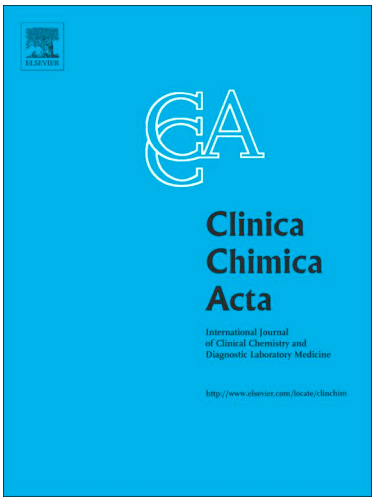

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Effect of vitamin D supplementation on clinical outcome and biochemical profile in South Indian population with vitamin D-deficient chronic urticaria- a randomized double-blind placebo controlled trial

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Abstract

Background: Chronic urticaria (CU) is a debilitating inflammatory skin disease. Prior studies have shown reduced concentrations of vitamin D in CU and there are limited reports of potential beneficial role for vitamin D supplementation in the treatment of subjects with CU. We assessed the effect of vitamin D supplementation in vitamin D deficient CU patients on the clinical outcome and inflammatory markers in South Indian patients with CU.

Methods: This randomized controlled trial involved 120 vitamin-D deficient CU patients. Urticaria activity score (UAS7) and autologous plasma skin test (APST) status was assessed in all cases. CU patients were supplemented with vitamin D with a dose of 60,000 IU fortnightly for a period of 12 weeks and those in the placebo arm received matched placebo. Five milliliters of blood was drawn from all study subjects at baseline and after 12 weeks for assessing inflammatory markers.

Results: We observed a significant reduction in UAS7 scores after 12 weeks in the vitamin D treated group in comparison to that of placebo. We also noted a significant reduction of the inflammatory cytokines in the vitamin D treated group.

Conclusion: Supplementation with vitamin D among patients with vitamin D deficient CU significantly decreases disease severity which is probably mediated through reduction of systemic inflammation.

Keywords: Vitamin D, chronic urticaria, interleukins, autoimmune, C-reactive protein, urticaria activity score, vitamin D binding protein, cytokines.

Introduction

Chronic urticaria (CU) is characterized by the spontaneous occurrence of wheals with or without angioedema lasting for more than six weeks. It has a lifetime prevalence of 1-3% and significantly impairs the quality of life [1]. Recent evidence suggests that CU mainly has a T-helper (Th)0 or mixed T-helper-1 (Th-1)/ T-helper-2 (Th2) profile. In CU, there is an increase in Th17 cell numbers, along with an upregulation of T-helper-17 (Th17) function [2]. This is accompanied by a reduction of regulatory-T (T-reg) cells, along with a defect in their function, which contributes to systemic inflammation [3].

Vitamin D is an immunoregulatory hormone, which skews the Th1/Th2 and Th17/Treg balance from a pro-inflammatory milieu to an anti-inflammatory one [3]. Vitamin D deficiency has been attributed in the etiopathogenesis of several autoimmune diseases like CU and atopic dermatitis. [1] Recent reports suggest that vitamin D is a negative acute phase reactant, reducing with progressive inflammation [4]. Prior studies have reported reduced concentrations of vitamin D in CU [2,5]. There are limited reports of a potential beneficial role for vitamin D supplementation in the treatment of subjects with CU [6-7]. Majority of the studies including one from our group [2,5] are on the estimation of vitamin D status on CU and there is only one study assessing the effect of vitamin D supplementation on outcome of CU, which was also limited by small sample size and subject diversity that was predominately overweight-obese, Caucasian females [6].

Nonetheless, vitamin D represents a relatively safe and inexpensive supplement, with clinical evidence to support its consideration and use as supplement for patients with CU. Given the above, there is an obvious need for clinical studies to understand the mechanisms of the potential clinical benefit of vitamin D. This is of particular relevance because the

Indian population is reported to have a widespread prevalence of varying degrees (50-90%) of vitamin D deficiency [2].

Methods

This was a randomized double-blind placebo controlled trial (RCT), conducted at JIPMER, a tertiary care hospital in South India. Vitamin-D deficient patients admitted with CU were eligible for this trial. JIPMER IEC reviewed and approved the study protocol. Informed written consent was obtained from all participants. This trial is registered on the Clinical Trials Registry-India. The protocol was submitted to the registry on 6 August 2015, before the first patient was enrolled.

Study population: Cases included 120 patients with CU conforming to the inclusion and exclusion criteria, who attended the urticaria clinic in the Department of Skin and Sexually Transmitted Diseases during the study period. The study included consenting patients with CU (presence of continuous or recurrent hives with or without angioedema for greater than 6 weeks duration) with vitamin D deficiency (serum vitamin D < 20 ng/ml) of both gender between the age group 20 and 50 years who attended the Dermatology Clinic during the study period. Patients with acute urticaria/physical urticaria / urticarial vasculitis, hereditary or acquired angioedema, symptoms of vitamin D deficiency such as musculoskeletal pain, fractures, hepatic or renal dysfunction, malignancies / infections, inflammatory cutaneous disorders, pregnant and lactating women were excluded from the study.

Sample size: Sample size was estimated using the formula for comparing two independent means and the estimation of all the study variables. The effect size in the difference in the severity score yielded higher sample size and therefore it was considered. In a previous study by Rorie et al. [6] which we used as reference to compute the sample size, the mean

difference in USS (Urticaria Symptom Severity Score) between the groups was 9 with a S.D of 15.64. In order to detect this difference with 80% power at 5% concentration of significance, the minimum sample size required for the study was estimated as 48 in each group. The sample size was further modified with an expected drop out of 25%. The modified sample size for the study was 60 in each group. This was calculated by using the Software "PS Power and Sample Size Programme version 3".

Outcome measure: The primary outcome was to assess the reduction in urticaria activity score (UAS7) following the administration of vitamin D in comparison to placebo at the end of 12 weeks. Urticaria severity score (USS) was difficult to assess among our patients as it involved many components and hence urticaria activity score was used to assess for response in our study. The urticaria activity score (UAS), used for seven consecutive days (UAS7), is an established and widely accepted tool to prospectively assess CSU activity. The UAS entails the assessment of the only two key urticaria signs and symptoms: wheals and itch. Total score, ranges from 0 (no disease activity) to 42 (maximal disease activity). It was documented by the patient in a diary over 7 days. [7]. The secondary outcomes include the effect of Vitamin D supplementation on the reduction in medication dosage and markers of systemic inflammation in CU.

Study course: Eligible patients with CU and serum vitamin D concentrations < 20 ng/ml were enrolled in the study and were randomized into two groups namely – Experimental group (vitamin D3 supplementation group) and placebo group. Experimental group (vitamin D supplementation group) received 60,000 IU of vitamin D3 fortnightly for a period of 3 months [3D Forte containing Vitamin D_3 60,000 IU] and those in the control

group received similar looking placebo fortnightly for a period of 3 months [Ostocalcium containing calcium 380mg + Vitamin D 200 IU]. Apart from this, patients in both the groups received standard treatment for CU with antihistamine (levocetirizine) ranging from 5mg to 20 mg/day based on the severity of CU, as advised by the dermatologist.

Randomization: Block randomization was done with a block size of 6 using random allocation software, by a person not directly involved with data collection, analysis or interpretation (SS). This randomization list was concealed from the investigators carrying out the study (AM, MM, LC and MR). Both the participants and the investigators were blinded to the intervention. Participants were not stratified in this trial by disease severity. Allocation concealment was done placing individual assignments (folded twice) in serially numbered opaque sealed envelopes by a research assistant not involved in the trial. Double blinding was done by using similar looking drug and placebo.

After enrollment, the following clinical data were obtained – demographic details, duration of the disease, routine diagnostic work up for urticaria, urticaria activity score (UAS7) [7] and medication use (type, frequency, dose). Autoreactivity was assessed by using the autologous plasma skin test (APST), by the dermatologist, during the routine diagnostic work up, as described by Asero et al. [8] after stopping antihistamines for a week. Those with a positive APST were diagnosed to have autoimmune urticaria. Clinical and anthropometric parameters were recorded, according to a predesigned proforma.

Blood collection and analysis: 5 ml blood sample was drawn from the antecubital vein following light application of a tourniquet. Routine parameters like serum urea, creatinine, calcium, phosphate, total protein, albumin and alkaline phosphatase concentrations were estimated in a clinical chemistry autoanalyzer (Beckman Coulter AU

680) and the concentrations of thyroid stimulating hormone (TSH) were assessed by chemiluminescence immunoassay (Advia Centaur, Siemens). TSH was measured to rule out autoimmune association with urticaria. Study parameters like total 25-hydroxy vitamin D were estimated using by chemiluminescence immunoassay (Advia Centaur, Siemens); VDBP (Bioassay Technology Laboratory, China); hs-CRP (Diagnostics Biochem Canada Inc, Canada) and concentrations of cytokines such as TGF-β using (RayBiotech, United States of America); IL-17 (Diaclone, France); IL-6 (Diaclone, France) were estimated by enzyme-linked immune-sorbent assay (ELISA).

Follow-up:

The patients were followed up at 12 weeks for recording urticaria activity score, medication usage and monitoring for side effects. The patients could not be followed up at 6 weeks for assessing disease severity due to their non-compliance. This was a limitation of the study. The collection of blood samples was done twice, at baseline and at 12 weeks for estimation of 25-hydroxy vitamin D, VDBP, hs-CRP and immunomodulatory cytokines: TGF-β, IL-17, IL-6. The effect of vitamin D supplementation in these patients were studied in terms of urticaria activity score and a change in levocetirizine requirement/dose. As a post-trial responsibility, after completion of the trial, the placebo group received 60,000 IU of vitamin D fortnightly for a period of 3 months free of cost.

Statistical Analysis:

Statistical analysis was performed using IBM SPSS statistics version 19 for Windows and GraphPad Prism version 6 for Windows. Both descriptive and inferential statistics were used to analyze the data. Baseline characteristics of the patients with CU were analyzed by descriptive statistics. The normality of continuous data (data related to variables such as age

of onset of urticaria, duration of disease, UAS7 score, hs-CRP, IL-17, IL-6, TGF-β, VDBP) was assessed by Kolmogrov-Smirnov test. The normally distributed data were described as mean ± standard deviation and median with inter-quartile range was used for non-Gaussian data. Unpaired data were compared by unpaired Student's t-test or Mann Whitney "U" test, as appropriate. Paired data were compared by paired Student's t-test or Wilcoxon's signed rank test, as appropriate. Analysis of covariance (ANCOVA) was performed to adjust for any baseline imbalances. Analysis was carried out at 5% concentration of significance and a $p<0.05$ was considered as statistically significant.

Results

One hundred and twenty five participants with CU were assessed for eligibility for this RCT. One hundred and twenty CU patients conforming to the inclusion and exclusion criteria were recruited. Out of the 120 participants, 25 were males and 95 were females (Fig. 1) The baseline clinical characteristics were comparable between the placebo group and the vitamin D supplementation group except for UAS7 and levocetirizine dose (Table 1). The median UAS7 was significantly greater in the Vitamin D group whereas the median levocetirizine dose was significantly higher in the placebo group (Tables 1 and 2). The concentrations of inflammatory cytokines were comparable in the groups except for IL-6 which was significantly higher in the vitamin D supplementation group. The 25-OH vitamin D concentrations and VDBP concentrations were not significantly different in the two groups (Table 3).

Across groups (at the end of 12 weeks): At the end of 12 weeks we observed a significant reduction in UAS7 scores and medication dosage in the vitamin D treated group in comparison to that of placebo (Tables 2 and 3). We also noted a significant reduction in all

the cytokines in the vitamin D supplementation group compared to that of placebo. The concentrations of 25-OH vitamin D and VDBP were significantly elevated in the vitamin D group in comparison to placebo at the end of 12 weeks.

Within groups (baseline and after 12 weeks): When we compared before and after treatment we noted a significant difference in the UAS7 score as well as medication dosage in both the groups. We also noted a significant reduction of all the inflammatory cytokines in the vitamin D treated group. However, in the placebo group there was no significant change in the cytokine concentrations. We observed a significant increase in 25-OH vitamin D and VDBP in the vitamin D treated group. However there was no significant change in 25-OH vitamin D and VDBP in the placebo group (Tables 2 and 3).

A one-way ANCOVA was conducted to compare the effectiveness of vitamin D supplementation on UAS7 at 12 weeks while adjusting for baseline UAS7 and medication usage. Levene's test ($p=0.57$) and normality checks ($p=0.06$) were carried out and the assumptions were met. There was a significant difference in 12 weeks UAS7 between the groups. The greatest influence on UAS7 at 12 weeks was the groups (sum of squares of 3691.775, Df=1 and $p<0.001$) followed by baseline UAS7 (sum of squares1572.35, Df=1 and $p<0.001$). The estimated marginal means in the vitamin D group was 16.84 (95% CI 15.47-18.22) and placebo group 29.11(95% CI 27.74 -30.49). The adjusted R square of the model was 0.56.

Discussion

We observed a larger reduction in UAS7 scores after 12 weeks in the vitamin D treated group in comparison to that of placebo. We also noted a significant reduction of the

inflammatory cytokines in the vitamin D treated group. There was a significant increase in the 25-OH vitamin D concentrations and VDBP in the vitamin D group, after 12 weeks. Though there was a basal imbalance in the UAS7 between the groups we noted that the scores were significantly higher in the vitamin D group. We found the difference to be significant in the ANCOVA model after adjusting for the basal imbalance in UAS7 score & and medication dose. Among the cytokines the concentrations of IL-6 were significantly elevated in the vitamin D group, which probably reflects the higher UAS7 score at baseline. When we compared within the 2 groups we noted a significant decrease in UAS7 and medication dose in both the groups. However, we observed a significant decrease in the inflammatory cytokines and increase in 25-OH vitamin D and VDBP only in the vitamin D treated group.

It has been reported that high-dose vitamin D3 supplementation might lessen the symptoms of CU which is in concordance with our findings [6]. In a previous study by Goetz et al. [9], a combination of symptoms such as pruritus, rash, and urticaria or angioedema with deficient vitamin D status was reported. Following vitamin D supplementation, the above symptoms resolved in 70% of CU patients with cutaneous manifestations and coincidental Vitamin D deficiency [9].

Vitamin D supplementation could lower the medication burden in CU [9]. We observed a significant reduction in the number of intake of levocetirizine tablets, in CU patients after vitamin D therapy. Contrary to our findings, Rorie et al. [6] did not find any reduction in the medication burden with vitamin D supplementation in CU.

We observed a reduction in the concentrations of inflammatory markers such as IL-17, IL-6, hs-CRP and TGF-β, whereas the concentrations of VDBP were found to be elevated. VDBP concentrations showed a significant rise after vitamin D supplementation, however the exact mechanism behind this rise is still unclear. The role played by vitamin D

as an immunomodulator is reflected by the reduction in the concentrations of the pro-inflammatory markers hs-CRP, TGF-β, IL-6 and IL-17, over a time period of 12 weeks. Vitamin D alters the Th1/Th2 and Th17/T-reg balance, causing immunosuppression and promotes an anti-inflammatory state in CU. Vitamin D contributes to the conversion of Tregs from CD4+ T cells, which have been shown to play a function in the quelling of pro-allergic mechanisms [10-12].

The active form of vitamin D downregulates the gene expression of Th1 cells, besides up-regulating the gene expression of Th2 cells [12-14]. Production of 1, 25 di-hydroxy vitamin D is affected adversely, owing to low-circulating 25-hydroxy vitamin D concentrations. This results in the suppression of VDR-dependent innate immune response. [13] There is contemporary evidence which supports the role for vitamin D-inducing T-regs in alleviating the inflammation in CU and this mechanism might be significant in understanding the role of vitamin D in CU. [12]

In our work, we did not encounter any side effects with the patients when a fortnightly dose of 60,000 IU vitamin D was consumed. We supplemented vitamin D_3 instead of vitamin D_2, as Vitamin D_3 has been studied extensively and found to be more potent in raising and maintaining steady-state 25-hydroxy vitamin D concentrations than Vitamin D_2 [15-17]. Our placebo-controlled trial was designed for a duration of 12 weeks in order to allow time for the appropriate attainment of a steady state 25-hydroxy vitamin D while using the recommended safe dosing regimen of 60,000 IU fortnightly.

This study was not without limitations. We failed to stratify the allocation of patients based on disease severity. We did estimate total 25-hydroxy vitamin D concentrations, but the assay could not differentiate between D_2 and D_3 concentrations. These were some limitations of our work. We could not document the change in clinical outcome and biochemical profile during follow-up at 6 weeks due to non-compliance from the participants.

Conclusion

In patients with vitamin D-deficient CU, there is significantly increased systemic inflammation, which showed significant positive correlation with disease severity. Supplementation with vitamin D reduced systemic inflammation, disease severity and medication intake, thereby improving the quality of life of patients with CU.

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Abstract

Background: Chronic urticaria (CU) is a debilitating inflammatory skin disease. Prior studies have shown reduced levels of vitamin D in CU and there are limited reports of potential beneficial role for vitamin D supplementation in the treatment of subjects with CU.

Aims & objectives: We undertook this study to assess the effect of vitamin D supplementation in vitamin D deficient CU patients on the clinical outcome and inflammatory markers in South Indian patients with CU.

Methodology: This randomized controlled trial involved 120 vitamin-D deficient CU patients. Urticaria activity score (UAS7) and autologous plasma skin test(APST) status was assessed in all cases. CU patients were supplemented with vitamin D with a dose of 60,000 IU fortnightly for a period of 12 weeks and those in the placebo arm received matched placebo. Five ml blood sample was drawn from all study subjects at baseline and after 12 weeks for assessing inflammatory markers.

Results: We observed a significant reduction in UAS7 scores after 12 weeks in the vitamin D treated group in comparison to that of placebo. We also noted a significant reduction of the inflammatory cytokines in the vitamin D treated group.

Conclusion: Supplementation with vitamin D among patients with vitamin D deficient CU significantly decreases disease severity which is probably mediated through reduction of systemic inflammation.

Keywords:

Vitamin D, chronic urticaria, interleukins, autoimmune, C-reactive protein, urticaria activity score, vitamin D binding protein, cytokines.

Author contributions:

Archana M: Investigation, Formal Analysis, Writing-Original draft preparation, L Chandrashekar: Conceptualization, Methodology, Resources, Supervision, Visualization, Formal Analysis, Data curation, Writing-Original draft preparation, Writing-Reviewing and Editing. M Rajappa: Conceptualization, Investigation, Resources, Formal Analysis, Validation, Funding acquisition, Project Administration, Supervision, Writing-Original draft preparation, Writing-Reviewing and Editing. M Munisamy: Investigation, Resources, Formal Analysis, Writing-Reviewing and Editing, JP Sahoo: Investigation, Resources, Writing-Reviewing and Editing, Sandhiya S: Investigation, Writing-Reviewing and Editing.

TABLE 1: Comparison of baseline characteristics between placebo group and vitamin D supplementation group

Parameter	Placebo Group (n=60) Mean ± SD/ Median (IQR)	Vitamin D Supplementation Group (n=60) Mean ± SD/ Median (IQR)	p value
Age (years)	36.71±11.01	38.80±12.54	0.34*
Gender (M: F)	13:47	12:48	-
BMI (kg/m^2)	23.4±4.82	23.7±5.67	0.75#
Disease duration	17.7±15.23	17.26±11.94	0.50#
UAS7	35(28-35)	35(35-42)	<0.0001#
Number of tablets of levocetirizine (5 mg)	3.6±0.58	3.4±0.49	0.01#
APST positive: APST negative. Number (%)	38(63.33%): 22 (36.67%)	43(71.67%): 17 (28.33%)	0.33$

* Independent Student's *t*-test, # Mann Whitney U-test $ χ^2 test

TABLE 2: Comparison of disease severity and medication usage at baseline and at 12 weeks in both the groups

Parameter	**Placebo Group (n=60) Median(IQR)**	**Vitamin D Supplementation Group (n=60) Median(IQR)**	**Across group p value #**
UAS 7 Baseline	35(28-35)	35(35-42)	<0.0001
UAS7 12 weeks	28(21-35)	21(14-21)	<0.0001
*Within group p value	P<0.0001	P<0.0001	
Number of tablets of 5 mg levocetirizine baseline	4(3-4)	3(3-4)	<0.0001
Number of tablets of 5 mg levocetirizine 12 weeks	4(4-4)	2(2-3)	<0.0001
*Within group p value	P=0.0026	P<0.0001	

Wilcoxon rank sum test *Wilcoxon signed rank test

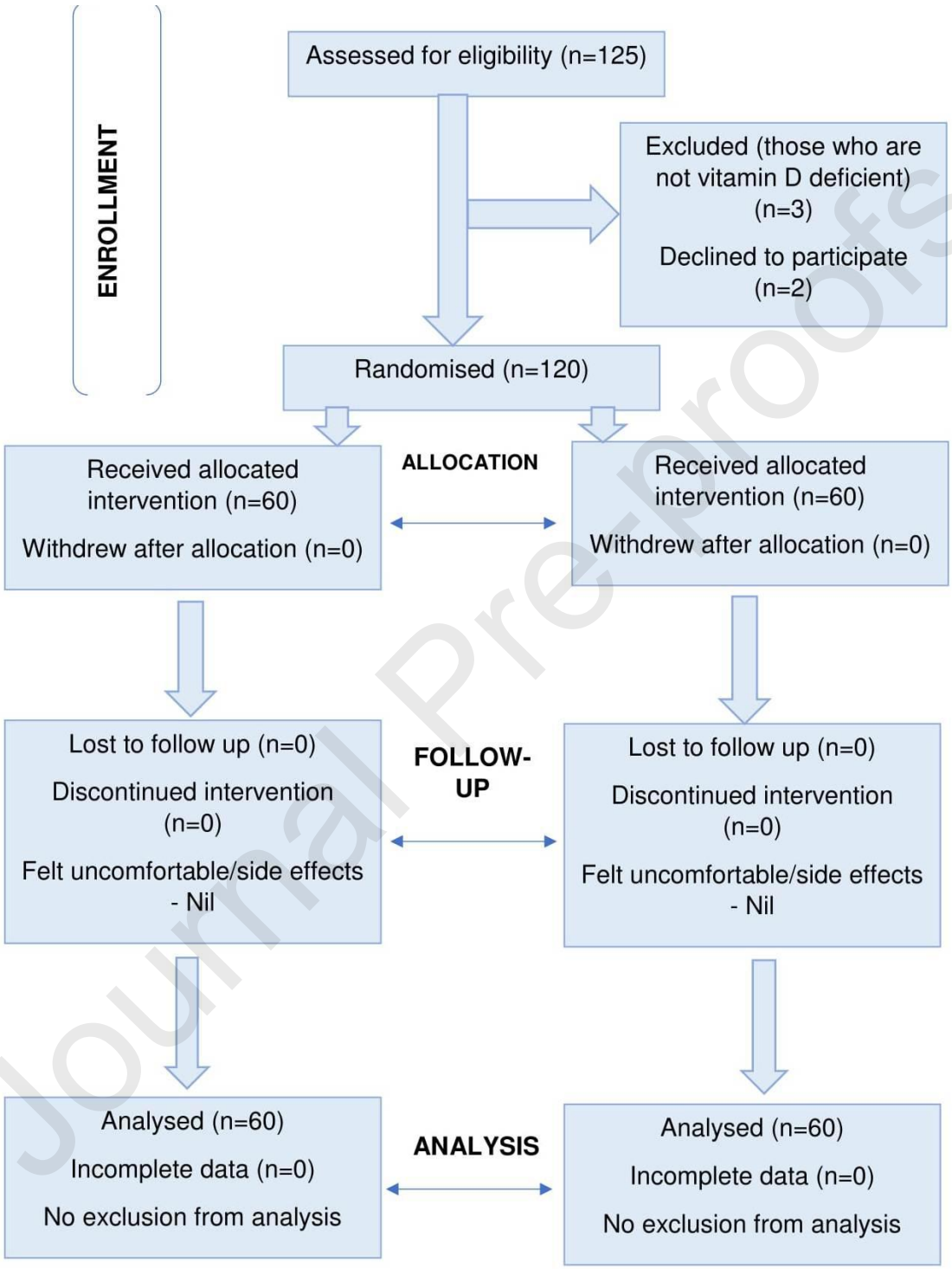
TABLE 3: Comparison of inflammatory markers at baseline and at 12 weeks in both the groups

	Placebo group n=60 Median(IQR)	**Vitamin D supplementation group Median(IQR) n=60**	**Across group p value[#]**
Baseline hs-CRP (mg/l)	16.88(13.7-18.9)	17.7(14.06-19.49)	NS
Follow up hs-CRP (mg/l)	15.97(12.24-18.63)	5.91(3.9-8.96)	<0.0001
***Within group p value**	*P=NS*	*P<0.0001*	
Baseline IL-6 (pg/ml)	18.97(14.16-27.47)	26.46(23-35.76)	<0.0001
Follow up IL-6 (pg/ml)	18.76(12.52-19.16)	16.47(12.52-19.16)	0.004
***Within group p value**	*P=NS*	*P<0.0001*	
Baseline IL-17 (pg/ml)	39.52(33.81-46.71)	43.2(29.2-58.22)	NS
Follow up IL-17 (pg/mL)	40.43(35.89-48.13)	18.25(11.81-22.61)	<0.0001
***Within group p value**	*P=NS*	*P<0.0001*	
Baseline TGF-β (ng/mL)	13.73(12.44-21.68)	18.46(13.74-24.41)	0.01
Follow up TGF-β (ng/mL)	16.11(13.74-20.65)	2.63(1.38-9.71)	<0.0001
***Within group p value**	*P=NS*	*P<0.0001*	
Baseline VDBP (µg/mL)	292.19(247.1-445.48)	323.95(247.96-364.87)	NS
Follow up VDBP (µg/mL)	300.5(243.64-446.79)	439.59(358.39-506.91)	<0.0001
***Within group p value**	*P=NS*	*P<0.0001*	
Baseline Vitamin D	13.7(12.02-15.76)	14.16(12.47-16.07)	NS
Follow up Vitamin D	14.33(12.35-16.7)	29.49(23.1-34.7)	<0.0001
***Within group p value**	*P=NS*	*P<0.0001*	

[#] Wilcoxon rank sum test [*]Wilcoxon signed rank test

Legend for Figure 1: Consort Diagram for the RCT

Figure 1: Consort Diagram of the RCT



- Chronic urticaria (CU) is a debilitating inflammatory skin disease.
- This study assessed the effect of vitamin D supplementation on outcomes in CU.
- Decrease in UAS7 and medication usage was seen in the intervention group, compared with placebo.
- Vitamin D supplementation led to decreased systemic inflammation, hence improving disease course and quality of life in CU.