in children with atopic dermatitis. In order to gather the information necessary, the blood samples of said children were collected via blood sampling and tested to measure the values required for this research. The data gathered was then analysed using various methods.

A total of 12 patients with Atopic Dermatitis were studied in this research. The subjects were made up of seven females and five males. The average age of the patients are 6 ± 2.96 months. The values obtained from the patients were the amount of Haemoglobins (measured in g/dL), Haematocrits (measured in percentage), Leukocytes (measured in / µL), Thrombocytes (measured in / µL), Basophils (measured in percentage), Absolute Basophils (measured in 10^3/µL) and Vitamin D 25 (OH) (measured in ng/mL).

The basic features of data in a study can be depicted via descriptive statistics. Descriptive analysis is the crucial first step that is used for carrying out statistical analysis. It helps to determine the distribution of the data collected which can be useful to detect many variables such as outliers. The calculations and data analysis for this research were done using the SPSS software for Windows version 23.

A descriptive analysis for this research contains various values such as, sex, age, Basophils and Vitamin D levels. The subjects were comprised of 5

females and 7 males. The age of the subjects ranges from two to 12 months. The mean age in months is 6.33. The amount of Basophils in the patients were measured in percentage. The average value of the Basophil percentage is 0.29%. In addition to that, the value of Absolute Basophils were also measured.

The mean value for Vitamin D was 19.15 ng/mL. The amount of Vitamin D measured enabled the subjects to be divided into three categories, which are insufficiency, deficiency and sufficiency. Out of the 12 subjects that were studied, the level of Vitamin D was deficient in seven people. Three people have a Vitamin D insufficiency while two people have sufficient amount of Vitamin D.

**Table 5.1 The Demographic Data and Clinical Data of Subjects**

| Parameter | Value |
|---|---|
| Sex % | |
| Female | 41.67 |
| Male | 58.33 |
| Age in months (mean ± SD) | 6.33 ± 2.96 |
| Basophils % (mean ± SD) | 0.29 ± 0.16 |
| Vitamin D levels % | |
| Deficient | 58.33 |
| Insufficient | 25.00 |
| Sufficient | 16.00 |

## 5.2 Vitamin D and Basophil in Research Subjects

The test of normality was done using the Saphiro-Wilk test to determine if there was a normal distribution of data. The value of the normality test is deemed significant if $p \geq 0.05$. Since $p = 0.2$, the result of the normality test provided a normal distribution of data. The criteria for a significant value from the homogeneity test is that $p \geq 0.05$. In this study, $p = 0.37$. Therefore, the test of homogeneity provided a homogenous range of data.

The numeric data collected was analysed using a statistical method, known as the Analysis of Variance (Anova) test. It is useful to access the differences between two or more groups. The value obtained from the one-way Anova test is significant if $p \leq 0.05$. In this research, the Anova test showed a significant difference between the sufficient, insufficient and deficient groups, where the value of p was 0.033. The Post Hoc test was conducted in order to confirm the difference between groups. There was a significant difference between the deficient and insufficient group as well as the insufficient and deficient group since the value of $p \leq 0.05$. However, the difference between the sufficient and insufficient group as well as the deficient and insufficient group were not significant since the value of $p > 0.05$.

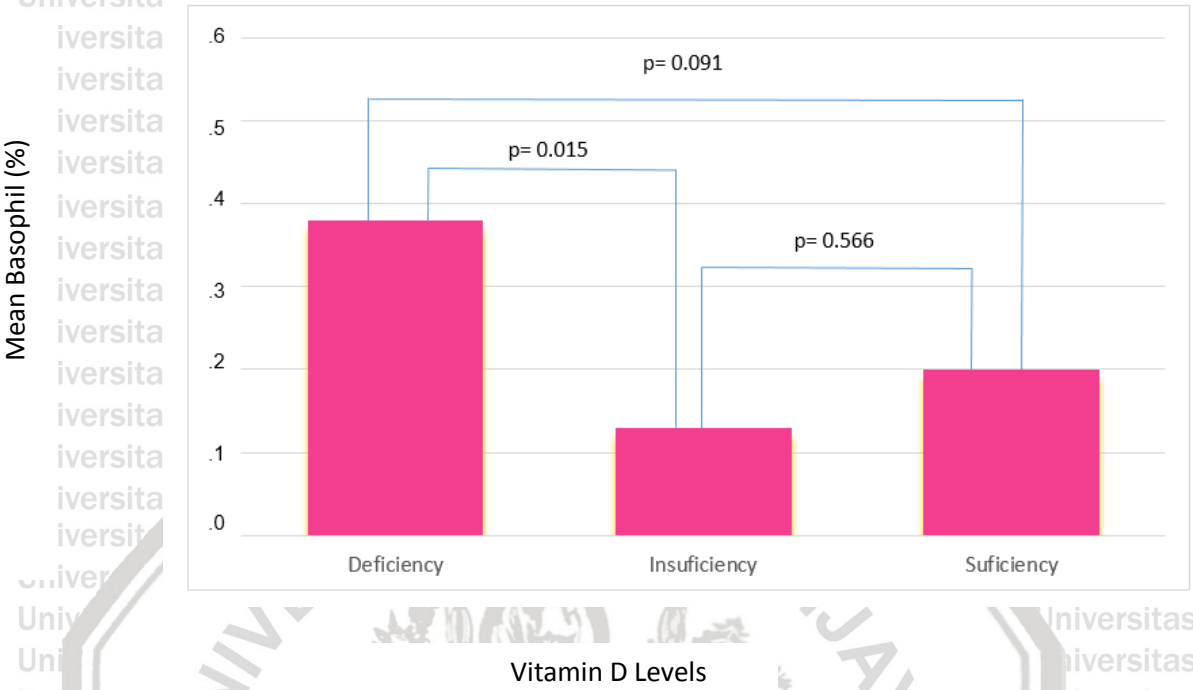


**Figure 5.1. Vitamin D Levels and Mean Values of Basophil**

The subjects of this research can be divided into three different categories based on their respective levels of Vitamin D. A total of seven patients (58%) were afflicted with Vitamin D deficiency (25 (OH) D <20 ng/mL). Meanwhile, the number of patients who had an insufficient amount of Vitamin D (25 (OH) D 20-30 ng/mL) were three (25 %). Finally, there were only two patients (17%) who had sufficient amount of Vitamin D 25 (OH) D >30 ng/mL). The amount of serum 25 (OH) D in each category in shown in a pie chart in figure 5.2.

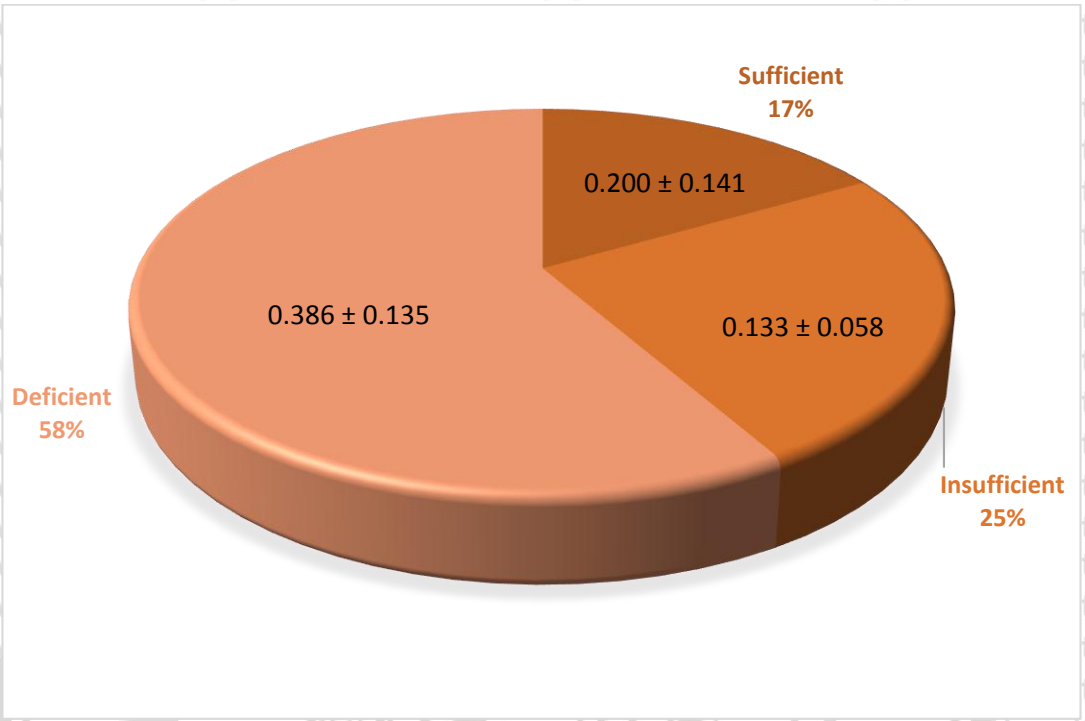


**Figure 5.2 The Level of Serum Vitamin D 25 (H) D and Their Categories**

### 5.3 The Correlation between Vitamin D and Basophil

In order to find out the linear relationship between the two quantitative variables, a Pearson correlation test was used. In this research, it is used to determine the correlation between Vitamin D levels and Basophil percentage. The important criteria that must be fulfilled is that the numeric data obtained in the research must be normally distributed. From the Pearson correlation test, the values obtained proved that there was a significant negative correlation between the level of Vitamin D and Basophil percentage, ($p = 0.029$, $r = -0.626$). The following graph shows the relationship between the 25 (OH) D levels (in ng/mL) and Basophil percentage, wherein the low levels of 25 (OH) D gave a corresponding high Basophil percentage.

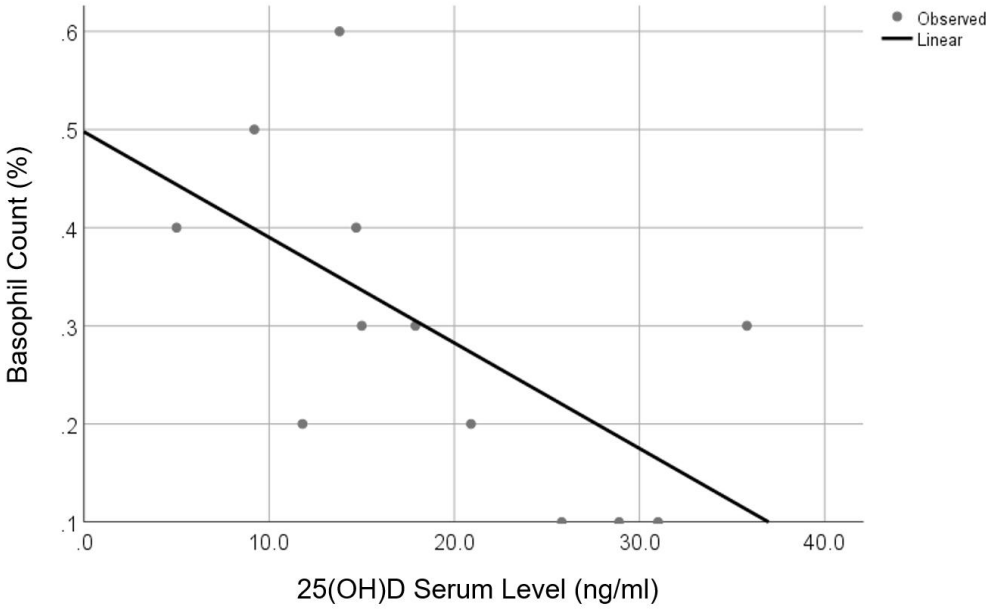


**Figure 5.3 The Graph of Correlation between the 25 (OH) D Serum Levels and Basophil Percentage**

# CHAPTER 6

# DISCUSSION

## 6.1 The Descriptive Data of Subjects

This cross-sectional study is aimed to determine the correlation between Vitamin D levels and Basophil percentage. The subjects were comprised of 12 patients. Of those patients, seven were females and five were males. The subjects' ages ranged from two to 12 month old patients who were diagnosed with Atopic Dermatitis.

There are many factors that contribute to the development of atopic dermatitis. Some of them include environmental and pharmacologic conditions as well as microbial exposure (Pandaleke, 2014). Dust mites and pollen can also trigger eczema flare ups. AD is a common occurrence in people who easily react towards chemicals or irritants such as cleaning products like soaps and detergents (Jones, 2018).

Many patients with atopic dermatitis experience symptoms stimulated by allergens such as dust mites, animal dander and pollen, foods like milk, eggs, nuts and fish. Environmental conditions including extreme temperatures, seasonal changes, humidity and smoke are responsible for triggering AD as well. Some of the irritants that cause flare ups encompass soaps, detergents, creams and topical medications, materials worn on the skin such as wool and synthetic fabrics. Other factors worth mentioning are hormonal changes in women especially during pregnancy or menstruation and infections caused by bacteria and virus (Chen *et al*., 2007).

According to a research conducted regarding the impact of gender and age on 25 (OH) D concentrations is so little that it was negligible (Vuistiner *et al*., 2015). In another study, it was proven that age and body mass index were two factors that provide remarkable effects in Vitamin D synthesis and bio-availability. Based on the NHANES 2001-2004 data, an estimated 30% of adults above the age of 50 years were known to have serum 25 (OH) D concentrations below 50 nmol/L or approximately 15.7 ng/mL. This is also because of a plummet in the skin's 7-dehydrcholesterol content which is age-dependent (Tsiaras and Weinstock, 2011).

**6.2 Vitamin D Levels in Patients with Atopic Dermatitis**

In this research, the amount of Vitamin D was only sufficient in two patients, leaving three patients with insufficiency and seven with deficiency. A person is said to have a sufficient amount of Vitamin D when he or she has a serum 25 (OH) D level above 30 ng/mL. Vitamin D insufficiency is defined as a serum 25 (OH) D level of 21-29 ng/mL. Meanwhile, Vitamin D deficiency is when the serum 25 (OH) D level is below 20 ng/mL.

There are a wide range of factors that influence the level of Vitamin D in humans. Some of them include genetics, exposure to sunlight, climate, and the changes in seasons, atmospheric components, skin pigmentation, age, obesity, chronic illnesses, personal factors such as clothing choices and the usage of sun protection (Tsiaras and Weinstock, 2011).

It is proven that there is an inverse correlation between body-mass index (BMI) and Vitamin D concentrations (Vuistiner *et al*., 2015). This is mainly a result

of decreased sun exposure which could be caused by little or no involvement in outdoor activities, insufficient nutrients in a poor diet and Vitamin D sequestration (Nowson *et al*., 2002). Skin pigmentation affects the concentration of Vitamin D in humans due to the fact that sunlight exposure is the most important source of Vitamin D. Studies that were conducted on people of African ethnicity revealed that they were indeed susceptible to Vitamin D deficiency since dark skin provides protection against UVB radiation (Shieh *et al*., 2017).

A study conducted in Hong Kong showed that genetic factors caused atopic dermatitis in around 70% of patient. The defect in Fillagrin gene can cause moderate to severe AD in up to one third of people in East Asia (Boguniewicz and Leung, 2013). Atopic dermatitis in adults is more common in Asia compared to western countries. In terms of gender, females are found to be slightly more susceptible to AD with a ratio of female: male ratio of 1.3:1 (Chan, 2006).

Many studies conducted on the relationship between breastfeeding and atopic dermatitis have shown controversial findings. However, the most studies have concluded that exclusive breastfeeding does not play a role in preventing atopic dermatitis. However, it has been known to significantly reduce the severity of AD in childhood. This is true especially when the infant has been breastfed exclusively for a minimum of three months (Lien and Goldman, 2011).

Vitamin D supplementation can result in a significant reduction in the amount of neutrophils present within white blood cells. Vitamin D plays a crucial role in both adaptive and innate immunity. Recently, vitamin D receptors (VDR) were discovered and detected in many tissues and cells in humans including almost all immune cells such as B cells, T cells, dendritic cells, neutrophils and

macrophages. In innate immunity, Vitamin D induces antimicrobial peptide synthesis but inhibits the expression of TLR (Toll-like receptor) and the pro-inflammatory cytokine production. In adaptive immunity, Vitamin D causes the reduction of Th-1 cytokine production and inhibition of T cell proliferation (Hoxha, 2014).

It is known that Vitamin D has the ability to repress inflammatory responses that occur in the body. It can enhance antimicrobial peptide activity and increase the integrity of the skin's permeability. Due to this reason, Vitamin D can be utilised as a therapeutic method to heal a number of skin disorders including but not limited to dry skin, psoriasis and of course; atopic dermatitis (Umar *et al*., 2018 ). In a study conducted to determine the effect of Vitamin D on winter- related atopic dermatitis, children who were given a Vitamin D supplementation for one month experienced a remarkable improvement in baseline score compared to the placebo group (Sidbury *et al*., 2008).

Atopic dermatitis was proven to be prevalent in patients with Vitamin D deficiency and Vitamin D serves as a method of prevention due to its immunomodulatory property. Since vitamin D is known to trigger the pathogenesis of atopic dermatitis, the supplementation of vitamin D can be useful for the recovery of atopic dermatitis (Chirumbolo *et al*., 2017). Studies and investigations in the field of immunology and epidemiology have proven that there is a relationship between Vitamin D deficiency and allergic skin diseases, in particular skin inflammation. Vitamin D deficiency is mostly involved with the increase in susceptibility to chronic skin diseases such as atopic dermatitis and chronic idiopathic urticarial (Oren *et al*., 2008).

Vitamin D contains the ability to induce the terminal differentiation as well as inhibit the proliferation of keratinocytes. As a result, it is used as a treatment for psoriasis. In addition to that, it was found that mice that were afflicted with an increased contact hypersensitivity response compared to those who have sufficient amount of Vitamin D. Immune cells express Vitamin D receptors or VDRs and their activation reduces inflammation. Vitamin D deficiency is also an implication in patients with autoimmunity (Umar *et al*., 2018).

Vitamin D has an important role in both adaptive and innate immunity (Hoxha, 2011). The active form of Vitamin D, which is a component called Calcitriol is synthesized by cells like monocyte-macrophages in the immune system where it performs a crucial role as a cytokine. It function is quite significant as it aids in the modulation of the body defences against microbes through a process which stimulated the innate immune system (Holick, 2009)

**6.3 Basophils in Patients with Atopic Dermatitis**

In this study, the mean value for Basophil percentage was 0.292. The maximum value obtained for Basophil percentage was 0.6 while the minimum value obtained was 0.1. The normal value of basophils in infants aged 2-12 months is <0.11 x 10^9/l.

Basophils in humans are capable of infiltrating skin lesions and are involved in the pathogenesis of skin diseases such as chronic idiopathic urticarial and systemic lupus erythematosus. Many studies have proven that skin lesions of patients who have been diagnosed with AD are enriched with basophils. Several years ago, a study proved that patients who were afflicted with food allergy and

severe atopic dermatitis had a remarkable increase in spontaneous basophil release of histamine (Borriello *et al*., 2014).

In addition to that, a study proved that the basophils from antigen-specific IgE-mediated activation were found in the peripheral blood of patients with atopic dermatitis (Tsiaras and Weinstock, 2011). When there is an increase in Basophils, it is mostly due to allergy, autoimmunity, and cancer or organ rejection. Basophils play an important role in the pathogenesis of atopic dermatitis among other diseases. In a research conducted with mice, it was concluded that IgE-dependent basophils played a crucial role in the pathogenesis of atopic dermatitis (Siracusa *et al*., 2013). They are also important in Ig-E independent allergic inflammation such as asthma induced by allergenic proteases, irritant contact dermatitis, eosinophilic esophagitis (Miyake and Karasuyama, 2017).

**6.4 The Relationship between Vitamin D level and Basophils**

The aim of this study is to prove that there is a relationship between the Vitamin D level and Basophil percentage in infants with Atopic Dermatitis. The percentage of basophils is high in patients with low levels of Vitamin D, therefore there is a negative correlation between Vitamin D levels and the percentage of basophils.

A study conducted in 2017 has proved that there is an inverse correlation between Vitamin D and the number of circulating basophils (Filho *et al*., 2017). Vitamin D supplement has been proven to decrease the number of basophils in circulation in mice with allergic airway diseases. In addition to basophils, Vitamin

D also shows an inverse correlation with the numbers of eosinophils and neutrophils in the circulation (Hollams *et al*., 2011).

Vitamin D has the ability to modulate the inflammation process in the body by regulating the inflammatory cytokines produced in immune-related diseases. It can also inhibit prostaglandins which are involved in inflammation in addition to immune cells such as B cells and T cells via VDR (Liu *et al*., 2018).

**6.5 The Limitations of the Study**

In this research, the relationship between Vitamin D levels and the percentage of basophils was studied. The most obvious limitation of this study is the small sample size. Although the subjects of this study were children who were diagnosed with atopic dermatitis, the effect of Vitamin D on them would be better examined by evaluating the symptoms experienced by them. Since the design of this study was cross-sectional, it is unknown if atopic dermatitis causes Vitamin D deficiency or vice versa. In order to determine this, a clinical study would be more useful. Another disadvantage of this study is the lack of clinical information regarding the subjects that were studied. There were no information on whether or not patients were breastfed exclusively or given additional liquid or solid food. As discussed before, there is indeed a relationship between breastfeeding and atopic dermatitis. It is also not known if the subjects suffer from other diseases. Finally, other factors that affect Vitamin D levels such as sunlight exposure, skin pigmentation and nutrition were not explored.

# CHAPTER 7

# CONCLUSION AND SUGGESTIONS

## 7.1 Conclusion

The following conclusions were obtained from this research:

1. The level of vitamin D in serum in children with atopic dermatitis in the paediatric department of Saiful Anwar Hospital is lower than the normal range
2. The percentage of basophils is higher in Vitamin D deficient children with atopic dermatitis in the paediatric department of Saiful Anwar Hospital
3. There is a negative correlation between the Vitamin D level in serum and the percentage of basophils in children with atopic dermatitis in the paediatric department of Saiful Anwar Hospital

## 7.2 Suggestions

The suggestions proposed in this research are as follows:

1. The sample size used in this study was too small
2. In this study, other factors that affect vitamin D levels such as sunlight exposure or nutrition were not evaluated
3. Since this is a cross-sectional study, it is not known whether atopic dermatitis caused Vitamin D deficiency or vice versa. In order to determine that, a clinical study should be conducted.

4. The effect of Vitamin D on patients with Atopic Dermatitis would have been better determined by evaluating the clinical manifestations present in patients

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## ATTACHMENTS

### 1. Ethical Clearance Form

KEMENTERIAN RISET, TEKNOLOGI, DAN PENDIDIKAN TINGGI
UNIVERSITAS BRAWIJAYA
FAKULTAS KEDOKTERAN
KOMISI ETIK PENELITIAN KESEHATAN
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KETERANGAN KELAIKAN ETIK
("ETHICAL CLEARANCE")

No. 237 / EC / KEPK / 10 / 2018

KOMISI ETIK PENELITIAN KESEHATAN FAKULTAS KEDOKTERAN UNIVERSITAS BRAWIJAYA, SETELAH MEMPELAJARI DENGAN SEKSAMA RANCANGAN PENELITIAN YANG DIUSULKAN, DENGAN INI MENYATAKAN BAHWA PENELITIAN DENGAN

| | | |
|---|---|---|
| **JUDUL** | : | **Pengaruh pemberian Suplementasi Vitamin D3 terhadap Profil Klinis dan Imunologis Pasien Anak dengan *Dermatitis Atopi.*** |
| **PENELITI UTAMA** | : | **Dr. dr. Wisnu Barlianto, Sp.A(K), M.Si.,Med** |
| **ANGGOTA** | : | **dr. Desy Wulandari, Sp.A, M.Biomed**<br>**dr. Tita Luthfia Sari, M.Biomed**<br>**dr. Vanisia Hayu Firdayanti**<br>**dr. M. Irfan Afandi**<br>**Siti Aminah Binti Patawari**<br>**Wika Septian Pratama**<br>**Yubandthra A/L Vebakaran**<br>**Jafrina Jasmin Binti Abdul Hakkeem**<br>**Nia Uswanti Binti Usman** |
| **UNIT / LEMBAGA** | : | **Fakultas Kedokteran – Universitas Brawijaya Malang.** |
| **TEMPAT PENELITIAN** | : | **Rumah Sakit Umum Dr. Saiful Anwar Malang** |

**DINYATAKAN LAIK ETIK.**

Malang,
Ketua

Prof. Dr. dr. Moch. Istiadjid ES, SpS, SpBS(K), SH, M.Hum, Dr(Hk)
NIK. 160746683

**Catatan :**
Keterangan Laik Etik Ini Berlaku 1 (Satu) Tahun Sejak Tanggal Dikeluarkan
Pada Akhir Penelitian, Laporan Pelaksanaan Penelitian Harus Diserahkan Kepada KEPK-FKUB Dalam Bentuk Soft Copy. Jika Ada Perubahan Protokol Dan / Atau Perpanjangan Penelitian, Harus Mengajukan Kembali Permohonan Kajian Etik Penelitian (Amandemen Protokol)

**2. Informed Consent Form**

**PENJELASAN UNTUK MENGIKUTI PENELITIAN**

1. Saya adalah Jafrina Jasmin, mahasiswa S1 Kedokteran Universitas Brawijaya Malang dengan ini meminta anak Bapak/ibu/sdr untuk berpartisipasi dengan sukarela dalam penelitian yang berjudul "Pengaruh Pemberian Suplementasi Vitamin D3 Terhadap Perbaikan Profil Klinis dan Imunologis pada Pasien Anak dengan Dermatitis Atopi".

2. Tujuan penelitian ini yang pertama adalah untuk mengetahui kadar Vitamin D pada pasien anak dengan Dermatitis Atopi yang dikaitkan dengan gejala klinis dan hasil laboratorium. Yang kedua, penelitian ini juga bertujuan untuk mengetahui pengaruh pemberian suplemen Vitamin D3 terhadap perbaikan gejala klinis, hasil lab, serta kualitas hidup pasien anak dengan Dermatitis Atopi. Hasil penelitian ini diharapkan dapat menjadi dasar dalam pengambilan kebijakan terkait terapi yang akan diberikan kepada pasien, terutama mengenai pentingnya pemberian supmentasi vitamin D pada pasien anak dengan Dermatitis Atopi.

3. Penelitian ini akan berlangsung selama kurang lebih 2 bulan untuk masing-masing pasien. Pada kunjungan pertama pasien akan dilakukan pengambilan data identitas, pengisian kuesioner, pengambilan sampel darah serta mendapatkan terapi. Setelah 2 bulan terapi, pasien kontrol untuk dilakukan evaluasi hasil terapi, pengisian kuesioner dan pengambilan sampel darah ulang.

4. Keuntungan yang Bapak/ibu/sdr dan anak Bapak/ibu/sdr peroleh dengan keikutsertaan anak Bapak/ibu/sdr dalam penelitian ini adalah dapat mengetahui perkembangan kondisi terkini terkait penyakit anak Bapak/ibu/sdr. Manfaat langsung yang Bapak/ibu/sdr dan anak Bapak/ibu/sdr peroleh adalah mengetahui kadar beberapa parameter laboratorium terkait dengan penyakit anak Bapak/ibu/sdr sehingga dokter dapat memberikan terapi yang tepat sesuai dengan kondisi pasien. Selain itu Bapak/ibu/sdr dan anak Bapak/ibu/sdr dapat mengetahui derajad penyakit anak Bapak/ibu/sdr sehingga dapat digunakan untuk monitoring dan Bapak/ibu/sdr dapat mengetahui apa saja yang harus dilakukan dan tidak boleh dilakukan untuk mencegah perburukan kondisi penyakit anak Bapak/ibu/sdr. Manfaat tidak langsung yang dapat diperoleh adalah memberikan informasi ilmiah mengenai pengaruh pemberian suplementasi vitamin D3 terhadap perbaikan gejala klinis dan hasil lab pada pasien anak dengan Dermatitis Atopi sebagai dasar dalam tatalaksana dan monitoring pasien Dermatitis Atopi secara umum.

5. Ketidaknyamanan/ risiko yang mungkin muncul yaitu pada saat prosedur pengambilan sampel darah mungkin menyebabkan rasa nyeri namn tidak perlu kuatir karena pengambilan sampel darah dilakukan oleh petugas profesional sehingga risiko/ kerugian yang dapat terjadi minimal. Terkait pemberian suplementasi vitamin D3 setiap hari selama 2 bulan, bila terjadi efek samping seperti mual, muntah, lemas, nyeri tulang dan gangguan perkemihan maka pasien dapat langsung menghubungi peneliti dengan nomor telepon yang tertera pada kartu kendali obat, untuk selanjutnya dilakukan pemeriksaan dan tindakan berikutnya.

6. Pada penelitian ini, prosedur pemilihan subjek yaitu pasien anak dengan diagnosis Dermatitis Atopi, usia 1 – 12 bulan, tidak mengkonsumsi vitamin D dalam 6 bulan terakhir, tidak ada kelainan absorbsi pada saluran cerna, serta tidak mengalami infeksi sekunder yang membutuhkan terapi antibiotik. Mengingat anak Bapak/ibu/sdr memenuhi kriteria tersebut, maka peneliti meminta kesediaan anak Bapak/ibu/sdr untuk mengikuti penelitian ini setelah penjelasan peneletian ini diberikan.

7. Prosedur penelitian yang harus diikuti yaitu pengisian data pasien dan kuesioner, pengambilan sampel darah, melaksanakan terapi sesuai petunjuk dokter serta kontrol 2 bulan berikutnya. Pengisian data dan kuesioner dilakukan oleh orang tua pasien dengan dibantu oleh tim peneliti. Pengambilan sampel darah pasien dilakukan oleh petugas yang telah berpengalaman dengan cara menusukkan jarum ke dalam pembuluh darah vena yang terletak di lipat siku bagian dalam kurang lebih sebanyak 5 cc. Terapi yang diberikan untuk pasien berupa krim topikal yang mengandung emolien dan steroid serta suplementasi vitamin D3 dalam bentuk sirup.

8. Setelah Bapak/ibu/sdr menyatakan kesediaan atas dan anak Bapak/ibu/sdr untuk berpartisipasi dalam penelitian ini, maka peneliti akan memastikan anak Bapak/ibu/sdr dalam keadaan sehat.

9. Sebelum pengisian kuisioner/ wawancara, peneliti akan menerangkan cara mengisi kuesioner kepada Bapak/ibu/sdr dan anak Bapak/ibu/sdr, selama kurang lebih 10 menit, dengan cara memberikan contoh secara langsung, sesuai dengan pengalaman yang anda alami dengan menggunakan tinta hitam.

10. Sebelum pengisian kuesioner/ wawancara, peneliti akan memberikan penjelasan mengenai setiap poin pertanyaan dalam kuesioner penelitian dan cara menjawabnya.

11. Selama pengisian kuesioner/ wawancara, diperkenankan bagi Bapak/ibu/sdr untuk menanyakan apabila ada yang belum dipahami dari isi kuisioner.

12. Setelah mengisi kuesioner/ wawancara, Bapak/ibu/sdr dapat melakukan tukar pengalaman dan tanya jawab dengan peneliti

13. Bapak/ibu/sdr dapat memberikan umpan balik dan saran pada peneliti terkait dengan proses pengambilan data dengan kuesioner/ wawancara baik selama maupun setelah proses pengisian kuesioner/ wawancara secara langsung pada peneliti.

14. Peneliti akan memberikan waktu satu hari kepada Bapak/ibu/sdr untuk menyatakan berpartisipasi/ tidak dalam penelitian ini secara sukarela, sebelum pengisian kuesioner/ wawancara.

15. Seandainya Bapak/ibu/sdr tidak menyetujui cara ini maka Bapak/ibu/sdr dapat memilih cara lain atau Bapak/ibu/sdr boleh tidak mengikuti penelitian ini sama sekali.

16. Jika Bapak/ibu/sdr menyatakan bersedia menjadi responden namun disaat penelitian berlangsung anda ingin berhenti, maka Bapak/ibu/sdr dapat menyatakan mengundurkan diri atau tidak melanjutkan ikut dalam penelitian ini. Tidak akan ada sanksi yang diberikan kepada Bapak/ibu/sdr terkait hal ini.

17. Nama dan jati diri anak Bapak/ibu/sdr akan tetap dirahasiakan, sehingga diharapkan Bapak/ibu/sdr tidak merasa khawatir dan dapat mengisi kuisioner sesuai kenyataan dan pengalaman Bapak/ibu/sdr yang sebenarnya.

18. Jika Bapak/ibu/sdr merasakan ketidaknyamanan atau dampak karena mengikuti penelitian ini, maka Bapak/ibu/sdr dapat menghubungi peneliti yaitu Dr. dr. Wisnu Barlianto, SpA(K), Masi. med dengan nomor Hp.08123319895.

19. Perlu Bapak/ibu/sdr ketahui bahwa penelitian ini telah mendapatkan persetujuan kelaikan etik dari suatu Komisi Etik Penelitian Kesehatan FKUB, sehingga Bapak/ibu/sdr tidak perlu khawatir karena penelitian ini akan dijalankan dengan menerapkan prinsip etik penelitian yang berlaku.

20. Hasil penelitian ini kelak akan dipublikasikan namun tidak terdapat identitas anak Bapak/ibu/sdr dalam publikasi tersebut sesuai dengan prinsip etik yang diterapkan.

21. Peneliti akan bertanggung jawab secara penuh terhadap kerahasiaan data yang Bapak/ibu/sdr berikan dengan menyimpan data hasil penelitian yang hanya dapat diakses oleh peneliti.

22. Jika Bapak/ibu/sdr bersedia menjadi partisipan penelitian ini, maka Bapak/ibu/sdr akan mendapatkan kompensasi berupa uang transport sebesar Rp 50.000,00.

23. Peneliti akan memberi tanda terima kasih berupa mainan untuk anak Bapak/ibu/sdr seharga Rp 50.000,00.

Peneliti Utama

Dr. dr.Wisnu Barlianto, SpA(K), Msi. med

**Pernyataan Persetujuan untuk**

**Berpartisipasi dalam Penelitian**

Saya yang bertandatangan dibawah ini menyatakan bahwa :

1. Saya telah mengerti tentang apa yang tercantum dalam lembar penjelasan dan telah dijelaskan oleh peneliti

2. Dengan ini saya menyatakan bahwa secara sukarela mengijinkan anak saya untuk ikut serta menjadi salah satu subyek penelitian yang berjudul "Pengaruh Pemberian Suplementasi Vitamin D3 Terhadap Profil Klinis dan Imunologis Pasien Anak dengan Dermatitis Atopi".

**Malang, .....,**

Peneliti

Yang membuat pernyataan

(.....)

(.....)

Saksi I

Saksi II

(.....)

(.....)

### 3. Clinical and Laboratory Data

**The Table of Clinical and Laboratory Data of Subjects**

| No | Sex | Age (months) | Hb (g/dL) | Hct (%) | Leu/µL | Tro/µL | Ba (%) | Ba Abs 10^3/µL | Vit D 25-OH (ng/mL) (Pre) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 5 | 10.0 | 30.2 | 6260 | 346000 | 0.6 | 0.10 | 13.8 |
| 2 | 1 | 12 | 11.5 | 33.2 | 8690 | 302000 | 0.3 | 0.03 | 35.8 |
| 3 | 1 | 2 | 9.5 | 26.7 | 7160 | 359000 | 0.3 | 0.01 | 15.0 |
| 4 | 1 | 7 | 13.4 | 38.7 | 4710 | 211000 | 0.2 | 0.01 | 20.9 |
| 5 | 2 | 7 | 10.3 | 30.3 | 9960 | 357000 | 0.1 | 0.01 | 28.9 |
| 6 | 2 | 6 | 11.2 | 34.5 | 7480 | 279000 | 0.3 | 0.03 | 17.9 |
| 7 | 1 | 3 | 11.1 | 32.3 | 13850 | 399000 | 0.4 | 0.02 | 14.7 |
| 8 | 2 | 10 | 11.5 | 33.9 | 8210 | 272000 | 0.5 | 0.01 | 9.2 |
| 9 | 2 | 6 | 11.6 | 33.2 | 8020 | 249000 | 0.2 | 0.02 | 11.8 |
| 10 | 1 | 6 | 10.6 | 33.3 | 7160 | 285000 | 0.1 | 0.01 | 25.8 |
| 11 | 2 | 3 | 10.6 | 31.3 | 7460 | 480000 | 0.4 | 0.03 | 5.0 |
| 12 | 2 | 9 | 12.5 | 34.7 | 8920 | 324000 | 0.1 | 0.02 | 31.0 |

Male: 1
Female: 2

## 4. Descriptive Analysis

**Descriptive Statistics**

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Age | 12 | 2 | 12 | 6.33 | 2.964 |
| Basophil | 12 | .1 | .6 | .292 | .1621 |
| Valid N (listwise) | 12 | | | | |

**Sex**

| | | Frequency | Percent | Valid Percent | Cumulative Percent |
|---|---|---|---|---|---|
| Valid | Male | 5 | 41.7 | 41.7 | 41.7 |
| | Female | 7 | 58.3 | 58.3 | 100.0 |
| | Total | 12 | 100.0 | 100.0 | |

**Classification of Vit D**

| | | Frequency | Percent | Valid Percent | Cumulative Percent |
|---|---|---|---|---|---|
| Valid | Deficiency | 7 | 58.3 | 58.3 | 58.3 |
| | Insufficiency | 3 | 25.0 | 25.0 | 83.3 |
| | Sufficiency | 2 | 16.7 | 16.7 | 100.0 |
| | Total | 12 | 100.0 | 100.0 | |

**Descriptives**

| | Class_of_VitD | | | Statistic | Std. Error |
|---|---|---|---|---|---|
| Basophil | Deficiency | Mean | | .386 | .0508 |
| | | 95% Confidence Interval for Mean | Lower Bound | .261 | |
| | | | Upper Bound | .510 | |
| | | 5% Trimmed Mean | | .384 | |
| | | Median | | .400 | |
| | | Variance | | .018 | |
| | | Std. Deviation | | .1345 | |
| | | Minimum | | .2 | |
| | | Maximum | | .6 | |

| | | | | |
|---|---|---|---|---|
| | | Range | .4 | |
| | | Interquartile Range | .2 | |
| | | Skewness | .352 | .794 |
| | | Kurtosis | -.302 | 1.587 |
| | Insufficiency | Mean | .133 | .0333 |
| | | 95% Confidence Interval for Mean | Lower Bound | -.010 |
| | | | Upper Bound | .277 |
| | | 5% Trimmed Mean | . | |
| | | Median | .100 | |
| | | Variance | .003 | |
| | | Std. Deviation | .0577 | |
| | | Minimum | .1 | |
| | | Maximum | .2 | |
| | | Range | .1 | |
| | | Interquartile Range | . | |
| | | Skewness | 1.732 | 1.225 |
| | | Kurtosis | . | . |
| | Sufficiency | Mean | .200 | .1000 |
| | | 95% Confidence Interval for Mean | Lower Bound | -1.071 |
| | | | Upper Bound | 1.471 |
| | | 5% Trimmed Mean | . | |
| | | Median | .200 | |
| | | Variance | .020 | |
| | | Std. Deviation | .1414 | |
| | | Minimum | .1 | |
| | | Maximum | .3 | |
| | | Range | .2 | |
| | | Interquartile Range | . | |
| | | Skewness | . | . |
| | | Kurtosis | . | . |

## 5. Normality and Homogenity Test

**Tests of Normality**

| | | Kolmogorov-Smirnov[a] | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|---|
| | Class_of_VitD | Statistic | df | Sig. | Statistic | df | Sig. |
| Basophil | Deficiency | .172 | 7 | .200* | .967 | 7 | .873 |
| | Insufficiency | .385 | 3 | . | .750 | 3 | .070 |
| | Sufficiency | .260 | 2 | . | | | |

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

**Test of Homogeneity of Variances**

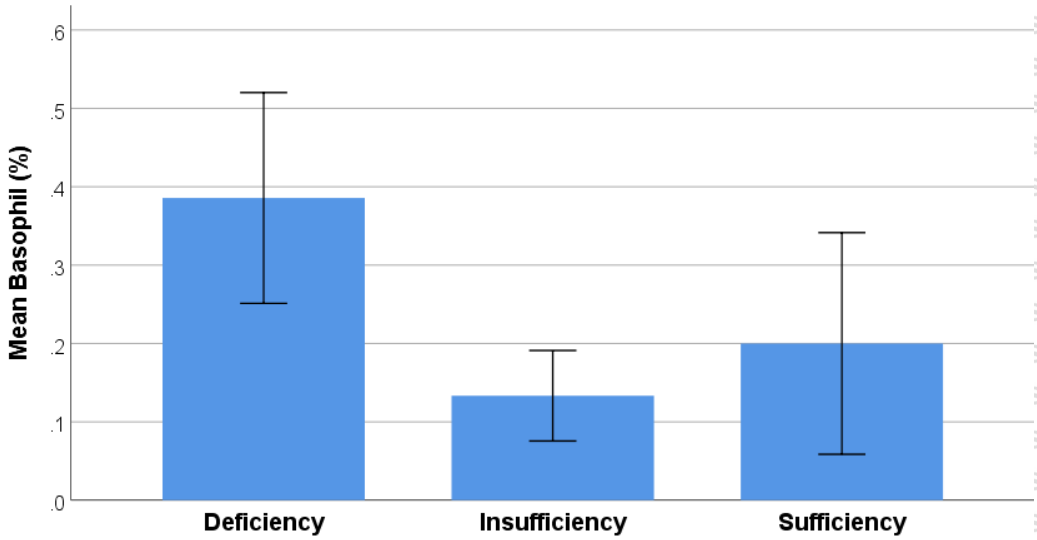
| | | Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|---|---|
| Basophil | Based on Mean | .908 | 2 | 9 | .437 |
| | Based on Median | .964 | 2 | 9 | .417 |
| | Based on Median and with adjusted df | .964 | 2 | 7.538 | .424 |
| | Based on trimmed mean | .937 | 2 | 9 | .427 |

## 6. One Way ANOVA Test

**ANOVA**

Basophil

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .154 | 2 | .077 | 5.122 | .033 |
| Within Groups | .135 | 9 | .015 | | |
| Total | .289 | 11 | | | |




## 7. Post Hoc Test

**Multiple Comparisons**

Dependent Variable: Basophil

LSD

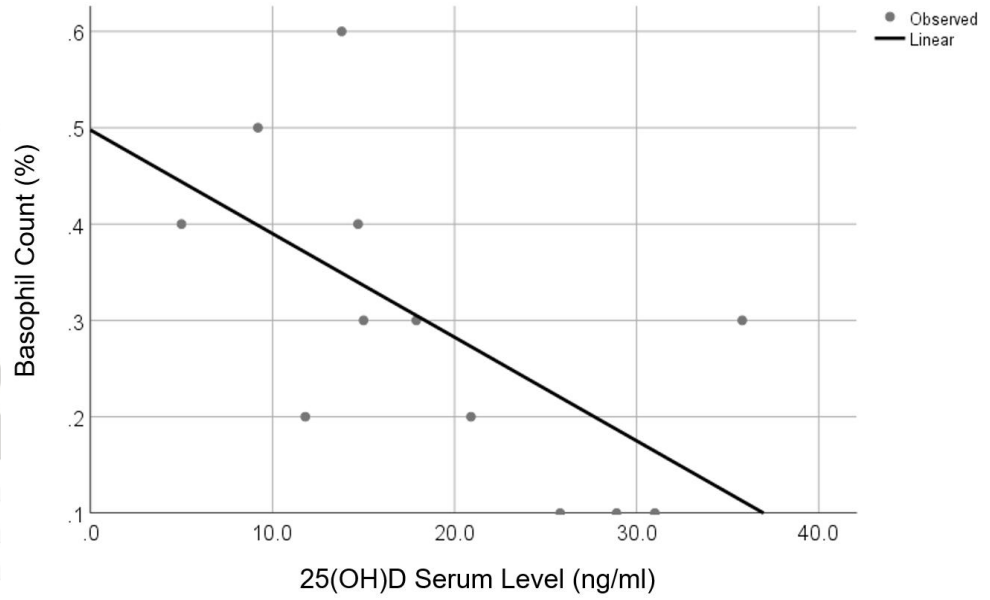
| (I) Class_of_VitD | (J) Class_of_VitD | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval Lower Bound | 95% Confidence Interval Upper Bound |
|---|---|---|---|---|---|---|
| Deficiency | Insufficiency | .2524* | .0846 | .015 | .061 | .444 |
| | Sufficiency | .1857 | .0983 | .091 | -.037 | .408 |
| Insufficiency | Deficiency | -.2524* | .0846 | .015 | -.444 | -.061 |
| | Sufficiency | -.0667 | .1119 | .566 | -.320 | .186 |
| Sufficiency | Deficiency | -.1857 | .0983 | .091 | -.408 | .037 |
| | Insufficiency | .0667 | .1119 | .566 | -.186 | .320 |

## 8. Pearson Correlation Test

**Correlations**

| | | VitD | Basophil |
|---|---|---|---|
| VitD | Pearson Correlation | 1 | -.626* |
| | Sig. (2-tailed) | | .029 |
| | N | 12 | 12 |
| Basophil | Pearson Correlation | -.626* | 1 |
| | Sig. (2-tailed) | .029 | |
| | N | 12 | 12 |

*. Correlation is significant at the 0.05 level (2-tailed).




**The Graph of Correlation between the 25 (OH) D Serum Levels and Basophil Percentage**