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STUDY OF GRWOTH HORMONE AND CORTISOL IN SEVERE ACUTE MALNUTRITION

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ABSTRACT

Development of local therapeutic nutritional intervention for malnourished children is recommended by WHO. Present study was designed to assess the efficacy of the study nutritional intervention in terms of hormone levels before and after the Nutritional Intervention Treatment (NIT) and to find correlation of age with growth hormone in malnourished children. This was Open label prospective parallel group active comparator interventional study,105 Study and 100 control SAM(Severe Acute malnutrition)children of 1 to 5 years of age and either sex were randomly enrolled. Study group was given NIT, providing 2.5 to 3gm Protein and 90-100 kcal/kg body weight/day for three months. Serum growth hormone, cortisol, total protein and albumin levels of both groups were estimated before and after the NIT. Before NIT **P** value for Growth Hormone and Cortisol were insignificant and after NIT both were significant**.** Correlation of Growth Hormone with Age was non significant and poor negative at **P**= 0.057 and pearson correlation coefficient **r** = -0.186 The investigators conclude that study nutritional intervention is the effective food supplement for the recovery of impaired harmones status in SAM children and growth harmone has nonsificant poor negative correlations with age.

KEYWORDS: Growth hormone, Cortisol, SAM, Correlations.

INTRODUCTION

Chronic child malnutrition is an ongoing, permanent emergency affecting 170 million children.[1] The children of India under the age of five are 48% stunted indicative of chronically malnourished. One out of five children in India is wasted as 19.8% of wasted children under five years are noticed in the country. Children under age five years are underweight for their age are 43%.[2,3] WHO have identified nine priorities in nutrition research, one of them is -The development of a sustainable community based nutrition intervention model [4] Considering the above vision of WHO it was noted that a malnourished children of study area genuinely required; a supply of need base therapeutic diet- to overcome the high prevalence and recurrence of malnutrition as well as to protect the children from opportunistic infections which usually due to low immunity. Their endocrinological study was also needed to reveal its role behind development of malnutrition. Therefore present study has been designed to monitor the serum Cortisol, Growth Hormone levels before and after the nutritional intervention treatment to SAM children, to assess the efficacy of the study nutrition intervention.

MATERIALS

1) **Cortisol kit:** ***Reference :*** **Product code:** LKCO1 : **Company:** Siemens.

Method: a solid- phase, two site chemiluminescent immunometric assay.[5]

Material supplied with the kit

- **Cortisol test Units:** Each barcode-labeled unit contains one bead coated with polyclonal rabbit anti cortisol antibody.
- **Cortisol reagent wedge :** One wedge with barcode,7.5 mL alkaline phosphtase(bovine calf intestine)conjugated to cortisol in buffer, with preservative.
- **Cortisol adjustors :** Two vials(Low and High) 3 mL each ,of cortisol in processed human serum, with preservative.[5]

2) **Growth Hormone kit:** ***Reference*****:** Product code : *LKGRH1*: **Company:** Siemens

Method: a solid- phase, two site chemiluminescent immunometric assay.[6]

Material supplied with the kit

- **GH test Units:** Each barcode-labeled unit contains one bead coated with murine monoclonal anti hGH antibody.
- **GH reagent wedge :** One wedge,7.5 mL alkaline phosphtase(bovine calf intestine)

conjugated to rabbit polyclonal anti-hGH antibody in buffer, with preservative.

- **GH adjustors:** Two vials(Low and High)containing lyophilized hGH in nonhuman serum, with preservative. Reconstitute each vial with 3mLdeionized water.[6]

3) Serum Total Protein kit : Company: Span Diagnostics Ltd., Surat, Gujarat.

Method: Modified Biuret, End point assay[7]

Material supplied with the kit: Biuret Reagent **;** Protein standard: 6.5 g/dL[7]

4) Albumin test kit: Company: Span diagnostics,

Method: Bromocresol Green,End point assay.[8]

Material supplied with the kit: i) Albumin reagent: 1x100 mL.,

ii) Albumin standard. 4 g/dL[8]

METHODOLOGY

From each test and control subjects morning fasting blood samples were collected in well labeled; plain vaccutainers,-such kind of blood collection was done at two different periods-first; at the time of enrollment and second; after three month's nutritional intervention treatment. All blood samples in plain vaccutainer were centrifuged within 1 hr to obtain serum. Estimation of Growth Hormone and cortisol were done on chemiluminescence machine-immulite-1000. Serum total protein and albumin were estimated on fully automated analyzer Olympus-AU-400 machine. Instructions provided by manufacturer in the all kits were followed. This was Open label prospective parallel group active comparator interventional study,105 Study and 100 control SAM children of 1 to 5 years of age and either sex were randomly enrolled. Study group was given NIT, providing 2.5 to 3gm Protein and 90-100 kcal/kg body weight/day for three months.

STATISTICAL ANALYSIS

Data was subjected to analysis by using SPSS S/W version -16 for variance, and differences were identified by Mean, S.D., S.E., 95 % C.I. P-value was obtained, $P< 0.05$ considered significant difference, $p < 0.000$ considered highly significant difference.

RESULTS

Table 1: Descriptive statistics of baseline characteristics Before treatment in study and control group.

Baseline chararcteristics	Groups	N	Mean	Std. Deviation	Std. Error Mean
Protein (T) Gm%	Study group	105	4.30	0.32	0.03
	Control group	100	4.30	0.33	0.03
Albumine (A) Gm%	Study group	105	2.54	0.22	0.02
	Control group	100	2.53	0.22	0.02
Globuline (G) Gm%	Study group	105	1.74	0.26	0.03
	Control group	100	1.75	0.27	0.03
Cortisol (morng) µg/dL	Study group	105	37.04	6.29	0.61
	Control group	100	37.60	5.10	0.51
Growth Hormon ng/mL	Study group	105	15.73	3.01	0.29
	Control group	100	15.84	2.99	0.30

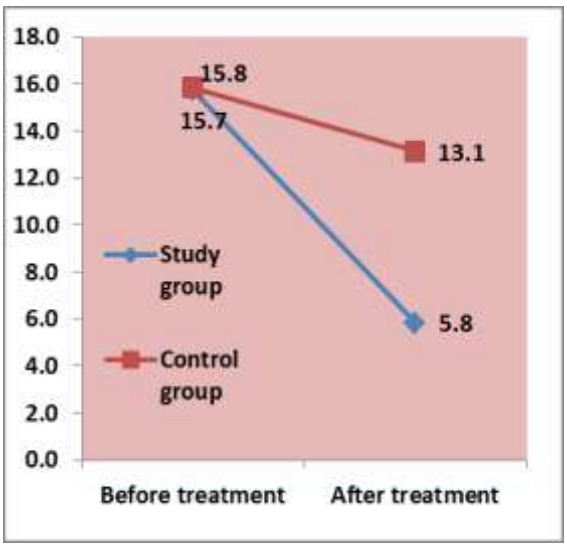

Figure – 1 Growth Hormon ng/mL
For total subjects (male&Female)

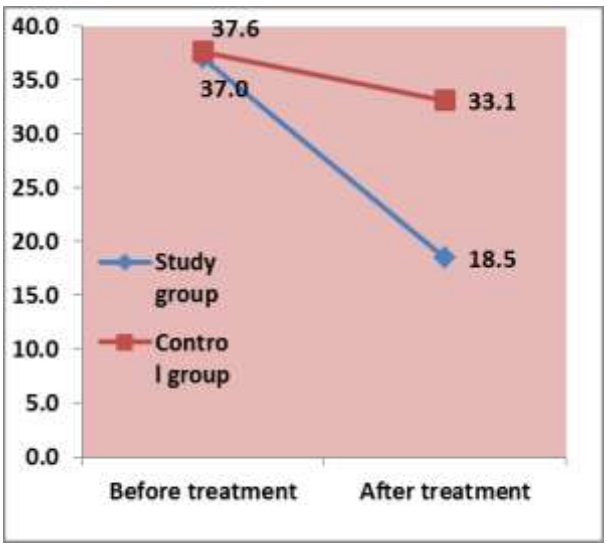

Figure - 2 Cortisol (morng) µg/dL
For total subjects(male & Female)

Table 2: Independent sample test for Before treatment in study and control group

Unpaired t-test for Equality of Means						95% C I of the Difference	
Baseline chararcteristics	Mean Difference	Std. Error Difference	t test value	df	p value	Lower	Upper
Protein (T) Gm%	0.003	0.045	0.063	203	0.950 (NS)	-0.087	0.092
Albumine (A) Gm%	0.007	0.030	0.234	203	0.816 (NS)	-0.052	0.067
Globuline (G) Gm%	-0.004	0.037	-0.113	203	0.910 (NS)	-0.077	0.069
Cortisol (morng) µg/dL	-0.558	0.802	-0.696	203	0.487 (NS)	-2.138	1.023
Growth Hormon ng/mL	-0.109	0.420	-0.261	203	0.794 (NS)	-0.937	0.718

Table 3: Descriptive statistics of baseline characteristics After treatment in study and control group.

Baseline chararcteristics	Groups	N	Mean	Std. Deviation	Std. Error Mean
Protein (T) Gm%	Study group	105	6.904	0.519	0.051
	Control group	100	4.805	0.287	0.029
Albumine (A) Gm%	Study group	105	4.117	0.487	0.048
	Control group	100	2.804	0.191	0.019
Globuline (G) Gm%	Study group	105	2.700	0.207	0.020
	Control group	100	1.930	0.259	0.026
Cortisol (morng) µg/dL	Study group	105	18.508	4.656	0.454
	Control group	100	33.100	4.705	0.471
Growth Hormon ng/mL	Study group	105	5.840	2.491	0.243
	Control group	100	13.130	2.482	0.248

Equal variances assumed

Table 4: Independent sample test for After treatment in study and control group.

Unpaired t-test for Equality of Means						95% C I of the Difference	
Baseline chararcteristics	**Mean Difference**	**Std. Error Difference**	**t test value**	**df**	**p value**	**Lower**	**Upper**
Protein (T) Gm%	2.119	0.059	35.954	203	**0.0001**	2.003	2.235
Albumine (A) Gm%	1.323	0.052	25.386	203	**0.0001**	1.220	1.426
Globuline (G) Gm%	0.807	0.033	24.759	203	**0.0001**	0.743	0.872
Cholesterol mg%	37.765	1.303	28.992	203	**0.0001**	35.197	40.333
Triglyceride mg/dL	31.883	1.037	30.746	203	**0.0001**	29.839	33.928
Cortisol (morng) µg/dL	-14.617	0.654	-22.352	203	**0.0001**	-15.906	-13.328
Growth Hormon ng/mL	-7.288	0.347	-20.978	203	**0.0001**	-7.973	-6.603

Table 5: Descriptive statistics for gender (Before treatment) in both groups.

Before treatment		Study group (N=105)			Control group (N=100)		
Characteristics	**Gender**	**N (Sample size of Gender)**	**Mean**	**Std. Deviation**	**N (Sample size of Gender)**	**Mean**	**Std. Deviation**
Growth Hormone ng/mL	Male	42	12.86	1.49	46	16.174	3.0341
	Female	63	17.64	2.12	54	15.552	2.9535

Table 6: Comparison in gender (Before treatment) for their characteristics in both groups.

Study group (Before treatment) (N=105)							
Characteristics	**Mean Difference**	**Std. Error Difference**	**t test value**	**df**	**p value**	**95% CI Lower**	**95% CI Upper**
Growth Hormone ng/mL	-4.778	0.377	-12.673	103	0.0001 (S)	-5.525	-4.03

Control group (**Before** treatment) (N=100)							
Characteristics	Mean Difference	Std. Error Difference	t test value	df	p value	95% CI Lower	95% CI Upper
Growth Hormone ng/mL	0.6221	0.6001	1.037	98	0.302 (NS)	-0.5688	1.8129

Table 7: Descriptive statistics for gender (After treatment) in both groups.

After treatment		**Study group (N=105)**			**Control group (N=100)**		
Characteristics	**Gender**	**N (Sample size of Gender)**	**Mean**	**Std. Deviation**	**N (Sample size of Gender)**	**Mean**	**Std. Deviation**
Growth Hormone ng/mL	Male	42	2.89	0.82	40	13.08	2.52
	Female	63	7.81	0.37	60	13.17	2.48

Table 8: Comparison in gender (After treatment) for their characteristics in both groups.

Study group (After treatment) (N=105)							
Characteristics	**Mean Difference**	**Std. Error Difference**	**t test value**	**df**	**p value**	**95% CI Lower**	**95% CI Upper**
Growth Hormone ng/mL	-4.915	0.118	-41.565	103	0.0001 (S)	-5.150	-4.681
Control group (After treatment) (N=100)							
Characteristics	**Mean Difference**	**Std. Error Difference**	**t test value**	**df**	**p value**	**95% CI Lower**	**95% CI Upper**
Growth Hormone ng/mL	-0.0917	0.5092	-0.18	98	0.857	-1.1021	0.9187

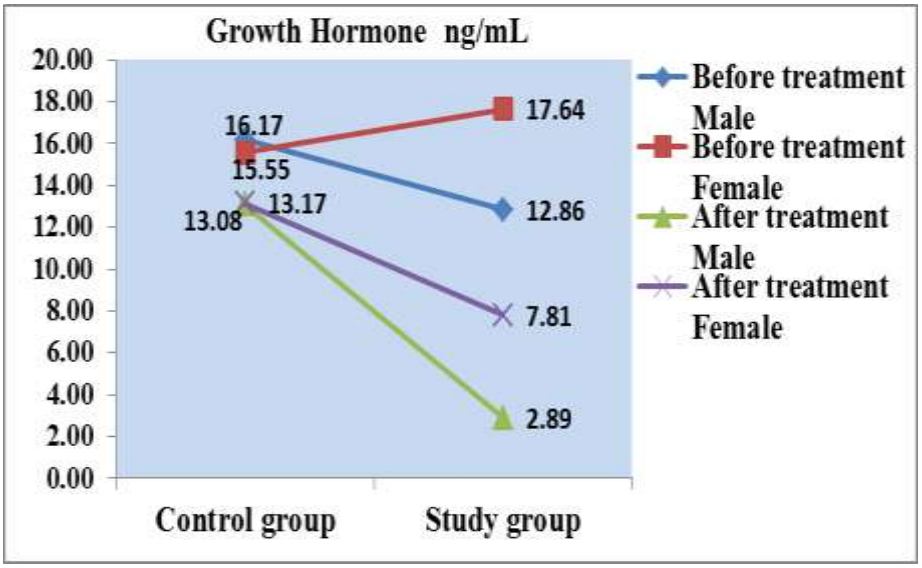

Figure 3: Growth Hormon (ng/mL) in male and Female.

DISCUSSION

Cortisol

In the metabolism of, lipids, proteins and carbohydrates Cortisol plays an important role. It helps to maintain blood pressure, also helps to regulate the immune system and affects blood glucose levels. Only a small percentage of cortisol is "free" and biologically active while most of the cortisol in the blood is bound to a protein. Free cortisol is present in the saliva and is excreted into the urine.[9] In a "diurnal variation" pattern the cortisol level in the blood rises and falls. In the early morning it peaks, while at midnight shows its lowest level, and throughout the day declines. When a disease either stimulates or limits production of cortisol then it can become disrupted.[10] The adrenal glands, produce and secretes Cortisol. The hypothalamus in the brain and by the pituitary gland production of the hormone is regulated. Corticotropin-releasing hormone (CRH) released by the hypothalamus when the blood cortisol level falls, by which the pituitary gland stimulates to produce ACTH (adrenocorticotropic hormone).[11]

The adrenal glands stimulated by ACTH to produce cortisol. The hypothalamus and both the adrenal glands and pituitary must be properly functioning ,then the cortisol is produced in appropriate amounts.Cortisol concentrations can influence cold, heat, trauma, infection, exercise, obesity,and debilitating disease. cortisol levels can be increased by, emotional and physical stress, Pregnancy and illness can increase[9]Adaptation to deficient nutrition depends primarily on the endocrine control of the metabolic processes involved. Kwashiorkor and marasmus have been described as forms of failure of adaptation and successful adaptation to protein energy malnutrition, respectively.[9,10] Failure of adaptation involves the inability of hormones to maintain normal metabolism because the malnutrition is too severe or the individual is physiologically unable to adapt to the dietary deficiency. Hormonal changes, such as increases in cortisol and growth hormone,have been reported by many workers[12,13,14] Some workers[12] describe the increase in plasma cortisol to the general stress of malnutrition, whereas others attribute it to infection. In both kwashiorkor and marasmus kinds of malnutrition, raised serum cortisol levels have been reported [14] Our intention in this study was to correlate serum cortisol levels in PEM, on admission and after treatment, with the absence of additional stresses resulting from complications such as infection. The proposed role of cortisol and Growth hormones in metabolic adaptation to malnutrition is also studied. Protein calorie malnutrition is well known to be multifactorial in its etiology, and it is suggested that variations in serum cortisol levels in this spectrum of conditions is

merely a biochemical marker of this multiple etiology, including infective processes, and of complications such as hypoglycemia.[12]

Following results were noted in the present study: Both groups had similar cortisol (p = 0.487) before nutritional intervention. Significant improvement in cortisol (p=0.0001) in study group as compared to control group was observed after nutritional intervention treatment. These results are in accordance with previous workers[12,14,15] These findings suggest that the increased catabolic action mediated by the high cortisol levels during nutritional deprivation allow proper breakdown of muscle protein necessary to provide the liver with the amino acids for gluconeogenesis and protein synthesis.[16] It was observed by previous workers that physiologically high levels of circulating cortisol have been shown to inhibit the stimulatory action of IGF-I on epiphyseal cartilage, and thus the growth of epiphyseal cartilage is impared during the period of malnutrition.[15]Adrenal function in protein energy malnutrition has been the subject of investigation for many years. Owing to contradictory reports between histological studies [10]and determinations of urinary steroid excretion,[11,12,13]

It was concluded that urinary excretion may be an unreliable guide to adrenal activity[10,12] and that the plasma cortisol concentration should be used. In general, raised concentrations of plasma cortisol in protein energy malnutrition, regardless of type have been reported,[16,14] although there have been conflicting results of adrenal activity being generally low in kwashiorkor, but high in marasmus:"[15,17] High adrenal activity may be essential for adaptation to protein energy malnutrition and that the main difference between the etiology of marasmus (successful adaptation) and kwashiorkor (failure of adaptation) is not of dietary origin, but depends mainly on the ability of the adrenal cortex to respond adequately to maintain metabolic integrity.[10,11,12] The plasma cortisol concentration is elevated in both types of protein energy malnutrition.The significant increase in cortisol levels of children with fatal protein energy malnutrition above those of surviving children, also makes it most unlikely for the magnitude of the adrenal response to be valid as an indication of successful adaptation.[18,11]

Moreover, high correlation was found between plasma cortisol concentration and the severity of the clinical features. The body mass deficit is correlated with cortisol concentration which is more elevated in kwashiorkor, the type of protein energy malnutrition that represents maladaptation.[14,19] Cortisol concentration is higher in kwashiorkor than in marasmus, and

still higher when the disease is fatal. It was also found that cortisol in experimental protein energy malnutrition (in pigs) was related to the clinical severity of the syndrome.[11,14,18]

Therefore, cortisol concentration is not so much an indication of an adaptive response, but imply represents the severity of the stress of protein energy malnutrition, In agreement with[20,21] the highest cortisol concentrations were found in children with high body mass deficits, and more so in children with kwashiorkor.[12,14]

Growth Hormone

Human Growth Hormone-HGH, Somatotropin are the other names of Growth Harmone. Formal name: Growth Hormone. By the action of hypophysis gland it is produced, while peptides known as somatomedins mediates it, of which the most important is the insulin-like growth factor I (IGF-1), also known as somatomedin-c and produced in the liver by the incentive of growth hormone GH.[22] Although Smith IF et.al. have reported increased GH levels and reduced Somatomedin-c levels in severely malnourished children these conditions caused by either a deficiency or overproduction of growth hormone(GH) and due to this reason growth of malnourished children was not adequate. To monitor the effectiveness of treatment for excess production of GH and to evaluate pituitary function GH test is used,[22] Insufficient GH production related to delayed maturational development, decreased bone density and muscle strength, and increased lipids. Excess amount of growth hormone in the blood may result to gigantism (in children) or acromegaly (in adults)[17,22] pituitary gland produces GH hormone. It is normally secreted into the bloodstream and its peak level is at night.[22] for a child's normal growth and development Growth hormone is essential from birth, it promotes proper linear bone growth. When GH production is insufficient children are smaller in size for their age and grow more slowly.[19,22] Gigantism with height of 7 or more feet tall results due to increased GH. GH play a role in regulating muscle mass, bone density, and lipid metabolism although it is not active in adults. Deficiencies can results to less muscle mass, altered lipid levels, decreased bone densities.[19, 23, 24]

In this study, elevated serum fasting GH levels have been noted before the nutritional intervention therapy and after the administration of therapy the levels were found to be normal. Both groups had similar GH ($p = 0.794$) levels before nutritional intervention. Significant improvement in GH ($p = 0.0001$) in study group as compared to control group was observed after nutritional intervention treatment.(Fig.1 Table.2,4)

GH status by Gender

Study Group: After comparison of the genders in study group before nutritional intervention treatment both gender had significant difference in GH status (P=0.0001).While after nutritional intervention treatment in study group both gender also had significant difference in GH status (p=0.0001). (Fig.3 ,Table.6,8)

Control Group: After comparison of the genders in control group before nutritional intervention treatment period both gender had non-significant difference in GH status (P=0.302).While after the treatment period, in control group both gender had non significant difference in GH status (p = 0.857). (Fig.2 ,Table.2,4)

Extensively basal GH levels have been studied in PEM.[22] In children with kwashiorkor despite impaired growth rates ,GH concentrations are elevated. The results are conflicting in marasmic children with of high and low GH levels[24,25] High GH and low IGF levels mediates effective lipolysis, which is adaptive mechanism of fuel (fatty acids) supply during nutritional deprivation for metabolism of peripheral tissues and brain.[26] High basal GH levels may indicate that their pituitaries may be near maximally stimulated in the basal state. The negative correlation between GH and cross-sectional fat are in agreement with the hypothesis that high GH levels in the presence of low somatomedin concentrations is an important adaptive mechanism to provide the organism with fuel (fatty acids), through lipolysis, during nutritional deprivation [23,26].Various alterations in hormonal levels ,act to defend the body against PEM. The elevated GH levels stimulate lipolysis, whereas the low IGF-I concentrations and impaired insulin secretion inhibit lipogenesis. Both processes assure fuel supply (fatty acids) to the brain and peripheral tissues through effective breakdown of adipose tissue.[19,26] Moreover, the increased muscle protein catabolism mediated by elevated basal cortisol levels and depressed anabolic activity due to the selective decrease in somatomedin levels assure an adequate supply of amino acids to the liver. This metabolic intermediate allows gluconeogenesis and protein synthesis to proceed and guards against the development of hypoglycemia and hypoalbuminemia in children with PEM. Thus, there is coordinated regulation of metabolism in PEM to defend the organism against hypoglycemia and to provide alternate substrates to sub serve the essential energy and biosynthetic requirements.[19,26,24,27] The increase in growth hormone is also related to changes in plasma amino acid concentration:' particularly alanine and valine.[26] The maladaptation to protein energy malnutrition (kwashiorkor) results from an inability of the adrenal cortex to respond

sufficiently to mobilize enough amino acids for use by an abnormally high secretion of growth hormone. The successful adaptation in marasmus results from an adequately responsive adrenal and relatively low growth hormone.[24,27,22] It is generally agreed that in severe kwashiorkor serum growth hormone concentrations are raised.[23,27,22]

In marasmus, growth hormone concentrations have been reported as either normal or raised.[27,22] The present results confirm those of that growth hormone concentrations are elevated in both kwashiorkor and marasmus. This elevated concentration of growth hormone is not significantly related to body mass deficit or to plasma concentrations of glucose or albumin.The present study shows that growth hormone concentration is markedly elevated in both types of protein energy malnutrition.Thus although hypoproteinaemia, and particularly hypo-albuminaemia, are expressions of the metabolic state during the maintenance of plasma albumin concentration and metabolic integrity in protein energy malnutrition, factors other than growth hormone and cortisol concentrations are primarily responsible for successful adaptation. This elevated concentration of growth hormone is not significantly related to body mass deficit or to plasma concentrations of glucose or albumin.

CONCLUSIONS

The investigators conclude that growth hormone concentrations are elevated in both kwashiorkor and marasmus. we further conclude that study nutritional intervention is the effective food supplement for the speedy recovery of impaired hormone status in the SAM children

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