Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2014; 2(4E):1455-1457 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com **DOI:** 10.36347/sjams.2014.v02i04.063

Research Article

Correlation between Vitamin C Deficiency and Hydroxyproline in Palestinian Children of Northern Part of Palestine

Ismail M. Masri
Arab American University, Dental College, Basic Science Department, P.O.Box 240, Jenin, Palestine

***Corresponding author**
Ismail M.Masri
Email: ismailmmasri@yahoo.com

Abstract: Vitamin C is the most mundane water soluble vitamin been found in citrus juice. Vitamin C participation in collagen synthesis is well established by hydroxylation of the amino acids, proline and lysine in the preprocollagen. This study designed to evluate the utility of the urine hydroxyproline excretion as marker for vitamin C defiency. Two groups of sample were analysed. 1st group consist of 25 male with age range 4-13 years, 2nd group consists 25 female with same age. The study was carried from the out patients clinic attended department of Orthdontics and Pediatric Dentistry at the Arab-American University, Jenin, Palestine.
Keywords: Hydroxyproline, Vitamin C, Scurvy, Chloramine T Reagent, Urine

INTRODUCTION

One of the most common vitamin C deficiencies is scurvy, ulceration of the gums, anemia and hemorrhage [1]. Since we live in a modern world full of artificial flavors and coloring school children in this age have gravitated for sweet juice with different flavors, but without any nourishment, such as vitamin C. Many of the clinical problems associated with its deficiency skeletal, infantile scurvy [2], the lesions of the gingiva [3-4], Impairment of wound rejuvenating and faulty of bon rejuvenating fractures [2, 5, 6] all are cognate to alteration in collagen synthesis. Vitamin C is one of the anabolic collagen. Hydroxyproline, is a common nonproteinogenic amino acid. It is found only in collagen and elastin mammals, derived from post-translational hydroxylation of proline, representing about 13% of the amino acid content of the various forms of collagen [1].

Vitamin C Deficiency, leads to hypo-hydroxyproline, to scurvy. Hydroxproline found in urine, from collagen metabolism in three forms: (a) Free which mostly reabsorbed by the kidney tubules, (b) Small and that represent 90% of urine hydroxyproline excretion and (c) Formed from newly synthesized collagen [7]. A random urine sample early morning (spot), gluten free diet is a sensitive marker.

MATERIALS AND METHODS

The study was conducted in the Physiology laboratory, College of Dentistry, at The Arab - American University. All patients advised to have free collagen free diet, and all types of juice-based beverages.

Determination of Urinary Hydroxyproline

It was done by hydroxyproline Colorimetric Assay Kit, (Catalog # K555-100; 100 assays; store at +4°C), made by Biovision [9]. Assay was performed according to the kit protocol in 10-200ppm range.

Reagent Preparation and Storage Conditions

Chloramine T Reagent

For each well to be analyzed, added 6 µl of Chloramine T. Concentrated to 94 µl of Oxidation Buffer and mixed well.

DMAB Reagent

For each well to be analyzed, added 50 µl of the DMAB. Concentrated to 50 µl of perchloric acid/isopropanol solution and mixed well. Kept on ice and protected from light.

Note: The reagent concentrates were stable as supplied. Once the concentrates had been diluted to working concentration, they were only good for 2-3 hours, so only made as much reagent as necessary for the number of samples and standards to be quantified.

Hydroxyproline Assay Protocol [8]

Urine samples hydrolyzed with equal volumes of concentrated HCl (~12 N; i.e. 100 µl Urine + 100 µl HCl) in a pressure-tight, Teflon capped vial at 120°C for 3 hrs. Clarified urine samples with activated charcoal by adding 4 mg of activated charcoal. Vortex

and centrifuge at 10000 x g for 3 min. to remove precipitate and activated charcoal. Repeat if needed. Transfer 10 µl of each hydrolyzed sample for a 96-well plate and evaporate to dryness under vacuum. Samples were performed in a pilot experiment & testing was done in different sample dilutions to ensure the readings are within the standard curve range. Endogenous compounds may interfere with the reaction. To ensure an accurate determination of hydroxyproline in the test samples, spiking samples with a known amount of standard (0.4 µg).

Standard Curve

Preparation

Hydroxyproline stock was prepared by adding 10 µl of the 1 mg/ml standard to 90 µl of dH_2O and was mixed well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells to generate 0.2, 0.4, 0.6, 0.8 & 1 µg/well hydroxyproline standard.

Reaction

100 µl of the Chloramine T reagent was added to each sample and standard and incubated at room temperature for 5 min. 100 µl of the DMAB reagent to each well and incubate for 90 min, at 60°C.

Measurement

Absorbance was measured at 560 nm in a microplate reader.

Calculation

Background correction was made by subtracting the value derived from the 0 hydroxyproline standard from all readings (The background reading can be significant and must be subtracted). Standard curve had been plotted. Samples had been read from standard curve to get the hydroxyproline amount in the reaction wells (B).

Sample hydroxyproline concentration (C) = B/V X D µg/µl

Where,

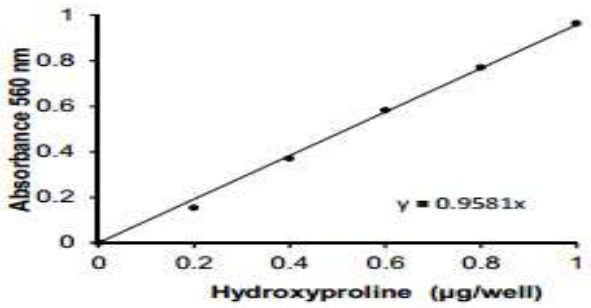
B is the amount of Hydroxyproline from Standard Curve (µg)

V is the sample volume added into the reaction well (µl)

D is the sample dilution factor.

Note

For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading. Hydroxyproline was estimated in ug per 100 ml of urine, while creatinine was aestimated in mg per 100ml. Values were expressed as mgs of hydoxyproline to mgs of creatinine excreted.



Urine creatinine

It was tested by by Jaffe's reaction.

Vitamin C Assay Protocol

Vitamin C (Ascorbic Acid) Assay was done by kit Drop-Count Iodometric titration method (Catalog # 3850) , made by HANNA INSRUMENT [10]. Assay was performed according to the kit protocol.

Urine Samples

10 ML of urine had been used for the assay with dH_2O dilution to the mark (50mL).

RESULTS

For healthy 20 male (age 3-14 years) the mean value of hydroxyproline: creation was 0.019±0.003, while the mean value for vitamin C was 23.85 mg/100ml. The mean value of vitamin C deficiency in 20% male was 9.5mg/100ml, while hydroxyproline: creatinine was 0.0064± 0.001. For healthy 15 female (age 3-14 years) the mean value hydroxproline: creatinine was 0.017 ± 0.002, while the mean value for the vitamin C was 39 mg/100ml. The mean value of vitamin C deficiency in 28 % of the female was 7.8 mg/100ml, while hyhydoxyproline: creatinine was 0.0042.

DISCUSSION

Vitamin C deficiency frequently found in children living on junk food and juice, leaving behind health quandaries mainly periodontal (defected collagen) and teeth, additionally as a vitamin, it is involved in the absorption of iron, anemia would be expected in the long term, as an antioxidant low immunity to viral and bacterial infection. Supplementing juice products in vitamin C, especially ones being sold in school children with a low price and a content of sugar, water and the color of (grape and orange) free of any minerals and vitamins, would overcome the deficiency of vitamin C.

CONCLUSION

The biochemical marker, of vitamin C in urine can be used as an early marker in school childrens, if deficiency is going on, and father complication might happen later such as scurvy, anemia Infections and loss of teeth, where the treatment bill will be high.

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