

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Studying the Relation Between V.D. Deficiency and Antioxidant Status in Urban Ricketic Egyptian Infants and Preschool Children

¹Hamed El-Khayat, ²Amany El-Wakkad, ¹Ehab Khairy Emam and ²Saadia Ibrahim

¹Department of Pediatric, Ain Shams University

²National Research Center, Dokki, Egypt

Abstract: This research was designed study the relation between vitamin D and selenium status as well as glutathione peroxidase activity as one of the antioxidant systems that may be a part of the immunodeficiency syndrome in rickets. The study was conducted on 33 patients diagnosed as active rickets on clinical, laboratory and radiological basis. Thirty age and sex matched apparently healthy infants and children were chosen to serve as controls. All studied infants and children were subjected to blood work to assess Complete Blood Picture (CBC), serum levels of 1, 25 (OH) 2 D, Calcium (ca), phosphorus (P), alkaline phosphatase (ALP) and selenium (Se) as well as Glutathione Peroxidase (GPX) enzyme activity in the whole blood. Patients were given intramuscular injection of Vitamin D in a shock therapy (600000 IU every 2 weeks for 3 doses) as well as oral Ca supplementation (800-1000 mg elemental Ca/day). These patients were reassessed clinically, radiologically and for the same laboratory parameters at 2 and 6 weeks after the start of treatment. The study revealed that the mean levels of serum Ca, P, active vitamin D, Se and GPX enzyme activity were significantly lower ($p < 0.001$) compared to control, while the mean serum level of ALP was significantly higher ($p < 0.001$) in rachitic patients at diagnosis compared to controls. The mean serum Ca and Se levels as well as GPX activity showed a significant increase after 2 weeks ($p < 0.001$) and a further significant increase after 6 weeks of treatment compared to levels at diagnosis ($p < 0.001$). On the contrary, the mean level of ALP showed a significant decrease after 2 weeks ($p < 0.001$) and a further significant decrease after 6 weeks of treatment compared to levels at diagnosis ($p < 0.001$). The serum active vitamin D levels had significant positive correlations with serum levels of Ca, Se and GPX activity ($p < 0.001$) and negative correlation with ALP levels. Similarly, serum Se level showed significant positive correlation with GPX activity ($p < 0.001$) and significant negative correlation ($p < 0.05$) with serum ALP levels. From the results of this study we can conclude that, patients with active rickets have evidently lower serum selenium levels which may be partly due to lack of absorption as a result of deficient vitamin D or as a part of multiple nutritional deficiency syndromes. Hence, the study recommends not only supplementing vitamin D and Ca but also Se, as it is mandatory for the promotion of the antioxidant activity of GPX with subsequent improvement of immune status in rachitic patients.

Key words: Rickets, vitamin D, antioxidant

INTRODUCTION

Nutritional rickets causes considerable disability among children. Though virtually eliminated from Europe and North America by the fortification of food with vitamin D, nutritional rickets remains prevalent in many parts of the world including Africa, India, Asia and the Middle East (Mughal *et al.*, 1999). Rickets has been ranked among the 5 most prevalent diseases among children in developing countries (Muhe *et al.*, 1997). Rickets is still prevalent in Egypt in spite of the advances in the socioeconomic status. The incidence of rickets among Egyptians during the first two years of life was reported to be 12-31.1% (El-Bishlawy *et al.*, 1992).

Vitamin D deficiency prevents the efficient absorption of dietary calcium and phosphorus. In a vitamin D deficient state, only 10-15% of dietary calcium and 50-60% of dietary phosphorus are absorbed (Holick, 2005).

Selenium is an essential trace element, which is incorporated as a selenoprotein into specific proteins in a regulated fashion and has antioxidant activity. Bone and other tissues rely on antioxidant system against potential cell and DNA damage through endogenous and environmental peroxides and reactive oxygen species. The optimized cell defense through antioxidant selenoproteins requires optimal Se supplementation (Jacob *et al.*, 1997; Chwan-Li *et al.*, 2001).

Mykkanen and Wasserman (1990) found that the uptake of selenite by purified brush border membrane isolated from the duodena of rachitic chicks is decreased and that selenite uptake was significantly enhanced by cholecalciferol treatment suggesting the effect of vitamin D on selenite uptake. Moreover, Kvicala (1999) stated that activated glutathione peroxidase enzyme through selenium is involved in many immune functions which all are important to overcome bacterial or viral infections. Thus the presence of selenium deficiency in cases of infantile rickets may have a role in defective immunity in such patients.

The aim of this study is to study the relation between vitamin D deficiency and selenium status as well as glutathione peroxidase activity as one of the facets of immunodeficiency syndrome in rickets.

MATERIALS AND METHODS

The National Research Center Ethical Committee approved the study. The parents of all subjects gave informed written consent to the participation of their children in the study.

Screening for cases having clinical manifestations of rickets was done in the period between January and April 2005 using cross section study conducted on 1000 infants and children aged between 2-36 months who were attending Pediatric Outpatient Clinic of Ain-Shams University Hospitals for follow up or with mild acute complaints.

Thirty three patients out of those diagnosed as active rickets were randomly selected to be enrolled in our study. They were 19 males and 14 females and their ages ranged between 6-36 months with mean age of 15.24 ± 7.69 months.

Thirty age and sex matched apparently healthy infants and children were chosen to serve as controls. They were 15 males and 15 females with mean age of 13.27 ± 8.34 months.

All patients were subjected to two phase study while controls were subjected only to the first phase.

First phase:

- Full history taking laying stress on personal and environmental data (age, sex, residence, housing and duration of exposure to sun light), full dietary history including type of milk, duration of exclusive milk feeding, time of weaning and type of food in weaned babies, history of vitamin D supplementation and clinical history including milestones of motor development, teething, chest infection, gastroenteritis and tetany.

- Evaluation of Socio-economic status according to Park and Park (1979).
- Thorough clinical examination including signs of rickets as well as signs of other vitamins and mineral deficiencies and signs of chest, throat or other infections.
- Anthropometric measures to assess growth.
- Laboratory investigations:
 - Serum calcium, phosphorus and alkaline phosphatase enzyme levels determined by the colorimetric manual method of Boehringer Mannheim Gm BH. The method used is O-cresolphthalein complexone, without deproteinization (David and Zafar, 1990).
 - Serum level of active vitamin D was measured quantitatively in the serum by using Biosource Radioimmunoassay Kit (Tom *et al.*, 1999).
 - Glutathione peroxidase enzyme activity was determined in the whole blood using the method described by Paglia and Valentine using Randox laboratories kits.
 - Serum selenium level was detected using Unicam 929 Atomic Absorption spectrometer attached with a graphite furnace.
 - Radiological investigations were done to confirm the diagnosis of active rickets.

Second phase: Patients were supplemented with vitamin D in a shock therapy (intramuscular injection of 600000 IU every 2 weeks for 3 doses as well as oral Ca supplementation (800-1000 mg elemental Ca/day).

Patients having chest infection were given antibiotics in the form of penicillin and gentamycin as combination therapy as well as chest physiotherapy.

Patients were reassessed at 2 and 6 weeks after the start of treatment by:

- Clinical examination for signs of rickets as well as signs of chest infection.
- Radiological assessment to assess healing of rickets.
- Laboratory measurements of serum levels of Ca, P, Se, active vitamin D and ALP enzyme as well as GPX enzyme activity in the whole blood.

Thirty age and sex matched apparently healthy infants and children were chosen to serve as controls. They were 15 males and 15 females with mean age of 13.27 ± 8.34 months. The controls were subjected only to the first phase.

RESULTS

The mean serum levels of Ca, P, active vitamin D and Se as well as GPX activity were significantly lower

Table 1: Mean serum levels of laboratory findings among patients with active rickets at diagnosis and at 2 and 6 weeks after vitamin D supplementation

Parameters	P0	P2	P6	P-value		
				P0 vs P2	P0 vs P6	P2 vs P6
Serum active vitamin D (pg mL ⁻¹)	13.18±4.44	21.55±8.74	33.85±10.27	<0.001	<0.001	<0.001
Serum Ca (mg dL ⁻¹)	8.89±1.12	9.27±0.62	9.67±0.61	<0.001	<0.001	<0.001
Serum P (mg dL ⁻¹)	3.21±0.59	3.31±0.41	3.42±0.46	<0.05	<0.05	<0.05
Serum ALP (IU L ⁻¹)	1509.55±992.19	1125.88±29.34	739.82±42.68	<0.001	<0.001	<0.001
Serum Se (ug mL ⁻¹)	20±1.3	23±3.5	27±3	<0.001	<0.001	<0.001
GPX activity (U L ⁻¹)	10.8±2.3	14.2±1.6	18.5±2.4	<0.001	<0.001	<0.001

P0 = Patients at diagnosis, P2 = Patients after 2 weeks of supplementation, P6 = Patients after 6 weeks of supplementation

Table 2: A comparison of mean levels of selenium and GPX activity of controls and patients at diagnosis, 2 weeks and after 6 weeks of therapeutic intervention

Parameters	Control	P0	P2	P6
GPX activity U L ⁻¹	20±2.7	10.8±2.3**	14.2±1.6**	18.5±2.4*
Selenium U gm L ⁻¹	29±1.3	20.0±1.3**	23.0±2.5**	27.0±3.0*

*p<0.05 **p<0.001 P0 = at diagnosis P2 = after 2 weeks P6 = after 6 weeks

Table 3: Correlation matrix (r-value) among variable laboratory parameters in patients with active rickets

Parameters	Ca	P	ALP	Hb	Vit. D	GPX	Se
Vit. D	0.31**	-0.15	-0.19	0.01	-	+0.44**	10.37**
GPX	+0.1	+0.17	-0.2*	+0.23*	+0.44	-	+0.48**
Se	0.13	-0.002	-0.2*	-0.01	0.37**	+0.48**	-

*p<0.05 **p<0.001

Table 4: The percentage frequency of chest infection and gastroenteritis at diagnosis of active rickets and 2 and six weeks after therapeutic intervention

Parameters	At diagnosis	2 weeks	6 weeks
		after treatment	after treatment
Chest infection	72.7%	40%*	15.2%*
Gastroenteritis	69.7%	42%*	21.2%*

*p<0.01

(p<0.001), while the mean serum ALP level was significantly higher in rachitic patients at diagnosis compared to controls (p<0.001).

The mean serum level of selenium was highly significantly lower in rachitic patients with chest infection compared to those without (p<0.001).

The mean levels of serum Ca, P, active vitamin D and Se as well as GPX activity in the whole blood showed a highly significant (p<0.001) increase at 2 weeks of treatment compared to the mean levels at diagnosis and a further significant increase (p<0.001) after 6 weeks of therapy, but still significantly (p<0.05) lower than control. Meanwhile, the mean levels of serum ALP enzyme showed a highly significant decrease (p<0.001) at 2 weeks and a further significant (p<0.001) decrease after 6 weeks of treatment (Table 1 and 2)

The serum vitamin D level showed significant positive correlation with serum levels of Ca, Se and GPX enzyme activity in the whole blood. Meanwhile, the GPX activity showed significant negative correlation with the serum level of ALP and positive with serum Se levels. Moreover, the serum Se levels showed a significant negative correlation with the serum level of ALP (Table 3).

A significant decrease (p<0.01) in the complication of tickets (chest infection and Gastroenteritis) after 2 weeks

of therapeutic intervention with further significant improvement after 6 weeks (p<0.01) (Table 4).

DISCUSSION

In this study, clinical rickets was found in 290 out of the 1000 examined patients (29%) who were attending the Outpatient Clinic in the Pediatric Hospital, Ain-Shams University. These patients were subjected to laboratory and radiological studies in order to confirm diagnosis and to determine the stage of rickets. It was found that 178 patients (17.8%), 38 patients (3.8%) and 74 patients (7.4%) had active, healing and healed rickets, respectively. So, the frequency rate of active rickets in our Outpatient Clinic was 17.8%. This is in agreement with, but slightly higher than the study of Awaad *et al.* (1975) who found the incidence of rickets among Egyptian infants during the first 2 years of life to be 12-13%. Thus, rickets is still a commonly recognized disease in Egypt. The factors responsible for occurrence of rickets in Egypt are the repeated poorly spaced pregnancies with lack of maternal vitamin D supplementation, the dusty atmosphere especially during winter and spring, the lack of health and nutritional education, the habit of excessive wrapping of infants and keeping them indoors without exposure to sunlight, poor housing, faulty weaning and the prevalence of gastroenteritis.

Vitamin D deficiency prevents the efficient absorption of dietary calcium and phosphorus. In a vitamin D deficient state, only 10-15% of dietary calcium and 50-60% of dietary phosphorus are absorbed. (Holick, 2005).

In this study, there were significant increases in the mean serum Ca and P levels after 2 weeks of therapeutic intervention, with vitamin D shock therapy and oral Ca supplementation, with further significant increase at 6 weeks. This could be attributed to increased Ca absorption. Vitamin D accomplishes this by interacting with its nuclear receptor, the Vitamin D receptor (VDR) in the small intestinal cells the 1, 25 (OH) 2 D-VDR structure complexes with retinoic acid X receptor (RXR) in the nucleus. The 1, 25 (OH) 2D-VDR-RXR complex binds to the vitamin D-responsive element (VDRE) for the epithelial calcium channel. The increased expression of the calcium channel permits more calcium to enter the cell, where the

vitamin D-dependent calcium-binding protein calbindin 9 k helps calcium's translocation into the blood stream. (Bouillon, 2001; Christakos *et al.*, 2003).

The decrease in serum calcium immediately recognized by the calcium sensor in the parathyroid glands, resulting in an increase in the expression, synthesis, secretion of parathyroid hormone. PTH decrease phosphorus reabsorption in the kidney causing loss of phosphorus into the urine, where increase of serum calcium level cause decrease in the secretion of parathyroid hormone with increase level of serum phosphorus (Holick, 2004). This coincides with present results as phosphorus level increased significantly after the intervention (vitamin D supplementation).

After therapeutic intervention, there was significant decrease of ALP level at 2 weeks with further significant decrease after 6 weeks ($p < 0.01$). This could be attributed to healing of rickets with bone mineralization and decrease of osteoblastic activity (Bacon *et al.*, 1990).

The mean serum Se level and the mean GPX enzyme activity of rachitic patients proved to be significantly lower compared to controls. In spite of the significant increase in both at 2 weeks after therapeutic intervention and the further significant increase at 6 weeks, yet, they were still significantly lower than controls.

The lower mean serum Se level in rachitic patients agrees with the results of Takada *et al.* (1992) who proved deficiency of trace elements in rachitic infants and Houssaini *et al.* (1997) who found a significant decrease of Se and other trace elements in malnourished patients compared to controls. The deficiency of Se in rachitic patients may be due to deficient intake of Se-rich foods as sea food, egg yolk, milk and meat which are usually deficient in the rachitogenic diet which includes mainly cereals and cow's milk that are deficient in Se (Alpers *et al.*, 1995). Another factor that may lead to Se deficiency in rickets is the defective Se absorption as a result of vitamin D deficiency. Mykkanen and Wasserman (1990) proved that vitamin D enhances uptake of selenite by the intestinal brush border membrane and that, vitamin D stimulated selenite uptake seems to be independent of the presence of phosphate and not influenced by the external sodium gradient and they found that, vitamin D had increased membrane bound SH-group either by change in the oxidation state of the SH-groups of the membrane proteins or by the transfer of the SH containing substances from the intracellular compartments to the brush border membrane. The covalent bonding between selenite and the membrane bound SH groups might well explain the increased uptake of Se by the brush border after vitamin D treatment. The effect of active vitamin D on absorption of Se is supported in this study by the presence of a significantly positive correlation between the serum levels of active vitamin D and that of Se in our patients. The significant increase in Se level after

therapeutic intervention in spite of lack of Se supplementation may be due to increased absorption as a result of vitamin D therapy.

It is well known that, GPX enzyme is made of 4 protein subunits, each of which contains one Se atom at its active site, thus Se is required for the enzyme activity and Se deficiency causes decreased activity (Sokol and Hoffenberg, 1996).

The significant decreased we found in the GPX activity could be explained on the basis of Se deficiency as supported by the significant positive correlation between the serum levels of Se and GPX activity. Present results showed increase in the activity of GPX with the increase of serum selenium level.

Karunasinghe *et al.* (2006) found a positive correlation between serum selenium and glutathion peroxidase activity in hemolysate, which coincide with our results.

Where EL-Khawaga (2005) stated that selenium enhances the endogenous antioxidant capacity of the cells by increasing the activities of superoxide dismutase, catalase, glutathion reductase and glutathion-transferase as well as increasing glutathion GSH.

In this study, 72.7% of rachitic patients had chest infection and 69.7% had gastroenteritis at the time of diagnosis. These percentage frequencies were significantly decreased to 15.2% for chest infection and 21.2% for gastroenteritis at 6 weeks after therapeutic intervention. The increased liability to infection in rickets is due to decrease of vitamin D.

It's now recognized that the kidney is not the only source of active vitamin D (1, 25 (OH) 2D, activated macrophages also express 25-hydroxy vitamin D-1 α -hydroxylase (CYP27B1) and thus produce 1, 25 (OH) 2D. Liu *et al.* (2006) reported that activation of TLRs with LPS resulted in the upregulation of expression of not only VDR but also the CYP27B1 gene. The local production of 1, 25 (OH) 2D induced expression of the antimicrobial peptide cathelicidin (LL-37), which is thought to be a key factor in the innate immune response when TLR is activated by any infective agent.

Selenium is one of the trace elements that is deficient in rachitic patients and its deficiency may lead to defective GPX enzyme activity with decreased antioxidant mechanisms and defective regulation of the biosynthesis of prostaglandins, leukotrienes and interferon's which may result in defective resistance to viral and bacterial infections (Kvicala, 1999). The role of selenium in the increased liability to infection in rachitic patients is greatly supported in our study by the presence of significantly lower mean serum Se level in patients having chest infection compared to those without and by the significant decrease in the percentage frequency of both chest infection and gastroenteritis at 6 weeks after therapeutic intervention concomitantly with the significant increase in the mean serum Se level.

From the course of this study, we can conclude that patients with active rickets have significant decrease in serum selenium levels which may be due to lack of absorption as a result of vitamin D deficiency or as a part of multiple nutritional deficiency syndromes. As consequence of this finding, glutathione peroxidase enzyme activity which is one of the important antioxidant enzyme systems is greatly reduced; that add on other factors that leads to the deficient immunity and liability to infection in cases of active rickets. Therefore, the study recommends not only supplementing vitamin D and Ca but we have to implement Se supplementation in the treatment of rickets so as to improve immune status of rachitic patients. This study also point out to further study of the pro-inflammatory cytokines in the ricketic patients and to evaluate the immune system in ricketic patients.

REFERENCES

- Alpers, D.H., W.F. Stenson and D.M. Ier, 1995. Minerals and Trace Elements. In: Manual of Nutritional Therapeutics Alpers, D.H., W.F. Stenson and D.M. Bier (Eds.), 3rd Edn., Little, Brown and Company, Boston, New York, Tronto, London, pp: 232-262.
- Awaad, S., A.S. Khalifa, M. Abul-Baga, K.A. Taoiba and R. Faris, 1975. A field survey in child health in rural community. *J. Trop. Med. Hyg.*, 178: 20.
- Bacon, G.E., M.L. Spence., N.J. Hopwood and R.P. Kelch, 1990. Vitamin D Deficiency Rickets. In: A practical Approach to Pediatrics Endocrinology. Bacon, G.E. (Ed.), 3rd Edn. Year Book Medical Publisher, Chicago, London, P: 253.
- Bouillon, R., 2001. Vitamin D: from Photosynthesis, Metabolism and Action to Clinical Applications. In: Endocrinology. DeGroot, L.J. and J.L. Jameson, (Eds.), W.B. Saunders. Philadelphia, Pennsylvania, USA., 1009: 1028.
- Christakos, S., P. Dhawan, Y. Liu, X. Peng and A. Porta, 2003. New insights into the mechanisms of vitamin D action. *J. Cell. Biochem.*, 88: 695-705.
- Chwan-Li, S., S. Woosun and C.P. Barbara, 2001. Interactions of selenium compounds with other antioxidants in DNA damage and apoptosis in human normal keratinocytes. *Cancer Epidemiol. Biomark. Preven.*, 10: 385-390.
- David, H.V. and I. Zafar, 1990. Alkaline Phosphatase. In: Clinical Methods. Keneth, H., W. Dalla and J. Willis (Eds.), 3rd Edn., Boston, London, Sydney, Singapore, Toronto and Wellington, 494-497.
- El-Bishlawy, A., N. Omaran, L. Ragab and M. El-Tobgi, 1992. Vitamin D metabolism and anemia in Egyptian children. *J. Pediatr.*, 119: 911-914.
- El-Khawaga, O.A., 2005. Role of selenium on antioxidant capacity in methomyl-treated mice. *J. Physiol. Biochem. Dec.*, 61: 501-6.
- Houssaini, E.A., M.R. Iraqi, J. Arnoud, M.J. Richara and A. Favier, 1997. Trace elements and protein calorie malnutrition in the fes area (Moroco). *Biol. Med. Pharmacotherap*, 51: 349-51.
- Holick, M.F., 2004. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease and osteoporosis. *Am. J. Clin. Nutr.*, 79: 362-371.
- Holick, M.F., 2005. Vitamin D. In: Modern Nutrition in Health and Disease. 10th Edn., Shils, M. *et al.* (Ed.), Lippincott Williams and Wilkins. Baltimore, Maryland, USA., pp: 329-345.
- Jacob, F., H. Mork, N. Schertze, I. Dreber, C. Schmutzler, B. Lex and J. Kohrle, 1997. Selenoproteins in bone, gastrointestinal tract and thyroid gland of the human. *Med- Klin.* 92 Suppl: 24-6.
- Karunasinghe, N., L.R. Ferguson, J. Tuckey and J. Masters, 2006. Hemolysate thioredoxin reductase and glutathione peroxidase activities correlate with serum selenium in a group of New Zealand men at high prostate cancer risk. *J. Nutr.*, 136: 2232-5.
- Kvicala, J., 1999. Selenium and the organism. *Cas. Lek. Cesk.*, 22: 99-106.
- Liu, P.T., *et al.*, 2006. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science*, 3: 1770-1773.
- Mughal, M.Z., H. Salama, T. Greenaway, I. Laing and E.B. Mawer, 1999. Florid rickets associated with prolonged breast feeding without vitamin D supplementation. *BMJ*, 318: 39-40.
- Muhe, L., S. Lu seged, K.E. Mason and E.A.F. Simoes, 1997. Case-control study of the role of nutritional rickets in the risk of developing pneumonia in Ethiopian children. *Lancet*, 349: 1801-4.
- Mykkanen, H.M. and R.H. Wasserman, 1990. Relationship of membrane bound sulfhydryl groups to vitamin D-stimulated uptake of (⁷⁵Se) selenite by the brush borer membrane vesicles from chick duodenum. *J. Nutr.*, 120: 882-888.
- Park, J.E. and K. Park, 1979. Textbook of preventive and social medicine. 7th Edn., Hessers Barner side. Ph Riblisher, 1268 Napier Town, pp: 81.
- Sokol, R.J. and E.J. Hoffenberg, 1996. Antioxidants in pediatric gastrointestinal disease. *Pediatric Clin. N. Am.*, 43: 471-484.
- Takada, M., M. Shimada, S. Hoson, M. Tauchi, M. Minato, S. Takahashi, M. Okuni and S. Takeuchi, 1992. Trace elements and mineral requirements for very low birth weight infants in rickets of prematurity. *Early Hum. Dev.*, 2: 333-8.
- Tom, D.T., R.F. Philip, M.P. John, O.L. Juliana, O.I. Christian, C.R. James and M.C. Gary, 1999. A comparison of calcium, vitamin D or both for nutritional rickets in Nigerian children. *N. Eng. J. Med.*, 341: 563-568.