Background: Asthma is a common chronic disorder of the airways that is complex and characterized by variable and recurring symptoms. It is characterized by the occurrence of bronchial hyper responsiveness, airflow obstruction and an underlying inflammation. Several studies have proposed the association of vitamin D with increased incidence of asthma symptoms. Aims and Objectives: This study aimed at determining the serum vitamin D status and biochemical parameters among asthmatic children in Sokoto-Nigeria. Materials and Methods: The study population consisted of 120 subjects including 80 asthmatic children and 40 age-and sex-matched apparently healthy controls from two tertiary Hospitals in Sokoto Metropolis. Serum vitamin D, calcium, parathyroid hormone (PTH), immunoglobulin E (IgE) and magnesium were measured using standard techniques. Results: Mean serum vitamin D and magnesium were significantly (p < 0.05) lower, while serum PTH and IgE were significantly higher (p<0.001) in asthmatic children than in controls. Vitamin D insufficiency was found to be prevalent among the asthmatic children. There was no significant relationship between the low level of vitamin D and asthma severity. Conclusion: This study observed that, hypovitaminosis D and hypomagnesaemia occurred among asthmatic children, and asthma was more prone in the younger children than in their older counterpart. It is recommended that in order to avoid or limit the rate of children having asthmatic episodes, vitamin D and magnesium supplements should be used in the management of asthmatic children.

Key words: Vitamin D status; Asthma; Children; Sokoto-Nigeria
in children. A greater burden of asthma and allergic diseases occur during childhood, at which time the rapidly rising rates of diseases are most evident in the population. This suggests that Vitamin D plays an important role in the protection against asthma and allergic diseases. However, the role of Vitamin D and other biochemical parameters in the pathogenesis of asthma has not been ascertained among asthmatic children living in Sokoto-Nigeria. The aim of the study was to assess the serum vitamin D status and some biochemical parameters among asthmatic children in Sokoto, North-Western Nigeria.

MATERIALS AND METHODS

A cross-sectional descriptive study that was carried out from October, 2017 to January 2018. The study population consisted of 120 subjects that comprised of 80 confirmed cases of asthma and 40 age-and sex-matched apparently healthy controls selected from Sokoto Metropolis. The study subjects were recruited at the Paediatric Pulmonology and Allergy Clinics of Usman Danfodiyo University Teaching Hospital (UDUTH) and the Paediatric Ward of Specialist Hospital, Sokoto, Nigeria. All asthmatic children (aged 1 to 15 years) were evaluated by the Consultant Chest Physicians at the Paediatric Pulmonology and Allergy Clinics of Usman Danfodiyo University Teaching Hospital (UDUTH) and the Paediatric Ward of Specialist Hospital, Sokoto, Nigeria. During enrolment of the study subjects, the structured interviewer-administered questionnaires were used to obtain information on the socio-demographic characteristics of the study subjects. Asthmatic children were placed on their regular treatment with oral or inhaled corticosteroids according to GINA classification. Subjects were ineligible if they presented with other chronic respiratory diseases or a history of consumption of any vitamin D supplement or drugs that can modulate the serum concentration of vitamin D. Study participants with no history of allergic diseases and negative atopic family history were enrolled as age-and gender-matched apparently healthy controls.

The children were weighed with minimum clothing to the nearest 0.1kg using a Salter scale or beam balance. Supine lengths of the children were measured to the nearest 0.1cm against a flat horizontal surface using a standard Stadiometer and body mass index was calculated using the following expression: BMI (kg/m²) = Body weight (kg)/height (m²).

About five milliliters (5ml) of whole blood was collected from each subject using standard techniques of venipuncture and transferred into a plain blood bottle, allowed to properly retract and the serum was separated after centrifugation at 3000g for 15 minutes in a closed container. The sera specimens were stored at -20°C and later analyzed. Serum vitamin D and parathyroid hormone were estimated by enzyme linked immunosorbent assay (ELISA). Total serum immunoglobulin E (IgE) by the method of. Serum calcium and phosphate were estimated by spectrophotometric method as described, while serum magnesium was estimated by the end point calorimetric method using xylidyl blue (Cobas 6000, Roche Diagnostics Limited Switzerland).

Statistical analysis

Data generated was analyzed using Microsoft Excel broadsheet and INSTAT® (Graph Pad Software Inc., La Jolla, CA, USA). Results were expressed as mean ± SEM. Pair wise comparison was made using student t-test. Chi (χ) - square test was used to assess the categorical variables between the groups. Results were also delineated according to severity of asthma and multiple comparisons made using analysis of variance (ANOVA). A P-value of equal to or less than 0.05 (P ≤ 0.05) was considered as significant.

RESULTS

The socio-demographic characteristics of the study population were shown in Table 1. The results indicated that the mean values for age, weight and BMI are similar between the asthmatic and control children (P = 0.709, P = 0.205, P = 0.208 respectively). The result indicated significant association between age group and being asthmatic. The result also revealed that children at the age

| Characteristics | Patients (n=80) | Controls (n=40) | P values |
|-----------------|----------------|----------------|----------|
| Age             | 5.81±0.39      | 6.05±0.45      | 0.709    |
| Age group       |                |                |          |
| (1-5) years     | 46 (57.5%)     | 19 (47.5%)     | 0.017*   |
| (6-10) years    | 22 (27.5%)     | 20 (50.0%)     |          |
| (11-15) years   | 12 (15.0%)     | 1 (2.5%)       |          |
| Height (m)      | 1.10±0.03      | 1.20±0.04      | 0.031*   |
| Weight (kg)     | 19.79±0.96     | 21.98±1.47     | 0.205    |
| BMI (Kg/m²)     | 18.47±1.44     | 15.69±1.15     | 0.208    |
| Gender          |                |                |          |
| Male            | 45 (56.2%)     | 22 (55%)       | 0.897    |
| Female          | 45 (43.8)      | 18 (45%)       |          |
| Ethnicity       |                |                |          |
| Hausa           | 63 (78.7%)     | 28 (70%)       | 0.520    |
| Fulani          | 7 (8.8%)       | 6 (15%)        |          |
| Igbo            | 2 (2.5%)       | 0 (0%)         |          |
| Yoruba          | 4 (5.0%)       | 4 (10%)        |          |
| Others          | 4 (5.0%)       | 2 (5%)         |          |

Values are in mean ± SEM, n and %. SEM = Standard error of mean, % = percentage and n = number of subjects. Values differ significantly at *P<0.05 using χ square, Values differ significantly at **P<0.05 using Student t-test.
group of 1 to 5 years are more prone to asthma, followed by those at the age group of 6 to 10 years and then 11 to 15 years. There were no significant associations between gender, ethnicity and being asthmatic. Gender wise, the predominantly affected subjects in this study are Hausa by tribe.

Figure 1 shows the serum vitamin D and biochemical analytes among asthmatic and control children. The mean serum levels of vitamin D and magnesium were significantly (P = 0.003 and P = 0.001 respectively) lower in asthmatic children compared with controls while, the mean serum levels of PTH and IgE were significantly (P = 0.001 and P = 0.001 respectively) higher in the asthmatic children compared with controls. However, there were no any significant (P = 0.720 and P = 0.663 respectively) differences in the serum Ca$^{2+}$ and PO$_4^{2-}$ levels between the asthmatic and control children.

The results of serum vitamin D levels based on different vitamin D deficiency groups among asthmatic and control children are shown in Figure 2. The mean serum vitamin D = 20 ng/ml (considered as deficient) was significantly (P = 0.002) higher in controls compared with the asthmatic children. However, there was no significant (P = 0.474 and P = 0.127 respectively) differences between serum levels of vitamin D, 20-30 ng/ml (considered as insufficient) and those > 30 ng/ml (considered as sufficient). The comparison of serum levels of vitamin D and biochemical parameters in the different grades of asthma severity among the asthmatic children and controls (Table 2) indicated no significant differences between the levels of the biochemical parameters in asthmatic children and controls. Table 3 shows the association between vitamin D deficiency group and asthma severity among asthmatic children based on the GINA classification. The result did not reveal any significant (P = 0.136) association between the vitamin D deficiency and asthma severity among the subjects studied.

**DISCUSSION**

In this study, the result indicated that asthma was more prone among children between the age of 1 – 5 years,
followed by those between the age of 6 - 10, years and then 11 - 15 years. Previous studies have reported the relationship between asthma and decreased serum levels of vitamin D in different populations.15-17

Table 2: Comparison of serum Vitamin D and biochemical analytes in different grades of asthma severity among asthmatic children

| Parameter                      | Mild intermittent (n=57) | Mild persistent (n=20) | Moderate persistent (n=2) | Severe persistent (n=1) | P values |
|--------------------------------|--------------------------|------------------------|--------------------------|-------------------------|----------|
| Vit D (ng/ml)                  | 22.07±1.25               | 19.46±1.70             | 33.70±1.37               | 12.25±0.00              | 0.118    |
| PTH (pg/ml)                    | 53.50±2.17               | 55.51±4.20             | 57.35±0.91               | 27.33±0.00              | 0.437    |
| IgE (µg/ml)                    | 1.35±0.10                | 1.36±0.19              | 2.45±1.17                | 1.08±0.00               | 0.280    |
| Ca²⁺ (mmol/L)                  | 2.27±0.03                | 2.30±0.05              | 2.35±0.05                | 2.30±0.00               | 0.940    |
| PO₄²⁻ (mmol/L)                 | 2.10±0.09                | 2.03±0.09              | 2.00±0.10                | 2.20±0.00               | 0.968    |
| Mg²⁺ (mmol/L)                  | 0.69±0.03                | 0.62±0.03              | 0.71±0.05                | 0.66±0.00               | 0.567    |

Values expressed as mean±SEM, n=number of subjects. Vit D=vitamin D, PTH=parathyroid hormone, IgE=immunoglobulin E, Ca²⁺=calcium, PO₄²⁻=phosphate, Mg²⁺=magnesium. P>0.05, not significantly different when compared with controls

Table 3: Association between Vitamin D deficiency grouping and asthma severity among asthmatic children based on the GINA classification

| Vitamin D deficiency grouping  | Mild Intermittent (n=57) | Mild Persistent (n=20) | Moderate Persistent (n=2) | Severe Persistent (n=1) | P values |
|--------------------------------|--------------------------|------------------------|--------------------------|-------------------------|----------|
| Deficiency (< 20 ng/ml)        | 29 (50.9%)               | 12 (60.0%)             | 0 (0.0%)                 | 1 (100%)                | 0.136    |
| Insufficient (20-30 ng/ml)     | 14 (24.6%)               | 6 (30.0%)              | 0 (0.0%)                 | 0 (0.0%)                |          |
| Sufficient (>30ng/ml)          | 14 (24.6%)               | 2 (10.0%)              | 2 (100%)                 | 0 (0.0%)                |          |

Values expressed as number of subjects with percentage in parenthesis
In this study, vitamin D insufficiency was found in both the control and asthmatic children, which shows that the incidence of vitamin D insufficiency is surprisingly high in the general population. This finding was in agreement with the previous studies of.\(^8\) \(^{17,18}\) Whereas Sokoto State being a tropical area with a hot semi-arid climate, vitamin D insufficiency was supposed to be uncommon due to the high ultra-violet radiation that penetrates the skin of the children when exposed to sunlight. The observed incidence of vitamin D insufficiency in the study population could be as a result of prolonged breastfeeding without vitamin D supplementation, maternal vitamin D deficiency or poor dietary intake. A valid observation from this study was the high incidence of vitamin D insufficiency. This may be due to typical dark skin complexion that majority of the study population have. This agreed with the observation by.\(^{19}\) that most dark-skinned people have low serum level of vitamin D due to their high melanin content that hinder the proper metabolism of vitamin D.\(^{20}\)

The low serum vitamin D levels observed in asthmatic children compared with their healthy counterpart was in agreement with previous studies.\(^8\) \(^{20,22}\) This may be the result of short-term exposure to sun light and inadequate access to food and supplements.\(^{23}\)

The findings in this study of increased serum levels of PTH among the asthmatic children was consistent with the previous studies.\(^{21,24,25}\) The increased serum PTH may be explained by the compensatory mechanism of the body to maintain normal serum levels of vitamin D and calcium. PTH increases with increase in asthma severity in order to stimulate the release of more vitamin D to combat the severity of asthma to a bearable minimum.\(^{25}\)

The finding of significantly increased serum levels of IgE among the asthmatic children compared with the controls also pinpoint the role of atopy in the expression of asthma in children in the study area. This finding is similar to the findings reported by previous researchers.\(^{23,26-29}\) This could be as a result of the well-known fact that IgE play a central role in the pathophysiology of allergic disorders such as asthma.\(^{28}\) IgE is major and first parameter that get elevated as soon as an activated T-memory cell is sensitized by allergens to cause an allergic airway inflammation. The mechanism involves the inducement of CD\(_4\) T-helper cell to produce type 2 (Th\(_2\)) cytokines such as interleukin 4 (IL-4), which then activate B cell isotype switching to IgE. Also CD8\(^+\) T cell may also lead to IgE class by switching via IL-13 rather than IL-4. All these events may lead to eosinophilic bronchitis, mucus hyper secretion and bronchial smooth muscle contraction as well as asthma.\(^{30}\)

There was significant decrease of serum levels of magnesium among asthmatic children compared with the control subjects. The result of this study agreed with the observation of previous studies.\(^{31,32}\) Low serum level of Mg\(^{2+}\) in asthmatic children may be related to either low Mg\(^{2+}\) intake or increase urinary loss of Mg\(^{2+}\) as a side effect of other therapy used for the treatment of asthma such as β\(_2\) agonist, corticosteroid and theophylline.\(^{33}\)

Magnesium ion has an inhibitory action on smooth muscle contraction, histamine release from mast cells and acetylcholine release from cholinergic nerve terminals. Magnesium has been shown to relax bronchial smooth muscles and influence the function of respiratory muscle. As such hypomagnesaemia have been associated with diminished respiratory muscles power which can aggravate the severity of asthma.\(^{32}\) Magnesium ion also play a role in the activation of vitamin D in the kidney, as such diminished level of this ion can lead to a decrease production of vitamin D which can also increase the severity of asthma.\(^{34}\)

The finding of high prevalence of vitamin D deficiency observed among the asthmatic children was in agreement with the previous studies of\(^{18,29}\) that reported vitamin D deficiency among asthmatic children. The increased prevalence hypovitaminosis D could be due to the fact that families whose children happen to be asthmatic wanted to keep their children at home always for fear of an asthma attack, if they went outdoor or engaged in any physical activities.\(^{35}\)

In this study, vitamin D deficiency, insufficiency or sufficiency was not associated with the severity of asthma, as was earlier reported by\(^{18}\) But this contrasted with the studies of\(^{36}\) that reported that vitamin D deficiency, insufficiency or sufficiency were associated with asthma severity.

This finding could be due to the fact that vitamin D deficiency or insufficiency has a down regulatory effect on glucocorticoid pathways which led to the need for increased steroid dosing which may itself increase asthma severity.

In addition, some studies have shown that respiratory infections such as rhinoviruses enhances allergic airway inflammation, reduce pulmonary function and increase asthma exacerbation severity, especially in asthmatic patient having vitamin D deficiency or insufficiency.\(^{37,38}\)

**CONCLUSION**

We observed that hypovitaminosis D and hypomagnesaemia occurred among asthmatic children, and asthma was more prone in the younger children than in their older

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counterpart. It is recommended that in order to avoid or limit the rate of children having asthmatic episodes, vitamin D and magnesium supplements should be included in the management of asthmatic children.

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Conflict of interest
None declared.

Ethics approval and consent to participate
The study protocol was approved by the Health Research and Ethics Committees of Usmanu Danfodiyo University Teaching Hospital and Specialist Hospitals, Sokoto (UDUTH/HREC, 2017/No. 635 and SHS/SUB 133/ Vol. I respectively) and written informed consent obtained from the children’s parents.

Consent for publication
All authors have consented for publication of the manuscript.

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Author’s Contribution:
MHY- Concept and design of the study, statistically analyzed, interpreted data and critical revision of the manuscript; MD- Concept and design of the study, reviewed the literature and preparation of first draft of the manuscript; MAS- Critical review of the study and revision of the manuscript; JNM- Concept and design of the study, helped in recruiting the patients and critical revision of the manuscript; GB- Helped in recruiting the patients, data collection and blood samples collection; YI- Helped in blood samples collection and analysis; BS- Data gathering and assisted in statistical analysis.

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