# Vitamin C and Thiobarbituric Acid Reactive Substances (TBARS) in Psoriasis Vulgaris Related to Psoriasis Area Severity Index (PASI)

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*Oxidative stress is caused by an imbalance between the production of pro-oxidants and the capacity of a biological system of rapid detoxification of free radicals. In this paper the level of pro-oxidants and antioxidants was quantified in patients with psoriasis vulgaris. The results of this study show that the level of oxygen reactive species dramatically increases and the physiologic antioxidant mechanisms are inefficient in patients with psoriasis vulgaris. These findings re-confirm that oxidative stress has a destructive and pathogenic potential in psoriasis.*

*Keywords: oxidative stress, biological system, psoriasis vulgaris*

Psoriasis is a chronic, inflammatory, immune-mediated, polygenic skin disorder characterized by the occurrence of erythematous, sharply demarcated plaques covered by thick white or yellow scales, mostly affecting the scalp, elbows, knees and sacrum. Lesions can however occur at any site. It is a rather common disorder, with a prevalence of approximately 2% in the world population. Several trigger factors, both endogenous and exogenous, can elicit psoriasis in predisposed patients. The most important triggers in such patients are streptococcal infections, medications like beta-blockers, angiotensin converting enzyme antagonists, antimalarials, nonsteroidal anti-inflammatory drugs, interferons, lithium or oral contraceptives, alcoholism, stress, hypocalcemia, HIV infection or cutaneous injury [1,2].

The severity of psoriasis can be assessed using the Psoriasis Area and Severity Index (PASI) and by calculating the percentage of body surface area affected (BSA). PASI however has the advantage that it better reflects the disease severity because it takes into account the erythema, induration and scaling (2). To calculate the PASI, the body is divided into four segments: head (H), trunk (T), upper extremities (U) and lower extremities (L). The erythema (E), induration (I) and desquamation (D) are assessed for each of the four segments separately, as follows: 0= none, 1= mild, 2= moderate, 3= severe, 4= very severe.

The affected area is assessed for each segment: 0 = none, 1= < 10%; 2= 10-30%, 3= 30-50%, 4= 50-70%, 5=70-90% and 6= 90-100%. The PASI is calculated using the following formula:

$$PASI=0.1\,(E_H + I_H+D_H)\,A_H + 0.3\,(E_T + I_T+D_T)\,A_T + 0.2\,(E_U+ I_U+ D_U)\,A_u +0.4\,(E_L + I_L+D_L)\,A_L$$

PASI can range from 0 to 72. Psoriasis is therefore considered mild when PASI is lower than 7, moderate when PASI is between 8 and 12 and severe when PASI is higher than 12 [2,3].

Psoriasis is a recurrent disorder of unknown etiology. Immune cells, especially T-lymphocytes, seem to have a very important role in the pathogenesis of psoriasis and the multiplication of keratinocytes. In recent years, however, researchers showed that oxidative stress might also have an important role in psoriasis. Skin is continuously exposed to visible and ultraviolet light, high concentrations of oxygen, airborne pollutants, dietary and drug metabolites, some of them being free radical generating agents. High levels of leukocytes increase reactive oxygen species and lipid peroxidation.

Oxidative stress is caused by an imbalance between the production of pro-oxidants and the capacity of a biological system to rapidly detoxify free radicals, remove altered molecules and repair the resulted prejudice [4]. Oxidative stress is not an affliction, a symptom or syndrome, but a biochemical process generated and maintained by endogenous and exogenous factors [5,6]. Lipid peroxidation is a major mechanism in the appearance of psoriatic lesions and implies oxidation of polyunsaturated fatty acids leading to the formation of toxic products (table 1). The main methods used for quantifying these noxious products are presented in table 1 [7-11]. The organism develops endogenous defense mechanisms which have the capacity to annihilate the produced free radicals [4,7-13]. The evaluation of enzymatic and non-enzymatic antioxidant components can be achieved through various analysis techniques (table 2).

In this paper we quantified the levels of pro-oxidants, expressed through the concentration of thiobarbituric acid reactive substances and antioxidants, assessed through the concentration of vitamin C in patients with psoriasis vulgaris.

## Experimental part

*Materials*

The urinary levels of lipo-peroxides (expressed through the concentration of thiobarbituric acid reactive substances- TBARS), creatinine and vitamin C were determined in 90 patients with psoriasis vulgaris and 50 controls.

-Inclusion criteria: optimum nutrient intake, adults, normocalcemic;

- exclusion criteria: the use of vitamin supplements, malabsorption, alcoholics, smokers, children, elders, patients requiring dialysis or infusions, Whipple disease or the irritable bowel syndrome, anemia, uricosuria, pregnancy, breastfeeding, allergy, diseases of the osseous system, blood coagulation disorders, physical and

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| *Markers* | *Biological specimens* | *Methods of detection* |
|---|---|---|
| F2 isoprostanes(8-iso-PGF2a) | urine, plasma, serum, tissues, cell lysates | GC-MS, GC-MS/MS, LC-MS, LC-MS/MS, Colorimetry. Fluorometry, ELISA, EIA, RIA, immunohistochemistry. |
| F4 Neuroprostanes | plasma, cerebrospinal fluid, tissues | colorimetry, fluorometry, ELISA, RIA, chromatography, immunohistochemistry. |
| Malondialdehyde(MDA) | variety of sample types | Colorimetry, fluorometry, spectrophotometry, immunoblot, ELISA, HPLC-fluorescence, GC/MS. |
| Thiobarbituric Acid Reactive Substances (TBARS) | variety of sample types | colorimetry, spectrophotometry, fluorometry, EIA,HPLC |
| 4-Hydroxynonenal Adduct (HNE-Adduct) | variety of biological sample | ELISA, GC-MS, HPLC |
| Acrolein-Lys Adduct | serum plasma, cerebrospinal fluid | ELISA |
| Oxidized Low density Lipoprotein (CML-LDL, HNE-LDL, MDA-LDL, OxPL-LDL) | serum ,plasma, other biological fluids | colorimetry, ELISA |
| Lipid hydroperoxide - Hexanoyl-Lysine adduct (HEL) | urine, serum, cultured cells | ELISA, HPLC, Western bloot, immunohistochemistry |

**Table1**
LIPID PEROXIDATION MARKERS

| Marker | Biological specimens | Methods of detection |
|---|---|---|
| Antioxidant enzyme systems | | |
| Catalase(CAT-E.C.1.11.1.6) | whole blood, RBCs, plasma, serum, cell lysates, tissue homogenates | colorimetry, fluorometry, spectrophotometry, ELISA |
| Superoxide dismutases (SODs-E.C.1.15.1.1) | plasma, serum, , RBCs, cerebrospinal fluid, cells, tissues | colorimetry, spectrophotometry, ELISA. |
| Glutathione peroxidase activities (GPXs-E.C.1.11.1.9) | serum, plasma, RBCs,tissue extracts, cell lysates | spectrophotometry, colorimetry, ELISA |
| Glutathione reductase activity(GR-E.C.1.8.1.7) | plasma, erythrocytes, tissues, cell lysates | colorimetry, spectrophotometry, ELISA |
| Glutathione-S-transferase activities(GSTs-E.C.2.5.1.18) | tissue homogenates, serum, plasma, cell lysates | colorimetry, spectrophotometry, ELISA |
| Thioredoxin reductase activity (TrxR-E.C.1.8.1.9) | tissue homogenates, cell lysates, cell culture supernatant, plasma, serum, urine | colorimetry, spectrophotometry, ELISA |
| Heme oxygenase activity (HMOX1-E.C.1.14.99.3) | cell culture supernatant, plasma, cerebrospinal fluid, cell lysates | fluorescence, HPLC, ELISA |
| Xanthine oxidase activity (XO-E.C.1.17.3.2) | cell culture supernatants, urine, serum, plasma, tissue extracts | fluorometry, colorimetry |
| Antioxidant non-enzyme systems | | |
| Alpha-Tocoferol(vitamin E) | plasma ,serum | HPLC |
| Carotenoides (alpha carotene, beta carotene, lycopene) | plasma, serum | GC/MS,HPLC-MS, reversed-phase HPLC |
| Retinol (vitamin A) | plasma, serum | GC/MS, HPLC-MS, reversed-phase HPLC |
| Retinol-binding protein (RBP) | plasma, serum | radial immunodiffusion |
| Ascorbic acid (vitamin C) | plasma, serum, urine | HPLC-CE, spectrophotometry, fluorometry |
| Glutathione | plasma, serum, saliva, urine,cell lysates, tissue extracts | colorimetry, fluorometry,HPLC |
| Uric acid | serum, plasma, urine | colorimetry, fluorometry, HPLC |
| Bilirubin | serum ,plasma | colorimetry |
| Albumin | serum, plasma, cerebrospinal fluid | colorimetry, spectrophotometry |
| Transferrin | serum, plasma , tissue homogenates, cell lysates, cell culture supernatant, tissues | immunoturbidimetry, spectrophotometry, flow cytometry, immunoblotting, ELISA, fluorescence-microscopy |

**Table 2**
ANTIOXIDANTS DEFENCE MECHANISMS

continuated table 2

| | | |
|---|---|---|
| Lactoferrin | cell culture supernatant, saliva, urine, serum, plasma | immunoturbidimetry, colorimetry, ELISA, immunohistochemistry, immunoblotting |
| Ferritin | serum, plasma, tissues ,cell culture supernatant, urine, cerebrospinal fluid | immunoturbidimetry, colorimetry, EIA, ELISA, Western blot, immunohistochemistry |
| Ceruloplasmin | serum, plasma , urine, tissue homogenates, cell culture supernatant | colorimetry, immunoturbidimetry , ELISA, CLIA, Western blot, immunohistochemistry |
| Minerals: copper, zinc, iron, manganese, selenium | variety of biological samples | flam photometry, spectrophotometry, PIXE, INAA |
| Antioxidant capacity assay | | |
| Cell Based exogenous antioxidant | tissues , cellular environment | fluorescence |
| Total Antioxidant Status (TAS) | plasma, cell fractions, tissue lysates | fluorometry, colorimetry |
| Trolox Equivalent Antioxidant Capacity(TEAC) | plasma, cell fractions, tissue lysates | fluorometry, spectrophotometry |
| Feric Reducing Antioxidant Power (FRAP) | plasma, cell fractions, tissue lysates | colorimetry, fluorometry |
| Asymmetric Dimethylarginine (ADMA) | plasma, cell fractions, tissue lysates | colorimetry, LC/MS, HPLC, ELISA |

*HPLC= high performance liquid chromatography, MS=mass spectrometry, GC=gas chromatography, LC-=iquid chromatography, TLC-=thin layer chromatography, ELISA= enzyme-linked immunosorbent assay, EIA=enzyme immunosorbent assay, CLIA=chemilumiscent immunoassay, RBC=red blood cell, PIXE=proton induced X ray emission, INAA= instrumental neutron activation analysis, RAGEs=receptors of advanced glycation end product, ECD=electrochemical detection, RIA=radio immuno assay*

intellectual exhaustion, infectious diseases, surgical interventions.

Biological samples. Urine sample collection: spontaneous urine samples (preferably the first urine collected in the morning) collected in sterile containers, with no conservatives. Urine specimens were centrifuged at 3000g for 10 min. The supernatant is used for determining the biological parameters of interest. Since creatinine excretion is relatively constant in a certain patient, the determination of the urinary creatinine concentration can be valuable in estimating the renal function. Therefore, the urinary creatinine concentration will be used as a reporting parameter of TBARS determined in the population included in the study.

*Laboratory methods*

Creatinine level (fig. 1) was assessed through colorimetry and is based on the reaction between creatinine and picric acid in an alkaline medium. Absorbance measured at 492 nm is directly proportional with the quantity of creatinine in the sample.

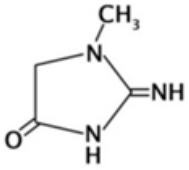


Fig. 1. Creatinine

Determination of vitamin C (fig. 2) was done using a spectrophotometric method and is based on the reaction between dinitrophenylhydrazine and dehydroascorbic acid, obtained through the reduction of ascorbic acid with copper sulphate. A coupling reaction takes place in the presence of thiourea, having as final product hydrazone, red coloured at 520 nm.

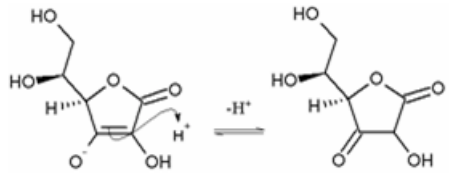


Fig. 2. Vitamin C

Determination of TBARS (fig. 3) was done spectrophotometrically using thiobarbituric acid. The reaction product is colorimetrically described at 532 nm.

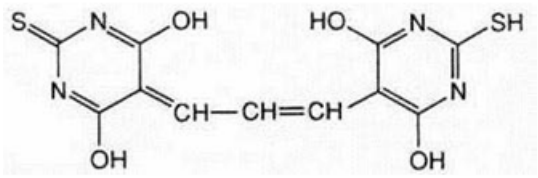


Fig. 3. TBARS

*Statistical analysis*

Comparison of quantitative variables for the two groups was performed by t test. It was chosen as a test of statistical significance 0.05 (5%), 95% confidence level showing that the decision is just. Correlations between variables were determined by linear regression and for the presentation of the relationship between two variables Pearson correlation coefficient was used. Data processing was made using SPSS software.

**Results and discussions**

Cutaneous disease severity was assessed using the PASI score. PASI <7, mild chronic plaque-type psoriasis (40 cases of psoriasis vulgaris in remission). PASI > 12 as severe chronic plaque-type psoriasis (50 cases of psoriasis vulgaris, active phase).

In the control phase, the level of vitamin C (mg/dl) was low in patients with psoriasis (11.3±6.8 versus 17.3 ±10.4, p=0.099) (table 3). Different values were also achieved between patients with active psoriasis and patients with psoriasis in remission (8.8+_3.7 versus 13.2+_8.7, p=0.062)(table 4). Statistically significant values between vitamin C levels (p=0.041) were achieved between patients with active psoriasis and controls (fig. 4).

The level of lipo-peroxides (ng TBARs/mg creatinine) presented considerable differences between psoriatic patients and controls (14.7± 6.2 compared to 7.5 ± 3.1, p=0.016) (table 3), between patients with active psoriasis and patients with psoriasis in remission (18.3 ± 8.2

| Variables | Psoriasis | Control | p value |
|---|---|---|---|
| Vitamin C(mg/dl) | 11.3+_6.8 | 17.3+_10.4 | 0.099 |
| TBARS/Creatinine(ng/mg) | 14.7+_6.2 | 7.5+_3.1 | 0.016 |

**Table 3**
EXPERIMENTAL DETERMINATIONS IN PATIENTS AND CONTROLS

| Variables | Psoriasis | | p value |
|---|---|---|---|
| | PASI<7 | PASI>12 | |
| Vitamin C(mg/dl) | 13.2+_8.7 | 8.8+_3.7 | 0.062 |
| TBARS/Creatinine(ng/mg) | 11.5+_3.2 | 18.3+_8.2 | 0.031 |

**Table 4**
TBARS AND VITAMIN C LEVELS IN PATIENTS WITH PSORIASIS GROUPED BASED ON PASI

| Variable pairs | Psoriasis | | | | Control | |
|---|---|---|---|---|---|---|
| | (active phase) | | (inactive phase) | | | |
| | R | p | r | p | r | p |
| Vitamin C/TBARS | -0.602 | 0.009 | -0.266 | 0.047 | 0.016 | 0.94 |
| TBARS/PASI | 0.422 | 0.028 | 0.012 | 0.299 | - | - |
| Vitamin C/PASI | -0.240 | 0.040 | 0.022 | 0.988 | - | - |

**Table 5**
STATISTICAL CORRELATIONS BETWEEN STUDIED PARAMETERS

compared to 11.5 ±3.2, p=0.031) (table 4). Statistically significant differences between TBARS values were obtained between controls and patients with active psoriasis (p=0.001), respectively patents with psoriasis in the inactive phase (p=0.014) (fig. 5).

Simple linear regression analysis revelaed a negative association between the concentration of vitamin C and the TBARS/creatinine ratio in patients with psoriasis in the active phase (r=-0.602, p=0.009) and in remission (r=-0.266. p=0.047) (table 5). The relation established between TBARS and PASI (r=0.422, p=0.028) and vitamin C and PASI (r=-0.240,p=0.040) is worth noticing only in patients active psoriasis (table 5).

The results of this study prove that vitamin C is rapidly consumed in patients with psoriatic lesions, this observation being supported by the negative relation obtained between vitamin C concentration and PASI. Furthermore, low levels of vitamin C play an important role in the exacerbation of lipid peroxidation, an effect explained through several mechanisms. First of all, vitamin C participates in maintaining capillary resistance. Second of all, vitamin C interferes with the modulation of the immune response and inflammation, regulation in the expression of adhesion molecules, inhibition of MAPKp38, NFKb, IL-1, IL-6, IL-33, TNF. Thirdly, vitamin C modulates carnitine synthesis, essential for transporting fatty acids in the mitochondria. Also, it plays an important role in the metabolism of lipids, vitamins. Vitamin C is a strong antioxidant agent (activates enzymatic detoxification in the liver, neutralizes free radicals and carcinogenic nitrosamines, assures the conservation and reinstatement of the antioxidant potential of vitamin E) and facilitates iron absorption in the digestive system. Moreover, vitamin C seems to be involved in the production of suprarenal hormones (glucocorticosteroids), collagen metabolism (promoting conversion of tropocollagen to collagen), folic acid, certain neurotransmitters (amination of some neuropeptides) and tyrosine (oxidative degradation). Vitamin C accelerates wound healing, inhibits melanin formation and increases cutaneous ceramide synthesis [12,13].

In the present study significantly increased values of TBARS were obtained in psoriasis, positively associated with PASI score. TBARS are intensely reactive molecules, formed in all mammalian cells after polyunsaturated fatty acid peroxidation and from arachidonic acid metabolism (prostaglandin and thromboxane biosynthesis). It binds covalently to the amino group of proteins, lipoproteins, phospholipids or DNA. Carbonyl groups react with the DNA to form malonildialdehyde-deoxyguanosine and deoxy-adenosine, but the main compound is pyrimido-purinone, which can significantly contribute to tissue alteration determined by lifestyle and dietary factors. Unlike free radicals, aldehydes are relatively stable and, as a consequence, they can diffuse through membranes and act at distance from the original location of the free radicals. Even though MDA can be inactivated by aldehyde-dehydrogenases, its production is accelerated by oxidative stress and, when concentrations reach critical levels, the dialdehyde can escape the detoxification process. MDA is toxic compound involved in a series of pathological processes. Some studies reported a high serum and tissue level of MDA in psoriasis [14].

## Conclusions

The results of the study show that the level of oxygen reactive species dramatically increases and the antioxidant physiologic mechanisms are inefficient in patients with psoriasis vulgaris. These findings reconfirm that oxidative stress has a destructive and pathogenic potential in psoriasis.

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