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 rephlux 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

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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, carbon-hydrates and nucleic acids and form new radical compounds, which can trigger chain reaction¹. Free radicals could be divided into endogenous and exogenous. While the firs ones are formed in organism during oxygen metabolism, phagocytosis, chemothaxis, 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 light²⁻⁴. 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 diseases⁵⁻⁷.

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 reflux⁸. 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 epithel⁹. The final stage is the development of the oesophageal adenocarcinoma (EADC) in the area of the metaplastic epithel¹⁰. The conditions that help the reflux of the stomach acid are obesity, full stomach, lying after eating, body ante flexion, hard objects elevating, pregnancy, and untreated obstructive lung diseases¹¹. Some drugs are also important in this respect like the muscle relaxations such as diazepam, the oral bronhodilatators such as teophilin, and the blockers of calcium channels¹².

Previous studies have shown that the antioxidants through their binding with the oxidants could prevent cell deterioration and cancer development^{13–15}. A number of experiments on smokers and non-smokers has proved the protective role of the vitamin C in the DNA oxidative destruction^{16,17}. Besides its inhibitive role in the DNA oxidation, the vitamin C is chemo-protective against the nitrosamine as a carcinogenic factor^{18,19}. Numerous epidemiological studies have proven the connection between the nutrition rich in fruit and vegetables and the reduced cancer $risc^{20,21}$. There is a prospective study which has proved that the patients with the intestinal polyposis and the colorectal carcinoma have lower β -carotene levels in the normal mucoses²². Furthermore, low levels of the vitamin C, retinol and β -carotene were measured in the persons with the higher risk of pulmonary carcinoma²³. Finally, significantly lower serum levels of the ascorbic acid and the β -carotene were experimentally proven in the patients with the abdominal cancer in the comparison with the healthy examinees²⁴. Bearing this on mind it is not surprising why the USA Food and Drug Administration (FDA) has advised that four important antioxidants: the vitamins A, C and E and the selenium should be added into food. On the other hand, there are studies which have shown that there is no protective effect of β -carotene and α -tocoferol or their combination in the cancer prevention²⁵. 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 EDAC.

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 $(53.04\pm14.41$ 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 organised as a semiguantitive 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 (Rephlux 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 examines have consumed more fruit and vegetables than the patients with GERD, BE and EADC. Similarly, the healthy examiners 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 studies^{26–30}. 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 hypothesised 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-

TABLE 1				
DISTRIBUTION OF ALL EXAMINEES REGARDING THE CONSUMMATION OF FRUITS AND VEGETABLES				

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

* Fisher's Exact Test

TABLE 2

DISTRIBUTION OF ALL EXAMINEES REGARDING THE CONSUMMATION OF VITAMINE AND MINERAL ADDITIVES

Additives consummation	Rephlux esophagitis, No (%)	Barrett's oesophagus, No (%)	Adenocarcinom oesophagus, No (%)	Control group No (%)	p*
Yes	12 (17.15)	0 (0.00)	2 (10.00)	27 (38.57)	< 0.001
No	58 (82.85)	20 (100.00)	18 (90.00)	43 (61.43)	< 0.001
Total	70 (100)	20 (100)	20 (100)	70 (100)	< 0.001

* Fisher's Exact Test

TABLE 3	
DISTRIBUTION OF ALL EXAMINEES REGARDING CONCENTRATION OF VITAMINS IN SERU	Μ

	Rephlux esophagitis, N=20	Barrett's oesophagus, N=20	Adenocarcinoma oesophagus, N=20	Control group, N=20	p*
vitamin A	$142.4{\pm}63.38$	$124.87{\pm}50.79$	$104.23{\pm}41.42$	168.52 ± 77.29	0.009
vitamin E	$7.58{\pm}1.66$	$6.82{\pm}1.49$	$5.60{\pm}1.23$	8.79 ± 1.24	< 0.001
vitamin C	6.73 ± 2.08	$4.83{\pm}1.54$	$3.97{\pm}1.67$	8.79 ± 2.33	< 0.001

* One-Way ANOVA

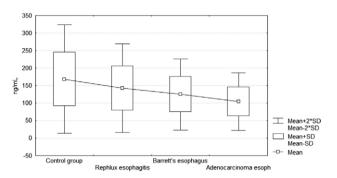


Fig. 1. Box and Whisker Plot diagram of the difference in the vitamin A concentrations between groups (p=0.009, One-Way ANOVA).

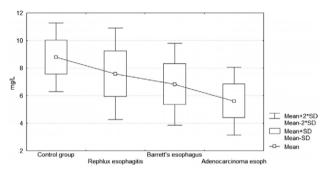


Fig. 3. Box and Whisker Plot diagram of the difference regarding the vitamin E concentrations between groups (p<0.001, One-Way ANOVA).

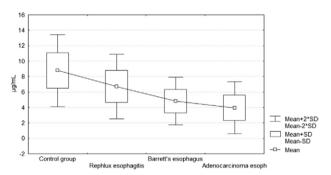


Fig. 2. Box and Whisker Plot diagram of the difference in the vitamin C concentrations between groups (p<0.001, One-Way ANOVA).

tially reduce the incidence of GERD, BE and EADC³². 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 inhibi-

tory effect of the vitamin C on the experimental oesophageal carcinogenesis induced by diethyl-nitrosamine in the Wistar rats³³. On this track, Rao et al. have proved the protective effect of quercetin, flavonoids alpha-tocopherol on experimental reflux oesophagitis induced in rats³⁴. 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 effect³⁵. 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 $100\%:65.7\%^{36}.$

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 vitamins³⁷. 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-tocopherol³⁸. 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 multivitamin additives was associated with a significantly reduced risk of EADC and markers of neoplastic progression development 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 patients with GERD, BE and EADC. Suzuki et al. have observed that during the acid reflux in the Barrett's segment as the anatomical site with maximal acidity, there was present an abundance of nitrites and thiocvanate and a lack of active ascorbic acid and total vitamin C^{40} . Fountoulakis et al. have analysed blood samples and endoscopic biopsies (squamous, Barrett's, and gastric mucosa) from 48 BE patients to determine the levels of vitamins A, C and E, and in the comparison with 48 age and sex matched healthy examinees, have observed the lower levels of vitamin C in both plasma and mucosa of the BE patients, while the levels of the vitamins A and E were average in both samples taken from the mentioned patients⁴¹. Clements et al. have observed that the patients with BE had significantly lower plasma concentrations of

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As for the conclusion, it should be said that all our results were statistically significant with p < 0.001. They have showed the striking differences in both the consummation of fruits and vegetables on one side, and vitamin and mineral additives on the other, between the healthy examinees 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 mentioned 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 refluksne bolesti (GERB), Barrettova ezofagusa (BE) i ezofagealnoga adenokarcinoma (EADC). On se koncentrira 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 ispitan 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 serumske koncentracije vitamina i stupnja refluksne bolesti. Rezultati su pokazali da su zdravi ispitanici konzumirali veće količine vitamina A, C i E, unesenih i prirodnim (voće i povrće) i nadomjesnim (industrijski vitaminski dodaci) 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 jutem kako bi se prevenirao razvoj GERB-a, BE-a i EADC-a.