Serum Zinc Concentrations in Cystic Fibrosis Patients With Ages Above Four Years: A cross-sectional evaluation

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Abstract

Aim: Assess the risk of zinc (Zn) deficiency in the older cystic fibrosis (CF) population.

Method: Cross sectional investigation of all CF patients above the age of 4 followed at the Ghent university centre between 2002 and 2003. Data on age, weight and height z-score, pancreatic and pulmonary functions, chronic pseudomonas infection and CF transmembrane conductance regulator (CFTR) mutations were collected. Serum Zn, vitamin A, E, retinol binding protein (RBP), albumin, sedimentation rate, total IgG and cholesterol were determined. Serum Zn was compared with a local healthy control group (1) and with literature data (3). *Results:* 101 patients (median age 16 years) were included. There was no difference in serum Zn concentration between CF patients and controls. In CF patients no difference in serum Zn concentration between pancreatic sufficient or insufficient patients was seen. Serum Zn was not associated to nutritional status or height Z-score. A significant association serum Zn to serum albumin ($p < 0.0005$) and to vitamin A ($p < 0.01$) was seen. No associations of

serum Zn to serum vitamin E, RBP, cholesterol or CFTR were present. There is a significant association serum Zn - forced vital capacity ($p < 0.01$). Serum Zn was not associated to inflammatory parameters or chronic pseudomonas infection. *Conclusion:* Comparison of CF patients with local controls revealed no significant differences. However, since persisting steatorrhoea increases Zn loss (2) and 12.6% of our population has a serum Zn below the P 2.5 value of the NHANES II study (3), there could remain an increased risk of Zn deficiency in some CF patients. Further on the association with pulmonary function needs more investigation.

Introduction

Assessment of marginal zinc (Zn) status in humans is problematic. The serum Zn is far from the ideal method since it lacks sensitivity and specificity to evaluate the Zn status. It is, however, a useful biomarker of a population's risk of Zn deficiency and can be used to determine whether interventions on the Zn status are needed (4). Zn deficiency was originally described in 1961 by Prasad et al. (5). The symptoms of Zn deficiency are stunted growth, delayed sexual maturation, disturbed immunity, poor appetite and diarrhoea each of which are frequently present in patients with cystic fibrosis (CF) (6-8).

In a previous contribution newly diagnosed, untreated CF patients were compared to healthy age-matched controls and no differences of serum Zn concentrations were observed in CF as well at diagnosis, as after one year of CF therapy (9). Since many of the older CF patients still present symptoms also commonly seen in Zn deficiency, a cross-sectional study of the serum Zn concentration of treated CF patients was performed here.

Subjects and Methods

This is a cross-sectional study of all CF patients over the age of 4 followed at CF centre Ghent during the period 2002-2003, $n = 101$, (female=48). From the age of 4 serum Zn is levelling in previously described normal controls (1). Therefore CF patients from the age of 4 are compared to 174 local controls and reference values (1, 3).

All were treated during at least 1 year. None of the patients took Zn supplements. Pancreatic insufficient (PI) patients (n= 90) received pancreatic exocrine replacement therapy and fat soluble vitamin supplements targeted to the serum values.

In patients above the age of 6 (n= 90) the pulmonary function was measured every 3 months on a Jaeger Masterscreen Body, a half year before and after the Zn determination. For evaluation of lung function the mean value of the 5 measurements was considered.

Laboratory analysis:

Blood was drawn by venapuncture after an overnight fasting of 8 hours and collected in a Zn free tube. After clotting and centrifugation, the serum was stored at -60°C until examination.

Zn was determined on a Perkin-Elmer 2380 flame atomic spectrophotometer, as described by Carter (11), adapted according to Dubrowski (12). Serum -tocopherol and retinol were analysed by isocratic high-pressure liquid chromatography (13). Total cholesterol was measured using an enzymatic colorimetric analysis as described by Allain (14). Serum albumin was determined by the Bromcresol green method on COBAS 6000 ® (Roche Diagnostics). The serum RBP concentration was measured on a Behring Nephelometer Analyzer II.

Genotypes were determined with the INNO-LiPA CFTR19 ® and INNO-LiPA CFTR17+Tn Update ® kit (Innogenetics N.V.) or sequencing of the CF transmembrane conductance regulator (CFTR) genome. CFTR gene mutations were classified as proposed by Welsh (10) using the CF mutation database (www.genet.sickkids.on.ca). Group A included patients with type I, II or III mutations resulting in no functional CFTR protein. Group B included patients with at least one type IV or V mutation resulting in a partially functional CFTR protein or those with unknown effect on the CFTR protein (table 1).

Statistics:

Nonparametric statistical methods (Mann-Witney U, Wilcoxon rank, Kruskal-Wallis and Spearman rank correlation) were used with Stat-View 5.1 (Abacus Concepts, Inc.

Berkeley,CA®) for Windows. Results are given as median and interquartile range (IQR) the significance of the differences between groups as tied p-values. The study protocol was approved by the Ethics Committee of Ghent University Hospital 2005/411.

Results:

Patients

There were 101 patients (48 females) included. Their median age was 16 years (IQR 11.2). The median age for gender was not different. Eleven patients were pancreatic sufficient (PS). The percentage of ideal weight for height (W/H %) was 98 % (IQR 14.95). There was no significant difference in W/H % according to gender or pancreatic function. The height z-score (H Z-score) was -1.3 (IQR 1.2). Girls were significantly more stunted than the boys with a median H Z-score of -1.6 (IQR 1.3) vs. -1.1 (IQR 1.03) ($P < 0.03$). None of the patients was vegetarian. The median forced expiratory volume in 1 second (FEV1%) was 82.8 % (IQR 39.1) and the forced vital capacity (FVC%) was 91.7% (IQR 27.25). There were 39 patients colonised with *Pseudomonas Aeruginosa*. They had a significantly worse pulmonary function ($P < 0.03$). FVC% was 96.6 % (IQR 25) for pseudomonas negative and 87.3 % (IQR 27.2) for the colonised patients.

The CFTR mutation subgroups are described in table 1. Group A includes mutations where no CFTR function is expected ($n = 68$). Group B includes mutations with decreased function or unknown effect on CFTR function ($n = 33$). All pancreatic sufficient patients were in group B. There were no significant differences in the clinical parameters between the genotype groups except for the H Z-score ($P < 0.02$). Group B (H Z-score -1.2, IQR 1.375) was significantly taller than Group A (H Z-score -1.4, IQR 1.43) ($P < 0.02$). Group B had higher serum cholesterol (145 mg/dL, IQR 48.75) than group A (132 mg/dL, IQR 50) ($P < 0.02$). The other laboratory data did not differ between the genotype groups.

Serum Zinc concentration

The median serum Zn concentration was 82 µg/dL (IQR 20). There was no difference between the CF patients and the healthy local controls (84 µg/dL (IQR 24)). However, the age

range in the control group was significantly lower with a median age of 6.5 year (4-13.8 year). Considering a fully age-match of CF patients (n= 42) similar results were obtained.

16.8 % of our CF population but also 12.6% of our healthy controls had a serum Zn below the lower cut-off (2.5 percentile = 65 µg/dL) of the NHANES II (3).

There was no difference according to gender or age. Dividing the CF patients in the age classes of NHANES II (<18, 18-25, >25 yrs), no differences were found.

Serum Zn is not associated with nutritional status expressed as W/H % or H Z-score. Even between patients with high or low (<65 µg/dL) serum Zn no differences in growth and nutritional status could be observed.

There was no statistical evidence of differences in serum Zn between pancreatic sufficient (n=11) (82.9 µg/dL (IQR 21)) and insufficient patients (n=90) (81.5 µg/dL (IQR 16.25)). However, none of the pancreatic sufficient CF patients had a serum Zn below 65µg/dL.

Comparing serum Zn of the genotype classes, no differences were seen. However, in group A 17.6 % (n=12) and in group B 15 % (n=5) had serum Zn below the lower cut-off ($P = 2.5 = 65 \mu\text{g/dL}$) of NHANES II (3).

Serum Zn and other serum values in CF patients

A significant association between serum Zn and albumin ($P < 0.0005$) and Zn- serum vit A ($P < 0.01$) were obvious. There was no association of serum Zn with cholesterol, RBP, vit E or with markers of inflammation (erythrocyte sedimentation rate, total IgG).

Serum Zn and pulmonary function

There was no difference in serum Zn between patients with or without pseudomonas colonisation. The pulmonary function in colonised patients (FVC% 87.3% IQR 27.3) was, however, significantly lower ($P < 0.004$). There was no association between Zn and FEV1% but a significant association was found with FVC% ($P < 0.01$) (fig 1).

Discussion

Zn has the second highest prevalence of all oligoelements in the human body (15). One could distinguish 3 main categories of Zn functions: catalytic, structural and regulatory. Zn is an essential component of the catalytic site of hundreds of different metallo-enzymes (16). It is further on an important structural element of gene regulatory proteins (17). The structure of these proteins is dependant on Zn chelation centres. At these sites Zn facilitates appropriate protein folding. These “Zn finger” proteins play a key role in formation and maintenance of all tissues (18). The last function of Zn is regulatory. It acts as an ionic signal in cells through gated membrane channels (19). More than 300 enzyme systems rely on its presence (20,21).

Therefore Zn deficiency symptoms are non-specific. They include loss of appetite, diminished sense of taste, growth retardation, disturbed immune functions, impaired wound healing and other skin changes (6-8, 22). Patients with CF can have similar symptoms.

Serum Zn remains the easiest and most widely used index of Zn status for population research. For determination of an individual Zn status, serum Zn lacks optimal sensitivity and specificity (4). Zinc is located in the cell and only a small portion is found in the circulation bound to plasma protein (15). As about 70% of the plasma Zn is bound to albumin (15), the correlation ($p < 0.0005$) found between serum Zn and albumin is not surprising.

Multiple aspects of the vit A status (absorption, metabolism, release, transport and utilisation) may be influenced by Zn (23). A significant association of Zn with vit A has been described in multiple other populations (24,25) and is confirmed in this study despite the treatment with high doses of vit A. Zn is required for hepatic synthesis of RBP, implying a regulatory role for Zn in mobilizing vit A within cells and from the liver (26). As observed by others (27), there was however no association of serum Zn and RBP in this CF population.

Although the influence of Zn on vit A absorption (23) opens interesting theories concerning the possibilities of improving the vit A status of CF patients by Zn supplementation, they were not confirmed in therapeutic trials (28).

Acrodermatitis enteropathica-like eruption is regularly described as presenting picture of CF (29-31). Since in our healthy controls (1) 12.6 % has a serum Zn concentration below the lower cut-off of the NHANES II study (3), they could be considered as a population at risk for Zn deficiency. Therefore it is interesting to have a closer look at populations with diseases known to cause increased Zn losses such as CF (2,32). Especially since the data on prevalence of Zn deficiency in the CF population are inconsistent (32-36). This study confirms the absence of a difference in serum Zn between treated CF patients and controls (33,35). Due to the small study size, the age related fluctuations described in NHANES II study are not consistent (3).

In general the severity of malabsorption is less pronounced in PS CF patients. In this population it is reflected by their higher H Z-score and serum cholesterol values. The group of Hambidge showed a decreased fractional absorption of Zn in PI CF and its increase by pancreatic enzyme supplementation (2). The faecal Zn loss correlated with faecal fat loss (32). From our CF population 16.8% had Zn values below the NHANES II cut-off. This was, however, exclusively seen in PI CF.

As observed by other groups (35, 37), the absence of association Zn nutritional status in this study population is not surprising, since malnutrition in CF is a complex multifactorial problem. It is influenced a.o. by nutritional intake, absorption and energy expenditure.

The relation between serum Zn and pulmonary function merits further investigation. Inflammation is more frequently present in patients with decreased pulmonary function. Inflammatory conditions are known to reduce serum Zn concentrations (38). There was, however, no association between serum Zn and red blood cell sedimentation rate or total IgG, both parameters of inflammation. Although patients colonised by *pseudomonas aeruginosa* had a significantly lower pulmonary function, there was no difference in serum Zn according to the colonisation status. Since Zn plays an important role in immunity (22) the decreased serum Zn in patients with decreased pulmonary function could enhance infection, leading to a vicious circle. Zn supplementation reduces pulmonary infections in many different conditions (39,40) it can be interesting to investigate in CF, especially in those patients with low serum

Zn values. The only double blind Zn supplementation study in CF did not select the population according to the initial serum Zn value. They were not able to observe differences in nutritional status or pulmonary function after the 8-weeks short duration of the Zn supplementation (28). Probably the effects of Zn supplementation could be more pronounced in the initially low Zn group.

CONCLUSION

A subgroup of CF patients shows marginal serum Zn and could be at risk of its major metabolic consequences. In this condition Zn supplementation needs to be thoroughly examined. Further on the decreased lung function of the low Zn group needs more attention.

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Abbreviations

Zn: Zinc PERT: pancreatic enzyme replacement therapy CF: cystic fibrosis W/H %: percentage of ideal weight for height

vit: vitamin FEV1%: forced expiratory volume in 1 second FVC%: forced vital capacity

CFTR: cystic fibrosis transmembrane

H Z-score: height Z-score conductance regulator

RBP: retinol binding protein PS: pancreas sufficient

PI: pancreas insuffucient

Table 1: Genotype of the 101 CF patients: details of the CF mutations and classification into 2 groups

Genotype Genotype Patients

Groups n°

A F508 47ΔF508/Δ

F508/E60XΔ 1

F508/G542XΔ 7

F508/N1303KΔ 3

F508/Q493XΔ 1

F508/1717-1GΔ ->A 1

F508/Y1092XΔ 1

F508/394delTTΔ 1

F508/R785XΔ 1

F508/R553XΔ 1

I507ΔF508/Δ 1

394delTT/394delTT 1

N1303K/N1303K 2

B F508/3849+10kbC-T 1Δ

TAGAΔF508/306Δ 1

F508/S1251NΔ 8

F508/L927PΔ 1

G458V/1717-1G->A 1

F508/I336KΔ 2

G542X/622-2 A->C 1

F508/G970RΔ 3

F508/3272-26A-Δ>G 2

H711R/805FΔ 2

F508/2789+5GΔ -> A 2

1717-1G->A/S1251N 1

G542X/G970R 1

394delTT/Y913C 1

N1303K/ deletion exon 19 1

unidentified/unidentified 2

3600+2insTA/2005 del T 1

F508/1898+1G-Δ>A 1

Deletion exon 2/ del exon 2 1

Fig 1: Correlation between serum Zn and forced vital capacity (FVC)