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Full Length Research Paper

Comparison of some anthropometric and biologic parameters in two groups of Tunisian infants (0 to 2 years)

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Malnutrition still remains a preoccupying health problem that affects the most vulnerable age group (less than five years). The aim of this research is to establish the nutritional profile of the Tunisian infants less than 2 years, to specify the principal deficiencies and the possible origins of these deficiencies. In our transverse exploratory study carried out in a period of 12 months, two groups of infants less than 2 year old; a control group (n = 18) and a malnourished group (n = 18) were used. Our data consolidate the impact of pregnant women's nutritional state, breastfeeding on the infant and the infant growth. In comparison with the control group, the malnourished group showed a significant reduction in weight/age, weight/height (p < 0.001), a significant reduction of the average values of cholesterol associated with high density lipoproteins (Chol-HDL) and the ratio of cholesterol associated with high density lipoproteins to cholesterol associated with low density lipoproteins (Chol-HDL / Chol-LDL). This shrinkage showed a positive correlation with the weight/height ratio which is an indicator of malnutrition gravity. The results of this study confirmed the existence of a remarkable change of the biological profile among malnourished infants. In addition, the study shows the necessity to target and develop means to fight against factors favoring malnutrition.

Key words: Malnutrition, infants, breastfeeding, protein, lipoprotein.

INTRODUCTION

In Tunisia, protein energy malnutrition (PEM) in preschool children is quite common, especially in rural areas (El Ati et al, 2002). According to the available epidemiological data, it was noticed that the prevalence of PEM varied between 11.2 and 23.4% in rural areas and 10.4 and 6.2% in urban areas (El Ati et al., 2002). PEM affects various significant functions such as the physical growth, mental development, training capacity, the organism deence capacity, in particular the immunizing response and antioxidant processes (Ahmed et al., 2009; Chen et al., 2009; Moynihan et al., 2009). The danger of malnutrition is especially serious among the young population, particularly children younger than 5 years.

A well regulated diet must satisfy not only the protein energetic needs, but also those various micro nutriments (vitamins, zinc, etc.), which interact with the pathogenneses of various diseases that are established. For example, it was reported that a deficit in zinc, selenium, iron, vitamins A, E and acid folic is able to alter the immunizing responses (Makonnen et al., 2003; Rubhana et al., 2004). Similarly, it was shown that there existed a significant relation between diarrhoea, measles, anaemia and deficit in zinc, vitamin A and iron, respectively (Rahman et al., 2002; Muller et al., 2003).

Malnutrition is also associated with biological disturbance. It is particularly accompanied by a reduction in

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Abbreviations: PEM, Protein energy malnutrition; **EDTA,** ethylenediamine tetra-acetic acid; **apo,** apolipoproteins; **TG,** triglycerides.

various proteins serum (albumin, prealbumin, retinol binding and apolipoproteins) and a reduction in the total cholesterol (chol), Chol-high density lipoprotein (HDL) and the ratio of Chol-HDL to Chol-low density lipoprotein (LDL).

The aim of this study is to look for ways of establishing the nutritional profile of the Tunisian infants of less than two years, examine the effect of malnutrition on this profile and specify the principal deficiencies affecting the young infants.

MATERIALS AND METHODS

Subjects

In our transverse exploratory study carried out during a period of 12 months (from January to September, 2007), thirty six infants under 2 years were recruited and divided into two groups: The first group is a control (n = 18), aged 11.3 ± 9.4 months, they were recruited among those frequenting the services of University Hospital Centre (UHC) of Sfax (large city in the south of Tunisia) through the regular postnatal control. They had a Z score for both their weight and height (between – 1 DS and + 1 DS).

The second group (n = 18), malnourished infants aged 7.8 ± 6.6 months, was recruited among those hospitalized in the service of paediatrics of this hospital. All the malnourished cases had a Z score < -2 D S (Pelletier et al., 1995). The protocol of research was made and approved by the ethical committee of the centre (UHC) and accepted by parents who gave written consent to the inclusion of their children in the study.

Socio-economic, anthropometric and dietary assessments

In the two groups, epidemiological characteristics (age and daily food consumption), clinics and anthropometrics parameters (height and weight) were noted. A socioeconomic survey, using a type closed standard questions was investigated. Moreover, the daily food intake of mothers was recorded according to the 24 h dietary recall method. The team also measured the index of body weight mass and the brachial perimeter.

Blood collection

Blood samples were taken from malnourished infants before any treatment. In both groups, fasting blood was drawn in vacationer tubes containing ethylenediamine tetra-acetic acid (EDTA) and centrifuged at 3000 rpm for 10 min, then the plasma was removed and conserved at 80°C until the analysis of different biological parameters were done.

Plasma biological parameters

Total proteins were quantified using the Biuret method (Biuret and Layne, 1957). The albumin, prealbumin, apolipoproteins AI (apo-AI) and B (apo-B) were determined by immunoturbidimetry on automates COBAS INTEGRA 400 (Roche Diagnostic Gmb H, Mannheim, Germany). The serum zinc and iron were determined after dilution of the sample in ultra-pure water by atomic absorption spectrophotometry using titrisol standard solution (Merck, Darmstadt, Germany) and PERKIN EL MER MODEL 305 B.

Vitamins

The serum vitamins A and E were realized using reversed-phase height-pressure liquid chromatography (RP-HPLC) according to the method of Jacob and Elmadfa (1995). Briefly, plasma proteins were precipitated with ethanol and lipids were extracted with n-hexane. After evaporation, the dry residue was redissolved with 150 µl of methanol-dichlormethane (85:15 v/v), mixed and then an amount of 100 µl of this solution was injected into a guard column (Merck LiChrospher 100 RP18 (10 µm), 250 x 4 mm). Samples were run at a flow rate of 1.0 ml/min on a Dionex HPLC system (Summit™ HPLC, USA). Absorption was monitored at 325 nm for retinol, at 295 nm for tocopherols, at 450 nm for carotenoids and at 270 nm for coenzyme Q_{10}. Concentrations were calculated from areas under the curve using an external calibration curve. The vitamins were determined by spectrophotometer detector in 340 nm for the retinol and the retinal- acetate and in 292 nm for the alpha-tocopherol.

Lipoproteins

The fraction of HDL lipoproteins was obtained after precipitation with phosphotungstic acid containing the apo B (LDL), by using the case marketed by Bio Merieux (France). The total cholesterol, triglycerides (TG) and HDL were determined by enzymatic methods by using the automat RA 1000 analyser (Bayer Diagnostic, München, Germany). The cholesterol LDL were calculated by using the formula of Friedward:

Chol LDL = CT- (Chol LDL + Chol VLDL)

The very low density lipoproteins were calculated by dividing the rates of TG by 5. All our subjects had concentrations of TG lower than 3 g/l, which is a necessary condition for the application of Friedewald et al., (1972) formula.

Statistical assessment of the data

The average and standard deviation of the different parameters studied were calculated. Students' t-test was used to assess the differences between the average values of these studied parameters in the groups. Statistical significance was considered for p values <0.05.

RESULTS AND DISCUSSION

Alimentary, socioeconomic and anthropometric data

The socioeconomic survey showed that fifteen percent of the malnourished infants' parents were illiterate and seventy-five percent of them have a level of primary studies. This study also showed that, their economic circumstances were mediocre (unemployed or daily labourers). The mothers of the malnourished infants had compared average values of index body weight and brachial perimeters with those of control group. They are respectively, 23.8 ± 3.3 kg/m^2 and 27.9 ± 3.3 cm for the first ones and 24.2 ± 3.5 kg/m^2 and 28.2 ± 3.3 cm for the second ones. Otherwise, the haemoglobin rate of the mothers of the malnourished (10.1 ± 0.7 g/dl) was lower than that observed in the mothers of the control group

Table 1. Comparison to the average values of the anthropometric parameters in the two groups of infants.

Parameter	Control (C)	Malnourished (M)	Significances (P)
Age (months)	11.32 ± 9.37	7.83 ± 6.62	NS
Weight at birth (kg)	3.14 ± 0.28	2.74 ± 0.45	P < 0.05
Weight (kg)	8.06 ± 2.86	5.13 ± 2.23	P < 0.02
Weight of reference (kg)	7.62 ± 2.53	7.62 ± 2.54	NS
Height at birth (cm)	50.39 ± 0.92	49.89 ± 0.96	NS
Height (cm)	66.43 ±10.10	61.08 ± 9.56	NS
Height of reference (cm)	67.39 ± 9.98	67.79 ± 9.54	NS
Weight/height	0.12 ± 0.03	0.08 ± 0.02	P < 0.001
Height/age	18.17 ±17.52	16.56 ±15.67	NS

ns: No significant difference

Table 2. Comparison of the mean values of the biological parameters in control and malnourished groups.

Parameter	Control (C)	Malnourished (M)	Significances(P)
Apolipoprotein AI (g/l)	1.00 ± 0.19	0.77± 0.13	P < 0.001
Apolipoprotein B (g/l)	0.59 ± 0.27	0.54 ± 0.09	NS
Proteins (g/l)	60.71±10.09	49.25 ± 9.85	P < 0.001
Albumin (g/l)	30.80 ± 5.86	24.62 ± 5.22	P < 0.001
Prealbumin (g/l)	0.14 ± 0.07	0.11 ± 0.05	NS
Vitamin A (µg/ml)	0.34 ± 0.16	0.21 ± 0.09	P < 0.01
Vitamin E (µg/ml)	10.07 ± 3.79	4.72 ± 4.14	P < 0.001
Iron (mg/ l)	40.91 ± 3.40	23.55 ± 3.65	P<0.001
Zinc (µg/dl)	98.63 ± 32.50	79.01 ± 38.69	P < 0.01
Total cholesterol (mmol/l)	3.50 ± 0.49	3.08 ± 0.40	NS
Triglycerides (mmol/l)	1.59 ± 0.47	1.23 ± 0.23	NS
Chol-HDL (mmol/l)	0.98 ± 0.23	0.71 ± 0.12	P < 0.001
Chol-LDL (mmol/l)	2.26 ± 0.46	2.09 ± 0.36	NS
Chol-HDL /Chol-LDL	0.45 ± 0.15	0.35 ± 0.09	P < 0.02

Chol-HDL: Cholesterol associated with high density lipoproteins; Chol-LDL: cholesterol associated with low density lipoproteins; Chol-HDL /Chol-LDL: ratio of Chol-HDL to Chol-LDL; ns: no significant difference.

(12.5 ± 0.6 g/dl; $p < 0.01$). These mothers are big tea consumers (120 to 150 cc/ day).

Contrary to the control group, few malnourished infants were breastfed (6 vs. 70%). 72% of them received infants' powder milk but in an insufficient quantity. Besides, the survey revealed an important frequency of gastro-intestinal infections (2 to 4 episodes/month) among these malnourished infants.

Anthropometric data

At birth as well as at the moment of recruitment, the two groups of infants had a comparable average of height. The average weight relative to these two times were, on the contrary, significantly less than the group of the malnourished ($p < 0.05$ at birth; $p < 0.02$ at the moment of recruitment). Moreover, the last group had a normal ratio height/age, but a ratio weight/height (0.08 ± 0.02 kg/cm) significantly lower ($p < 0.001$) than that observed in the control group (0.12 ± 0.03 kg/cm) (Table 1). The same differences were found before and after the age of 6 months.

Biological parameters

Table 2 shows a comparable level in total cholesterol, TG, LDL –Chol, Apo-B and pre-albumin among the two groups. The data in Table 2 however shows significant differences between the two groups in some other biological parameters. Indeed, the malnourished group had a

Table 3. Correlate coefficients of weight/height ratio with other parameters.

Parameter	Weight/height
Age	0.69 (***)
Weight at birth	0.37 (*)
Apolipoprotein AI	ns
Apolipoprotein B	ns
Proteins	0.68 (***)
Albumin	0.49 (***)
Prealbumin	0.70 (***)
Vitamin A	0.66 (***)
Vitamin E	0.77 (***)
Zinc	ns
Iron	0.67 (***)
Total cholesterol	0.37 (*)
Triglycerides	0.48 (***)
Chol- HDL	ns
Chol-LDL	0.37 (*)
Chol-HDL /Chol-LDL ratio	-0.04

Chol-HDL; Cholesterol associated with high density lipoproteins; Chol-LDL: cholesterol associated with low density lipoproteins; Chol-HDL /Chol-LDL: ratio of Chol-HDL to Chol-LDL.
*p < 0.05, ** p < 0.01, ***p < 0.001, ns: no significant difference.

decrease in vitamin A, zinc ($p < 0.01$), Chol-HDL / Chol-LDL ratio ($p < 0.02$), Apo-AI, total proteins, albumin, vitamin E, HDL and iron ($p < 0.001$).

In our study, we examined the relationship between the ratio weight/height (at the moment of recruitment) with the various examined parameters. Our data (Table 3) show that, some parameters correlated significantly with the ratio weight/height at the moment of recruitment, except the Apo AI, Apo-B, zinc and Chol-HDL.

Our results show that the malnutrition of the infants induced a delay of the growth and perturbation of the biological parameters. Nutritional status was assessed using weight-for-height as an indicator to the present state of nutrition and height-for-age as an indicator of past nutrition. The growth of our groups was evaluated from the study of the ratios weight/height and height/age. The decrease in the first ratio is due to a fast thinning, whereas the decrease in the second ratio can be explained by a delay in the height growth. Only the first ratio has been found to be significantly decreased in the malnourished group, comparatively with the control group. The non variation of the second ratio can be explained by the age of our subjects which was of few months (7.83 ± 6.62 months).

The borderline grade weight/height ratio among malnourished infants is probably attributable to the less privileged socioeconomic conditions of the community they belonged to. In addition, the abnormal state of our patients would be, in part, due to a prenatal origin as it is suggested by their weight at birth, less than that found in the control group. This hypothesis is confirmed by the existence of a positive correlation between their weight at birth and the weight/height relationship at the time of their admission in the hospital.

The interaction between the nutritional state of the mother and that of the foetus has been established. It has been notably reported that a mother's iron deficiency is explained, within the newborn baby, by an increasing anaemia (Sibeko et al., 2004). Besides, it has been proved that the satisfaction of the needs of the foetus for calcium requires an adequate amount of calcium and vitamin D by pregnant women. In the default case, the infant would have been born with a deficit of calcium and a weak reserve of vitamin D. This relationship between mother-infant also appeared clearly after the quantify-cations of vitamins A and E in the mother's blood as well as in that of the umbilical cord (Schulpis et al., 2004).

The alimentary survey realized in our subjects revealed that, many postnatal factors were thought to aggravate the delay of the growth and biological disturbances. The absence of breast-feeding in our patients (94%) and the contribution of an insufficient need of artificial milk clearly showed a nutritional deficit. Besides, the great loss in weight and height should also be taken into consideration since our patients suffered from gastro-intestinal problems. These problems could result from inadequate complementary foods taken by babies in/before due time (before 4 to 6 months), due to the insufficient amounts of milk they have had. Other factors could interfere in the growth, especially the problems of infection as a result of the bad hygienic conditions. Such state was also likely to occur in underfed babies who are protected by various defensive factors existing in the mother's milk (IgA, lysozyme, macrophages and granulocytes) (Hanson and Soderstrom, 1981).

Therefore, it was of much importance to study parameters relating to oxidative stress (antioxidant vitamins and lipid peroxidation products). In fact, in malnourished infants, levels of plasma antioxidant vitamins A and E did significantly differ from levels observed in a similar control group. In addition, plasma levels of antioxidants were significantly lower in these malnourished infants. These observations are in agreement with other studies (Golden and Ramadath, 1987; Houssaini et al., 1977; Mc Laren et al., 1969). From the course of this study, it is evident that there is a significant correlation between enzymatic anti-oxidants and the antioxidant vitamin levels that reflect the pathological features of nutritional program rehabilitation. Therefore, there should be special emphasis on supplying antioxidant agents during any nutritional rehabilitation programme such as vitamins (A, E and C) and trace elements (calcium, magnesium, potassium and iron). Consideration should also be given to supplying therapeutic enzymatic antioxidants in order to reduce free radical generation and enhance antioxidant status. These measures may help to reduce the mortality and morbidity associated with malnutrition.

Other studies attempted to investigate the combination of several antioxidants as supplements (zinc, copper, selenium, vitamin A and E, etc.). It appears that, anti-oxidants work together to help protect the body from potentially harmful effects of free radicals (Gauche and Hausswirth, 2006). On the other hand, the malnourished group compared to the control group, showed a significant reduction of other various plasmatic parameters as vitamins A and E, proteins, apo-AI and trace element (Table 3). In accordance with our findings, a reduction of serum concentrations of albumins and vitamin E has been observed in Moroccan infants suffering from severe under nutrition (Squali et al., 2001). However, the concentration of TG which increases in these babies was found to be stable in our patients. A study on young Tunisian population suffering from stagnation reported a reduction of the apo-AI but not in the cholesterol, triglycerides, chol-LDL or chol-HDL concentrations (Sibeko et al., 2004).

The alteration of the biological profile observed in the malnourished group can result from the insufficient food, the over loss and an unsatisfactory absorption. This can be the result, notably of a quantitative imbalance of feeding. A deficit in vitamin D (a favourable factor for calcium absorption) or an excess in phosphorus (accelerating calcium) can, for example, lead to a hypocalcaemia despite the resulted sufficient calcium. The resulted irons under a form of weak absorptions (such as vitamin C) can produce hyposideremy, even if the resulted iron is sufficient.

Other markers of nutritional status, the haemoglobin and albumin levels were also reduced in malnourished group. At this stage, it is interesting to notice that, the mothers of these infants consume a lot of tea, which can aggravate anaemia, as reported in other surveys (Cournot and Hercberg, 1993; Hamdaoui et al., 1995). The change in the iron rate, observed in the malnourished group would then have started since the foetal stage as a result of an interaction of the mother's state with that of foetus. This hypothesis could be seen as decrease in the rates of haemoglobins found in the mothers of the malnourished young.

Our results show the existence of the relationship between the growth delay and the biological disturbance. Indeed, in the malnourished group, the ratio of weight/ height correlated positively with some biological parameters as protein, albumin, prealbumin, vitamin A, vitamin E, iron, total Chol, TG and LDL Chol. Such effects were reported by other studies concerning young malnourished subjects. These people particularly showed a low rate of apo-AI (13 and 23) and a stable level of these parameters (Sibeko et al., 2004; Slimane et al., 1992; Truswell, 1975). However, their apo-B remained unchanged (Sibeko et al., 2004).

Conclusion

The results of this study confirmed the impact of pregnant women nutritional state and breastfeeding on the infant and infant growth. Our data showed a significant alteration in the level of several anthropometric and biological parameters in the young malnourished when compared to the control. Such effects show the necessity of establishing a program to fight against the factors favouring malnutrition in our country. These results also show that, it is necessary to take the supplementation of anti-oxidants agents such as vitamins (A, E and C) and trace elements (calcium, magnesium, potassium and iron) during any nutritional rehabilitation program into consideration.

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