Antioxidants and Pancreatic β-cell Function in Malnourished Infants: A Causal Relationship.

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Abstract:

The study was carried out on 30 infants between 6 and 24 months of age. They were equally divided into age and sex matched three groups of normally growing infants, marasmus, and kwashiorkor cases. Fasting levels of blood glucose, C-peptide, zinc, and vitamins A, E, and C were estimated. Blood glucose and C-peptide levels were serially estimated ½ and 2 hours after IV administration of 10 ml/kg of glucose 10% given as a bolus.

None of the malnourished cases developed fasting hypoglycemia. Malnourished infants showed a postprandial diabetic- like curve after IV glucose challenge test concomitant with inappropriate increase in C- peptide secretion. They had also a significantly lower C- peptide /glucose ratio than the normally growing infants. Malnourished cases had a lower serum levels of zinc, vitamins A, E, and C. The glucose intolerance- manifested by low C-peptide /glucose ratio- was more evident among kwashiorkor cases who had also significantly lower levels of serum zinc and vitamin A than cases of marasmus. We suggested an association between glucose intolerance, poor βcell response to IV glucose challenge test and the low serum levels of the antioxidants- zinc, vitamins A, E, and C among malnourished infants especially kwashiorkor cases that showed significantly lower results.

Introduction:

Although much information is available on the effect of protein-energy malnutrition (PEM) on various metabolic processes, data presenting its influence on carbohydrate metabolism are scanty and contradictory. The exact cause of hypoglycemia, which had been encountered in malnourished children, is still unclear. Is it-impaired gluconeogenesis? Delayed insulin clearance? Defective pancreatic beta(β)- cell function ? Or is it all of these factors acting together in harmony?

Endocrines had been reported to be greatly affected in PEM including high serum levels of growth hormone and cortisol, and low level of circulating insulin.[1,2] Ultrastructural study of islands of Langerhans in childhood PEM showed variable degrees of degeneration of all cell types with membrane damage, loss of ribosomes, vesiculation and mitochondrial swelling. These destructive β-cell changes may be related to chronic hypoproteinemia and/or free radical damage and possibly accounting for low serum insulin levels reported by many workers.[3] However, other investigators reported normal insulin secretion and suggested insulin resistance by insulin antagonists especially in kwashiorkor.[4] C-peptide estimation in the peripheral blood is an accurate measure of insulin secretion from the pancreatic β-cells as both; C-peptide and insulin are secreted in an equimolar amount under different circumstances. Also, while the liver uptake of insulin is considerable, C-peptide uptake by the liver is negligible.[5]

This work was aiming at: [1]. The assessment of β-cell function in the malnourished children by measuring serum C-peptide levels before and after IV glucose challenge test; and [2]. The estimation of serum levels of zinc, vitamins A & E and ascorbic acid.

Subjects and methods:

The study was carried out on 30 infants between 6 and 24 months of age. They were equally divided into age and sex matched three groups (healthy controls, marasmus and kwashiorkor) All the infants were subjected to:

1. Clinical examination which revealed the presence of mild to moderate infections in nine of the malnourished cases in the form of thrush stomatitis, gastroenteritis, and bronchitis. They received the proper care and improved.
2. Laboratory investigations including fasting levels of: (a). Blood glucose (BGL) by oxidase test; (b). Blood C-peptide by radio-immunoassay.[6]; (c). Serum zinc by absorption spectrophotometer.[7]; (d). Serum vitamins A&E by fluorometric micromethod.[8]; (e). Plasma ascorbic acid by 2,6 Dichlorophenol indol phenol titration.[8].

Blood glucose and C- peptide levels were serially estimated ½ and 2 hours after infusion of 10 ml/ kgm body weight of glucose 10%- given as a bolus.

Results:

- None of the malnourished cases developed fasting hypoglycemia (Blood glucose <45mg/dl). No significant difference between healthy controls and malnourished infants in respect to the fasting blood glucose and C- peptide levels. Blood glucose levels were significantly higher in malnourished than control infants at ½ and 2 hours after the glucose challenge test and Cases of marasmus had the highest levels. C-peptide levels were significantly lower among the malnourished than the control infants. The lowest levels were reported in kwashiorkor cases ½ hour after the challenge test and in cases of marasmus 2 hours after the challenge test.(tables I, II)
- Malnourished infants showed a postprandial diabetic- like curve after IV glucose challenge test concomitant with inappropriate increase in C-peptide secretion. (figures 1,2)
- Malnourished infants had a significantly lower C-peptide/glucose ratio in the fasting state and after glucose challenge test than control infants.(Table III)
- Malnourished cases had significantly lower serum levels of zinc vitamins A,E, and ascorbic acid in comparison to the healthy control infants (Table IV)
- The glucose intolerance- manifested by low C-peptide /glucose ratio- was more evident among kwashiorkor cases who had also significantly lower levels of serum zinc and vitamin A than cases of marasmus.(tables III,IV).

Table I: Blood glucose levels (mg/dl) at different time intervals.

	Fasting BGL Mean ± SD	BGL - ½ hr after G challenge test Mean ± SD	BGL - 2hrs after G challenge test Mean ± SD	t of difference between fasting & ½ hr BGL	t of difference between½ & 2 hrs BGL
Control (n=10)	64.2 ± 1.6	83.9±3.4	65.4±1.4	5.30*	5.05*
Marasmus (n=10)	70.1±4.2	142±18.8	111±16.1	3.74*	1.25
Kwashiorkor (n=10)	65±1.9	128.1±7.2	87.9±4.4	8.51*	4.80*
F test values & sites of significance	19.35 ---	50.65* (1,2) (1,3) (2,3)	23.95* (1,2) (1,3) (2,3)		

**Significant at 5% level.*

Table II: Serum C-peptide levels at different time intervals.

	Fasting C-peptide X±SD	C-peptide- ½ hr after G challenge test X±SD	C-peptide -2 hrs after G challenge test X±SD	t of difference between fasting & ½ hr C-peptide levels	t of difference between ½ &2 hrs C-peptide levels
Control (n=10)	1.81±0.12	2.6±0.11	1.63±0.17	4.82*	7.70*
Marasmus (n=10)	0.63±0.14	1.55±0.49	0.54±0.54	1.84	2.10*
Kwashiorkor (n=10)	0.51±0.18	0.73±0.31	1.16±0.30	1.05	1.79
F test values & sites of significance	3.68	16.58* (1,3) (2,3)	86.25* (1,2) (1,3) (2,3)		

**Significant at 5% level.*

Table III: Comparison between C-peptide (ug/dl) / blood glucose (mg/dl) ratio among the studied groups(10^{-3})

	Control (n=10)	Marasmus (n=10)	Kwashiorkor(n=10)	F test values
C-peptide/G ratio - at fasting g ± SD	0.027±0.005	0.005±0.009	0.005±0.008	16.001* (1,2) (1,3)
C-peptide/G ratio -½ hr after G challenge test g± SD	0.031±0.005	0.007±0.018	0.003±0.005	15.20* (1,2) (1,3) (2,3)
C-peptide/G ratio - 2 hr after G challenge test g± SD	0.025±0.004	0.003±0.003	0.009±0.008	16.36* (1,2) (1,3) (2,3)

*g: Geometric mean *: Significant at 5% level*

Table IV: Comparison between the mean values of serum zinc, vitamins A&E and ascorbic acid in malnourished and healthy control infants

	Control (n=10)	Marasmus (n=10)	Kwashiorkor (n=10)	F test values
Serum zinc (ug/dl): Mean±SD	99.01±18.01	50.23±16.42	27.32±12.56	5.36* (1,2) (1,3) (2,3)
Serum vitamin A (mg/dl): Mean ±SD	24.82±3.56	15.91±4.22	8.78±5.42	4.15* (1,2) (1,3) (2,3)
Serum vitamin E (mg/dl): Mean ±SD	1.91±0.78	0.97±0.42	0.49±0.42	2.1* (1,2) (1,3)
Serum Ascorbic acid(mg/dl): Mean±SD	6.01±0.82	2.91±0.83	2.88±0.94	3.12* (1,2) (1,3)

** Significant at 5% level*

Fig 1. The mean values of blood glucose in normal, marasmus & kwashiorkor at fasting & after challenge tests (* = Significant at 5 % level)

Fig 2: Mean values of C-Peptide levels in the fasting state & after the challenge tests (*=significant at 5%)

Discussion:

The insignificantly different fasting BGL among control and malnourished infants was in agreement with the results of many workers. [1,2] Also, the development of hypoglycemia in none of our malnourished -even kwashiorkor cases - was in accordance to the report that hypoglycemia can be seen only in terminal cases with severe long standing course of illness. [9] On the other hand, some investigators reported low fasting BGL and more frequent hypoglycemia among malnourished cases when compared to normal controls. [10] The failure of blood glucose to reach the basal level -2 hours after the IV glucose challenge test- and the impairment of C- peptide secretion in response to glucose stimulation demonstrated some degree of glucose intolerance most probably caused by inadequate response of pancreatic β cells to the increased blood glucose. This was in harmony with the reports of investigators who found sluggish response of serum insulin after glucose or arginine stimulation.[4] Some investigators had been attributed such glucose intolerance to poor insulin release as a result of pancreatic endocrinal insufficiency secondary to deficiency of β cells cytotrophic. [11]

The impaired glucose tolerance observed in kwashiorkor cases was explained by poor functional response of pancreatic β cells due to diminished rate of protein synthesis secondary to amino acids deficiency.[12] In addition to the poor initial response of βcells, kwashiorkor cases showed a sustained low C- peptide secretion after the IV glucose challenge test, suggesting presence of insulin antagonism on top of sluggish inadequate response of β-cells. In fact, this finding is compatible with the presence of insulin antagonists such as growth hormone and cortisol, being generally elevated among cases of PEM. [4] The pancreatic dysfunction observed in the malnourished cases, in addition to the poor C-peptide secretion, was also manifested by low C-peptide /glucose ratio after IV glucose challenge test. This was in agreement with other workers who reported low insulin/glucose ratio in cases of kwashiorkor at different circumstances. [4,11]

The demonstrated lower serum zinc levels among malnourished especially kwashiorkor cases when compared to control infants was in agreement with Golden and Ramadath [13] who found lower plasma zinc concentration in association with nutritional edema. As well as having catalytic, structural and regulatory roles in enzymes that participate in the metabolism of carbohydrate, protein, lipids and nucleic acids, zinc has also been found to have regulatory functions in the binding of insulin to the cell membrane. In humans, Zinc is also associated with presecretory insulin hormones in the pancreatic β-cells, and its repletion in patients with total parenteral nutrition is associated with increased insulin secretion. [13,14,15] The estimated significantly lower levels of vitamin A&E and ascorbic acid among the malnourished cases was in harmony with the proposal that uncontrolled oxidative damage to tissue components by oxygen derived free radicals may be an important factor in the etiology of kwashiorkor and that certain vitamins particularly carotenoids and vitamins E&C may play a protective role. [13,16]

We assume that there is a considerable association between glucose intolerance, poor βcell response to glucose challenge and low serum levels of zinc, vitamin A, E and C observed in the studied malnourished especially kwashiorkor cases. In support of this view, among experimental animals, which were maintained on low protein high carbohydrate contents, plasma insulin levels were low[12] . Also, Brooks et al [3] suggested that the islet cell changes in PEM may be related to free radical damages secondary to depletion of glutathione and other antioxidants, as well as relative deficiency of zinc. They added that the effects of Agonal anoxia and a short fixation delay after death must be considered. [3,17] However, this suggestion needs reassessment of βcell function after dietary rehabilitation and supplementation of the possibly claimed deficient vitamins and trace elements. The next few years will undoubtedly present a clarification of free radical -nutrient and tissue interactions, and may help to put the concept on a sounder footing.

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