AL-AZHAR
Dental Journal
For Girls

The Official Publication
of The Faculty of Dental
Medicine For Girls,
Al-Azhar University
Cairo, Egypt.

Print ISSN 2537-0308 • Online ISSN 2537-0316

ADJ-for Girls, Vol. 8, No. 2, April (2021) — PP. 307:314

The Effect of Chewable Chitosan on Phosphate Levels in Serum and Saliva In Hemodialysis Pediatric Patients

Mahy G. Salem [1*], Mervat I. Fawzy [2], Mohamed H. Mostafa [3], Moftah M. Rabea [4]

Codex : 17/21.04

azhardentj@azhar.edu.eg

http://adjg.journals.ekb.eg

DOI: 10.21608/adjg.2021.20612.1206

Pediatric Dentistry & Orthodontics
(Pediatric Dentistry, Orthodontics)

ABSTRACT

Purpose: To evaluate the effect of a salivary phosphate binder "Chitosan" in a chewable form on serum and salivary levels of phosphate, calcium and urea in a group of hyperphosphatemic, hemodialysis pediatric patients suffering from end stage renal disease. **Subjects and Methods:** The study comprised 30 children on hemodialysis; (14 males and 16 females), aging 7-16 years, selected from the hemodialysis units of Al-Azhar University Hospitals. All the children chewed chitosan loaded chewing gum for one hour, twice daily, during the fasting hours between meals throughout the trial period (3 months), while maintained on oral phosphate binders. **Results:** There was a statistically significant decrease in the mean serum levels of phosphate and urea as well as an increase in serum calcium means levels. There was a significant decrease in mean levels of salivary phosphate and urea and increase in mean levels of salivary calcium. **Conclusion:** Chitosan loaded chewing-gum can be safely and effectively used for management of hyperphosphatemia in hemodialysis pediatric patients, as an addition to the oral phosphate binders.

KEYWORDS

Chitosan, Chewing gum, Hyperphosphatemia, Hemodialysis

INTRODUCTION

Hyperphosphatemia is a common complication of end stage renal disease (ESRD) and hemodilysis (HD) [(1)]. Hyperphosphatemia is a life threatening condition that leads to death from cardiovascular calcifications [(2)]. Furthermore, hyperphosphatemia along with calcium

- Paper extracted from Doctor Thesis titled "The Effect of Chewable Chitosan on Phosphate Levels in Serum, Saliva in Hemodialysis Pediatric Patients."

1. Pediatric Dentist, Air-Force Specialized Hospital,Cairo,Egypt.
2. Professor of Endodontics, Faculty of Dental Medicine for Girls, Al-Azhar University,Cairo,Egypt.
3. Associate Professor and Head of Pedodontics and Oral Health Department, Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt.
4. Professor of Pediatrics and Pediatric Nephrology, Faculty of Medicine for Boys, Al-Azhar University,Cairo,Egypt.

* Corresponding author email: mahygamil57@yahoo.com

depletion lead to the development of secondary hyperparathyroidism, renal osteodystrophy, renal bone disease and metabolic bone disease [3]. In children, these disorders are associated with growth problems, and a variety of oral manifestations, including delayed eruption of the permanent dentition, enamel hypoplasia and the development of malocclusion [4-7].

The current strategy for the management of hyperphosphatemia is based on dietary phosphate restriction, increasing frequency and length of dialysis sessions, and the administration of orally administered phosphate binders[8]. Oral phosphate binders (e.g. Calcium carbonate, Savelamer hydrochloride, lanthanum etc.), that work by reducing the absorption of dietary phosphates in gastrointestinal tract [9].

Despite intensive hemodialysis therapy, by increasing the length and frequency of the dialysis sessions, dietary phosphate restriction, and the administration of oral phosphate binders; many hemodialysis patients fail to achieve the recommend serum phosphate levels [10, 11].

With deterioration of renal function; there will be a compensatory increase of phosphates excreted in saliva, since phosphates are normally excreted via parotid gland and pancreatic juice [12, 13]. An increased salivary excretion of phosphates has been reported in patients with chronic kidney disease and hemodialysis patients and independent on dietary phosphate intake [14].Salivary phosphates are ingested with saliva, become absorbed via GIT, to form an additional load to the dietary phosphate intake, and hinder the action of oral phosphate binders [15].

A novel approach targeting salivary phosphates by salivary phosphate binders has been proposed as an addition to oral phosphate binders. Therefore, adding salivary phosphate binders in a chewable form during fasting periods to the oral phosphate binders, taken with meals could optimize the efficacy of the latter, thereby facilitating reduction of serum phosphate towards the recommended level [15, 16]. Studies have shown that "Chitosan" in a chewable form can be used safely and effectively in adults as a salivary phosphate binder to reduce salivary and serum phosphate levels [17].

Chitosan is a deacetylation product of "Chitin"; the principal component of the exoskeleton of crustaceans (Crabs and shrimps), a natural polymer of glucosamine [18]. Chitosan is nontoxic, biocompatible, biodegradable, insoluble in water, and is not cleaved by digestive enzymes, nor is it absorbed via the alimentary tract, skin or any other rout. Chitosan is food and drug administration (FDA) approved as dietary supplement [19]. The aim of this study was to evaluate the impact of long term effect of a salivary phosphate binder "Chitosan" in a chewable form on phosphate, calcium, and urea levels in serum and saliva in hemodialysis children.

SUBJECTS AND METHODS

30 male and female patients aging 7-16 years were selected to participate in this study, from a pool of 59 children with ESRD undergoing regular hemodialysis in the pediatric nephrology units, Al-Azhar University Hospitals, Cairo. The inclusion criteria included serum phosphate levels > 6mg/dL, free from other concomitant systemic diseases and dialysis period > 1 year. Exclusion criteria included presence of known allergy to shell fish (Crabs, Shrimps). All Children received regular hemodialysis sessions (3essions/week), each session lasted 4 hours using the same type of dialysis machine (Fersineus 4008A) and filter (F5). Patients continued to receive their usual medications: Phosphate binding drugs, vitamin D and iron.

Ethical Consideration: Research approval was obtained before research implementation from the Ethical Research Committee of Faculty of Dental Medicine for Girls, Al-Azhar University. The objectives and the aim of the study were explained to the parents of the eligible children. A written informed consent was obtained, signed by the parents of each child.

Study Design:

Prospective-observational, interventional single –blind, uncontrolled Clinical Trial.

Medical and Dental History:

Socio – demographic data, history of the renal disease and dialysis data were obtained from the patients files. Dialysis efficiency, and blood chemistry were obtained from the monthly data sheet, and mean values calculated.

Base-Line Data:

a) Blood chemistry:

Pre-dialysis blood samples were obtained from all the children through the venous catheter placed for hemodialysis, and analyzed for serum phosphates, calcium and urea just before starting the clinical trial.

b) Sialochemistry :

Predialysis samples of unstimulated whole saliva (UWS) were collected from the participants by the spitting method [20]. The saliva samples were analyzed for salivary phosphate, calcium and urea.

Study Medication:

A phosphate binding agent in a slow release oral delivery system comprising chewing gum and an organic acid. The phosphate binding agent used in this study was chitosan (Chito – lab) ® Chitosan-Egypt Co., with degree of deacetylation > 95, medium viscosity, high molecular weight (30000Da) in powder form.

Preparation of the Chewable Formula:

20 mg chitosan were loaded in a commercially available; sugar free chewing gum (Trident) as cold compressed 3 layered cuboid, patented tablets measuring 1.7 cm^3 each. The patented tablet is composed of two external layers of the chewing gum, and an inner core. The inner core is composed of medium – viscosity, chitosan powder premixed with gum arabic as an excipient (binder), and acidified with 2% acetic acid in a ratio of 1:4 weights by volume [21]. The inner core was placed between two layers of the chewing gum and compressed thus acting as a delivery system. The chewing gum was provided in 3 different flavors: strawberry, mint and tropical fruits and was distributed according to the individual preference of the participants. The chewable formula was prepared by a pharmacist in a registered lab, under aseptic precautions, and all the measures of drug dispensing were perfectly undertaken.

Clinical Trial:

All children received chewable tablets, containing 20mg chitosan per-piece. All children were requested to chew one piece of the gum for 1 hour during the fasting period between breakfast and lunch, and another one between lunch and supper and spit it at the end of the chewing session; daily and uninterruptedly for a period of 3 months. Regular weekly checking of the stock of the tablets delivered to each child was done. As per dialysis protocol, all the participants had their oral phosphate binder daily dosing, and diet remained unchanged for the duration of the clinical trial.

Post-trial study parameters:

a) Blood chemistry:

Pre-dialysis blood samples were obtained from all subjects through the venous catheter placed for hemodialysis, and analyzed for serum phosphates, calcium and urea.

b) Sialochemistry:

Pre-dialysis unstimulated whole saliva samples were collected from all the children and the saliva samples were analyzed for salivary phosphates, calcium and urea, using the biochemical autoanalyzer.

Statistical Analysis

Data were presented as mean, standard deviation (SD), mean difference and 95% Confidence Interval (95% CI) for the difference values. For parametric data, Paired t-test was used for comparisons between different variables before and after the trial used to compare between different variables before and after the trial. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

RESULTS

This study was conducted on 30 hypersphosphatemic hemodialysis pediatric patients. 2 subjects (6.6%) died during the study; and the study group comprised only 28 participants aged 7-16 years: 13 males (46.4%) and 15 females (53.6%). The mean (SD) values for age were 10.7 (2.2) years old with a minimum of 7 and a maximum of 16 years. There was a statistically significant direct correlation between salivary urea and serum urea at base line. There was a statistically significant direct correlation between salivary phosphate and serum phosphate.

Post-Trial Changes:

a) Blood chemistry:

There was a statistically significant decrease in mean serum urea levels at the end of the trial; there was a statistically significant decrease in mean serum Phosphate levels at the end of the trial. There was a statistically significant increase in mean serum Calcium levels at the end of the trial and there was a statistically significant decrease in mean Calcium-Phosphate Product levels at the end of the trial. A comparison between the mean values of the serum parameters of the study group before and after the clinical trial is shown (Table1).

b) Sialochemistry:

There was a statistically significant decrease in mean salivary urea levels at the end of the trial, a statistically significant decrease in mean salivary phosphate levels at the end of the trial. There was a statistically significant increase in mean salivary calcium levels at the end of the trial and no statistically significant change in mean calcium-phosphate Product levels at the end of the trial. A comparison between the mean values of the sialochemical parameters is presented in (Table 2).

Table (1): *Comparison between serum parameters of the study group patients before and after the trial.*

Serum Parameter	Base line		After 3 months		Mean Difference	95% CI for Difference		Test value	P-value	Effect size
	Mean	SD	Mean	SD		Lower bound	Upper bound			
Urea (mg/dl)	188.2	45.4	163.4	28.7	-24.8	-36.8	-12.7	t = -4.231	<0.001*	d = 0.578
Phosphate (mg/dl)	7.5	1.6	5.6	1.9	-1.9	-2.4	-1.5	t = -8.341	<0.001*	d = 1.031
Calcium (mg/dl)	7.7	1.3	8.7	1.8	1	0.6	1.4	t = 5.539	<0.001*	d = 0.594
Calcium-Phosphate Product	56.8	12.7	46	14	-10.9	-14.8	-6.9	t = -5.612	<0.001*	d = 0.809

**: Significant at P ≤ 0.05*

Table (2): *Descriptive statistics and results of paired t-test and Wilcoxon signed-rank test for comparison between salivary parameters of the study group before and after the trial:*

Salivary Parameter	Base line		After 3 months		Mean Difference	95% CI for Difference		Test value	P-value	Effect size
	Mean	SD	Mean	SD		Lower bound	Upper bound			
Urea (mg/dl)	133.4	26	122.1	25.5	-11.2	-19.8	-2.7	t = -2.686	0.012*	d = 0.436
Phosphate (mg/dl)	27.5	5.2	19.4	6.6	-8.1	-9.3	-6.9	t = -13.601	<0.001*	d = 1.222
Calcium (mg/dl)	1.9	1.1	3.1	1.5	1.2	0.8	1.7	z = -4.522 †	<0.001*	r = 0.855
Calcium-Phosphate Product	51.4	30.2	58.4	28.3	6.9	0.5	13.4	z = -1.867 †	0.062	r = 0.353

**: Significant at P ≤ 0.05, †: Wilcoxon signed-rank test*

DISCUSSION

In the current study, regarding the pre-trial (base line) data, salivary phosphates and urea were higher than those of blood (3.7 times the serum level), while salivary calcium was less, in agreement with earlier studies [7-12]. The positive correlation between blood and saliva levels of phosphates, Ca and urea, observed in this study were consistent with previous reports [24].Several studies have demonstrated high salivary phosphate levels in hemodialysis patients compared to controls. Salivary phosphate correlated well also with serum creatinine, and was 3 times higher in hemodialysis patients compared to control subjects [22, 23] .According to another study, salivary urea is the best known, currently used, and most promising, salivary parameter of renal function with potential use in both screening and monitoring of renal function [24].

To our knowledge; this is the first study on the long term effect of a chewable phosphate binding agent (Chitosan) on salivary and serum phosphate levels in hemodialysis pediatric patients. The few previous studies on the effect of chitosan on salivary and serum phosphate levels were performed on adult and /or elderly hemodialysis population for a brief period of time (2-3 weeks). Since there are no similar studies on the effect of chewable chitosan on salivary and serum phosphate levels in hemodialysis pediatric patients are available for comparison, the results of the present study were compared with the results of previous studies on adult hemodialysis population [22-25].

In the present study; reduction of mean serum phosphate concentration after 3 months of using chitosan loaded chewing gum was 25.3 % from base-line value. This significant decrease in serum phosphate concentration approximates to the goal serum phosphate levels recommended by the national kidney foundation – kidney disease improving global outcome (NKF- KDIGO) for the fifth stage of chronic kidney disease [26]. Meanwhile the mean serum calcium level showed a 12% increase, approximating to the normal serum calcium levels. However, in children, determination of serum phosphate levels is based on serum calcium levels as per the recommendations of (NKF- KDIGO) guide lines [26]. Comparable results on adults showed a 30% decrease of mean serum phosphate levels after chewing a gum containing 20 mg chitosan for 1 hour, two times per day for 3 weeks [27]. In the present study, salivary phosphate levels decrease was 29.4% after the trial whereas the same study on adults reported a higher decrease of 54% in salivary phosphate in 3 weeks [27]. In the

current study salivary urea showed a decrease of 8.4% and serum urea decreased 13%. These results are comparable with that presented by another study, which showed reduction of serum urea of 21% after administration of 45mg chitosan daily for 12 weeks [28].

The advantage of chewing gum is being kept in the mouth for prolonged periods and the chitosan in the chewing gum is slowly released and effectively comes in contact with phosphates present in the saliva of the user. After binding phosphates in the saliva, the phosphate bound chitosan may be swallowed and thus passing through the gastrointestinal tract; without being absorbed into the blood stream Another advantage of chewing gum is that phosphate bound chitosan may be still present in the chewing gum and by the disposal of the chewing gum after the chewing session; swallowing of more phosphates is avoided [28].This may be advantageous when compared to other delivery vehicles such as tablets, lozenges etc.

It has been reported that the phosphate binding ability of chitosan is improved by combining chitosan with an organic acid, which can reduce the level of free phosphates in the saliva [29]. Since binding of phosphate by chitosan results from the ionic interaction between positively charged amine moieties $(NH)^{++}$ of the glucosamine polymer and negative charges of phosphate ($PO4^{-}$), combining chitosan with an acid provides an extra positively charged hydrogen ion (H^{+}) to the amine group, thus increasing its capacity to bind phosphate ,thus it has been advised that the chewable formulation should comprise an organic acid as an enhancer of the phosphate binding ability of chitosan [29].In the present study; 2% acetic acid was used as an enhancer of the phosphate binding ability of chitosan as described in other studies, which recommended that the oral delivery system should contain chitosan and an organic acid in a ratio of 1:4 weight by volume [30].

In the present study; gum arabic was used as a binder excipient to chitosan powder. Gum arabic is a natural polymer that mixes well with chitosan. It is chemically inert, biocompatible and water soluble. It dissolves slowly in saliva assuring a continuous slow release of the principle active ingredients in the chewing gum; thus establishing a suitable slow release oral delivery system [30, 31].

No interventional trials in the literature have demonstrated the impact of chewable chitosan of salivary and serum phosphate levels in hemodialysis pediatric patients. Thus, until similar studies are performed on hemodialysis pediatric patients; are performed this study will stand alone, waiting for further researches in the future. Finally, it should be mentioned that the present study is a preliminary study on the effect of chewable chitosan on both salivary and serum phosphate levels in hemodialysis pediatric patients. Further double – blind, placebo controlled studies are needed, however, to confirm the results of this study.

CONCLUSION

Chitosan in a chewable formula can be safely and effectively used for the management of hyperphosphatemia in hemodialysis pediatric patients, as an addition to oral phosphate binders.

RECOMMENDATIONS:

1. The use of chitosan - containing flavored chewing gum as an easy, convenient, well accepted supplement that can be used to control phosphate levels, alleviate thirst sensation, help compliance to fluid restriction, control of IDWG and improve the quality of life of hemodialysis population.
2. Encourage the use of salivary biomarkers instead of conventional invasive skin pricking blood tests in the diagnosis and monitoring disease activity in hemodialysis pediatric patients.
3. Rising the awareness of the importance of oral and dental hygiene among the hemodialysis population through education and motivation is

mandatory as well as recommending periodic dental checkups. Oral hygiene measures and instructions of oral home care habits for all children on hemodialysis as well as their families and /or guardians are necessary.

4. Esthetic restorations may be needed in cases of disfiguring enamel defects or hypoplasia to improve self-image and confidence of the hemodialysis child.

DECLARATION

There was no conflict of interest

No fund was received for this study.

REFERENCES

1. National Institute for Health and Clinical Excellence NICE.Renal replacement therapy. NICE guideline.2018; 2-29.
2. Zheng CM, Lu KC, WuCC, Hso YH, Lin YF.Association of serum phosphate and related factors in ESRD- Related vascular calcification. 2012; 2011: 1-8.
3. Schmitt CP, Mehls O. Mineral and bone disorders in children with chronic kidney disease Nature Review Nephrol 2011 7: 624-34.
4. Hussin R, Alvaris – Elias AC, Toppng A, Raimann IG. Cross – sectional study of growth and metabolic bone disease in a global cohort undergoing chronic hemodialysis. J. Pediatr 2018; 202: 44.
5. Woodhead JC, Nowak AJ, Crall JJ, Robillard JE. Dental abnormalities in c children with chronic renal failure pediat Dent 2018; 4: 281-85.
6. Moussa SA, and Mostafa N, The oral and dental health status in children under hemodialysis. Pediatric Dentistry 2019; 2: 145-56.
7. Honarmond M, Mollashahi LE, Nakaee A, Sargdazaie E. Oral manifestations and salivary changes in renal patients undergoing hemodialysis. J Clin Exp Denl 2016; 9 : 207-10.
8. Shaman AM.,and Kowalski SR. Hyperphosphatemia management in patients with chronic kidney disease. Saudi Pharmaceut J 2016; 24: 494-505.
9. Tonelli M, Pannu N, Manns B. Oral phosphate binders in patients with kidney failure. NEng J Med 2010; 362: 1312-15.
10. Gutekunst L. An Update on Phosphate Binders: A Dietition's Perspective. J Renal Nutr 2018; 26: 209-18.
11. NHS: Notengham University Children Hospitals, Hemodialysis for chronic kidney disease Guide lines.2018; 1-21.
12. Savica V, Billighrini G, Calo L. Salivary Glands, A third kidney for phosphate excretion. Blood Purification 2009; 28: 364.
13. Savica V, Calo LA, Santoro D, Monardo P, Santoro G, Mura U, Bellinghieri G Salivary Glands: A new player in phosphorus metabolism J Renal Nutrition. 2011; 21 : 39-42.
14. Savica V, Calo L, Santoro D, Monardo P, coranata A, Bellinghieri G. Salivary Phosphate Secretion in chronic Kidney disease. J Renal Nutrition 2008; 18: 87-90.
15. Savica V, Calo L, Granata A, Caldarera R.A new approach to evaluate hyperphosphatemia in chronic kidney disease . Clin Nephrol 2007, 68 : 216-21.
16. Savica V, Calo LA, Caldarera R, Cavateri, A, Granata A, Santero D et al. Phosphate salivary secretion in hemodialysis patients: Implications for treatment of hyperphosphatemia Nephron Physiol 2007; 105: 52-5
17. Savica V, Calo L, Monard P. Salivary Phosphate. Binding chewing Gum Reduces Hyperphosphatemia in Dialysis Patients. J Am Soc Nephrol, 2009; 20: 639-44.
18. Zargar V, Azghari M, Dashti A.A. Review on chitin and chitosan polymers: Structure, chemistry, solubility, derivatives and applications.Chem Biol Eng Rev 2015; 2: 204-26.
19. FDA Food and Drug Administration: Center of food safety and applied nutrition, Center of Additive Safety (HFS-255) GRAS. Notice (GRN) No.433-Chilo Clear ® Shrimp derived Chitosan Feb.2012.
20. Barreto EC, Barreto DV, Massy Z, and Druke T. Strategies for phosphate control in patients with CKD.Kidney Int Rep. 2019; 4: 1043-56.
21. Steenberg, LCK, Nielsen, KD, and Bruun, HZ. Chewing gum comprising chitosan for use in reduction of the level of free phosphorus compounds in the digestive juice. World International Property Organization.International Publication number W0 2012/143011A1 (26.10.2012).
22. Calo L, Savica V, D;Anglo A, Piccoli A, Fusaro M, and Davis PA. Reduction of hyperphosphatemia is related to reduction of C. Reactive protein in dialysis patients. Study in Savelamer-resistant hemodialysis patients treated by

chitosan chewing gum as salivary phosphate binder. J. Am Soc Nephrol 2009; 20: 639-44.

23. Akizawa T., Tsuruta Y, Okada Y, Miyanchi Y, Suda A, Kasahara H. Effect of chitosan chewing – gum on reducing serum phosphorus in hemodialysis patients: a multi – cender, randomized, double – blind, placebo controlled trial. BMC Nephrol 2014; 15: 98-106.
24. Al Jomai IA, Abdulkareem SA, and Hossami, TM Saliva: A diagnostic tool for oral health. J Pharm Res Allid Sci 2018; 7: 25-32.
25. Boughton B. Chewing Chitosan added gum reduces phosphate levels in ESRD patients. J Am Soc Nephrol 2009, 20: 639-44.
26. NKF-KDIGO 2017 "Guide line update Recommendations for CKD mineral and Bone Disorders (CKD-MBD) in children with CKD.
27. Zhang H, Yu M, and Zhang, H. Chitosan: a desirable candidate for treating hyperphosphatemia. J Pharmac Pharmacol 2016; 4: 99-104.
28. Kaats GR michalek JE, Preuss: HG: Evaluating efficacy of chitosan product using a double blinded, placebo controlled protocol. J Am Coll Nutr 2006; 25: 389-94.
29. US patent Application Publication. Phosphate – binding chitosan and uses there of. Pub No.: US2012/0277183A1. Nov.1, 2012.
30. Olady O. Processing and Modification of Gum Arabic in Specific Applications. In: Gum Arabic, Elsevier 2018; 112-29.
31. Wei, T.M.M., Lu, W.M. and Khutoryanskiy.V.V. Chitosan and its derivatives for application in mucoadhesive drug delivery Systems- Polymers.2018; 10: 267.