

Correlation between Vitamin C Deficiency and Hydroxyproline Amino Acid in Young Children of Northern Part in Palestine

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Abstract

Vitamin C is a water soluble vitamin found in many natural sources, citric fruits, vegetables, particularly as antioxidants and as a factor to catabolism and metabolism. The aim of this article is to correlate the level of vitamin C and the hydroxyproline level in urine samples among young palestinian children from age 5 - 14 years. Materials and Methods: Urine samples of 34 individuals of both sexes, their ages ranged from 5 - 14 years were collected and analyzed for determination of Urinary Hydroxyproline by using Modified Neuman and Logan Method. The result of this study shows a significant correlation between Vitamin C and Hydroxyproline at level 0.01. The conclusion of this study was that low vitamin C intake was found among young palestinian children from age 5 - 14 years, Vitamin C should be supplemented in the drinks taken by young palestinian children from age 5 - 14 years as well as further research and investigation with large samples required to include all children from middle and southern Palestine.

Keywords

Vitamin C, Hydroxyproline, Palestinian Children

1. Introduction

Vitamin C is a water soluble vitamin found in many natural sources, citric fruits, vegetables a particularly as an

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tioxidants and as a factor to catabolism and metabolism [1].

From the earliest descriptions of scurvy, the disease caused by vitamin C deficiency, it was evident that there were effects on connective tissues, especially in healing wounds [2]. The history of experiments that led to the conclusion that collagen metabolism was affected by the disease and that formation of hydroxyproline, amino acid found almost uniquely in collagen, might be involved was reviewed extensively [3] [4].

Many of the clinical problems associated with ascorbic acid deficiency, such as abnormalities of the skeleton seen in infantile scurvy [5], the lesions of the gingiva [6] [7], as well as the impairment of wound healing and faulty healing of bony fractures [5] [8] [9], are related to alterations in collagen metabolism.

The relationship between vitamin C and collagen is well established and lies in the hydroxylation of proline and lysine residues in the preformed protocollagen molecule [10] [11].

The aim of this article is to correlate the level of vitamin C and the hydroxyproline level in urine samples among young palestinian children from age 5 - 14 years.

2. Materials & Methodology

This study has been carried out on 34 individuals of both sexes their ages ranged from 5 - 14 years between 2013 and 2014. The sample randomly selected from out patients attending department of orthodontics and pediatric Dentistry at the faculty of dentistry—Arab American university—jenin in northern part of Palestine.

Inclusions criteria were healthy patients older than 5 years till 14 years, with ASA class I and no supplemental vitamin C intake. Unhealthy patients with ASA class II, III, IV, V and using supplemental vitamin c drugs were considered as exclusion criteria.

Ethical approval, this study registered and approved by the biomedical department at dental faculty of Arab American university in which all records of the samples were examined. Parental informed consent was carried out for each subject involved in this study by using a written form.

Case history had been taken for each subject and Clinical Examination carried out on dental chair for each subject who seated in reclining position. Plain mirrors, blunt probes (0.6 - 0.7 mm in diameter). Arab American university-jenin Assessment forms (2010) used.

The collected Urine samples were analyzed as the following:

Determination of Urinary Hydroxyproline:

Method Modified Neuman and Logan [12].

Principle: Hydroxyproline is treated with CuSO_4 and H_2O_2 in alkaline solution, this results in the formation of pyrroline-4-carboxylic acid.

Acidification is converted to pyrrole-2-carboxylic acid.

The later condenses with P-dimethylaminobenzaldehyde to give a coloured complex which is measured at 540 nm (Table 1).

Reagents:

1) Copper sulphate (0.01 M): Dissolve 0.159 g of it in 100 ml Distilled water.

Table 1. Procedure.

S.N	Reagent/Sample	Blank	Standard	Test
1	Urine			1 ml
2	Standard		1 ml	
3	Distilled Water	1 ml		
4	CuSO_4 (0.01) M	1 ml	1 ml	1 ml
5	NaOH (2.5) M	1 ml	1 ml	1 ml
6	6.6% H_2O_2	1 ml	1 ml	1 ml
7	3N H_2SO_4 (with agitation)	4 ml	4 ml	4 ml
8	5% P-dimethylamionbenzaldehyde	2 ml	2 ml	2 ml

- 2) Sodium Hydroxide (2.5 N): Dissolve 10 g of it in 100 ml distilled water.
- 3) 6% hydrogen peroxide
- 4) Sulphuric acid (6 N).
- 5) P-dimethylaminobenzaldehyde (5% solution in n-propanol).
- 6) Hydroxyproline standard: A series of it corresponding to 5, 10, 15, 20, 25, 30, 35, 40, 45 microgram of it are prepared.

The solutions are mixed and shaken occasionally during a period of 5 minutes, and then placed in water bath at 80°C for 5 minutes with frequent vigorous shaking. The heating and shaking destroys the excess of peroxide. Traces of peroxide, which remain, will decrease color formation and produce an orange-red hue. The tubes are chilled in an ice water bath and then added.

The tubes are placed in water bath at 70°C for 16 minutes and then placed in tap water. Read the color at 540 nm.

- 1) Hydroxyproline is estimated in µg/dl of urine and creatinine is estimated in mg/dl of urine.
- 2) Urine creatinine is estimated in micro cuvette by Jeff's reaction.

Statistical assessment was carried out using SPSS software version 20.0 (SPSS Inc., Chicago, IL, USA). Pearson Correlation test was used to define the correlation between vitamin C and hydroxyproline amino acid.

3. Results

This study aimed to correlate the level of vitamin C and the hydroxyproline level in urine samples among young Palestinian children from age 5 - 14 years.

The lab value measurements of vitamin C and Hydroxyproline among children from 5 - 14 years of both sexes were taken as shown in **Table 2**.

The mean value of urine hydroxyproline: creatinine ratio in 35 healthy children of both sexes aged 5 - 14 years old was 0.01 ± 0.004 .

The findings of this study show a significant correlation between Vitamin C and Hydroxyproline at level 0.01 as shown in **Table 3**.

4. Discussion

The study reported here represents the first comprehensive analysis that correlate the level of vitamin C and the hydroxyproline level in urine samples among young Palestinian children from age 5 - 14 years.

Vitamin C has many vital functions in the body. Humans cannot make vitamin C (ascorbic acid or ascorbate) and must obtain it through the diet or as supplements [13].

A low vitamin C intake agrees with the observation of direct relationship between vitamin C and the hydroxyproline which the results of this study confirmed and ascertained as the results of other studies [10] [11].

Modern life from high technology to fast food among young generation health diet raising children from age 5 - 14 years who suffering vitamin C deficiency, most probably due to the artificial flavor and coloring had been supplemented to different types of snacks which had been consumed in large quantities by children in school and playing games on TV.

In addition this particular age group of people from 5 to 14 year old has longer outdoors stay at this age leading to greater consumption of in between meals snack food full of fillers, artificial flavor and colors and consequently to be considered at high risk in terms of Vitamin C deficiency.

The extent of the generalisability of the findings from a reasonable small sample is considered to be as a potential limitation and which will lead to further investigations in the future.

This paper is important for pediatric dentists for the following reasons:

- 1) Dietary advice is an essential part of oral care of children.
- 2) As our children constantly drinking and snacking, it is very important to be able to give sensible practical advice regarding drinks.

The basic advice is straight forward Vitamin C should be supplemented in the drinks taken by young children.

5. Conclusions

- 1) A low vitamin C intake was found among young Palestinian children from age 5 - 14 years, so there should be increase intake of Vitamin C.

Table 2. Lab value measurements of vitamin C and OH-PR among children from 5 - 14 years of both sexes.

Paient No.	Age years	Gender	Vitamin C N-R 20 - 30 mg/dl	OH-PR N-R 0.01 ± 0.004	Diseases
1	5	M	22	0.03	
2	8	F	35	0.03	G6PD
3	35	M	18	0.012	
4	10	F	20	0.015	
5	10	F	32	0.021	
6	9	M	18	0.01	
7	6	M	27	0.02	
8	13	M	24	0.015	
9	9	M	32	0.012	
10	9	M	29	0.032	
11	10	M	25	0.032	
12	12	F	20	0.0145	
13	10	M	22	0.021	
14	7	F	25	0.033	
15	14	M	25	0.025	
16	7	M	21	0.015	
17	9	F	29	0.0135	
18	8.5	F	31	0.021	
19	3.5	M	31	0.012	
20	9	M	33	0.013	
21	13	F	25	0.015	
22	11	F	29	0.0135	
23	8.5	M	38	0.044	
24	7	M	27	0.02	
25	10	M	25	0.016	
26	8	F	5	0.003	Vitamin C Deficiency
27	11	F	15	0.008	Vitamin C Deficiency
28	9	F	2	0.002	Vitamin C Deficiency
29	12	M	10	0.02	Vitamin C Deficiency
30	13	F	5	0.0015	Vitamin C Deficiency
31	13	M	7	0.0091	Vitamin C Deficiency
32	9	M	3	0.005	Vitamin C Deficiency
33	7	F	8	0.004	Vitamin C Deficiency
34	5	M	5	0.002	Vitamin C Deficiency

Table 3. Correlation between Vitamin C and hydroxyproline amino acid.

		Vitamin C	OH-PR
Vitamin C	Pearson Correlation	1	0.696**
	Sig. (2-tailed)		0.000
	N	34	34
OH-PR	Pearson Correlation	0.696**	1
	Sig. (2-tailed)	0.000	
	N	34	34

**Correlation is significant at the 0.01 level (2-tailed).

2) Vitamin C should be supplemented in the drinks taken by young palestinian children from age 5 - 14 years as drinks highly consumed among young palestinian children.

3) Further research and investigation with large samples required to include all children from middle and southern Palestine.

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