The Impact of the Vitamins A, C and E in the Prevention of Gastroesophageal Reflux Disease, Barrett’s Oesophagus and Oesophageal Adenocarcinoma

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ABSTRACT

This paper aims at evaluating the impact of vitamins intake in the prevention of gastroesophageal reflux disease (GERD), Barrett’s oesophagus (BE), and oesophageal adenocarcinoma (EADC). It concentrates primarily on the antioxidant vitamins A, C and E. There were 180 subjects included in the trial, 109 males and 71 females, which were divided in the four groups (70 patients with GERD, 20 patients with BE, 20 patients with EADC, and 70 healthy examinees composing a control group). Their antioxidant vitamins intake was investigated through the usage of the dietary questionnaires. Concentration of the mentioned antioxidant vitamin in serum was detected by HPLC method, and although there were no major statistical differences in their levels between four groups, there existed a correlation between the vitamin serum concentration and the reflux disease degree. The results showed that the healthy examinees had consumed the greater quantities of the vitamins A, C and E, through both the natural (fruits and vegetables) and the supplementary (industrial vitamin additives) way, than the patients with GERD, BE and EADC. This was reflected in the higher serum levels of the mentioned vitamins in the first group in the comparison with the second group. Based on this, the intake of the vitamins A, C and E through both the natural and the supplementary ways is suggested in order to prevent the development of the GERD, BE and EADC.

Key words: antioxidant vitamins, dietary questionnaire, gastroesophageal reflux disease, Barrett’s oesophagus, oesophageal adenocarcinoma

Introduction

Antioxidant micronutrients are a heterogeneous group of different molecules which have in common a functional possibility for the binding of oxygen radicals. In this respect they function as reducers. The mentioned group is composed of vitamins A, C and E, coenzyme Q, glucose, ubiquinon, bilirubine, urates, flavonoids, some amino-acids and certain peptides. All of them reduce the inflammatory effect of free radicals. Free radicals are...
chemical substances with one or more unpaired electrons in the outer electron circle. This enables them with the great chemical reactivity with low reagents specificity, which results in a chemical reaction named oxidation, in which they react with various proteins, lipids, carbohydrates and nucleic acids and form new radical compounds, which can trigger chain reaction. Free radicals could be divided into endogenous and exogenous. While the first ones are formed in organism during oxygen metabolism, phagocytosis, chemotaxis, apoptosis, coagulation, hypoxia or hyperoxia, the second ones come from cigarette smoke, drugs, food, pesticides, radon, ozone, nitrogen oxide, sulphur oxide, radioactive elements and UV light. The abundance of free radicals causes oxidative stress. The disruption of the equilibrium between oxidative stress and anti-oxidative protectives causes numerous acute and chronic diseases.

One of them is gastroesophageal reflux disease (GERD) as the most frequent oesophageal disease, which can be defined as any clinical symptomatic condition or histopathological change of oesophagus, which is a consequence of repeated episodes of gastroesophageal reflux. The next stage would be the development of the Barrett’s oesophagus (BE), which is a precarcinomatous condition characterised with the metaplasia of the typical squamous epithel into the atypical cylindrical epithel. The final stage is the development of the oesophageal adenocarcinoma (EADC) in the area of the metaplastic epithel. The conditions that help the reflux of the stomach acid into the body are obesity, full stomach, lying after eating, body anteflexion, hard objects elevating, pregnancy, and untreated obstructive lung diseases. Some drugs are also important in this respect like the muscle relaxations such as diazepam, the oral bronhodilatators such as theophilin, and the blockers of calcium channels.

Previous studies have shown that the antioxidants through their binding with the oxidants could prevent cell deterioration and cancer development. A number of experiments on smokers and non-smokers has proved the protective role of the vitamin C in the DNA oxidative destruction. Besides its inhibitive role in the DNA oxidation, the vitamin C is chemo-protective against the nitrosamine as a carcinogenic factor. Numerous epidemiological studies have proven the connection between the nutrition rich in fruit and vegetables and the reduced cancer risk. There is a prospective study which has proved that the patients with the intestinal polyposis and the colorectal carcinoma have lower β-carotene and α-tocoferol or their combination in the cancer prevention. Due to the mentioned opposite results, we have decided to determine the possible connection between the serum levels of the vitamins A, C and E with the level of the oesophageal destruction in GERD, BE and EADC.

Sample and Methods

The study lasted from September 2000 until June 2002. There were 180 subjects included in it, 71 women and 109 men, who were chosen randomly and divided into four groups (70 patients with GERD, 20 patients with BE, 20 patients with EADC, and 70 healthy examinees as a control group). They ranged from youths to elders (53.04±14.41 years, range=17–83 years). The subjects all lived in the region of Eastern Croatia. They underwent oesophagogastroduodenoscopy in the Department for Endoscopy at the Clinic for Internal Medicine of the Osijek University Hospital Center, and an endoscopic diagnosis was made for each patient.

Dietetic research was conducted through the individual interview based on the dietary questionnaire. Its aim was to establish the possible influence of dietary habits in the development of the reflux conditions. It was organized as a semiquantitative questionnaire containing the questions on the consummation of fruits, vegetables and synthetic vitamins. Serum concentrations of vitamins A, C and E were determined by the method of fluid chromatography of high efficiency in the Laboratory for the pharmacokinetics of the Department for the pharmacokinetics and analytical toxicology in the Clinical department for the laboratory diagnostics of the Zagreb University Hospital Centre. The blood for the analysis was taken from the patients directly after the oesophagogastroduodenoscopy in the Osijek University Hospital Center, centrifuged in the following 8 to 14 hours, and analyzed in 24 to 48 hours for the vitamin C and 30 to 60 days for the vitamins A and E. Finally, the consumption of the vitamins and the minerals, regarding the quality and the quantity of their usage, was evaluated during 2001 in the pharmacy shop "Mursa".

Statistical analysis

Data from the questionnaire are presented as absolute and relative frequencies summarized in the tables and the concentrations of the vitamins are presented with the Box and Whisker Plot diagrams. Differences in groups (Reflux esophagitis, Barrett’s esophagus, Adenocarcinoma esophagus and Control group) were tested with Fisher’s Exact test and One-Way ANOVA (with Dunnett’s T3 post hoc test) with significance level set at p<0.05.

Statistical analyses were conducted using SAS software (version 9.1, SAS Institute Inc., Cary, NC, USA) while diagrams were made using STATISTICA software (version 8, StatSoft Inc., Tulsa, OK, USA).
Results

Data on the consummation of fruits and vegetables, the consummation of vitamin and mineral additives and the concentration of vitamins A, C and E in the serum of the examinees with gastroesophageal reflux disease (GERD), Barrett’s oesophagus (BE), and oesophageal adenocarcinoma (EADC), and the control group are presented in Tables 1, 2 and 3 and illustrated in Figures 1, 2 and 3. One can see that the healthy examinees have consumed more fruit and vegetables than the patients with GERD, BE and EADC. Similarly, the healthy examinees have taken more vitamin and mineral additives than the described patients. Finally, the greater concentration of vitamins was found in the serum of the healthy examinees. The Fischer’s exact test has showed that all the differences are statistically significant with p<0.001.

Discussion and Conclusion

This study represents a continuation of our previous study which has proved the role of the nutrition in the pathogenesis of GERD, BE and EADC. It was again composed as a multidisciplinary approach, which is typical in the nutrition studies26–30. Other studies have already proved that the ecological diet with organic food can improve health and prevent diseases due to its higher content of antioxidants in plant products and a higher content of fat-soluble vitamins and omega-3 fatty acids in animal products. On this track, Davitt et al. have hypothesized that GERD could be explained as an equivalent of xerophthalmia due to the observed lack of vitamin A in the serum of the patients affected with it31. Based on their prospective epidemiological study, Veugelers et al. have suggested that diets high in vitamin C may substan-

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### TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Reflux esophagitis, No (%)</th>
<th>Barrett’s oesophagus, No (%)</th>
<th>Adenocarcinoma oesophagus, No (%)</th>
<th>Control group, No (%)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit consummation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>more than ones per day</td>
<td>3 (4.29)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>35 (50.00)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3-7 times per week</td>
<td>37 (52.85)</td>
<td>1 (5.00)</td>
<td>10 (50.00)</td>
<td>33 (47.14)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>less than ones per week</td>
<td>21 (30.00)</td>
<td>13 (65.00)</td>
<td>7 (35.00)</td>
<td>2 (2.86)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>none</td>
<td>9 (12.86)</td>
<td>6 (30.00)</td>
<td>3 (15.00)</td>
<td>0 (0.00)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vegetable consummation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>more than ones per day</td>
<td>3 (4.28)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>12 (17.14)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3-7 times per day</td>
<td>38 (54.29)</td>
<td>10 (50.00)</td>
<td>11 (55.00)</td>
<td>56 (80.00)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>less than ones per week</td>
<td>23 (32.86)</td>
<td>4 (20.00)</td>
<td>8 (40.00)</td>
<td>2 (2.86)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>none</td>
<td>6 (8.57)</td>
<td>6 (30.00)</td>
<td>1 (5.00)</td>
<td>0 (0.00)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total</td>
<td>70 (100)</td>
<td>20 (100)</td>
<td>20 (100)</td>
<td>70 (100)</td>
<td></td>
</tr>
</tbody>
</table>

* Fisher’s Exact Test

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### TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Reflux esophagitis, No (%)</th>
<th>Barrett’s oesophagus, No (%)</th>
<th>Adenocarcinoma oesophagus, No (%)</th>
<th>Control group, No (%)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Additives consumption</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>12 (17.15)</td>
<td>0 (0.00)</td>
<td>2 (10.00)</td>
<td>27 (38.57)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No</td>
<td>58 (82.85)</td>
<td>20 (100.00)</td>
<td>18 (90.00)</td>
<td>43 (61.43)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total</td>
<td>70 (100)</td>
<td>20 (100)</td>
<td>20 (100)</td>
<td>70 (100)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Fisher’s Exact Test

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### TABLE 3

<table>
<thead>
<tr>
<th></th>
<th>Reflux esophagitis, N=20</th>
<th>Barrett’s oesophagus, N=20</th>
<th>Adenocarcinoma oesophagus, N=20</th>
<th>Control group, N=20</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>vitamin A</td>
<td>142.4±83.38</td>
<td>124.87±50.79</td>
<td>104.23±41.42</td>
<td>168.52±77.29</td>
<td>0.009</td>
</tr>
<tr>
<td>vitamin E</td>
<td>7.58±1.66</td>
<td>6.82±1.49</td>
<td>5.60±1.23</td>
<td>8.79±2.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>vitamin C</td>
<td>6.73±2.08</td>
<td>4.83±1.54</td>
<td>3.97±1.67</td>
<td>8.79±2.33</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* One-Way ANOVA
tially reduce the incidence of GERD, BE and EADC. The results of our study are in accordance with the described studies, because they have showed the low intake of vitamins A, C and E through both the fruit and vegetable diet and vitamin and mineral additives which was reflected in their low serum levels in the patients with GERD, BE and EADC on one side, and their high intake through the both mentioned ways and consequently their high serum levels in the healthy examinees.

The influence of different vitamins in the prevention of GERD, BE and EADC was investigated on the rat models as well. Ramos et al. have determined an inhibitory effect of the vitamin C on the experimental oesophageal carcinogenesis induced by diethyl-nitrosamine in the Wistar rats. On this track, Rao et al. have proved the protective effect of quercetin, flavonoids alpha-tocopherol on experimental reflux oesophagitis induced in rats. This was also proved by Hao et al. who have determined that alpha-tocopherol inhibits the development of EADC formation in the oesophago-gastro-duodenal anastomosis rat model, alone and in the combination with N-acetylcysteine as another anti-oxidant, and in contrast with Omeprazole, as the most used medication against GERD, which showed only a slight non-significant inhibitory effect. It was conformed on the patients as well, in the study conducted by Pereira on the Brazilian population, who showed that a dietary supplementation containing melatonin, L-tryptophan, vitamin B6, folic acid, vitamin B12, methionine and betaine was more helpful to the patients with GERD in comparison with omeprazole as in ratio 100%:65.7%

Regarding the epidemiological studies, Kubo et al. evaluated the associations among antioxidants, fruit and vegetable intake and the risk of BE among the residents of the State of California. 296 patients with BE cases were matched to 308 patients with GERD and 309 healthy examinees. The results showed that dietary antioxidants (vitamin C, beta-carotene and especially vitamin E) from fruits and vegetables were inversely associated with the risk of BE, while no association was observed for the supplement intake of the mentioned vitamins. This is partially in accordance with our results, which have showed the positive influence of both natural and supplementary intake of the vitamins. The observed differences in the effect of the supplementary vitamin intake could be explained with the small amount of examinees from our sample, who were taking the mentioned supplements, precisely only 41 out of 180.

Terry et al. studied intakes of vitamin C, beta-carotene and alpha-tocopherol in a nationwide population-based case-control study in Sweden. They have compared 185, 165, and 258 cases of EADC, oesophageal squamous-cell carcinoma, and gastric cardia adenocarcinoma with 815 healthy examinees. A high parallel intake of vitamin C, beta-carotene and alpha-tocopherol showed a 40–50% decreased risk of both histological types of oesophageal cancer compared with the subjects with a low parallel intake. On contrary, the antioxidant intake was not associated with the risk of gastric cardia adenocarcinoma. Separately, vitamin C and beta-carotene reduced the risk of oesophageal cancers more than alpha-tocopherol. The mentioned results are in accordance with our findings of the preventive influence of vitamins A, C and E in the development of EADC.

Dong et al. have evaluated the association between the use of supplemental vitamins and minerals and the risk of neoplastic progression of BE into EADC among the citizens of the USA. During a mean follow-up of 5 years, there were 37 patients with EADC, 42 with tetraploidy and 34 with aneuploidy. This cohort study has showed that the use of vitamins A and C and multivita-
min additives was associated with a significantly reduced risk of EADC and markers of neoplastic progression de-
velopment among the patients with BE39. This is again in
accordance with our findings. More importantly, due to
the similarity in sizes of the observed patients, 111 to
180, we could go so far to claim the positive effect of the
supplementary vitamin intake on the development of
EADC.

Finally, a number of papers has analysed the level of
vitamins in both the plasma and the mucosa of the pa-
tients with GERD, BE and EADC. Suzuki et al. have ob-
erved that during the acid reflux in the Barrett’s seg-
dment as the anatomical site with maximal acidity, there
was present an abundance of nitrites and thiocyanate
which as the anatomical site with maximal acidity, there
was present an abundance of nitrites and thiocyanate.

As the conclusion, it should be said that all our re-
results were statistically significant with p<0.001. They
have showed the striking differences in both the consum-
ration of fruits and vegetables on one side, and vitamin
and mineral additives on the other, between the healthy
examines and the patients with GERD, BE and EADC.

Based on this, the consummation of vitamins A, C and E
through both the natural (fruits and vegetables) and the
supplementary (industrial vitamin additives) ways could
be advised in order to prevent the development of Gerd,
BE and EADC in a healthy population on one hand, and
to improve the life quality of the patients with the men-
tioned diseases on the other hand.

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UTJECAJ VITAMINA NA PREVENCIJU GASTROEZOFAGEALNE REFLUKSNE BOLESTI, BARRETTOVA EZOFAGUSA I EZOFAGEALNOGA ADENOKARCINOMA

SAŽETAK

Ovaj rad nastoji evaluirati utjecaj uzimanja vitamina u prevenciji gastroezofagealne refluksnale bolesti (GERB), Barrettova ezofagusa (BE) i ezofagealnoga adenokarcinoma (EADC). On se koncentriratira prvenstveno na antioksidacijske vitamine A, C i E. U studiju je bilo uključeno 180 ispitanika, 109 muškaraca i 71 žena, koji su podijeljeni u četiri skupine (70 pacijenata s GERB-om, 20 pacijenata s BE-aom, 20 pacijenata s EADC-om, te 70 zdravih ispitanika u kontrolnoj skupini). Njihov unos antioksidacijskih vitamina je ispitavan pomoću prehrambenih upitnika. Koncentracija spomenutih antioksidacijskih vitamina u serumu je određena pomoću HPLC metode, te je iako nije bilo veće statističke razlike u njihovim razinama između četiri promatrane skupine ispitanika, ustanovljena povezanost između serumskih koncentracija vitamina i stupnja refluksnih bolesti. Rezultati su pokazali da su zdravi ispitanici konzumirali više količine vitamina A, C i E, unosenih i prirodnim (voćem i povrćem) i nadomjesnim (industrijski vitaminski dodacima) putem, nego pacijenti s GERB-om, BE-aom i EADC-om. To se također odrazilo u većim serumskim razinama spomenutih vitamina u prvoj skupini u usporedbi s ostalim skupinama. Na temelju toga, može se savjetovati unos antioksidacijskih vitamina A, C i E i prirodnim i nadomjesnim putem kako bi se prevenirao razvoj GERB-a, BE-a i EADC-a.