Original article

Serum trace elements in dysphagic gastrostomy candidates before endoscopic gastrostomy for long term enteral feeding

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SUMMARY

Background & aims: Patients who underwent endoscopic gastrostomy (PEG) present protein-energy malnutrition, but little is known about Trace Elements (TE), Zinc (Zn), Copper (Cu), Selenium (Se), Iron (Fe), Chromium (Cr). Our aim was the evaluation of serum TE in patients who underwent PEG and its relationship with serum proteins, BMI and nature of underlying disorder.

Methods: A prospective observational study was performed collecting: patient’s age, gender, underlying pathology and nutrition status. All patients underwent head and neck malignancies (HNC) and neurological causes (ND). TE Levels were assessed by Atomic Absorption Spectroscopy for Se/Cr. The patients were divided into head and neck cancer (HNC) and neurological dysphagia (ND).

Results: 146 patients (89 males), 21–95 years: HNC-56; ND-90. Low BMI in 78. Low values mostly for Zn (n = 122) and Fe (n = 69), but less for Se (n = 31), Cu (n = 16), Cr (n = 7); low albumin in 77, low transferrin in 94 and 66 with both proteins low. Significant differences between the groups of underlying disease only for Zn (t = 4.032; p < 0.01) and a correlation between proteins and TE respectively (r = 0.197, p = 0.025, and albumin and Fe (r = 0.415, p = 0.000).

Conclusions: When gastrostomy was performed, patients display low serum TE namely Zn, but also Fe, less striking regarding others TE. It was related with prolonged fasting, whatever the underlying disease. Low proteins were associated with low TE. Teams taking care of PEG-patients should use Zn supplementation and include other TE evaluation as part of the nutritional assessment of PEG candidates.

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1. Introduction

Essential Trace Elements (TE) such as Zinc (Zn), Copper (Cu), Selenium (Se), Iron (Fe), and Chromium (Cr) are required in small amounts for normal metabolism and are linked together in cytosolic defense against reactive oxygen and nitrogen species [1]. Deficiency in TE has adverse health effects in immune status, inflammation, oxidative damage, and lymphocyte function. Zn is involved in three major functions, catalytic, regulatory and structural [2–4]. The World Health Organization highlighted Zn deficiency as one of the 10 major factors contributing to disease in developing countries [5]. Se is an essential non-metallic trace element, the only to be specified in the genetic code. It is required for normal metabolism and is incorporated as selenocysteine at the active site of selenoproteins with antioxidant functions, anti-inflammatory, antitumorigenic, antiangiogenic, antiatherogenic and immunomodulatory effects [6–12]. Twenty five essential selenoproteins are grouped into glutathione peroxidases, thioredoxin reductases and iodothyronine deiodinases [13–16]. Deficiencies result in deleterious repercussions, including organ...
dysfunction; poor wound healing and altered immunity [17–19].

Cu is the cofactor of several fundamental enzymes, including cytochrome-c oxidase, CuZn superoxide dismutase and ceruloplasmin. Fe acts as a catalytic centre for a broad spectrum of metabolic functions, as haemoglobin and myoglobin; it is stored as ferritin and hemosiderin and is found as a transit chelate with transferrin. Cr is critical for insulin performance that is enhanced by Cr, though influencing carbohydrate, lipid and protein metabolism [20,21]. Chromium deficiency may lead to elevated serum cholesterol and triglycerides and decreased high-density lipoprotein cholesterol. Beyond clinical apparent deficiencies of TE, which are seldom diagnosed, subclinical deficiencies may impair metabolism and must be accounted for, particularly in patients and groups of risk.

The prevalence of swallowing disorders is very high in patients with acute or chronic neurological disease, and head or neck cancer. Whatever the underlying disease, dysphagia reduces the oral intake, leading to depletion of macronutrients and micronutrients [22,23]. Percutaneous endoscopic gastrostomy (PEG) is the gold standard for enteral feeding longer than 3 weeks. When patients are referred to the PEG procedure for long term enteral feeding they have already several weeks with low ingestion and frequently present with protein-calorie malnutrition. As well as protein-calorie malnutrition, TE deficiencies may occur, but monitoring TE in dysphagic patients is infrequent and there are few systematic studies of TE in PEG patients [24].

2. Aims

The aims of our study were:

1. Evaluation of serum TE concentration (Zn, Cu, Se, Fe and Cr) at time of the procedure in dysphagic patients referred to gastrostomy;
2. Comparison of serum TE concentration between two groups of long term dysphagic patients (head or neck cancer; neurological dysphagia) that underwent PEG;
3. Association between serum TE concentration and Body Mass Index categories (BMI).
4. Association between serum TE concentration and markers of malnutrition and/or inflammation (serum albumin and transferrin).

3. Material and methods

We performed a prospective observational study that evaluated serum TE concentration. In consecutive adult patients that were referred to and underwent endoscopic gastrostomy in order to have nutritional support for long term dysphagia (more than 3–4 weeks). All adult patients were invited to participate. The only exclusion was age <18 years and refusal to be included in the study. All subjects were informed of the purpose and procedures of the study and gave their informed consent. This study was approved by the Hospital Ethics Committee.

According to the underlying disease causing dysphagia, patients were split in two groups [1]: head and neck cancer (HNC) included oral cavity, pharyngeal, laryngeal, oesophageal proximal cancer and cervical cancers arising from other organs or tissues and [2] neurological dysphagia (ND) including acute and chronic disorders.

Collected data included patient's age, gender, clinical indication for enteral feeding through, Nutritional Risk Screening, Body Mass Index (BMI), and serum albumin, transferrin and TE concentration.

3.1. Nutritional risk identification

For nutritional screening we used the tool recommended by E.S.P.E.N., the Nutritional Risk Screening — NRS 2002.

3.2. Global nutritional assessment

As most of these patients have important speech difficulties due to neurological disorders or head and neck cancer, nutritional assessment tools depend on oral communication were unsuitable. Global nutritional assessment relied mostly in objective evaluation, using anthropometry - Body Mass Index (BMI) - and serum data, including albumin and transferrin. Albumin <35 g/l and transferrin <2.0 g/l were considered suggestive of malnutrition. Global Nutritional Assessment relied mostly in objective evaluation (anthropometry data) and serum data, including albumin and transferrin. BMI was obtained in most patients and expressed as body weight/height squared (kg/m²). If patients were bedridden and could not stand up for weight and height evaluation, BMI was estimated using the Mid Upper Arm Circumference and regression equations described by Powell-Tuck/Hennessy, which was previously been used by our group [25]. Malnutrition was defined as a BMI <18.5 kg/m² for adults patients younger than 65 years and <22 kg/m² for patients with 65 years or older [26]. Although serum proteins may be influenced by non-nutritional factors, albumin <3.5 g/l and transferrin <200 mg/dl were considered suggestive of malnutrition. A dietary recall from the previous weeks was obtain from patients, family or caregivers.

3.3. TE blood samples assays

From patients that underwent endoscopic gastrostomy, a blood sample was obtained minutes before the gastrostomy procedure. Blood samples were obtained between 8:00 and 10:00 AM following at least 12 h of fasting. Part of the blood sample of each patient was used for the standard PEG-patient evaluation, including serum proteins. Other part of the blood sample was split into specifically designed metal-free tubes for Zn, Se, Cr and Cu assessment. After centrifugation serum samples were kept frozen (−80 °C) until the analysis. Serum Fe was evaluated through hospital routine using ferrozine colorimetric method (R1: citric acid 200 mmol/L; thiourea: 115 mmol/L; detergent: R2: sodium ascorbat: 150 mmol/L; ferrozine: 6 mmol/L: preservative). Serum concentrations of other TE were analyzed and reported from the laboratory (REQUIMTE - Rede de Química e Tecnologia Departamento de Química/Faculdade de Ciencias e Tecnologiada Universidade Nova de Lisboa). Serum Zn and Cu samples were assayed using ICP-AES — Inductively Coupled Plasma-Atomic Emission Spectroscopy (washing solution 5%HNO3 in water, Fluka® patterns 1000 mg/L) and serum Se and Cr were assayed using GFAAS — Furnace Atomic Absorption Spectroscopy (5%HNO3 in water with diluting solution 0.1%HNO3(v/v)0.1%Triton X 100, Fluka® patterns 1000 mg/L). We considered reference values for the TE: Zn: 70–120 µg/dl; Se: 8–27.2 µg/dl; Cu: 70–140 µg/dl; Cr: 0.05–0.2 µg/dl; and Fe: 45–160 µg/dl [27].

3.4. Statistical analysis

It was used the Statistical Package for Social Sciences (IBM SPSS Statistics), version 22.0. The results are considered significant at a 5% significance level. To assess the normality of the data we used the Shapiro–Wilk test. Descriptive statistics were used to evaluate TE levels (Zn, Cr, Se, Fe and Cu) in dysphagic patients. Independent Samples t-test was used (since the assumption of normality was verified) to compare TE levels and proteins (albumin and transferrin) between the two groups (HNC and ND). To study the
relationship between TE levels, albumin and transferrin, Pearson correlation was used. To compare TE levels between BMI categories, Kruskal–Wallis test was used, since the assumption of normality of data in BMI categories is not verified. To compare protein concentration between BMI categories, ANOVA was used, since the assumption of normality of data in BMI categories is verified.

4. Results

4.1. Characteristics of the study population

This study included 146 dysphagic patients who were admitted for PEG. 89 men and 57 women, ranging in age from 21 to 95 years with a mean age of 68.2 years (SD = 14.2). Of these, 90 were aged 65 years old. According the underlying disease, patients were divided into two groups: 1-HNC group, with head and neck cancer (n = 56) and 2- ND group, with neurological dysphagia (n = 90). HNC cancers were, mostly, located in the oral cavity (n = 10), larynges (n = 15), pharynges (n = 20), and proximal oesophagus (n = 11). The ND patients comprises strokes (n = 29), dementias (n = 20), neurosurgical injuries (n = 24), amyotrophic lateral sclerosis (n = 6) and other neurological diseases (n = 11) causing dysphagia. (Table 1)

Before the PEG procedure, all patients had dysphagia for at least one month after the diagnosis of the underlying disease. Prior low oral intake was mostly variable according with the underlying disease, ranging from a few weeks to several months. Likewise, weight loss before the pre-gastrostomy evaluation was widely variable. Nevertheless, all patients had prior intake under 50% of daily caloric needs and Nutritional Risk Screening – NRS 2002 – presented a score ≥3 in every patient, signalling the nutritional risk. All patients were clinically stable at the moment of PEG and sample collection, (unstable patients are excluded or postponed).

During the endoscopic gastrostomy procedure, before the PEG tube placement, all patients underwent a complete upper GI diagnostic endoscopy evaluation. No one displayed any major disorder or any potential bleeding lesion.

4.2. Body mass index

Most of the patients had the BMI calculated from Quetelet's equation kg/m\(^2\). Only in 62 (42.5%) cases (53 ND, 9 HNC) BMI was estimated using the Mid Upper Arm Circumference and regression equations described by Powell-Tuck/Hennessy. From 146 patients, 78 (53%) showed low BMI (<18.5 kg/m\(^2\) for patients younger than 65 years and <22 kg/m\(^2\) for patients with65 years or older). When we divided the study population according to the cause of dysphagia, 47 patients from the ND group presented a low BMI (52%) while in HNC group 31 patients also presented a low BMI (55%). Older patients group presented more malnutrition (n = 58; 64%) compared to younger (n = 20; 36%).

4.3. TE and proteins serum concentrations

4.3.1. TE concentration

**Zinc:** One hundred forty six patients were evaluated regarding Zn in the range 31–114 µg/dl (normal range: 70–120 µg/dl), with a mean 53.94 ± 16.20 µg/dl. From these patients, 122 (84%), showed low Zn, while 24 (16%) presented normal values, albeit close to the lower bound. From these 24 patients, 17 (71%) were from neurosurgery ward, presenting acute brain lesions from trauma or surgery. From the remaining 7 (29%) patients, 4 had HNC and 3 suffered from progressive neurological disorders. HNC patients presented on average a lower level than ND patients. A statistically significant differences were detected between these groups (t\(_{140,326} = -2.642, p < 0.01\)).

**Selenium:** One hundred forty six patients were evaluated regarding Se in the range 0.2–61.5 µg/dl (normal range: 8–27.2 µg/dl) with a mean 10.22 ± 3.67 µg/dl. From these patients, 115 (79%) had normal Se; 31 (21%) had low values between 0.2 and 7.9 µg/dl. From these 19 (21%) were from the ND group and 11 (19.6%) were from the HNC group. No statistically significant differences were detected between these groups (t\(_{142} = -0.386, p = 0.700\)). Elderly patients group presented 13% of low values equal to the younger group.

**Copper:** One hundred forty six patients were evaluated regarding Cu in the range 42–160 µg/dl (normal range: 70–140 µg/dl), with a mean 97.23 ± 25.6 µg/dl. Most of patients, 130 (89%) were within the normal range, and the remaining 16 (11%) presented low values, under 70 µg/dl. When divided into two groups, 13 ND patients and 3 HNC showed low Cu. No statistically significant differences were detected between the two groups (HNC and ND) (t\(_{144} = 0.059, p = 0.953\)). Elderly patients group presented 13% of low values, more than the younger group (6%).

**Iron:** One hundred forty six patients were evaluated regarding Fe in the range 7–243 µg/dl (normal range: 45–160 µg/dl), with a mean of 53.97 µg/dl ± 32.38 µg/dl. Globally, 77 patients (53%) were within the normal range while 69 (47%) had low values (7–44 µg/dl). When divided according to the cause of dysphagia, 41% (n = 37) of ND group and 57% (n = 32) of HNC group showed low concentrations. No statistically significant differences were detected between these two groups (t\(_{144} = -0.112, p = 0.911\)). 0.45 patients from the elderly group (50%) and 25 from the younger group (45%) presented low values.

**Chromium:** One hundred twenty seven patients were evaluated regarding Cr in the range 0.01–7.5 µg/dl (normal range: 0.05–0.2 µg/dl), with a mean of 0.907 µg/dl ± 1.37 µg/dl. Only 7 patients (6%) had low values, 4 from HNC group and 3 from ND group. No statistically significant differences were detected between the two groups (t\(_{125} = 0.235, p = 0.814\)). Five patients with low values were from the elderly group, two from the younger patients group. (Tables 2 and 3)

4.3.2. Albumin and transferrin serum concentrations

Albumin and transferrin were evaluated in 144 patients. Regarding albumin, we obtained a mean of 3.4 g/dl ± 0.35 ranging

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**Table 1**

<table>
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between malnourished (mean according to BMI (mean 4.5). Relationship between BMI and serum proteins concentrations between other groups.

Obviously, a statistically significant correlation in positive direction between albumin and Cr (r = 0.460, p = 0.000) was identified. Moreover, a significant correlation in positive direction was found between transferrin and Fe (r = 0.415, p = 0.000). Also, we found a significant correlation in positive direction between albumin and Cr (r = 0.217, p = 0.012). No significant differences were observed between other groups.

### 4.4. Relationship between Albumin, transferrin and serum TE concentrations

Regarding the relationship between TE and albumin and transferrin, significant correlations were detected. A significant correlation in positive direction between albumin and Zn (r = 0.197, p = 0.025), albumin and Fe (r = 0.415, p = 0.000) was identified. Obviously, a significant correlation in positive direction was found between transferrin and Fe (r = 0.460, p = 0.000). Also, we found a significant correlation in positive direction between albumin and Cr (r = 0.217, p = 0.012). No significant differences were observed between other groups.

### 4.5. Relationship between BMI and serum proteins concentrations

No statistically significant differences in proteins were found between malnourished (mean = 6.419 g/dl) and nourished patients according to BMI (mean = 6.367 g/dl) (t126 = 0.375, p = 0.708). Only 35 patients (24.3%) showed simultaneously low BMI, albumin and transferrin.

### 4.6. Relationship between BMI and serum TE concentrations

Regarding TE, statistically significant differences were detected only in Fe between malnourished (mean = 48.68 μg/dl) and nourished (mean = 60.26 μg/dl) patients (t110.602 = −2.039, p = 0.044) (Table 4).

### 5. Discussion

Swallowing perturbations are frequent in patients with HNC and ND. Besides dysphagia, individual characteristics as alcoholism and tobacco consumption, cancer anorexia and therapy side effects contribute to the development of severe malnutrition. Our patients suffered from long standing dysphagia, for more than 1 month, with daily oral intake below 50% of nutritional needs, thus having indication for immediately nutritional support by enteral feeding for long time and the Guidelines recommended the use of PEG procedure. Nutritional assessment tools that need oral communication with the patients are frequently inadequate in the context of our study, because the same disorders that induce dysphagia also lead to impaired speech capacities. We used quantitative measurements, anthropometry and laboratory assays, in order to obtain objective data to approach the nutritional status. The long standing dysphagia had reflex in the weight and in the albumin and transferrin levels, and we want to know what happened about trace elements.

Our patients presented low BMI in 53% (n = 78). These results could be worse if we had not used the usual cut-off 18.5 kg/m² instead of 20 kg/m² corresponding to NRS 2002, however 18.5 kg/m² is the limit adopted by the World Health Organization and it is the mostly widely used. During dysphagia, the patients lost weight according to low intake but, certainly, in some cases this period was not long enough to decrease BMI. This could explain our results, with 47% patients having a normal BMI. According the underlying diseases we obtained similar percentage of low BMI from the two groups, 53% (n = 47) from the ND and 54% (n = 31) from the HNC, with no statistical significant differences. We found lower BMI in the oldest group, so we believe that aging may increase the malnutrition risk.

Looking for laboratory data, we found 77 (53%) patients with low albumin and nearly two-thirds, 94 (65%) presented low levels of both proteins and only 35 displayed low BMI, albumin and transferrin simultaneously, all values suggestive of malnutrition. The lack of association between BMI, albumin and transferrin suggests the presence of an important inflammatory process contributing for the decrease of these proteins. So, in some patients the inflammatory process may have an important contribution, triggering a complex process of stress and starvation.

Regarding TE we expected to find similar low values. Zn was severely decreased in 122 (84%) patients. From remain 24 (16%), 17 (71%) were from neurosurgery ward, and the other 7 (29%), 4 had HNC and 3 suffered from progressive ND. Most of them with normal Zn suffered from a sudden or rapidly progressive dysphagia, meaning the intake had been normal up to a few weeks before the gastrostomy and so there was not enough time to observe a decrease in the values. This could mean that the reduction of Zn seems unrelated with the nature of the underlying disease but with the duration of the starvation period induced by dysphagia. Iron were normal in 77 patients (53%) and 69 (47%) were low. From these 69 patients 45 (65%) were from the elderly group.

Most of patients displayed normal values for remain TE. For Se, unlike Zn, only 30 (21%) patients (19ND group; 11 HNC group)
showed low values. Our data suggest that Se can be maintained despite reduced intake of food. At least in part, this may occur because Se absorption from the diet is very efficient so that the low caloric diet prior to gastrostomy seems to be sufficient in most of patients [2]. No differences were detected according age. For Ca most of patients displayed normal values. Only 16 (11%) presented low values. However concerning age, we found 12 patients from the oldest group, suggesting that aging can influence the Ca concentration. According to literature, Cu deficiency is rare in short time semi-starvation [2]. We found similar results in our study, meaning that the period of dysphagia of our patients was not enough to cause Cu deficiency. For Cr, we obtained results from 127. Only 7 (6%) patients showed low Cr, 4 from HNC group. Concerning age, we found worse results in the oldest group (5 patients).

Concerning the relationship between TE and the underlying disorders, we found similar results for most TE. Regarding Se, no statistically significant differences were detected between HNC and ND patients. Regarding Fe, we found low values in 37 patients (41%) from ND and in 32 patients (57%) from HNC. As mentioned before all these samples were obtained just before the moment of PEG procedure. There was no endoscopic major findings in any of the patients that explained these results. No statistically significant differences were detected between these two groups. Cu deficiency was found only in 16 patients (11%), 13 from ND and 3 from HNC, without significant statistical differences. Globally, low Te seems to be unrelated with the nature of the underlying disorder causing dysphagia.

Comparing TE with BMI categories, we detected statistically significant differences only in the Fe between malnourished (mean = 48.68 µg/dl), suggesting that low Fe should be considered as a usual feature of malnutrition in these patients.

The last aim was to assess the relationship between TE and albumin and transferrin, serum markers of malnutrition and/or inflammation. A significant correlation was detected in positive direction between albumin and Zn, signifying that higher levels of albumin are associated with higher levels of Zn. Decreased Zn was found in patients with normal and low albumin and transferrin but decreased Zn cannot be only ascribed to reduced albumin binding capacity, because patients with normal serum proteins may also display low Zn. Most likely because the lack of major Zn reserves it seems to be more sensitive to shorter starvation periods than albumin or transferrin. Also a significant correlation in positive direction between Fe and albumin and transferrin (expectably) was identified. The same correlation was found between albumin and Cr. These results indicate that higher levels of albumin and transferrin are associated with higher levels of Zn, Fe and Cr. On the other hand, no relationship was found between Se and albumin and transferrin. Probably, Se may remain stable until its main enzyme, glutathione peroxidase, decrease regardless albumin and transferrin. Most of our patients displayed low proteins reflecting the reduced dietary intake and the activity of the underlying diseases. From these patients only 15% with low albumin and 14% with low transferrin were the same who presented low Se. Globally, Se variation cannot be ascribed to the concentration of albumin and transferrin. Also, no relationship was found between Cu and albumin and transferrin. Cu variation cannot be ascribed to the concentration of albumin and transferrin.

The association between proteins and Zn, Fe and Cr may be present due to several reasons. Decreased Te and proteins may result from low intake or from disease activity. Whatever the causes, dysphagic patients with low proteins should be considered as a greater risk of TE deficiency.

Our results indicate that low Zn are the rule in long term dysphagic patients and low Fe are very frequent, but not Se, Cu, and Cr. We believe that deficiencies need longer periods of low ingestion before being detectable not observed in most of our patients.

In our experience most dysphagic patients had low Te when gastrostomy was performed and intensive nutritional support begun. More striking regarding Zn, but was also frequent with Fe. It seems to be related with prolonged fasting, whatever the nature of the underlying disease. We suggest that teams taking care of PEG-fed patients should systematically use Zn supplementation, and include evaluation of other TE, mainly Fe as part of the nutritional assessment of PEG-patients when starting the nutritional support through gastrostomy.

Conflict of interest

All authors declare no conflict of interest.

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Statement of authorship

All authors have made substantial contributions and final approved the conception, writing, and final version. Author contribution: Santos C. conceived and design the study, collected and conducted the data analysis, drafted the manuscript, and final approval de version to be submitted; Fonseca J. analysed and interpreted data, revised and contributed to final approval of the version to be submitted; Carolino E analyzed and played of data, revised and contributes to final approval, Guerreiro AS, conceived and design the study, revised and contributed to final approval of the version to be submitted. All authors had read and approved the manuscript.

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