Do Calcium and Vitamin D Levels in Blood Predict Clinical Features and/or Response to Treatment in People with Psoriasis?

by

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Declaration

I, Dr Sihan Waghid, hereby declare that the work on which this thesis is based is my original work (except where acknowledgments indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university. I empower the university to reproduce for research either the whole or any portion of the contents in any manner whatsoever.

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Abstract

Context: Psoriasis is an immune-mediated skin disease which is chronic and inflammatory, with multiple clinical types and variable severity. It is renowned for its significantly negative effect on quality of life and presents with recurrent relapses and remissions. The selection of treatment of psoriasis is dependent on the psoriasis type, impact on the individual and the severity of the disease. Variable factors that remain incompletely understood influence response to systemic treatment. It is of interest to establish whether calcium or vitamin D serum levels are linked with response to treatment in people with variable (moderate to severe) psoriasis in our population.

Aims: This study aims to establish whether:

1. Clinical type or severity of psoriasis is correlated with serum calcium or vitamin D;

2. Calcium or vitamin D serum levels and response to treatment with systemic agents, including methotrexate, cyclosporine, acitretin and ultraviolet light, are associated in people with variable (moderate to severe) psoriasis;

3. Calcium and vitamin D serum levels differ in psoriatic patients compared with such levels in a control group.

Materials and methods: As a case control (prospective) study, one hundred people (n = 100) were recruited, fifty having psoriasis and fifty control subjects. The psoriatic participants began a new systemic treatment and/or ultraviolet light for moderate to severe psoriasis. On the basis of a PASI score at both baseline and a three-month follow-up, response to treatment was assessed. The calcium and vitamin D serum levels were measured in psoriatic patients and controls. The collected blood samples were processed by the National Health Laboratory Services. Data collected from interviews, examination and laboratory results were recorded on a data capture sheet.

The study was planned to be conducted for one year, but, due to the coronavirus pandemic, it was extended into a second year. For the psoriasis group, visits at one

month and three months were recorded. Standard psoriasis treatment was provided by the dermatology health care professionals at Groote Schuur Hospital.

ANOVA statistics and the Chi-squared test were applied to determine whether there is a correlation between variables.

Results: No significant difference in calcium serum levels was found in psoriatic patients with different types of psoriasis ($p = 0.63$) or in those with varying severity of psoriasis ($p = 0.48$). A significant difference in the vitamin D serum levels in relation to different types of psoriasis ($p = 0.62$) or the severity of psoriasis ($p = 0.31$), was also absent.

Psoriatic patients' response to treatment were less in those patients with low vitamin D serum levels. People with a higher vitamin D level had a significantly greater change in the PASI score ($p = 0.01$). No correlation was seen between calcium serum levels and changes in PASI score at follow-up. In other words no relationship between serum calcium level and response to treatment in psoriasis ($p = 0.49$) was found.

This study exhibited no difference in calcium levels between the control group and psoriatic group ($p = 0.79$). However, there were significantly lower vitamin D levels in the psoriatic group compared to the controls ($p = 0.01$). The mean vitamin D level was 49,92 nmol/L in the control group (SD 20.69) and the mean vitamin D level in the psoriatic group equalled 39.82 nmol/L (SD 19.49).

Conclusion: We established that psoriatic patients had lower vitamin D serum levels than controls and psoriatic patients with higher vitamin D serum levels responded better to systemic treatment. However, our findings are limited by small numbers and further studies need to be performed to corroborate this result. It is possible that treatment with Vitamin D could improve outcomes in our patients. This study demonstrated that calcium serum levels did not differ between the two groups and did not correlate with response to systemic treatment.

Keywords: psoriasis treatment, calcium and vitamin D levels, psoriasis area severity index (PASI)

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6. List of Abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body Mass Index
HIV	Human Immunodeficiency Virus
PASI	Psoriasis Area Severity Index
TBSA	Total Body Surface Area
UVA	Ultraviolet A Light
UVB	Ultraviolet B Light
IL2	Interleukin 2
IL6	Interleukin 6
IFN-gamma	Interferon-gamma
mRNA	Messenger Ribonucleic Acid
T cells	T cells
NK cells	Natural Killer cells
25(OH)D	25 Hydroxyvitamin D or Hydroxycholecalciferol

7. List of Statistical Graphs

Chapter 1: Introduction and Literature Review

1.1 Introduction

Psoriasis is an immune-mediated skin disease which is both chronic and inflammatory, with multiple clinical types and variable severity. It is renowned for its negative impact on quality of life and presents with recurrent relapses and remissions.[1] Five diverse types of psoriasis have been described: psoriasis vulgaris, guttate psoriasis, flexural psoriasis, pustular psoriasis (either palmar-plantar or generalised), and erythrodermic psoriasis.[2] The common form, psoriasis vulgaris, is characterised by erythematous, well-circumscribed plaques with a silvery scale. The scale represents a hyperproliferation of the epidermis with premature maturation of keratinocytes and incomplete cornification.[3] Psoriasis can affect any site but has a predilection for extensor surfaces. A common occurrence in psoriasis is nail involvement which includes 5-10 percent of patients without cutaneous involvement. Psoriatic patients are at a growing risk of developing comorbid diseases, such as metabolic syndrome, cardiovascular disease, psychiatric disease, non-alcoholic fatty liver disease, Crohn's disease, lymphoma, and thyroid disease.[2]

Pathogenesis of the disease was initially regarded as a hyperproliferative disorder of the epidermal keratinocytes [4], but it is now recognised to be more complex. For several years disputes about the fundamental process occurred and whether the initial event was hyperplastic keratinocytes with secondary immune activation or *vice versa.*[5] Ongoing studies suggest that there is an initial dysregulation in the immune system.[3] It is likely that an interplay between keratinocytes, T-cells, dendritic cells, neutrophils and cytokines maintain the cutaneous inflammation of psoriasis.[3] In addition to its having a genetic predisposition[6], it is also associated with multiple triggering factors, including trauma, sunburn, chemical irritants, infections, and certain medications.[2] Some reports suggest that prescribing calcium channel blockers exacerbated the occurrence of psoriasis.[7]

1.1.1 Outcome measures in psoriasis

Multiple outcome measures were developed for classifying psoriasis severity. Menter, *et al*. classified the severity of psoriasis by using the total body surface area (TBSA). TBSA ranges from mild (< three percent body surface area involved), moderate (> three percent but less than 10 % body surface area involved), to severe (>10% body surface area involved).[8] Psoriasis area severity index (PASI) is a more refined tool used to quantify the severity of the disease. When recording PASI score the affected area and the lesion characteristics are formularised, resulting in a score from 0 to 75. Characteristics taken into account include erythema, scale, and plaque thickness. It is usually used to monitor disease in clinical trials, with a required improvement of 75% from baseline to determine the success of treatment (PASI = 75).[8] Although the PASI score is an appropriate validated psoriasis severity assessment tool,[9] it does not consider issues of impact on quality of life, pain, bleeding, or pruritus.[10] Another widely used metric is the Physician Global Assessment (PGA). The PGA subjectively assesses all psoriatic lesions, based on erythema, scale, and induration without considering body surface area and lesion location.[9]

1.1.2 Systemic treatment of psoriasis

Psoriasis treatment is dependent on psoriasis type, severity of the disease and the preferences and general health of the patient. Mild disease can be treated with topical treatments, which include salicylic acid, vitamin D analogues, topical corticosteroids, tar, calcineurin inhibitors and dithranol. Moderate to severe disease, not controlled with topical treatment, may be treated with ultraviolet light treatment (UVA and UVB) or systemic treatment (methotrexate, retinoids, cyclosporin or biological agents). Usually, systemic treatment is considered for patients with more than 10% of their body surface exposed, have psoriatic arthritis or have three or more nails involved and if it significantly impacts their quality of life.[11] Psoriasis has an unpredictable course with variable responses to systemic treatment. Potential side effects of most

systemic medications determine the usefulness of pre-treatments test that could predict the response.[12]

Sometimes systemic agents that are used to treat variable (moderate to severe) psoriasis are linked to adverse short and long-term side effects. Methotrexate, a folic acid antagonist, remains the first-line systemic treatment for people with moderate to severe psoriasis.[13] Although the precise mechanism of action of methotrexate is unknown, it is understood to act via its anti-proliferative, anti-inflammatory, and immunosuppressive effects. It inhibits epidermal hyperplasia by hindering DNA synthesis and reduces the chemotaxis of neutrophils. It is blamed for the decrease of important pro-inflammatory cytokines.[14] Methotrexate is commonly used due to its low cost, availability, long term safety profile and efficacy. However, it is recognised that not all patients will respond equally. A meta-analysis found that only 40% of patients achieve a PASI 75 (improvement of 75 % from baseline) by twelve weeks of methotrexate use and 45% by 52 weeks of methotrexate use.[15] Furthermore, methotrexate is known for side effects such as vomiting and nausea, abdominal pain and, very rarely, liver or lung fibrosis.[15] With the reported variation in efficacy and side effect profile of systemic treatments, researchers aim to find predictors for using systemic treatment in psoriatic patients. Studies looked at genetic markers[16] as well as baseline patient characteristics such as BMI that can contribute to poor response to treatment.[17] However, both these studies focus on biological therapy rather than other systemic treatments. A study completed in 2015 describes low serum calcium as being a predictor of poor response to psoriasis treatment, particularly to methotrexate.[12] In the same study, it is suggested that methotrexate may act through calcium-dependent mechanisms. The study showed that there was a synergistic effect of calcium and methotrexate and that calcium supplementation strengthened the inhibitory effect of methotrexate on epidermal proliferation. Pre-treatment calcium correlated the highest with methotrexate efficacy. This might be useful to improve treatment results in psoriasis. This study was performed in China and it would be interesting to know whether their findings could be replicated in South Africa.

Other conventional systemic medications that are used to treat psoriasis include cyclosporine and acitretin. Acitretin is thought to act in psoriasis via its effects on cell proliferation, keratinization, inflammation, and immune dysregulation.[18] Studies confirmed the efficacy of acitretin in treating different forms of psoriasis; pustular and erythrodermic psoriasis respond better to acitretin than does psoriasis vulgaris. Minor adverse effects, such as dryness of skin and mucosae, headache and soft tissue aching, are common in people taking acitretin. A major concern is that acitretin is highly teratogenic, which limits its use in women.

Since the 1990s, cyclosporine as a calcineurin inhibitor was used for the treatment of psoriasis. The mode of action of cyclosporine in psoriasis has still not been fully elucidated, but its efficacy has been shown in many randomised controlled trials.[19] Renal impairment is the main limiting factor of cyclosporine as long-term continuous treatment.

Over the last decade, biological therapy has transformed the treatment of moderate to severe psoriasis. According to a meta-analysis, biological therapies such as adalimumab, secukinumab, ustekinumab, infliximab, and ixekizumab are more efficacious than methotrexate or controls at three to four months.[20] However, biological agents are extremely expensive, which limits their availability in low and medium income countries. Furthermore, biological agents may not be tolerated, and have additional risks, including hypersensitivity reactions and the development of serious infections, including tuberculosis.

1.2 Normal role of calcium and vitamin D in keratinocyte differentiation and implications in psoriasis

Calcium and vitamin D are known to regulate the differentiation of keratinocytes.[21] A calcium gradient is found in the epidermis of the skin that effects keratinocyte differentiation. The basal layer has a lower gradient than the granular layer.[22] The increased calcium level in the granular layer promotes differentiation into the

corneocytes found in the stratum corneum.[23] It is important to understand how abnormalities in calcium metabolism potentially contribute to the development of psoriasis. Keratinocytes present in people's skin with psoriasis undergo incomplete differentiation and there are defects in the calcium gradient between the basal and cornified layer in the epidermis of both lesional and non-lesional psoriasis skin.[26] Psoriatic keratinocytes have slightly lower calcium stores than normal keratinocytes.[24]

1.2.1 Calcium and psoriasis

Several studies have reported low serum calcium levels in people with psoriasis, including those with chronic plaque psoriasis, erythrodermic psoriasis and pustular psoriasis.[25] There have also been reports that prescribing calcium channel blockers exacerbated psoriasis, and triggered the onset of new psoriasis.[7] Hypocalcaemia has been recorded particularly in more severe kinds of psoriasis, such as erythrodermic psoriasis and pustular psoriasis.[25]

It has been suggested that a disturbance in the homeostasis of calcium could be present in developing new psoriasis or exacerbating existing psoriasis.[26] The rationale behind this suggestion is that hypocalcaemia can damage cadherins, which are cell adhesion molecules. As has been alluded to, calcium differentiates and proliferates keratinocyte, and cell adhesion requires calcium-dependent cadherins. Furthermore, case reports verify the treatment of psoriasis with calcium supplementation.[26]

However, an investigation into serum calcium and/or vitamin D levels predictive of response to treatment with systemic agents, including ultraviolet light, methotrexate, cyclosporine, and acitretin, in people with variable (moderate to severe) psoriasis, has not been done in South Africa as yet.

1.2.2 Vitamin D in psoriasis

Vitamin D analogues appear to have beneficial therapeutic effects in some patients, when used topically in psoriasis.[27] The exact mechanical action is not known but studies suggest that their efficacy in psoriasis is due to the anti-proliferative effect, effect on increasing cellular differentiation and their immunomodulating properties.[27] The topical use of vitamin D analogues in treating psoriasis has promoted further research into the relationship between psoriasis and vitamin D. Low levels of vitamin D potentially implicate the pathogenesis of psoriasis.[28] Vitamin D-3 acts on the vitamin D receptor in keratinocytes to regulate growth and differentiation. It also affects the immune function of T-cells and dendritic cells. It has been suggested that vitamin D-3 inhibits important pro-inflammatory cytokines such as IL2, IL6, IFN-gamma, granulocyte-macrophage colony-stimulating factor mRNA and the ability to inhibit cytotoxic T-cells and NK cells.[28] The role of vitamin D in psoriasis may be further supported by the beneficial effect of UVB in psoriasis treatment. Phototherapy is known to increase the serum levels of 25(OH)D and calcium in psoriatic patients.[12] Several factors can alter vitamin D levels, including diet, sun exposure, malabsorption of vitamin D, and certain medications. Although controversial, a study in Italy correlates between severity of psoriasis and vitamin D deficiency.[28] An exploratory investigation supported this finding.[29] The possible relationship has not been investigated in South Africa.

1.3 Impact of skin type, UVR, and diet on vitamin D and calcium levels

The phenotype, loosely recognised as skin colour, has been defined in the Fitzpatrick skin typing system, described in 1975 by Thomas Fitzpatrick. [30] The system records the skin type according to an individual's exposure to sun, in terms of the degree of skin tanning or burning. Six Fitzpatrick skin phototypes have been described: (I) never sun tans and always burns; (II) burns easily and tans minimally with difficulty; (III) burns discreetly, tans discreetly and evenly; (IV) burns marginally, tans discreetly and effortlessly; (V) described as a skin colour of brown, rarely burns and tans profusely, and (VI) is described as dark brown or black, never burns and tans abundantly.[31, 30]

The active form of vitamin D is acquired by means of cutaneous exposure to ultraviolet light and through diet. In the skin vitamin D activation is determined by the degree of skin pigmentation as well as the season, and length of exposure to UV light.[32] In turn, vitamin D levels influence the blood levels of calcium. People with uniformly distributed cutaneous melanin (Fitzpatrick skin types 5-6) are potentially at greater risk of having low levels of vitamin D and calcium, as melanin distribution affects the absorption of ultraviolet light. In South Africa, several studies have assessed whether the South African population, categorised according to age or colour of skin, or place of living, is vitamin D deficient. None of these studies could be considered as providing a complete reflection of vitamin D serum levels in South Africa.[33]

Currently, the limits for vitamin D deficiency, insufficiency and sufficiency are not precisely defined. The Revised South African Guidelines for the diagnosis and management of osteoporosis[34] describes the assessment of vitamin D status as follows: < 30nmol/L equals deficient, 30-50nmol/L considered as insufficient, > 50nmol/L as sufficient and, 125-150nmol/L is the safe upper limit.

A methodical review of the literature between 1983 and 2000 concluded that, in South Africa, the mean calcium intake varied between 400 and 500mg/day.[35] This makes the calcium intake of South Africans half the recommended daily allowance of 1000mg a day according to the National Institutes of Health.

In South Africa, there seems to be no guidance available on how much time for sun exposure to activate sufficient vitamin D. Likewise, no distinction is made among people with fair and darkly pigmented skin. Scientifically validated data of agreed safe threshold levels of sun exposure that allows for optimal vitamin D synthesis without increasing the risk of cutaneous malignancy, are non-existent. [33] Studies of levels of vitamin D in certain countries have recommended exposure of 10-15 minutes of daily sunshine, which could potentially increase vitamin D levels,[36] although the latitude must be considered. As a result of cultural, behavioural, and geographical differences among populations, increased UV radiation may be insufficient in maintaining a

nutritious vitamin D level, especially during winter. Dietary vitamin D intake may be very important in some populations.

Food sources of vitamin D and calcium include, oily fish, fish liver oils, wild mushrooms, egg yolk, some bread and dairy products, such as cheese and yoghurt. But even in these foods, regarded as rich in vitamin D, levels are variable. All these factors could potentially influence the severity of psoriasis.

To date, there are no simple, reliable and affordable laboratory tests that can predict which patients might be at risk of poor response to any form of systemic psoriasis treatment. Influential factors in response to systemic treatment most likely include genetic factors[16] and baseline patient characteristics such as BMI.[17] However, both these studies focus on biological therapy rather than other systemic treatments.

A study in China[12] showed that response to treatment may be less effective if the patient has a low serum calcium level. In the same study, it was also noted that psoriatic patients had lower serum calcium levels than the controls. Due to calcium and vitamin D's relationship, it is interesting to establish if serum levels of either calcium or vitamin D are low or can predict response to systemic psoriasis treatment in our population.

1.4 Summary

Calcium and vitamin D are renowned to regulate the differentiation of keratinocytes.[21] Keratinocytes in psoriatic people's skin undertake incomplete differentiation with calcium gradient deficits in psoriatic skin.[24] Psoriasis keratinocytes have slightly lower calcium rations than normal keratinocytes.[22] It has been suggested that abnormalities in calcium metabolism contribute to the development of psoriasis. Furthermore, several studies have reported low serum calcium and vitamin D levels in psoriasis patients that may have implications in the pathogenesis.[28] Although controversial,

some studies suggest vitamin D deficiency correlates with the severity of psoriasis.[29,28]

Chapter 2: Hypothesis, Aims, Materials, and Methods

2.1 Primary hypothesis

The examined hypothesis for this study can be formulated as follows: Do calcium and vitamin D levels in blood predict clinical features and/or response to treatment in people with psoriasis?.

2.2 Aims

This study aims to establish whether:

1. The clinical type or severity of psoriasis is correlated with calcium and/or vitamin D serum levels;

2. Calcium and/or vitamin D lserum evels are predictive of response to treatment with systemic agents, including ultraviolet light, methotrexate, cyclosporine, and acitretin, in people with fluctuating psoriasis (moderate to severe);

3. Calcium and vitamin D serum levels of people with varying (moderate to severe) psoriasis differ from an age-, race- and gender-matched control group.

2.3 Materials and Methods

2.3.1 Study Population

This is a case-control (prospective) study that involves people with psoriasis, attending the Dermatology outpatient clinic or admitted to ward G23 at Groote Schuur Hospital, a tertiary medical appointment centre in Cape Town, between September 2019 and September 2021. Fifty psoriasis patients (n = 50) and fifty controls (n = 50) participated in the study. Patients were recruited when they were about to start new systemic

treatment or ultraviolet light for psoriasis under the care of a dermatologist. All patients with varying psoriasis (moderate to severe) were evaluated and eligible ones were included (Appendices III & IV) commensurate with criteria for the initiation of systemic treatment and the provision of informed consent to ensure participation. The Human Research Ethics Committee of the University of Cape Town (Reference No.: 514/2019) approved the study. All participants' data related to their clinical history, including previous medical history and current medications, clinical examination, including PASI score, and investigations were recorded. Privacy of personal information was ensured by using coded identities and the data were stored on a computer with password protection.

The inclusion criteria for the psoriatic group were:

- Age over 18 years;
- Informed consent provided;
- Clinical diagnosis of patients with varying psoriasis (moderate to severe); and
- Clinical decision to commence systemic treatment and/or ultraviolet light therapy.

The exclusion criteria were:
- Patients who are pregnant or breast-feeding; and
- Patients unable or not willing to give informed consent.

At initiation, the study involved a control group (n = 50) without psoriasis that participated simultaneously with the psoriatic patients (n = 50) in the study.

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2.3.2 Sample Collection and Processing

For the psoriasis group (n = 50), at the first visit, participants were asked about the duration of psoriasis, distribution of psoriasis, if they had any other medical illnesses and if they were on any medication. Diet was assessed, in terms of specific foods rich in calcium and vitamin D, as adequate or inadequate.

Examination findings included the type of psoriasis and the extent of psoriasis, using a PASI score. A PASI score was performed by dividing the body into four areas; head, arms, trunk to the groin, and legs to buttocks. An average score for erythema, thickness, and scale for each of the four areas was generated as 0 = absent, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe. The scores for erythema, thickness, and scale for each area were added together. A percentage was generated for skin covered with psoriasis for each area and converted to a 0-6 scale (0 = 0%, 1 = <10 %, 2 = 10-<30%, 3 = 30-<50%, 4 = 50-70%, 5 = 70-<90%, 6 = 90-100%). The sum of the scores of erythema, thickness, and scale was multiplied by the scale 0-6 generated for skin covered with psoriasis for each area and multiplied by 0.1, 0.2, 0.3, and 0.4 for the head, arms, trunk, and legs. The sum of the scores results in a PASI score between 0 and 75.

After a complete assessment of the patient, the following blood samples were obtained: For patients initiated on methotrexate: creatinine, albumin, AST, ALT, HIV, hepatitis B, and C serology and Full Blood Count; for patients initiated on acitretin: pregnancy test, fasting lipids, and glucose, Full Blood Count, AST, ALT, creatinine; for patients starting cyclosporine: serum creatinine, performed on two separate days; and for all patients, calcium and vitamin D serum levels. The samples for serum calcium and vitamin D were coded with a labelling system that anonymised patients' identity.

The age, sex, and race-matched controls were recruited from Dermatology outpatients with a clinical history, including their previous medical history and current medications. Calcium and vitamin D serum levels were measured.

All collected blood samples were processed by the National Health Laboratory Services. Serum calcium was measured spectrophotometrically and Vitamin D by electrochemiluminescence, on the Roche Cobas 6000.

Data collected from personal history, examination, and laboratory results were recorded on a data capture sheet (Appendix 5). Separate data capture sheets were completed for the 50 psoriasis patients and the 50 controls .

Although the study was initially planned to be conducted for one year, with a visit at one-month and at three-months for the psoriasis group, however the emergence of the coronavirus pandemic derailed the process. The investigation was extended over another year to ensure that an adequate number of patients and controls had been recruited. During the follow-up visits, outpatients were given standard psoriasis treatment provided by dermatology health care professionals. Participation in the study did not influence the treatment of patients. The control group was seen once, with no follow-up.

All participants were apprised with the study's goals and required to provide consent for data collection. Collected data (Appendices III & IV) included type of psoriasis, duration of psoriasis in years, serum calcium and vitamin D levels, PASI score at baseline and follow-up, systemic treatment, (including either methotrexate, acitretin, cyclosporine, and light therapy). Gender, Fitzpatrick skin type, calcium and vitamin D levels were collected for the patients and the controls. [Appendix VIII: Data Sheet].

A data capturing sheet facilitated the statistical analyses of the findings.

Statistical Analysis:

Quantitative data were expressed using central tendency, mean and median and dispersion scales with standard deviation. The following statistical data techniques were used:

- Histograms and box-and-whisker plots that graphically demonstrated the frequency with which various data scores of multiple variables occurred in data sets without analysis.

- Chi-Squared test and analysis of variance (ANOVA) to analyse the calcium and vitamin D serum levels of both the controlled and psoriatic groups. ANOVA graphs enabled us to deduce statistical differences by comparing the means of the sample groups (controls and patients).

- Scatter plot visualisation graphs to demonstrate the calcium and vitamin D serum levels of psoriatic patients, specifically focusing on analysing the existence and strength of the relationship between calcium/vitamin D serum levels and patients' responses to treatment.

- A p-value <0.05 is significant.

Chapter 3: Data Analysis and Results

3.1 Participants

Participants in this study included people with psoriasis (n = 50), the patient group, and people without psoriasis (n = 50) the control group.

3.2 Patient demographics and disease characteristics

(Table 1) The mean age for the psoriasis group was 48.12 and that of the control group was 47.6. In the psoriasis group there were 21 females and 29 males, whereas in the control group there were 33 females and 17 males. In the psoriasis group the Fitzpatrick skin types were as follow: Fitzpatrick 1 (n = 0); Fitzpatrick 2 (n = 6); Fitzpatrick 3 (n = 35); Fitzpatrick 4 (n = 5); Fitzpatrick 5 (n = 3); and Fitzpatrick 6 (n = 1). In the control group, Fitzpatrick 1 (n = 0); Fitzpatrick 2 (n = 5); Fitzpatrick 3 (n = 28); Fitzpatrick 4 (n = 11); Fitzpatrick 5 (n = 5); and Fitzpatrick 6 (n = 1). Comorbidities were varied as indicated in Table 1 below.

Between 1 September 2019 and 1 September 2021 fifty adults were clinically assessed as having severe psoriasis, i.e. a PASI score of > 10. Psoriasis vulgaris was the most frequent form of psoriasis seen (n = 40), followed by erythrodermic psoriasis (n = 8) and only two persons had severe pustular psoriasis (n = 2). The mean duration of disease in years in the psoriasis group was 13.22 [Fig. 1]. At baseline, the mean PASI score was 29.92 [Table 2].

Overall 22% of the patients had psoriatic arthritis, confirmed by a rheumatologist, and 44% had a comorbid disease. Patients' demographics and disease characteristics are summarised in Table 1. Thirty patients were started on light therapy, (22 as sole

additional therapy). Methotrexate was the most common systemic agent initiated (n = 31) whereas nine of the patients received acitretin. Two patients received cyclosporine. Seven patients were non-adherent to the systemic treatment and 5 patients were lost to follow-up [Fig. 3: Treatment of Psoriatic Patients].

Table 1: Demographics and clinical characteristics of patients with psoriasis vs controls for this study.

Variables	Psoriasis (n = 50)	Controls (n = 50)	p-values
Age (in years) mean (standard deviation)	48.12 (15.4)	47.6 (16.3)	0.88
Females	21	33	0.02
Males	29	17	0.02
Fitzpatrick skin type n (%) 1 2 3 4 5 6	 0 (0%) 6 (12%) 35 (70%) 5 (10%) 3 (6%) 1 (2%)	 0 (%) 5(10%) 28(56%) 11(8%) 5(10%) 1(2%)	0.42
Inflammatory arthritis No *comorbidities or **skin disease only 1-2 comorbidities 2-3 comorbidities >4 comorbidities	11 28 15 5 1	0 38 4 1 0	<0.01 0.82
Serum Calcium mmol/L Mean (SD)	2.35 (0.13)	2.35 (0.12)	0.79
Serum Vitamin D nmol/L Mean (SD)	39.82 (19.49)	49.92 (20.79)	0.01

**Comorbidities included: non-alcoholic fatty liver disease, hypertension, diabetes mellitus, hypercholesterolaemia, obesity, gout, gastroesophageal reflux disease, thyroid disease, emphysema/ asthma, lung malignancy, polycystic ovarian syndrome, and bipolar mood disorder.*

***Skin diseases included: n = 1 acne; n = 3, keloids; n = 1, lipoma; n = 6, hair loss; n = 2, urticaria; n = 4, eczema (contact eczema, venous stasis eczema, atopic eczema); n = 4, actinic keratosis; n = 1, hidradenitis suppurativa; n = 1, vitiligo; n = 1, Kaposi sarcoma; n = 1, vasculitis; n = 1, venous malformation; n = 4, skin tags; n = 1, neurofibromatosis; n = 1, furuncles; n = 2, onychomycosis; n = 1, previous drug reaction; n = 1, lymphoma; n = 2, acanthosis nigricans; n = 1, lichen planus; and n = 1, pruritus*

Table 2: Disease related characteristics of the psoriasis patients

Variables	**n = 50**
Mean psoriasis duration (SD)	13.22 (9.46)
Psoriasis subtypes n (%) Psoriasis vulgaris Erythrodermic psoriasis Flexural psoriasis Pustular psoriasis	 N = 40 (80%) N = 8 (16%) N = 0 (0) N = 2 (4%)
Therapy planned in addition to topical n (%) Light therapy only Methotrexate Acitretin Cyclosporine	 N = 8 (16%) N = 31 (62%) N = 9 (18%) N = 2 (4%)
Mean PASI at baseline (SD)	29.92 (11. 58)
Mean PASI at follow-up (SD)	16.76 (17.36)

*SD = standard deviation. PASI = Psoriasis Area Severity Index

3.3 Calcium and vitamin D levels in psoriatic patients vs controls

Among the patient group the mean serum calcium level was 2.35 (SD 0.13) and in the control groups the mean calcium level was 2.35 (SD 0.12). The mean serum vitamin D level in the psoriatic group was 39.82 (SD 19.49) and the mean vitamin D in the control group was 49.92 (SD 20.79) [Figs. 10 & 11]. In the psoriasis group, 42 of 49 patients had low serum vitamin D levels (one was rejected). Among the 42 patients with low serum vitamin D levels, 14 (29%) were considered deficient (<30nmol/L), 28 (57%) were insufficient (30nmol/L-50nmol/L) and 7 (14%) were normal (>50nmol/L – p = <0.01). Twenty-nine patients (n = 29) in the control group had low vitamin D levels, with six (12%) being deficient (30nmol/L), 23 (46%) insufficient (30nmol/L-50nmol/L), 21 (42%) were normal (>50nmol/L) (Fig 14).

Seven of the patients in the psoriatic group had low serum calcium levels, in comparison with the control group, in which only one person had a low calcium level. However this difference was statistically insignificant (p = 0.79) (Fig. 4).

3.3.1 Does the clinical type and severity of psoriasis correlate with serum calcium or vitamin D?

This findings indicate that serum calcium levels do not seem to differ among patients with various clinical types of psoriasis such as erythrodermic psoriasis and psoriasis vulgaris (p = 0.63) [Fig.6]. Although there were two patients with pustular psoriasis, they were not included in the statistical analysis due to a limited sample size. Equally, the study suggests that vitamin D does not correlate with different types of psoriasis (p = 0.62) [Fig. 6].

No correlation was found between calcium serum levels and severity of psoriasis (p = 0.48). A Pearson correlation test showed a trend towards a negative correlation, that is, as the calcium level increased, the PASI tended to decrease (Fig. 7).

Vitamin D levels did not correlate with the severity of psoriasis (p = 0.31). However, there was a trend based on a negative (Pearson) correlation, that is, as vitamin D increases the PASI score appears to decrease (Fig. 8).

3.3.2 In patients with severe psoriasis is there an association between calcium and vitamin D levels and response to treatment with systemic agents, including ultraviolet light, methotrexate, cyclosporin, and acitretin?

A significant positive correlation between vitamin D levels of patients and response to treatment was found. Patients with increased vitamin D serum levels responded better to treatment, as demonstrated in the scatter plot (p = 0.01) (Fig. 9.1).

No correlation was found between calcium and response to systemic treatment (p = 0.49) (Fig. 9.2).

The mean change in PASI score in percentage was 70%.

The mean PASI at baseline was 29.92 and the mean PASI at follow-up was 16.76 (p = <0.01).

Summary of PASI improvement from baseline to follow up in patient group:

Change in PASI score	n
• No change in PASI or worsened PASI	11
• PASI progress <25%	6
• PASI progress 25-49%	0
• PASI progress 50-74%	2

• PASI progress >75	21
• Patient lost to follow up, non-adherent or stopped medication due to side effects	10

Of the 11 patients who had an unchanged PASI score, 6 (55%) had deficient vitamin D (<30nmol/L) and 5 (45%) had insufficient vitamin D (>30nmol/L-50nmol/L) and, none of the patients had a normal vitamin D serum level. Patients who had a PASI improvement of less than 25%, had insufficient and deficient vitamin D serum levels and none had normal vitamin D serum levels.

Ten participants in the patient group were lost to follow-up, non-adherent to medication, or stopped the medication due to significant adverse events.

3.3.3 How do the calcium and vitamin D serum levels of people with moderate to severe psoriasis compare with an age-, race- and gender-matched control group?

There was no difference in calcium serum levels (p = 0.79) between the control group (SD = 0.12) and the psoriatic group (SD = 0.13). However, a significant difference in vitamin D serum levels between the control group and the psoriatic group (p = 0.01) emerged. The mean vitamin D serum levels were 49,92 in the control group with SD equal to 20.69. The mean vitamin D serum levels were equivalent to 39.82 in the patient group, (SD = 19.49).

According to the National Osteoporosis Foundation of South Africa (NOFSA) 2017 guidelines, a sufficient level of vitamin D is 50nmol/L, which means that the psoriatic group had insufficient vitamin D serum levels and the levels in the control group were higher, but still considered insufficient as the mean serum vitamin D level was less than 50nmol/L.

3.3.4 Other descriptive data derived from the study

1. Do the duration of psoriasis and calcium and vitamin D serum levels correlate?

We found no correlation between calcium serum levels and duration of psoriasis ($p = 0.37$). Similarly, vitamin D serum levels did not correlate with the duration of psoriasis ($p = 0.49$).

2. Does a correlation between Fitzpatrick skin type and calcium and vitamin D serum levels exist?

A trend emerged, suggesting that people with skin type 2 had higher vitamin D serum levels, which was insignificant ($p = 0.81$). There was no relationship between Fitzpatrick skin types and serum calcium levels ($p = 0.81$).

Similarly, there seemed to be a trend that in Fitzpatrick type 2 skin type the control group had lower calcium serum levels than the patient group, however this was statistically insignificant ($p = 0.15$).

However, Fitzpatrick skin type 2 patients had higher vitamin D levels ($p = <0.01$) compared to Fitzpatrick skin types 3, 4, and 5 patients.

Chapter 4: Discussion

4.1 Discussion

This was a prospective study to investigate calcium and vitamin D serum levels in psoriatic patients and to explore their effects on response to treatment.

Response to systemic treatment in psoriasis is variable. Data regarding pre-treatment tests to predict response to systemic treatment in psoriasis are limited. Studies have looked at genetic markers[16] as well as baseline patient characteristics such as BMI that can contribute to poor response to treatment.[17] However, both these studies focus on biological therapy rather than other systemic treatments. Retrospectively, a study of 77 patients in China[12] indicated that there was better response to methotrexate in psoriatic patients with enhanced pre-treatment calcium levels. The retrospective analysis looked at effects of methotrexate and calcium on keratinocyte growth inhibition in vitro and on a psoriasis-like mouse model in vivo.

As an initial prospective study investigating a possible correlation between calcium and vitamin D serum levels and response to systemic treatment, this study is substantial. Unlike the study in China, this study did not show a correlation between calcium serum levels and response to treatment. We did not find lower calcium serum levels in the psoriatic group compared to age and race matched controls. This is inconsistent with a previous case control study conducted in Iran. They found 37.2% of the psoriatic patients had low calcium serum levels, and 62.8% had normal calcium serum levels.[29] The retrospective study in China[12] showed similar results to the study conducted in Iran. Our study identified seven psoriasis patients with low serum calcium levels and one patient in the control group with a low serum calcium level ($p = 0.79$). This was statistically insignificant.

When studying the relations among calcium and different types of psoriasis, our case control study demonstrated no difference in serum calcium levels among patients with either plaque or erythrodermic psoriasis. We showed no correlation between calcium serum levels and the severity of psoriasis. This finding is inconsistent with the results of a previous study,[25] which showed that hypocalcaemia is more prevalent in people with increased forms of psoriasis, i.e. patients with erythrodermic psoriasis and pustular psoriasis had hypocalcaemia more frequently than patients with psoriasis vulgaris. The study, similar to our study, was a case-control one, based in Iran over a span of two years. The calcium serum levels were measured in 98 hospitalised psoriatic patients and compared with 100 patients without psoriasis.

Low vitamin D serum levels were identified more in our psoriatic than the control group. This finding is aligned with the study in Italy,[28] which showed that their patients with psoriasis had significantly lower vitamin D serum levels than controls and that their patients had a higher risk of deficiency in vitamin D than people without psoriasis. There is a scarcity of studies that accurately describe serum vitamin D levels in people in South Africa.[32] However, based on global guidelines, this study found that people with and without psoriasis have low vitamin D serum levels, with psoriasis patients being more deficient in vitamin D than people without psoriasis.

This study did not find a correlation between vitamin D serum levels and the type of psoriasis ($p = 0.62$). This result is consistent with a case-control study in Syria,[37] consisting of 88 patients with psoriasis and 86 controls ($p>05$). That study showed a significant negative association between psoriasis severity and vitamin D serum levels. However our study showed no such correlation between psoriasis severity and vitamin D serum levels. The cross-sectional study in Italy,[28] which consisted of 145 patients with chronic plaque psoriasis, showed similar results to our study.

There was a significant connection between vitamin D serum levels and the mean duration of diseases in our study, as also demonstrated by a previous study.[37] However, an important finding not demonstrated before, is that psoriasis patients with higher vitamin D levels responded better to treatment. Our study is the first to have

demonstrated that vitamin D serum levels could be a pre-treatment test in predicting response to treatment.

This is a real-world prospective study and its focus on serum level determination of psoriatic patients in relation to their treatment in routine clinical practice, makes it significant. This is so, especially when we have suggestive evidence that changing vitamin D serum levels potentially impact response to treatment of psoriatic patients.

In conclusion, although our sample size is small, its findings are inconsistent with those of previous studies that showed the presence of low calcium serum levels in psoriatic patients and that serum calcium may be a predictor of response to treatment. However, this study is consistent with other studies that corroborated low vitamin D serum levels in psoriatic patients.[29, 12] To our knowledge, this study serum is novel in serum its investigation of calcium and vitamin D serum levels combined in a real-world cohort of psoriatic patients and to explore how it effects the response to treatment.

4.2 Summary of results

This study found that:

- Psoriatic patients had notably lower vitamin D serum levels than the controls;
- Calcium serum levels were normal in our psoriatic patients;
- There seems to be no correlation between calcium and vitamin D serum levels and severity or different types of psoriasis;
- There is insufficient evidence to suggest that pre-treatment calcium would strengthen the effect of systemic agents such as methotrexate, cyclosporine, or acitretin; and
- If vitamin D intake of psoriatic patients were to be increased, people with psoriasis might respond better to treatment.

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4.4 Limitations of the study

1. Due to the coronavirus pandemic, the proposed follow-up time, which was set at one-month and three-months, could not be applied due to a restriction on the clinic visits. This also affected the treatment of patients because they could not always receive treatment on time. Mostly, the inability to admit patients due to coronavirus restrictions can be considered as a major shortcoming of this study.

2. The patient and control groups were not perfectly matched, as there were more females in the control group.

3. Some participants were non-adherent, were lost to follow-up, and had medication side effects that were not reported, possibly as a result of Coronavirus restrictions.

4. The sample size of the two groups was small. The vast majority of patients had psoriasis vulgaris or erythrodermic psoriasis and only two patients had pustular psoriasis. Therefore, the pustular psoriasis group was considered too small to be included in the statistical analysis.

5. The higher the PASI, the higher the likelihood that the patient would be admitted to the Dermatology ward. In this case, they received optimal topical treatment by nursing staff, together with phototherapy and a systemic agent, which would ultimately change the PASI score more significantly. Fifteen (n =15) of the patients who had a PASI >75 were hospital admissions.

4.5 Recommendations

Impending studies probing predictors of response to treatment should preferably enrol a larger group of patients.

It is of interest to ascertain as to whether the findings in this study can be replicated independently, that is with calcium serum and vitamin D serum levels separately. In fact, this study offers an opportunity to focus more on the ramifications of increasing vitamin D serum levels in psoriatic patients, in particular how higher levels of vitamin D serum levels might modify response to treatment of patients with psoriasis.

We should consider giving these patients a vitamin D oral supplementation when they are not responding to treatment or are being offered a systemic treatment. This should be in addition to encouraging sun exposure and a diet rich in vitamin D.

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5. Appendices

Appendix 1: Ethics Approval

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

05 August 2019

HREC REF: 514/2019

Dr S Jessop
Division of Dermatology
G23, NGSH

Dear Dr Jessop

PROJECT TITLE: DO LEVELS OF CALCIUM AND VITAMIN D IN BLOOD AND HAIR PREDICT CLINICAL FEATURES AND/OR RESPONSE TO TREATMENT IN PEOPLE WITH PSORIASIS? PHD-CANDIDATE - DR SIHAN WAGHID

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 August 2020.

Please update all references to Helsinki Declaration 2008 to 2013.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the following student: Dr S Waghid will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.

Appendix II: Protocol Amendment

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

Form FHS006: Protocol Amendment

HREC office use only (FWA00001637; IRB00001938)			
☐ Approved	☐ Type of review: Expedited	☐ Full committee	
This serves as notification that all changes and documentation described below are approved.			
Signature HREC Chairperson / Designee		Date	

Note: All **Major** amendments must include a **Cover Letter** and a local **PI Synopsis** justifying the changes for the amendment. Please note that incomplete amendment submissions will not be reviewed.

Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za with subject line: FHS006 + (HREC Reference number).

Sort

The latest forms are found on our website.

http://www.health.uct.ac.za/fhs/research/humanethics/forms

Please also clarify your plan for research-related activities during COVID-19 lockdown.

Comments from the HREC to the Principal Investigator:
Note: The approval of this protocol amendment does not grant annual approval. Please complete the FHS016 / FHS017 form for annual approval at least one month before study expiration.

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	26 July 2021
HREC REF Number	514/2019
Protocol Title	**Do calcium and vitamin D levels in blood predict clinical features and/or response to treatment in people with psoriasis?**
Protocol Number (if applicable)	
Principal Investigator	Dr Susan Jessop

Department / Office Internal Mail Address	G23 New Groote Schuur Hospital Observatory, 7925 Cape Town South Africa	
1.1 Is this a major or a minor amendment? (see FHS006hlp) Major (tick box) Minor (tick box)	☐ Major	✓ Minor
1.2 Does this protocol receive US Federal funding?	☐ Yes	✓ No
1.3 If the amendment is a major amendment and receives US Federal Funding, does the amendment require full committee approval? Note: Any protocol amendments for **Full Committee Review** MUST be submitted on the monthly HREC submission dates. (Please email an electronic copy to hrec-enquiries@uct.ac.za)	☐ Yes	✓ No
1.4 Did the initial study require UCT No-Fault Insurance	☐ Yes	✓ No

2. List of Proposed Amendments with Revised Version Numbers and Dates

Please itemise on the page below, all amendments with revised version numbers and dates, which need approval.
This page will be detached, signed and returned to the PI as notification of approval. Please add extra pages if necessary.

1. **Title:**

Do calcium and vitamin D levels in blood predict clinical features and/or response to treatment in people with psoriasis?

Previous Title:

Do levels of calcium and vitamin D in blood and hair predict clinical features and/or response to treatment in people with psoriasis

2. **Aims extended to include:**

- To compare the calcium and vitamin D serum levels of people with moderate to severe psoriasis with an age-, race- and gender-matched control group.

3. **Method amended:**

This is a case-control study.

Previously: [a prospective quantitative study]

Inserted under method is the following:

Calcium and Vitamin D blood levels will be measured in a group of age, sex and race-matched controls recruited from the Dermatology outpatient's department. A clinical history will be taken to exclude conditions that could influence calcium or Vitamin D levels, including previous medical history and current medications.

3. Protocol status (tick ✓)

☐	Open to enrolment
☐	No participants have been enrolled
	Closed to enrolment (tick ✓)
✓	Research-related activities are ongoing
☐	Research-related activities are complete, long-term follow-up only
☐	Research-related activities are complete, data analysis only

4. Proposed changes will affect: (tick ✓ all the categories that apply)

Protocol	
✓	Study objectives, design (including investigator's brochure, clinical activities, study length)
☐	Study instruments, questionnaires, interview schedules
✓	Sample size
✓	Recruitment methods
☐	Eligibility criteria (inclusion and exclusion criteria)
☐	Drug/device (composition, amount, schedule, route of administration, combination with other drugs/devices, safety information)
☐	Data collection/ analysis
☐	Principal Investigator. (Please attach revised conflict of interest and PI declaration statements. Refer: sections 7 and 8.4 in the New Protocol Application Form FHS013)
✓	Consent form and information sheet
☐	Recruitment materials (e.g. advertisements)
☐	Administrative (e.g. change in sponsor's name, change in contact information)
☐	Other. Please specify:

**Note: Amendment changes involving study length, sample size, additional sites and eligibility criteria (i.e. inclusion of minors and /or pregnant woman) need to be declared to the Insurance office. Please liaise via fhs.sponsorship@uct.ac.za regarding the required documentation and information to be submitted to obtain an updated UCT No-fault Insurance Certificate- it should be included herewith*

4.1 In your opinion, will there be any **increase** in risk, discomfort or inconvenience to participants?	☐ Yes	✓ No
If yes, please provide a detailed justification/explanation:		

4.2 What follow-up action do you propose for participants who are already enrolled in the study?	
☐	Inform current participants as soon as possible
☐	Re-consent current participants with revised consent/assent forms (append)
✓	No action required
☐	Other. Please describe:

5. Detailed description of the change(s)

Please attach, for each amendment, a summary of all changes which clearly indicates:

i. Old wording (e.g. ~~strikethrough~~ text, CHANGED FROM and CHANGED TO)

ii. New wording (e.g. *italicized*, **bold**, tracked)

iii. Detailed rationale/ justification/ explanation for each change

***Please note**: There is no change to the protocol, except for the addition of a control group. We believe this to be a minor amendment.*

6. Ethics Review for Amendment Levy – cost including vat

Amendment Review Costs including VAT

Please tick amount to be billed:

Submission Type	Description	New fee (Vat Incl.)	tick ✓
Research funded solely from UCT departmental/ divisional/group budget	Major/ Minor Amendments	R0,00	✓
Non-sponsored student research for degree purposes at UCT/Other Universities & Colleges	Major/ Minor Amendments	R0,00	☐
Protocol amendment - Major (FHS006 Form)	Clinical Trial & International Grant Funded Research - Any changes to the protocol that requires Full Committee review	R8 000.00	☐
Protocol amendment - Major (FHS006 Form)	Clinical Trial & International Grant Funded Research - Any change to the protocol that requires Expedited review that does not require Full Committee Review	R5 000,00	☐
Protocol amendment - Minor (FHS006 Form)	Clinical Trial & International Grant Funded Research - Minor amendments, administrative changes that do not affect study design e.g. changes to informed consent form, changes in study staff, etc.	R2 250,00	☐
Protocol amendment - Major (FHS006 Form)	National grant funded research - Any change to the protocol that requires Full Committee review	R7 000,00	☐
Protocol amendment - Major (FHS006 Form)	National grant funded research - Any change to the protocol that requires Expedited review that does not require Full Committee review	R2 500,00	☐
Protocol amendment - Minor (FHS006 Form)	National grant funded research - Minor amendments, administrative changes that do not affect study design e.g. changes to informed consent form, changes in study staff, etc.	R1 000,00	☐

NB: Protocols funded by UCT (e.g. departmental funding / student research) and by certain grant funding organizations (e.g. MRC, NRF, CANSA,) are exempt from these charges.

Please provide details for Invoicing, either complete section 1 or 2 :

1. Invoice billing – Directly to Sponsor	
Sponsor's name	
Billing Address of Sponsor:	
Vat Number:	
Contact person:	
Telephone number:	
Email Address:	
2. Internal Journal Billing:	
Fund Number:	
Cost Centre Number:	
Account Holder Name:	
Division of Account Holder:	

7. Amendment Submission checklist (tick ✓)

7.1 Please tick that all the documents are attached before submitting to the HREC. NB: Incomplete submissions will not be processed	
✓	Latest FHS006 form completed with all sections completed as per our website
✓	Cover Letter
☐	PI Justification/ Summary for the reasons for the amendment ***N/A minor amendment***
✓	Protocol - Track changes & Clean Copy (where necessary)
✓	Informed Consent Forms (ICF), if applicable (Any changes made to ICF tracked & clean copy)
✓	Any other additional documentation in support of amendment
☐	Updated no fault insurance certificate (if applicable)

Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za with subject line: FHS006 + (HREC Reference number). The latest forms are found on our website.

8. Signature

My signature certifies that I will maintain the anonymity and/ or confidentiality of information collected in this research. If at any time I want to share or re-use the information for purposes other than those disclosed in the original approval, I will seek further approval from the HREC.

Signature of PI		Date	26th July 2021

Appendix III: Informed Consent Form - Control Group

UNIVERSITY OF CAPE TOWN: Faculty Of Health Sciences

Informed consent form: (Control group)

Dear participant:

We, at the Department of Health Sciences, Division of Dermatology and Chemical Pathology at the University of Cape Town, are conducting a study on blood sample levels of calcium and vitamin D in persons attending the dermatology outpatient clinic.

Why is the study being done?/ what is the purpose of the study?
The study is being done to find out if you have low levels of calcium and vitamin D in your blood.

Why are you being asked to take part in this study?
Being part of this research study will aid you and other patients by seeing if there is a need to add calcium and vitamin D supplements to your diet or whether we should encourage you to have a higher calcium and vitamin D diet.

How many people will take part in this study?
20 participants will be part of the study.

How many times will you have to be seen at Groote Schuur Hospital for the study?
No further testing is necessary unless clinically indicated.

What are the risks of the study?
There are no risks to you in this study..

Are there any benefits to you for being part of the study?
There are no additional benefits in the study. You will be treated as any other patient attending the dermatology outpatient clinic.

Will your test results be shared with you?
Yes, all investigations will be shared with you.

Will any of your blood be stored and used for research in the future?
Yes.

Who will see the information, which is collected about you in the study?
Your personal information, clinical examination, blood results will be kept confidential to the people involved in the study.

Will you receive payment for taking part in the study?
No.

What will happen when the study is over?
You will continue with your treatment and will have routine follow ups at Groote Schuur Hospital as needed.

Who do you speak to if you have any questions about the study?
Supervisor: Dr. Susan Jessop:
Researcher: Dr. Sihan Waghid (021) 404-6138.

If there are any ethical concerns about your rights as a participant in this research study you can contact UCT's Faculty of Health Sciences Human Research Ethics Committee on (021 406-6338).

Appendix IV: Informed Consent Form - Patient Group

UNIVERSITY OF CAPE TOWN: Faculty Of Health Sciences

PATIENT DATA CAPTURE SHEET (Control group)

Patient initials

Folder no.

Date of birth dd mm yyyy

Gender M F

First Visit:

Background medical history

……

……

……

……

Co-morbidities	
Metabolic syndrome	
Cardiovascular disease	
Non-alcoholic fatty liver disease	
Thyroid disease	
Chron's disease	

LABORATORY INVESTIGATIONS:

Date	
Serum calcium level	
25 (OH) vitamin D level	

Other medical illnesses:

……

……

Medication

……

……

Diet: (Please indicate how many times a week you eat the following foods):

Foods	**Times per week**
Oily fish (salmon, sardines, tuna)	
Egg yolk	
Fish liver oils	
Wild mushrooms	

Foods rich in calcium	**Times per week**
Milk	
Cheese	
Yoghurt	
Leafy greens	
Orange juice	
Breads	

……

Sun exposure: (Tick where appropriate)

Less than 10 minutes a day ……

10-15 minutes daily ……

More than 15 minutes a day ……..

UNIVERSITY OF CAPE TOWN: Faculty Of Health Sciences

Do calcium and vitamin D levels in blood predict clinical features and/or response to treatment in people with psoriasis?

Informed consent form:

Dear participant:

We, at the Department of Health Sciences, Division of Dermatology and Chemical Pathology at the University of Cape Town, are conducting a study on whether blood and hair sample levels of calcium and vitamin D are related to severity of psoriasis and response to treatment. Psoriasis is a chronic skin disease with different clinical types and severities. The cause of the disease is complex and not clearly understood. The treatment of psoriasis depends on the type and severity of disease. Mild psoriasis, where less than 3% of the body is involved, can be treated with ointments or creams such as salicylic acid, dovobet, steroid creams, tar and dithranol. Moderate and severe psoriasis, where more than 3% of the body is involved, can be treated with ultraviolet light (UVA or UVB) and medication like methotrexate, acitretin and cyclosporine.

Why is the study being done?/ what is the purpose of the study?
The study is being done to find out if you have lower levels of calcium and vitamin D in your blood and hair. The lower calcium and vitamin D levels may aid in predicting response to your treatment (such as methotrexate, acitretin, cyclosporine) i.e. if you have lower levels of calcium and vitamin D you may respond more poorly to your treatment than if you had higher levels of calcium and vitamin D. Treating you with supplements might help you to respond better to your treatment.

Why are you being asked to take part in this study?
Being part of this research study will aid you and other patients with psoriasis by seeing if there is a need to add calcium and vitamin D supplements to your diet or whether we should encourage you to have a higher calcium and vitamin D diet.

How many people will take part in this study?
50 participants with moderate to severe psoriasis, who require systemic treatment will be part of the study.

How many times will you have to be seen at Groote Schuur Hospital for the study?
After your first visit, you will be asked to follow up at one month and at three months to assess if your skin is improving on treatment.

What are the risks of the study?

There are no risks to you in this study. We will still use the appropriate treatment for you, based on the severity of your psoriasis. The blood tests we will do are part of routine blood tests for the medication you are starting. We will only be adding a calcium and vitamin D level to the tests.

Are there any benefits to you for being part of the study?
There are no additional benefits in the study. You will be treated as any other patient with psoriasis who requires systemic medication to assist you in controlling your psoriasis and improving your quality of life.

What other choices do you have?
If you are not happy with taking systemic medication and prefer topical medication only (dithranol, topical steroids, salicylic acid, and dovobet) you can decide not to take systemic medication and therefore not be part of the study.
If at any stage in the study you wish to withdraw, you are welcome to do so. We will continue to treat you in the usual way.

Will your test results be shared with you?
Yes, all investigations including blood results will be shared with you.

Will your blood sample be stored and used for research in the future?
Yes

Who will see the information, which is collected about you in the study?
Your personal information, clinical examination, blood results will be kept confidential to the people involved in the study.

Will you receive payment for taking part in the study?
No

What will happen when the study is over?
You will continue with your treatment and will have routine follow ups at Groote Schuur Hospital, depending on how controlled or poorly controlled your psoriasis is.

Who do you speak to if you have any questions about the study?
Supervisor: Dr. Susan Jessop:
Researcher: Dr. Sihan Waghid (021) 404-6138.

If there are any ethical concerns about your rights as a participant in this research study you can contact UCT's Faculty of Health Sciences Human Research Ethics Committee on (021 406-6338).

Appendix V: Patient Data Capture Sheet

UNIVERSITY OF CAPE TOWN: Faculty Of Health Sciences

PATIENT DATA CAPTURE SHEET

Project title: Do calcium and vitamin D levels in blood predict clinical features and/or response to treatment in people with psoriasis?

Patient initials

Folder no.

Date of birth dd mm yyyy

Gender M F

First Visit:

Background medical history

Duration of Psoriasis:

……

……

Distribution:

……

……

Extracutaneous involvement (joints, scalp and nail):

……

……

Co-morbidities	
Metabolic syndrome	
Cardiovascular disease	
Non-alcoholic fatty liver disease	
Thyroid disease	
Chron's disease	

Other medical illnesses:

……

……

Medication

……

……

Diet: (Please indicate how many times a week you eat the following foods):

Foods	**Times per week**
Oily fish (salmon, sardines, tuna)	
Egg yolk	
Fish liver oils	
Wild mushrooms	

Foods rich in calcium	**Times per week**
Milk	
Cheese	
Yoghurt	
Leafy greens	
Orange juice	
Breads	

……

Sun exposure: (Tick where appropriate)

Less than 10 minutes a day ……

10-15 minutes daily ……

More than 15 minutes a day ……..

Type of psoriasis:

Psoriasis Vulgaris	
Guttate Psoriasis	
Flexural psoriasis	
Pustular psoriasis	
Erythrodermic psoriasis	

TBSA:

TBSA	
Mild (< three percent body surface area)	
Moderate (>three percent but less than <10 % body surface area)	
Severe (> 10% body surface area)	

PASI SCORE:

	Head	Arms	Trunk to groin	Legs to buttocks
1)Redness				
2)Thickness				
3)Scale				
4)Sum of rows 1,2,3				
5) Area score				
6) Score of row 4 multiplied by row 5 multiplied by the area multiplier				
7)Sum row 6 for each column for PASI score				

CURRENT TREATMENT OR RECENT (Last Three Months) TOPICAL TREATMENT: (circle treatment used)

Topical treatment: salicylic acid, vitamin D analogues, topical corticosteroids, tar, calcineurin inhibitors, dithranol

Ultraviolet light: UVA, UVB, how many sessions per week? And for how long have you had lights?

……

Alternative treatments:

……

LABORATORY INVESTIGATIONS:

Date		
FBC		
Albumin		
AST/ALT		
Hepatitis studies:		
HIV		
Serum calcium level		
25 (OH) Vitamin D level		
Fasting lipogram		
Fasting Glucose		
Serum creatinine		

SYSTEMIC TREATMENT STARTED:

Systemic medication	Date
Methotrexate	
Cyclosporine	
Acitretin	
Other	

FOLLOW-UP VISIT: (AT THREE MONTHS)

TBSA	
Mild (< three percent body surface area)	
Moderate (>three percent but less than <10 % body surface area)	
Severe (> 10% body surface area)	

PASI SCORE:

	Head	Arms	Trunk to groin	Legs to buttocks
1)Redness				
2)Thickness				
3)Scale				
4)Sum of rows 1,2,3				
5) Area score				
6) Score of row 4 multiplied by row 5 multiplied by the area multiplier				
7)Sum row 6 for each column for PASI score				

1)The body is divided into four areas: head, arms, trunk to groin, and legs to buttocks.
2) An average score for erythema, thickness, and scale for each of the four areas is generated. 0=absent, 1=mild, 2=moderate, 3=severe,4= very severe
3) The scores of erythema, thickness, and scale for each area are added together.
4) A percentage is generated for skin covered with psoriasis for each area and converted to a 0-6 scale (0=0%, 1=< 10 %, 2=10-<30%, 3=30-<50%, 4=50-70%, 5=70-<90%, 6=90-100%).
5) Multiply score of (3) above times (4) above for each area and multiply that by 0.1, 0.2, 0.3, and o.4 for head, arms, trunk, and legs
6) Add these scores to get the PASI score

Appendix VI: Response to the Ethics Committee's Query on Amended Protocol

3rd August 2021

HREC: 514/ 2019 PI response.

Dear Professor Blockman,

Thank you for the response from the HREC regarding ethnic attribution in our study.
We respond to the following questions below.

1. *Please provide a full scientific as well as ethical justification for the use of race as a variable for this research.*
2. *Please also discuss how race will be assigned.*

Vitamin D is activated in human skin, under the influence of ultraviolet light. Vitamin D levels, in turn, influence the blood levels of calcium. People with uniformly distributed cutaneous melanin (Fitspatrick skin types 5-6) may be at greater risk of having low levels of vitamin D and calcium, as melanin distribution affects the absorption of ultraviolet light. This, in turn, may influence the type and severity of psoriasis. Skin vitamin D activation is influenced by the degree of skin pigmentation as well as the season, and the length of exposure to UV light (Dawson-Hughes, B., 2004, p 1765)

In this study, calcium and vitamin D blood levels will be measured in people with psoriasis and in a group of normal controls. The groups will be matched according to age, sex and phototype, to control for possible variables in Vitamin D metabolism.
Skin colour will be decribed by Fitpatrick skin type 1-2, 3-4 or 5-6 (Gupta V, Sharma VK. 2019.

We have modified our protocol to reflect Fitzpatrick skin type, rather than ethnic group. Fitzpatrick skin type is determined on history, by degree of tanning in response to ultraviolet light.

References

Gupta V, Sharma VK. 2019. Skin typing: Fitzpatrick grading and others. *Clin Dermatol* Sep-Oct 2019;37(5):430-436.

Dawson-Hughes, B., 2004. Racial/ethnic considerations in making recommendations for vitamin D for adult and elderly men and women. *The American Journal of Clinical Nutrition*, *80*(6), pp.1763-1766.

Yours sincerely

SJessop

Sue Jessop

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

Form FHS006: Protocol Amendment

HREC office use only (FWA00001637; IRB00001938)		
☑ Approved	☑ Type of review: Expedited	☐ Full committee
This serves as notification that all changes and documentation described below are approved.		
Signature HREC Chairperson / Designee		Date 4/8/21

Note: All **Major** amendments must include a **Cover Letter** and a local **PI Synopsis** justifying the changes for the amendment. Please note that incomplete amendment submissions will not be reviewed.

Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za with subject line: FHS006 + (HREC Reference number).

The latest forms are found on our website.

http://www.health.uct.ac.za/fhs/research/humanethics/forms

Please also clarify your plan for research-related activities during COVID-19 lockdown.

Comments from the HREC to the Principal Investigator:
Thank you for your response received 4/8/2021
Note: The approval of this protocol amendment does not grant annual approval. Please complete the FHS016 / FHS017 form for annual approval at least one month before study expiration.

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	26 July 2021
HREC REF Number	514/2019
Protocol Title	**Do calcium and vitamin D levels in blood predict clinical features and/or response to treatment in people with psoriasis?**
Protocol Number (if applicable)	
Principal Investigator	Dr Susan Jessop

Appendix VII: Annual Progress Report / Renewal

HUMAN RESEARCH ETHICS COMMITTEE
22 OCT 2021
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30-10-2022
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	23/10/2021

Note: Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	21 October 2021		
HREC REF Number	514/209	Current Ethics Approval was granted until	30 August 2021
Protocol title	Do calcium and vitamin D levels in blood predict clinical features and/or response to treatment in people with psoriasis?		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?		☐ Yes	☑ No
If yes, could you please provide the HREC Reference number for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Dr Susan Jessop		

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Western Cape Government

GROOTE SCHUUR HOSPITAL

Enquiries: Dr Bernadette Eick
e-mail: GSHReserach.Request@westerncape.gov.za

DR SUSAN JESSOP
MEDICINE: DERMATOLOGY

E-mail: susan.jessop@uct.ac.za

Dear Dr Jessop

RESEARCH PROJECT: Do Levels of Calcium and Vitamin D in Blood and Hair Predict Clinical Features and/or Response to Treatment in People with Psoriasis? (PhD Candidate: S Waghid)

Your recent letter to the hospital refers.

You are granted permission to proceed with your research, which is valid until **30 October 2022**.

Please note the following:

a) Your research may not interfere with normal patient care.
b) Hospital staff may not be asked to assist with the research.
c) Confidentiality must always be maintained.
d) No additional costs to the hospital should be incurred as indicated in your Annexure 2 i.e. Lab, consumables or stationery. **If access to TRACK Care/NHLS is required, kindly attach our letter of approval to the application form and approach Information Management to assist with data.**
e) **No patient folders may be removed from the premises or be inaccessible.**
f) Please provide the research assistant/field worker with a copy of this letter as verification of approval.
g) Should you at any time require photographs of your subjects, please obtain the necessary indemnity forms from our Public Relations Office (E45 OMB or ext. 2187/2188).
h) Should you require additional research time beyond the stipulated expiry date, please apply for an extension.
i) Please discuss the study with the HOD before commencing.
j) Please introduce yourself to the person in charge of an area before commencing.
k) On completion of your research, please forward any recommendations/findings that can be beneficial to use to take further action that may inform redevelopment of future policy / review guidelines.
l) Please contact Michelle Riley (Patient Fees) at ext. 2276 to ascertain if there will be charges for conducting the Research and to obtain a quote or to discuss charges
m) Kindly submit a copy of the publication or report to this office on completion of the research.
n) At no time should any posters encouraging patients to partake in research, be displayed within a clinical area.
o) Please adhere to ALL COVID-19 regulations and Groote Schuur Hospital policies.

I would like to wish you every success with the project.

Yours sincerely

DR BERNADETTE EICK
CHIEF OPERATIONAL OFFICER
Date: 15 November 2021

C.C. Mr L. Naidoo, Prof. N. Ntusi, Prof. N. Khumalo ,Mr. A. Mohamed

Appendix VIII: Data Sheet (Statistician)

Project Title: Do Calcium and Vitamin D Levels in Blood Predict Clinical Features and/or Response to Treatment in People with Psoriasis?

		Lab Investigations								Systemic treatment							
Patients no.	Type pf psoria	Calcium	Vitamin D	TBSA %	(base	PASI Score	(ba	Follow-up TBS	Follow-up PAS	Methotrexate	Acitretin	Cyclosporine	Light therapy	Age	Gender	Fitzpatrick	Duration of psoriasis (in years)
40	psoriasis vulga	2.31	63.7		20	15.3		5	2.5		50mg		yes	51	F	3	26
34	psoriasis vulga	2.48	49.7		25	18.2		5	3	15mg				43	M	3	20
36	psoriasis vulga	2.49	46.9		25	18.6		30	23.4	15mg				32	M	5	2
15	psoriasis vulga	2.30	29.8		30	13.6		b					yes	78	M	2	11
19	psoriasis vulga	2.45	35.7		30	12.8		28	22.6	15mg				62	M	3	43
23	psoriaiss vukgi	2.24	32.3		30	12.6		25		22	15mg			49	F	3	20
11	psoriasis vulga	2.54		36	35	16.8		5	6.7	15mg				62	M	3	10
20	psoriasis vulga	2.4	103.2		35	19.6		5	0.4	15mg				26	M	3	5
50	psoriasis vulga	2.34	34.2		35		21	15	9.9	15mg				36	F	3	9
6	Psoriasis Vulga	2.45	67.9		40		21	a	a				yes	23	F	2	23
14	psoriasis vulga	2.30	29.8		40	22.8		40		23	15mg			70	M	3	20
18	psoriasis vulga	2.3	25.6		40	20.4		30		25	15mg			38	M	3	30
21	psoriasis vukgi	2.38	28.6		40	26.6		25		24	15mg			41	F	3	14
25	psoriasis vukgi	2.33	54.6		40		24	b					yes	41	M	5	10
5	Psoriasis vulga	2.53	29.8		50	28.7		50	29.9		20mg		yes	70	F	3	5
22	psoriasis vulga	2.30	49.5		50	29.9	c	c			15mg			60	M	3	30
27	psoriaisis vulgi	2.40		32	55	28.2		60	40.6		75mg			44	M	3	10
9	Psoriasis Vulga	2.19	23.6		60	36.3		5	3.2		50mg		yes	56	F	3	32
10	psoriasis vulga	2.12	28.8		60	34.7		5	2.8	15mg			yes	58	F	3	5
13	psoriasis vulga	2.31	23.1		60	33.1	e	e					yes	28	M	4	16
31	psoriasis vulga	2.42	47.5		60	33.3		5	2.6	15mg			yes	47	M	4	2
3	psoriasis vulga	2.45		49	65		30	3	2.3	15mg			yes	63	M	2	3
12	psoriasis vulga	2.24	34.6		65	36.8		2	3.5	15mg				64	M	3	4
42	psoriasis vukgi	2.47	117.4		65	33.6	b	b					yes	60	M	2	10
32	psoriasis vulga	2.42	38.8		67	27.7		10	5.9		50mg		yes	56	F	3	8
2	Psoriasis vulga	2.53	29.8		70	34.8		70	36.6	15mg			yes	58	M	4	5
29	psoriaiss vulga	2.32	30.2		70		30	5		2.5	15mg			31	F	3	22
37	psoriasis vulga	2.27	38.2		70	35.2		2	2.1				yes	46	F	3	2
38	psoriasis vulga	2.39	32.1		30	17.2	b	b					yes	31	F	3	2
39	psoriasis vulga	2.24	49.8		35	16.1		2	1.6	15mg			yes	51	F	3	1
41	pustular psoria	2.19	32.5		32	27.3		1	2.9	15mg			yes	58	M	4	20
43	psoriasis vulga	2.37	49.4		35	22.4		2	5.4		50mg			68	F	3	4
45	psoriasis vulga	2.36	33.2		30	17.8		5	3.9	15mg				36	F	3	10
46	psoriasis vulga	2.30	45.6		33	23.5	b	b		15mg				56	M	3	10
47	psoriasis vulga	2.45	22.4		35	15.5		2	1.8	15mg			yes	32	M	3	10
8	Psoriasis Vulga	2.46	REJECTED		40		28.6	b	b	15mg				58	F	3	12
28	psoriasis vulga	2.21	33.2		60		35	3	1.9	15mg			yes	78	M	3	7
44	psoriasis vulga	2.50	50.6		60	32.9	b	b		15mg			yes	36	M	3	2
49	psoriasis vulga	2.46	39.2		65	31.4		30	33.4		50mg			55	M	3	7
1	Erythrodermic	2.4	32.5		90	54.0		90	51.6	25mg			yes	57	F	3	20
4	Erythrodermic	2.49	36.1		95		53	90	51.2			225mg	yes	30	M	2	22
7	erythrodermic	2.36	20.6		95	55.6		95		57		250mg	yes	37	F	5	18
16	erythrodermic	2.31	42.5		90	45.8		8	5.2				yes	23	F	2	13
17	erythrodermic	2.42	14.4		90	45.8		28		35	15mg			20	F	3	13
24	psoriaiss vulga	2.18	9.6		95		37	40		45		50mg	yes	41	M	6	8
26	psoriasis vulga	1.83	15.2		90	32.8		90		30	15mg		yes	60	M	3	15
30	erythrodermic	2.19	36.4		90	47.6		85	40.9	15mg			yes	20	M	3	8
33	Erythrodermic	2.46	71.7		92		46	5	1.2		50mg		yes	54	M	4	20
35	pustular psoria	2.19		33	90		45	2	3.3		75mg		yes	68	F	3	30
48	erythrodermic	2.37	40.7		90	50.4		5	4.8	15mg			yes	44	M	3	12

Controls no.																	
51	2.37		48											36	F	3	
52	2.35	26.1												54	F	3	
53	2.28	17.9												44	F	5	
54	2.40	35.8												64	M	4	
55	2.26	28.3												38	F	5	
56	2.44	40.2												52	F	3	
57	2.31		37											47	F	4	
58	2.29		30											27	F	4	
59	2.31	56.3												53	M	3	
60	2.44	38.8												55	F	3	
61	2.47	57.3												46	F	4	
62	2.46	59.5												60	F	3	
63	2.36	119.8												72	M	2	
64	2.47	30.5												20	F	3	
65	2.30		40.4											45		4	
66		2.45	71											34	M	3	
67	2.40	81.6												75	F	3	
68	2.41	58.1												30	F	3	
69	2.27	52.3												55	F	3	
70	2.24	28.5												50	F	4	
71		2.45	55.6											49	F	3	
72	2.36	83.9												20	F	5	
73		2.85	41											32	F	5	
74	2.31	84.8												73	M	2	
75		2.28	49.8											50	F	3	
76	1.95	44.1												65	F	3	
77		2.23	52.3											22	F	3	
78		2.4	36.9											32	F	3	
79	2.38	35.9												73	F	4	
80	2.45		48.2											52	M	3	
81	2.36	55.2												46	M	3	
82	2.39	33.2												32	F	4	
83	2.29	34.8												72	F	2	
84	2.34	26.7												49	F	3	
85	2.30	31.4												20	M	6	
86		2.21	37.6											44	F	5	
87	2.28	44.1												37	F	3	
88	2.31	57.8												18	F	3	
89	2.44	69.9												34	M	3	
90	2.32	101.1												60	M	2	
91	2.22	32.5												75	M	4	
92	2.52	65.2												66	M	4	
93	2.36	48.6												30	M	3	
94	2.48	50.4												66	M	3	
95	2.28		27.1											29	M	3	
96	2.55		34											62	M	3	
97	2.36	44.6												63	F	3	
98	2.33	64.8												51	F	4	
99	2.25	60.1												44	F	3	
100	2.22	87.1												57	F	2	

Figures

Fig. 1: Duration of Psoriasis

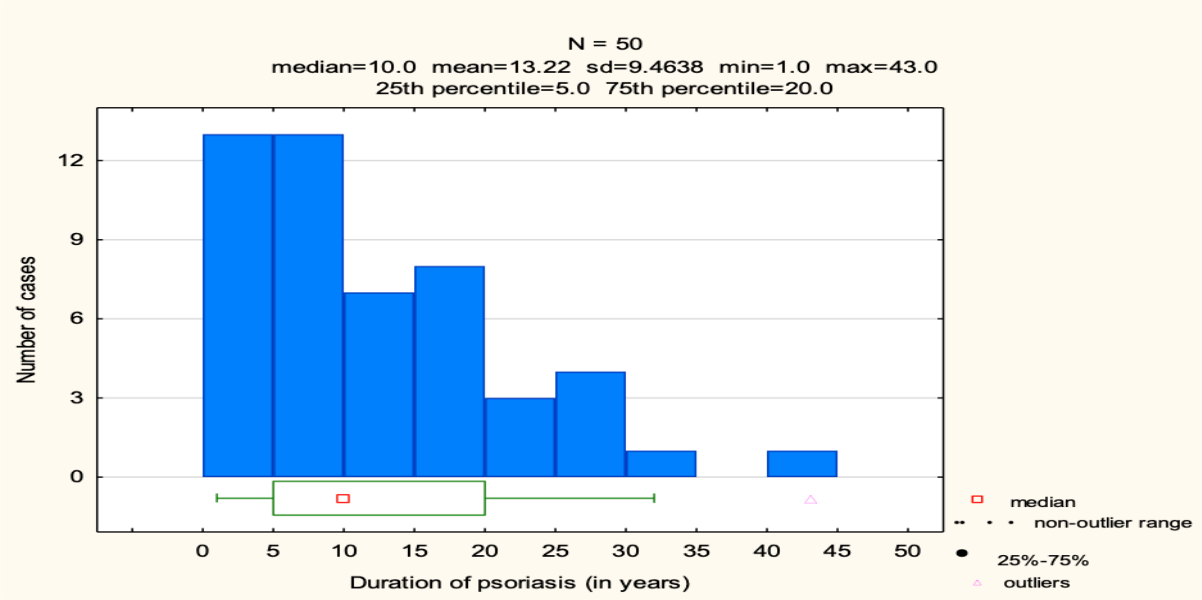

Fig. 2: PASI Scores: Baseline vs Follow-up

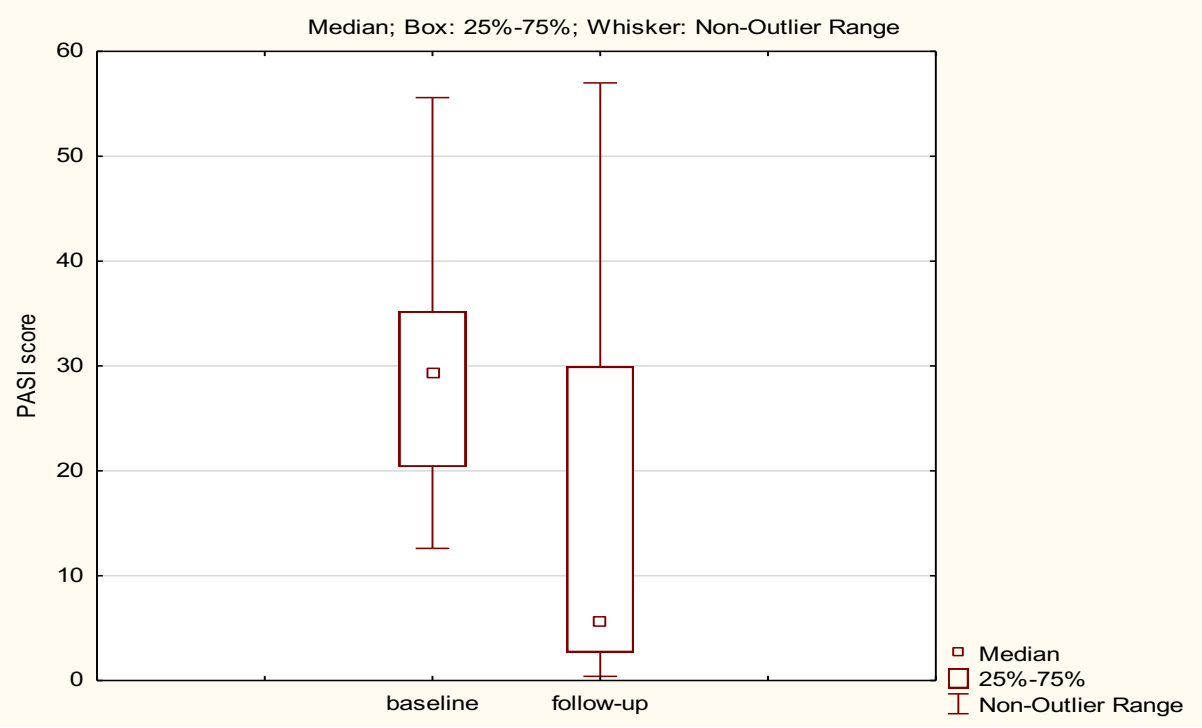

Fig. 3: Treatment initiated

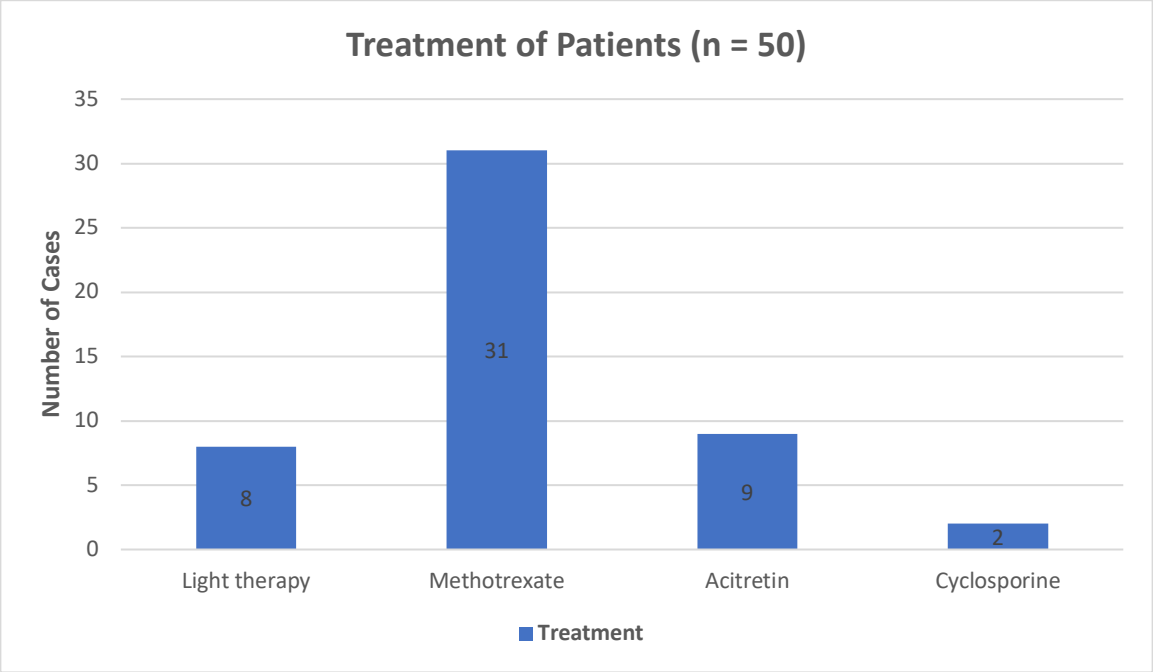

Fig. 4: Serum Calcium Levels of Controls vs Patients [p = 0.79]

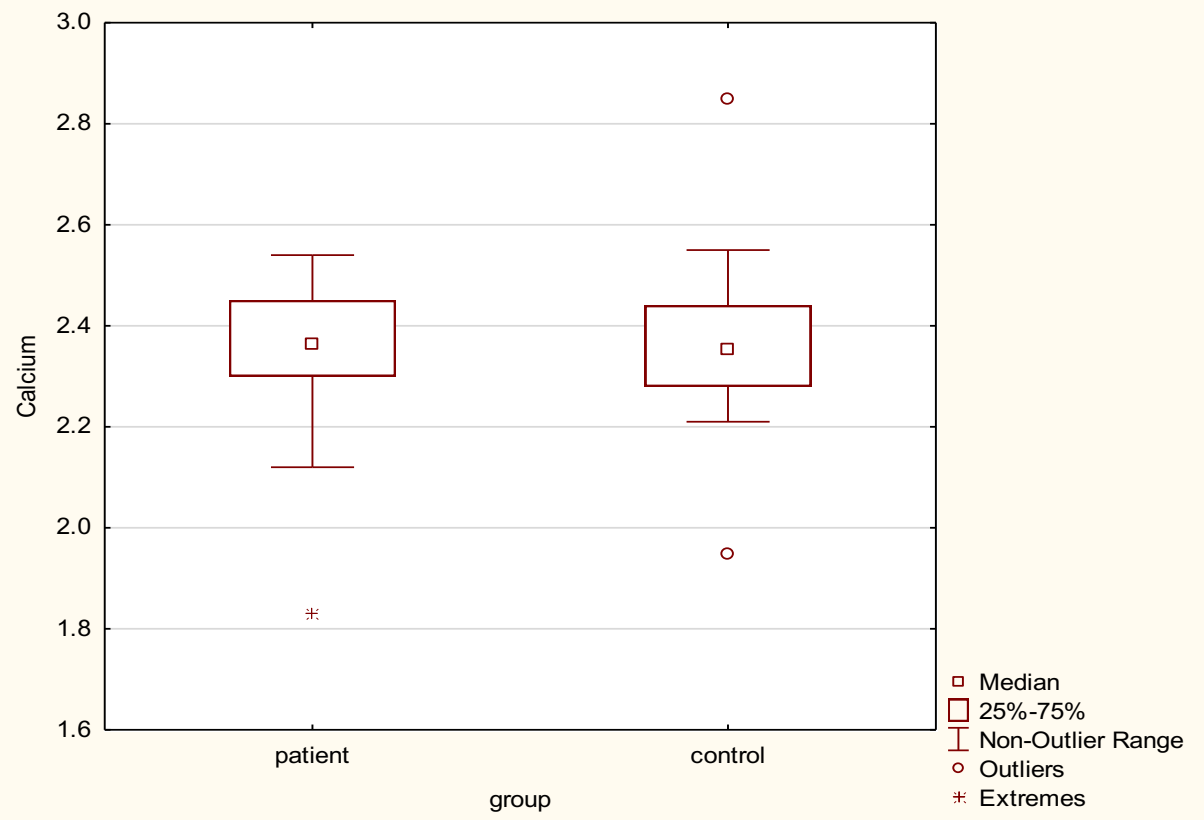

Fig. 5: Serum vitamin D Levels of Controls vs Patients [p = 0.01]

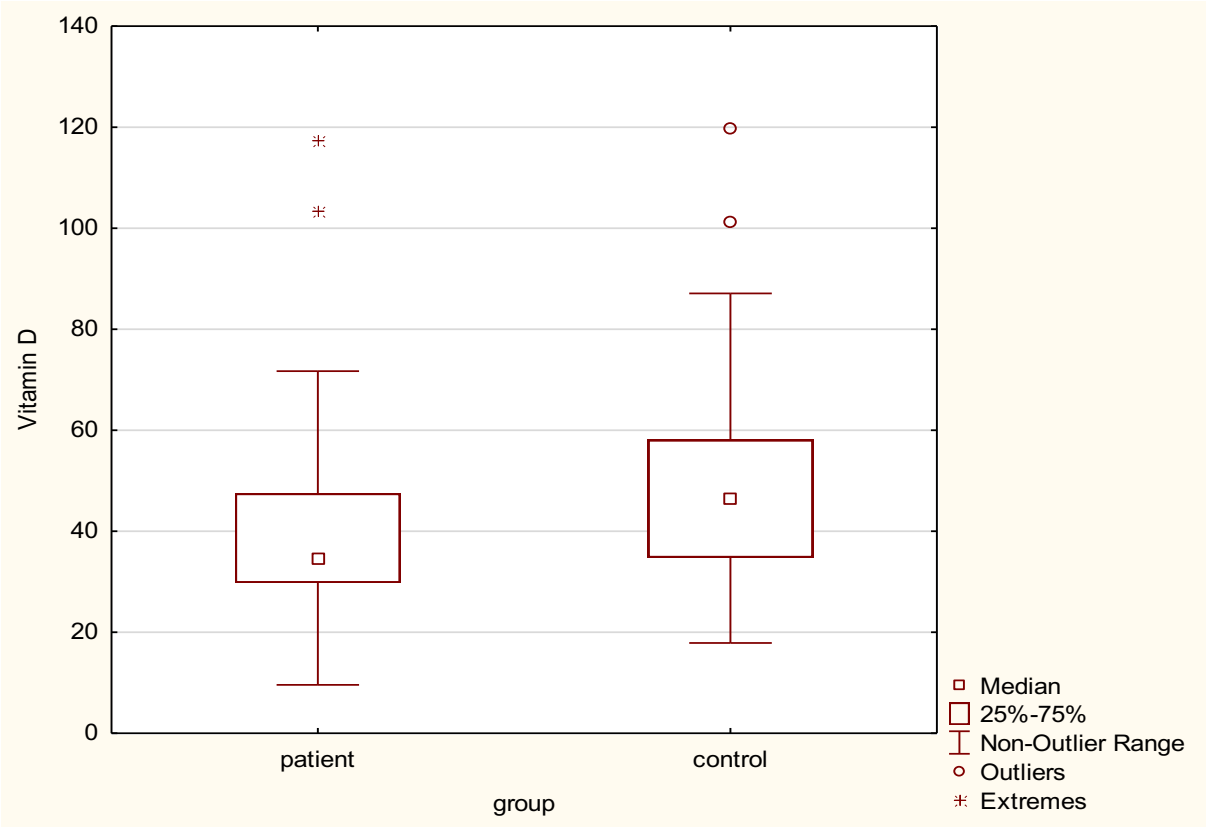

Fig. 6: Correlation between Calcium and Vitamin D Serum Levels and Types of Psoriasis

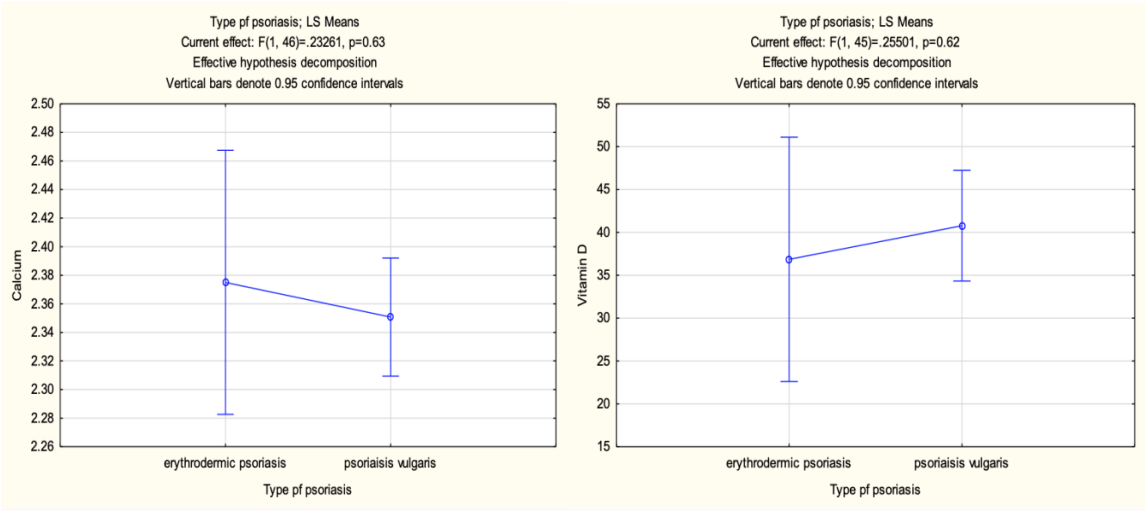

Fig. 7: Calcium and severity of psoriasis measured by PASI

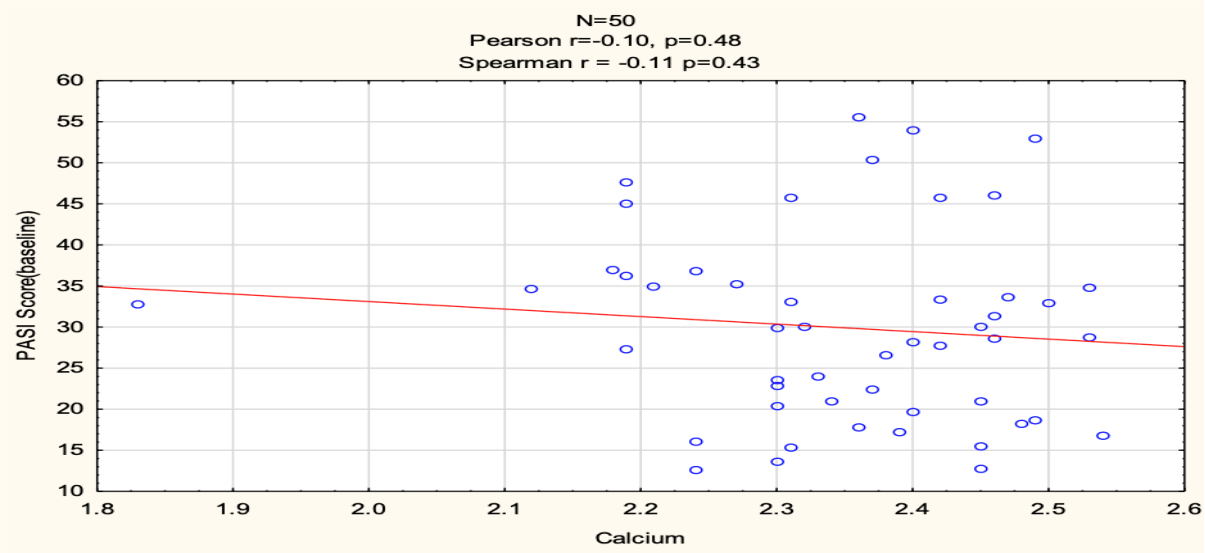

Fig. 8: Vitamin D and severity of psoriasis measured by the PASI

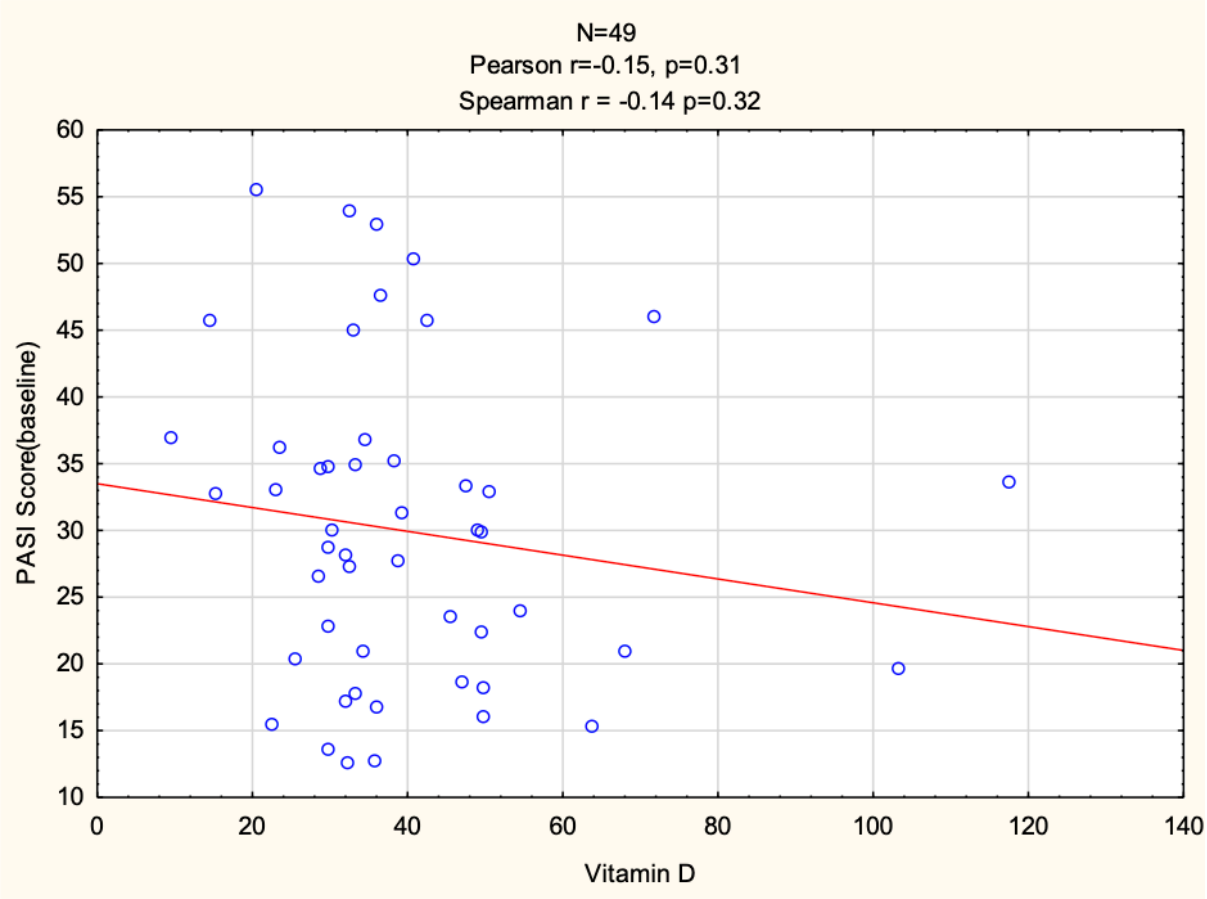

Fig. 9.1: Calcium and Vitamin D and Change in PASI Score

Response to Treatment

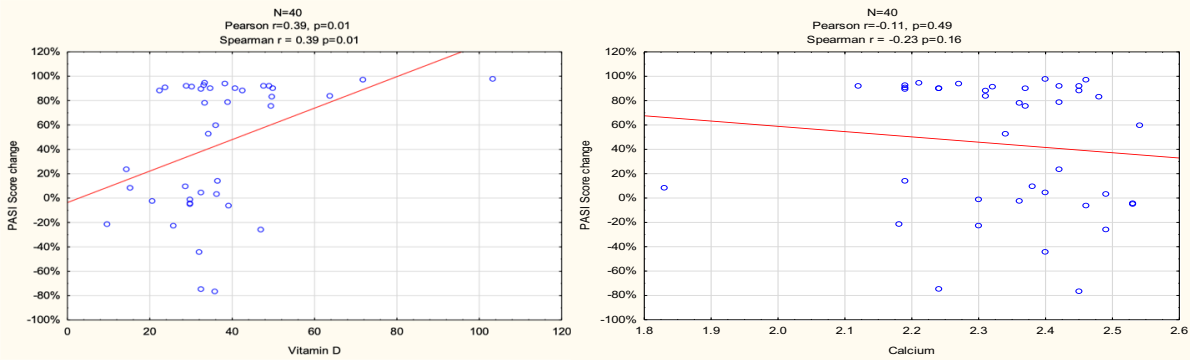

Fig. 9.2: Vitamin D and Change in PASI Score

Response to Treatment

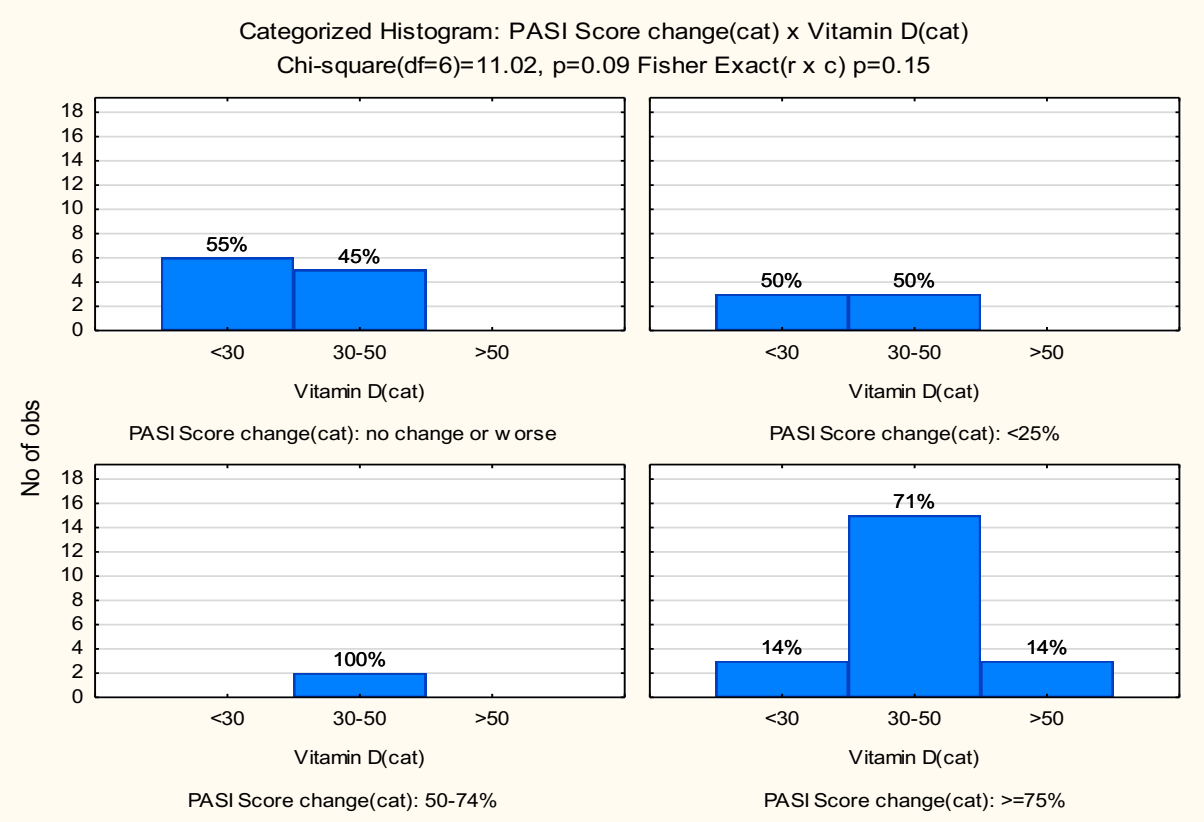

Fig. 10: Fitzpatrick skin type and calcium levels

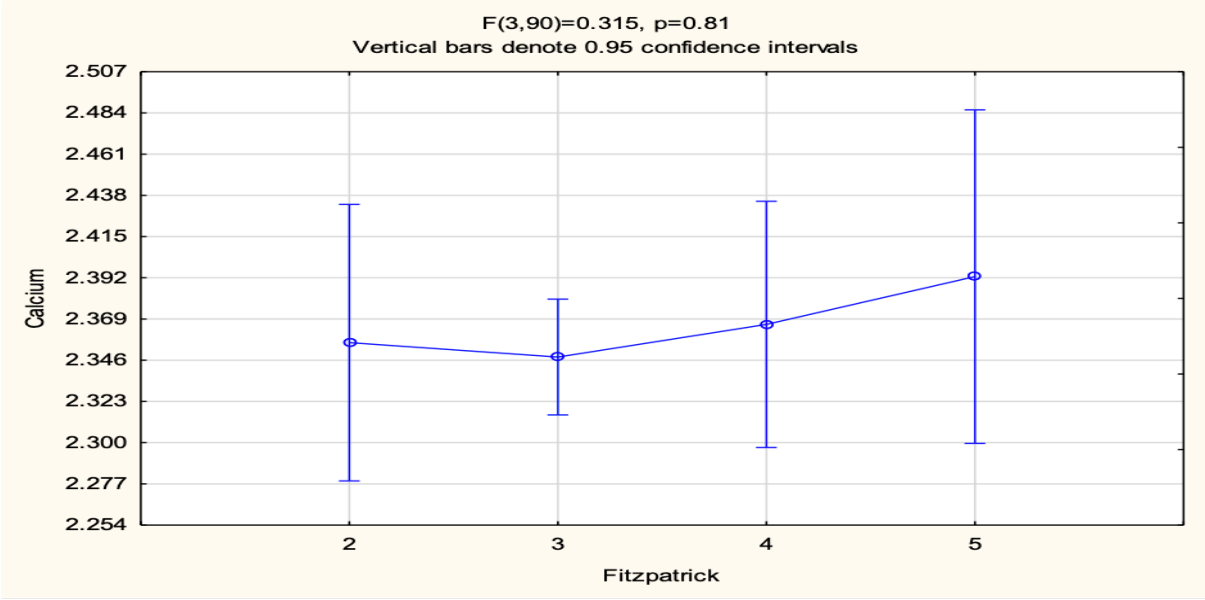

Fig. 11: Fitzpatrick skin type and serum calcium

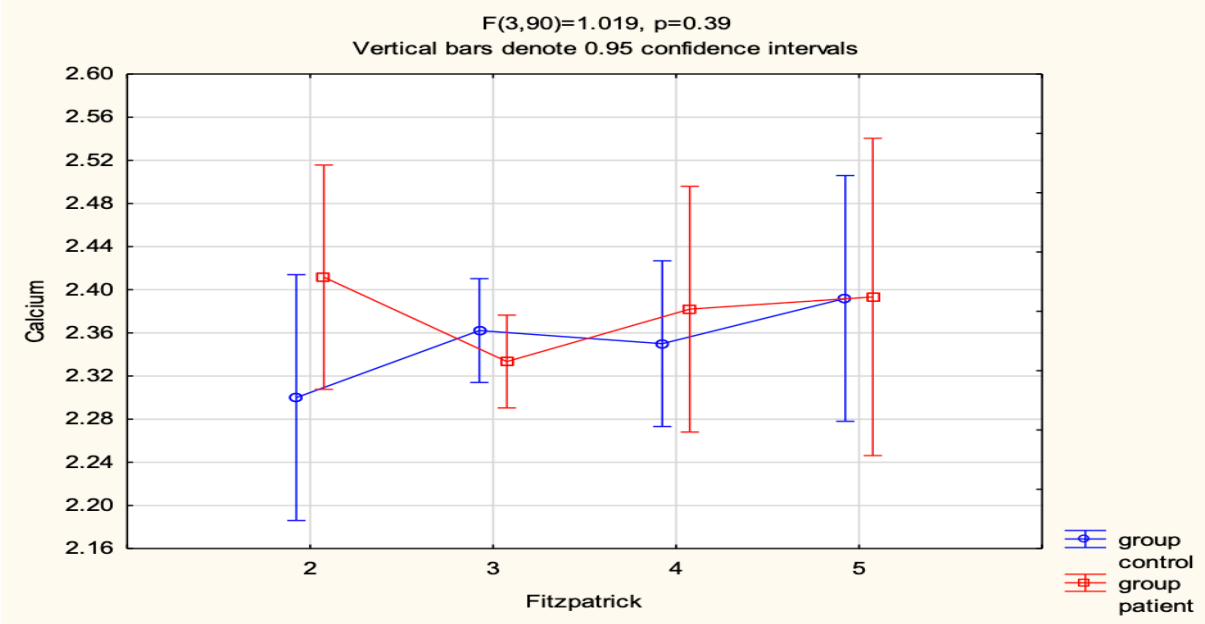

Fig. 12: Fitzpatrick skin type and serum vitamin D

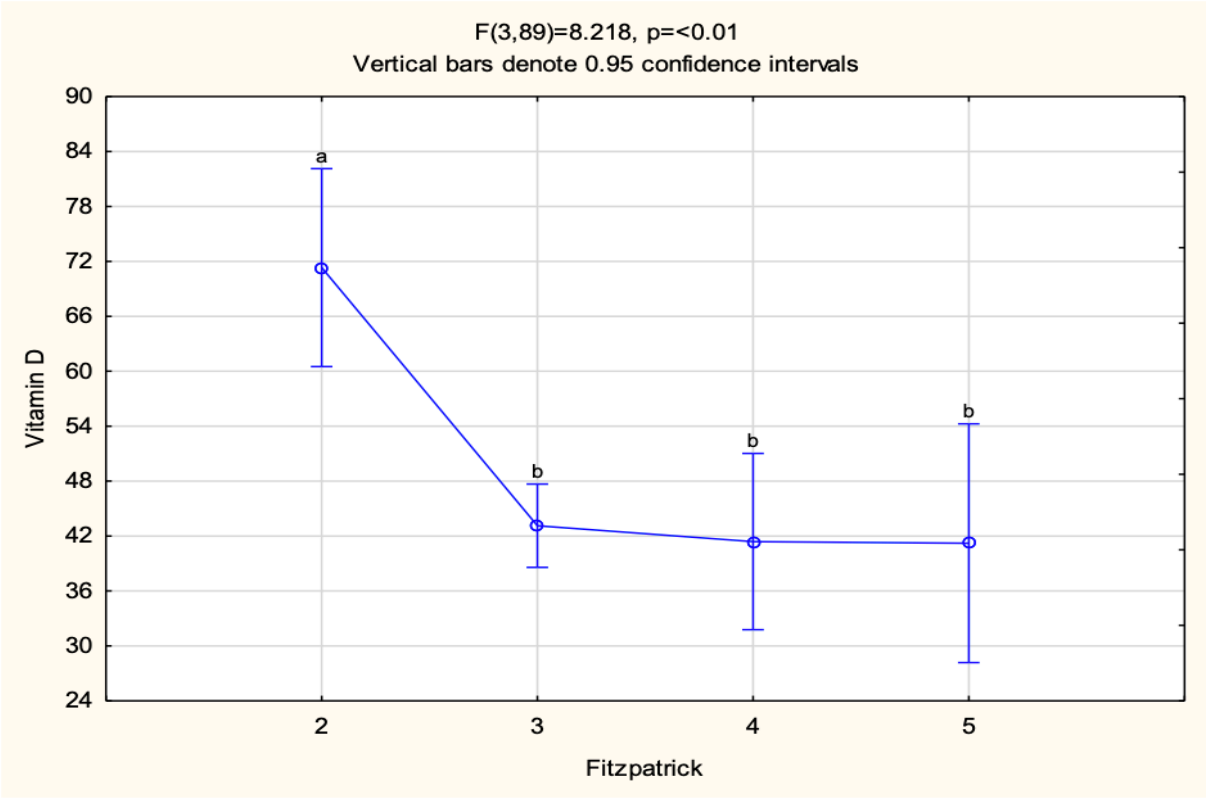

Fig. 13: Change in PASI at follow-up

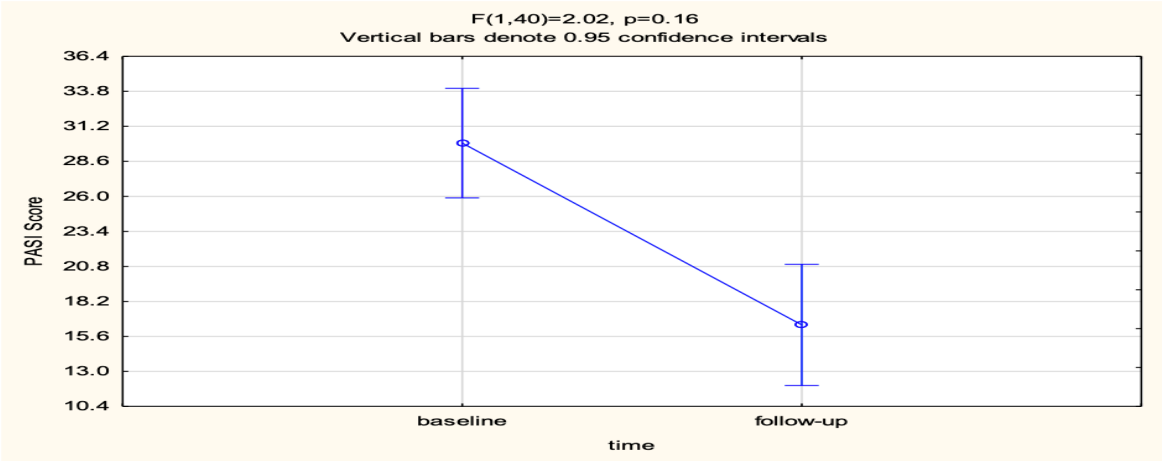

Fig. 14: Vitamin D Levels in patients and controls