

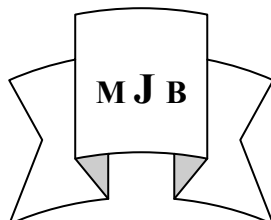
A New Keynote of Hemorrhoids Patients as Oxidative Stress Indicators

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Abstract

This study evaluated the effect of hemorrhoids on the levels of antioxidants such as vitamin E and C, as well as the malondialdehyde(MDA) levels as the end product of lipid peroxidation and peroxynitrite (ONOO⁻) levels as a parameter of peroxidative stress for 35 patients(20 males,15 females) affected by hemorrhoids, before and after rubber band ligation by using baron band ligation device compared with healthy control groups of 35(20 males, 15 females) .

It has been found that the levels of vit. E and C are significantly decreased, where as the MDA and peroxynitrite levels are increased among patients (males and females) before the treatment compared with controls, while the vitamins values are significantly increased when the MDA and peroxynitrite(ONOO⁻) levels are non significantly decreased after 7 days of treatment which is safe and easy procedure. The results suggest that the measurement of lipid peroxidation and peroxidative product matched with evaluation of antioxidants, may be a best indicators for that hemorrhoids induce oxidative stress compared with healthy controls and the treatment, due to decrease of oxidative stress and tissue damage.

الخلاصة

تطرق هذا البحث إلى دراسة مضادات الأكسدة مثل فيتامين C و E وعلاقتها بالمالون ثنائي الالديهيد كونه الناتج النهائي لتأكسد الدهون وعلاقتها كذلك بالبيروكسي نائترت كونه احد العوامل المسببة للجهد التأكسدي لمرضى البواسير الشرجية، تضمنت الدراسة ٣٥ مريضاً (٢٠ ذكر و ١٥ أنثى)، تتراوح أعمارهم بين ٢٠ و ٥٠ سنة، سحبت عينات الدم من المرضى قبل المعالجة وبعد ٧ ايام من إجراء المعالجة باستعمال جهاز بارون لوضع الحلقات البلاستيكية، مقارنة بمجموعة السيطرة المكونة من ٢٠ ذكر و ١٥ أنثى، تتراوح أعمارهم بين ٢٠ إلى ٥٠ سنة.

نتائج هذه الدراسة دلت على حصول زيادة في إنتاج الجذور الحرة أدت إلى ارتفاعاً معنوياً في مستويات المالون ثنائي الالديهيد والبيروكسي نائترت وانخفاضاً معنوياً في مستويات مضادات الأكسدة (فيتامين E و C) قبل إجراء المعالجة كنتيجة لزيادة الجهد التأكسدي مقارنة بمجموعة السيطرة، وارتفعت قيم مضادات الأكسدة وانخفضت قيم المؤكسدات مقارنة بمجموعة السيطرة بعد المعالجة بواسطة الحلقات البلاستيكية والتي تمت بدون مضاعفات تذكر .

Introduction

Hemorrhoids are one of the most common gastrointestinal disorder seen by general practitioners. It has been estimated that they can occur at any age and can effect both women and men, also common among pregnant women, The natural evolution of hemorrhoids is benign but

hemorrhoids tend to get worse over time, and disease should be treated as soon as it occurs[1,2].

Hemorrhoids diseases have similar symptom to those of more serious anorectal pathologies, particularly, carcinoma and inflammatory bowel disease[3]. Hemorrhoids are divided into internal and external Hemorrhoids

and the internal variety is further sub divided into four stages, based principally on the degree of the prolapse.[4]

First degree hemorrhoids are pathogenically enlarged but never prolapsed, but they may be give blood during defecation asymptomatic, they can be seen only with proctoscope. Second degree hemorrhoids cannot be seen on external examination, but the patients give history of the prolapse with defecation, In third degree hemorrhoids, the prolapse occurs with every bowel motion, occasionally with straining and exertion specially when standing, while the fourth degree of hemorrhoids are permanently prolapsed and thus prone to thrombosis, they are painful and often bleed profusely, the over lying mucosa often becomes keratinized[5]. number of methods may be used to remove or reduce the size of internal hemorrhoids. These techniques include: Rubber band ligation: A rubber band is placed around the base of the hemorrhoid inside the rectum, Sclerotherapy and surgical excision are another alternative ways of treatment[4].

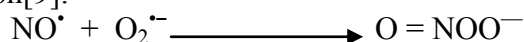
Free radicals are important in living organisms _include hydroxyl(OH[•]), superoxide(O₂^{•-}), and peroxy(LOO[•]) and

peroxynitrite(ONOO⁻), oxidative stress is the term referring to the imbalance between the generation of reactive oxygen species(ROS) and the activity of antioxidant defenses[6]. ROS can attack polyunsaturated fatty acids (PUFA) in cell membrane leading to a chain lipid peroxidation, fatty acids breakdown results in formation of various oxidatively modified products which are toxic to cells and finally converted into stable end products. It is possible to measure the extent of peroxidative damage estimating the stable end products of lipid peroxidation such as malondialdehyde[7].

There are many types of antioxidants, they can be classified by their mechanism of action, preventative antioxidants include peroxide decomposers, while chain breaking antioxidants intercept chain carrying radicals. Many chain breaking antioxidants donate a hydrogen atom to the chain carrying radical thereby stopping the oxidation process, this results in an antioxidant radical. α -Tocophrol protects against lipid peroxidation which is a chain reaction, it breaks the propagation by donation a hydrogen atom to the chain carrying peroxy radical, thereby stopping the oxidation process[8].



Vitamin C plays a major role in free radical scavenging which produce during oxidative stress such as OH[•], LOO[•] and protecting against lipid peroxidation, can also interact with the plasma membrane by donating electrons to α - TocO[•], recycling of α - tocopherol by ascorbat helps to protect membrane lipids from Peroxidation[9].



Nitric oxide radical has been identified as a biologically important molecule involved in a number of physiological processes, NO[•] can react rapidly with superoxide anion(O₂^{•-}) to produce the potent oxidant peroxynitrite(ONOO⁻), which is very reactive species initiating oxidation and nitration [10, 11].

The aim of the present study is to assess the effect of hemorrhoidal disease on levels of vitamin C, E and malondialdehyde and peroxynitrite(ONOO⁻) in males and females compared with healthy controls.

Patients and Methods

35 patients, 20 males and 15 females aged 20 to 50 years and 35 healthy subjects 20 males and 15 females aged 20 to 50 years as a control group, blood samples were collected from patients with hemorrhoids of various degrees before they undergoing rubber band ligation which done as out patients by the same surgeon in private clinic, and after seven days of the treatment, second sample were collected. After clotting, serum was separated by centrifugation, the analytical determinations described below were either performed immediately, or serum was stored at -20°C and used within 72 hours.

Methods

Vitamin E was measured using the methods described by Toro et al.[12] α -tocopherol react with α,α -dipyridyl to produce a complex, which has λ_{max} at 520 nm, vit E concentration was expressed as mg/ L. Vitamin C concentration was evaluated in serum by using chemical analysis, briefly, ascorbic acid in the protein free supernatant is oxidized by Cu⁺² ions to dehydroascorbic acid and diketogulonic acid. These ketones react with 2,4-dinitrophenyl hydrazine in H₂SO₄ to form the phenyl hydrazone products, which absorb at 520 nm,[13] vit. C concentration was expressed in mg/ L.

MDA levels were analyzed according to method described by Bueg(14), to 1ml of the resulting supernatant were added 2 ml thiobarbituric acid reagent (15% W/V trichloroacetic acid, 0.37% thiobarbituric acid and 0.25 HCl), the solution was then heated at 100°C for 15 min. After cooling, the precipitate was removed by centrifugation at 3000 xg for 5 min., and the absorbance of the supernatant determined at 530 nm. While the determination of peroxynitrite concentration described by Vanuffelen B.E., the principles was based on the radical of peroxynitrite(ONOO⁻) mediates nitration of phenol to form nitrophenol compound which is detected at 412 nm[15].

Statistical analysis

All results are expressed as a mean $\pm$ SD(standard deviation), comparison between patients and controls were preformed by the student's t-test. Person's correlations were used to determine relationship between parameters studied taken $P \leq 0.05$ as the lowest level of significant.

Results

The vitamin E concentration of males and females patients are significant increase ($P=0.00, 0.004$) respectively, after the treatment compared with these patients before the treatment shown in Fig(1), and the vitamin C concentration for males and females patients are significant increase ($P=0.05, 0.02$) respectively, after the treatment compared with these patients before treat, Fig.(2) and Table(1 and 2).

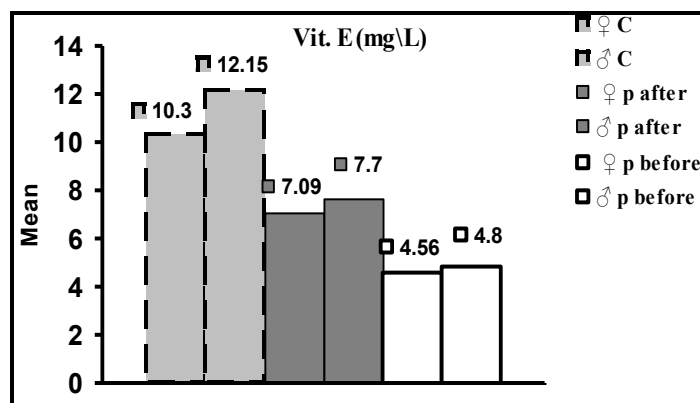


Figure 1 The levels of vitamin E in mg/L for patients and controls. (♂c, ♀c) male and female control, (♂p, ♀p) male and female patient after and before the treatment

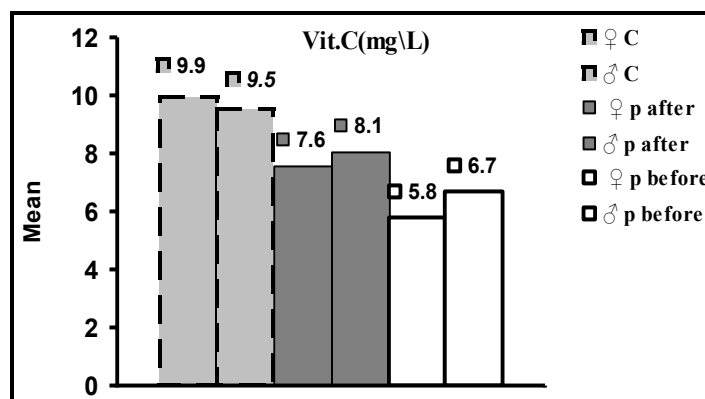


Figure 2 The levels of vitamin C in mg/L for patients and controls. (♂c, ♀c) male and female control, (♂p, ♀p) male and female patient after and before the treatment

While the analysis showed a decrease in the MDA(Fig.3) and Table(1, 2) to be statistically no significant for treatment patients,

males and females (P= 0.1, 0.4) comparison to these patients before treatment.

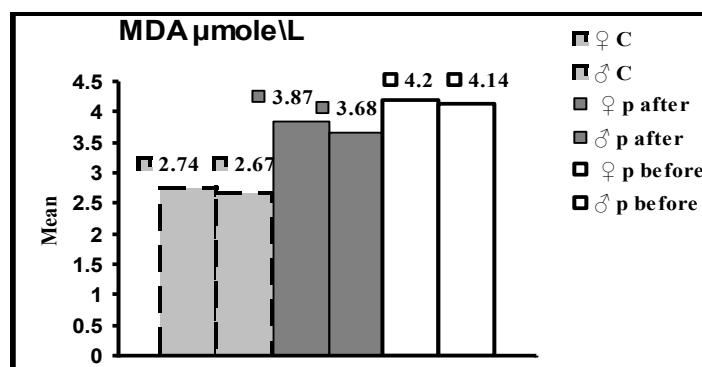


Figure 3 The levels of MDA in μmole/L for patients and controls. (♂c, ♀c) male and female control, (♂p, ♀p) male and female patient after and before the treatment

The levels of peroxynitrite in serum of hemorrhoids patients(before and after) are non significantly increase compared with controls shown in Fig.(4), while the levels of males patients($P=0.02$) after the treatment are significantly decreased compared with

these patients before the treatment, so the levels of peroxynitrite of females patients $P=0.06$ after the treatment are non significantly decreased compared with females patients before the treatment, Table (1, 2).

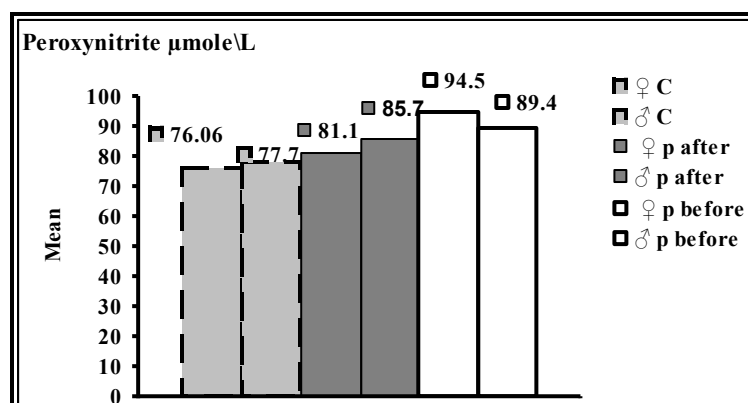


Figure 4 The levels of MDA in $\mu\text{mole/L}$ for patients and controls. (♂c , ♀c) male and female control, (♂p , ♀p) male and female patient after and before the treatment.

Table 1 The levels of Vitamin E, C in mg/L and MDA, ONOO^- in $\mu\text{mole/L}$ for males patients before and after treatment compared with males controls.

	n	♂patient before	♂patient after	♂ control	n	P value
Vit E	25	4.8±1.9	7.7±3.6	12.15 ± 4.3	20	0.00*S 0.002**S
Vit C	25	6.7±3.2	8.1±3.6	9.5±2.5	20	0.00*S 0.001**S
MDA	25	4.14±1.6	3.68±1.7	2.67±0.49	20	0.00*S 0.01** S
ONOO ⁻	25	89.4±35.3	85.7±34.2	77.7±21.5	20	0.2* 0.02**S

Results are expressed as mean± SD, S: significant. * P value between patients and controls before treatment, ** P value between patients and controls after treatment.

Table 2 The levels of Vitamin E, C in mg\L and MDA, ONOO⁻ in μ mole\L for females patients before and after treatment compared with females controls.

	n	♀patient before	♀patient after	♀ control	n	P value
Vit E	15	4.56±2.07	7.09±3.4	10.3 ± 3.4	15	0.00*S 0.006**S
Vit C	15	5.8±2.3	7.6±3.9	9.9±2.8	15	0.00*S 0.004**S
MDA	15	4.2±1.8	3.87±1.7	2.74±0.18	15	*0.00 S 0.003**S
ONOO ⁻	15	94.5±37.9	81.1±21.0	76.06±21.9	15	0.2* 0.4**

Results are expressed as mean± SD, S: significant, * P value between patients and controls before treatment, **P value between patients and controls after treatment.

Discussion

The change in values of serum vitamin E concentration in hemorrhoidal disease sign to reduction of the activity of antioxidant system and the depletion of vit.E concentration in patients are indicated hyperconsumption as a radicals scavenger and poly unsaturated fatty acids protector and other component of cell membranes and low density lipoprotein to guard against oxidative damage in cells and the complications due to hemorrhoids disease. This may be leading to imbalance between oxidant and antioxidant in favor of the prooxidant thus leading to potential damage and dominant risk factor of many diseases[16].

Evidence for increased oxidation in hemorrhoidal patients compared with controls were also provided by the presence of increased prooxidant production in blood[17], vitamin E and C are lower in patients before the treatment can be suggest for consumption of vit E as a scavenger and recycle it by the vit C, thus, it protect cell membranes from external oxidants, moreover vitamin C can detoxified of free radicals formed in body fluids in patients, mainly acts by scavenging reactive oxygen species(ROS) directly, and among

these species probably the most important are superoxide and peroxynitrite, causing ascorbate oxidized rapidly[1,18], this may explain the depletion in vitamin C concentration in the present study. Accordingly in this report we investigated the tissues damage due to the oxidative stress that occur in subjects with hemorrhoids.

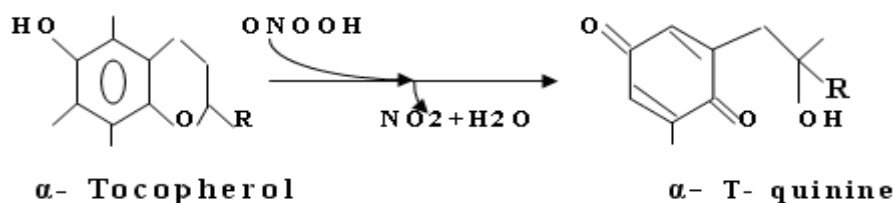
The variation in antioxidant levels of hemorrhoidal patients can be due to the difference in age, sex, degree of disease and the period of disease.

Disease produce ROS such as H₂O₂, O₂ • and OH• which are potentially harmful to the cell membrane with its high content of polyunsaturated fatty acids[19], increment in MDA might be due to generation of reactive oxygen species and to the excessive oxidative damage generated in these patients, ROS in turn can oxidize many other important biomolecules, including membrane lipid[20]. The increase of MDA levels in the serum indicates the ongoing oxidative stress in hemorrhoids patients. The serum antioxidant defense are overwhelmed and organs are no adequately protected and undergo oxidation[21,22].

The elevated of peroxynitrite levels of hemorrhoids patients could be the essential cause to depletion of vitamin

E concentration because α - tocopherol was regarded as a defense substrate against peroxynitrite attack, the

protective action of the α - tocopherol was shown below[23, 24].



Lower levels of vitamin E and C and higher levels of MDA and peroxynitrite have been associated with a complication of disease which is released a free radicals species including lipid peroxy radicals ($\text{LOO}\cdot$), $\text{O}_2^-\cdot$ and ONOO^- [1, 25, 26].

These results expected since our hemorrhoidal patients under conditions of oxidative stress and explain how the treatment of hemorrhoids can be decrement of oxidative stress of these patients.

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