Effects of UV Radiation and Oxidative DNA Adduct 8-hydroxy-2'-deoxiguanosine on the Skin Diseases

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Excessive sun exposure may be associated with increased oxidative stress and oxidative damage of DNA. Based on this hypothesis, the authors focused on investigating the causal role of UV-induced genomic damage in the pathophysiology of porphyria cutaneatarda (photosensitive acquired metabolic disorder with autosomal dominanttransmittion). In this study we demonstrated that serum levels of 8-hydroxy-2'-deoxyguanosine, analteredgene product, was correlated with body iron content(hemoglobin, sideremia, transferrin, ferritin) in patients with porphyria cutanea tarda. Ironis involved in malignant processes by determining oxidative damage of DNA. Reduction of body iron content and genetic manipulation could have positive implications in prevention and progression of diseases mediated by oxidative stress.

Keywords: UV radiation, DNA adduct, 8-hydroxy-2'-deoxyguanosine (8-OHdG), iron store proteins, porphyria cutanea tarda

Excessive exposure to ultraviolet light (UV) is the main risk factor for skin cancer , sunburn, premature skin aging, some skin disorders associated with photosensitivity (porphyria, lupus erythematosus, vitiligo, rosacea, acne vulgaris), view disorders, decreased immune competence, worsening of pre-existing pathological conditions (collagenosis, psoriasis, cardiovascular diseases, rheumatoid arthritis) [1-5]. These findings were the base for specialists to investigate the role of genomic lesions induced by UV radiation in production of porphyria cutanea tarda (PCT).

UV spectrum of sunlight contains fields UVC (190-290nm), UVB (290- 320nm), UVA (320- 380nm). Stratospheric ozone retains full UVC radiations and partial UVB radiations. UVA and a fraction of UVB radiations reach the Earth's surface.

UVB rays have a higher energy than UVA . UVB rays are absorbed directly by a number of cell components (nucleic acids, proteins, urocanic acid), exert mutagenic effects and are responsible for skin cancer development. UVA radiation acts on cellular constituents (nucleic acids, proteins, lipids, polysaccharides), exerting a weaker mutagenic effect than UVB.

UVA radiations operate on the body by oxidative mechanisms, correlated with reactive oxygen species formation. Typically, reactive oxygen species are grouped in:

- species with radical character: superoxide anion $O_2^{\cdot-}$, hydroxyl radical $HO^{\cdot}$, Nitrogen monoxide NO;
- nonradical species: oxigen singlet O_2, hydrogen peroxide H_2O_2, hypochlorous acid HOCl, lipid hydroperoxides.

UV photons are absorbed by the nucleic acids, proteins and various chromophores in the cell environement, such as quinones, steroids, porphyrins, flavin coenzymes, heminin proteins (cytochromes, peroxidase, catalase). The absorption of UVA photons by chromophores results in formation of reactive oxygen species or by transfering the energy to certain target molecules [5-12].

H_2O_2 is formed by UV irradiation of tryptophan. $O_2^{\cdot-}$ anions are produced by UVA irradiation of NADH and NADPH. In the presence of iron ions, H_2O_2 and $O_2^{\cdot-}$ can participate in Haber -Weiss reaction, and thus, resulting the production of hydroxyl radicals.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + HO^{\cdot}$$
$$Fe^{3+} + O_2^{\cdot-} \rightarrow Fe^{2+} + O_2$$

Reactive oxygen species generated by UVA irradiation react with almost all molecules in biological systems: carbohydrates, phospholipids, nucleotides, organic acids, amino acids.

An important target of UVA are DNA macromolecules [1-4, 6, 8, 10, 11]. It is considered that UVA radiations interact with DNA by indirect mechanisms mediated by endogens photosensitisers. UVA induce multiple types of DNA lesions, cleavage of phosphodiester bonds at the level of a single strand, the formation of DNA-protein cross-links, dimerizationadjacentpyrimidine (fig. 1). Cleavage of single-strandedphosphodiester bondsby cleavageof DNA is a major change during irradiationUVA comparing with photons UVB and UVC.Pyrimidine dimer formation is not a major change in DNA during irradiation with UVA, compared with irradiation with UV B and UVC.

UVA rays ca cause transcriptional activation of genes encoding hemeoxygenase -1 (HO -1), metallothionein, CL100 phosphatase and collagenase [4 , 9, 14]. Activation of HO-1 is an mechanism that controls the intracellular level of iron ions. High levels of iron ions promotes lipid peroxidation dependent on iron. Hemeoxygenase -1 and -2 are involved in heme and heme proteins degradation. Product of heme degradation, bilirubin, is a powerful antioxidant, while the compounds catalysed by hemeoxygenase, porphyrins, act as photosensitizers in terms of UVA

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irradiation, generating $O_2^{\cdot}$. In dermis and epidermis the mechanisms of constitutive (HO-2) and inducible (HO -1) protection are closely correlated, being modulated by intracellular levels of ferritin. HO-1 expression in response to the action of oxidant agents and to the decrease of reduced glutathione concentration in tissues not involved in hemoglobin degradation, suggest that heme degradation and heme proteins constitutes a protective mechanism against oxidative stress.

Metallothionein, activated by UVA radiation is involved in protection against oxidative stress by: scavengeraction of reactive oxygen species, superoxide radicals and series of organic radicals capture, ions binding Zn, Cd, Cu, blocking Fenton reaction by chelating iron ions.

CL100 gene encodes Tyr/Thrphosphatase that inactivates MAP kinase involved in the oxidative stress response.

Collagenase activation secondary to UVA irradiation represents a destructive response that causes degeneration of interstitial collagen phenomenon of fotosenescence..

In eukaryotic cells, there were identified pathways for reparing damaged DNA (fig. 1). In humans the studies proved the effectiveness for the next mechanisms for reparing genomic lesions: repair pathways, base excision repair (BER), nucleotide excision repair (NER), recombinational repair (homologous recombination/Non-homologous End-Joining-HR/NHEJ), mismatch repair (MMR), direct reversal - (methyl guanine methyl transferase, MGMT). Therefore, determination of oxidative stress markers in intact cells or tissues must be interpreted as a balance between level of aggression and rate of DNA repair at the time of obtaining the sample. One of the indicators used to assess the effect of oxidative stress on nucleic acids is represented by 8-hydroxy-2'-deoxyguanosine (fig. 2) [4, 8, 10]. Deciphering fundamental mechanisms of oxidation/DNA repair would allow identification of new therapeutic targets in diseases mediated by oxidative stress.

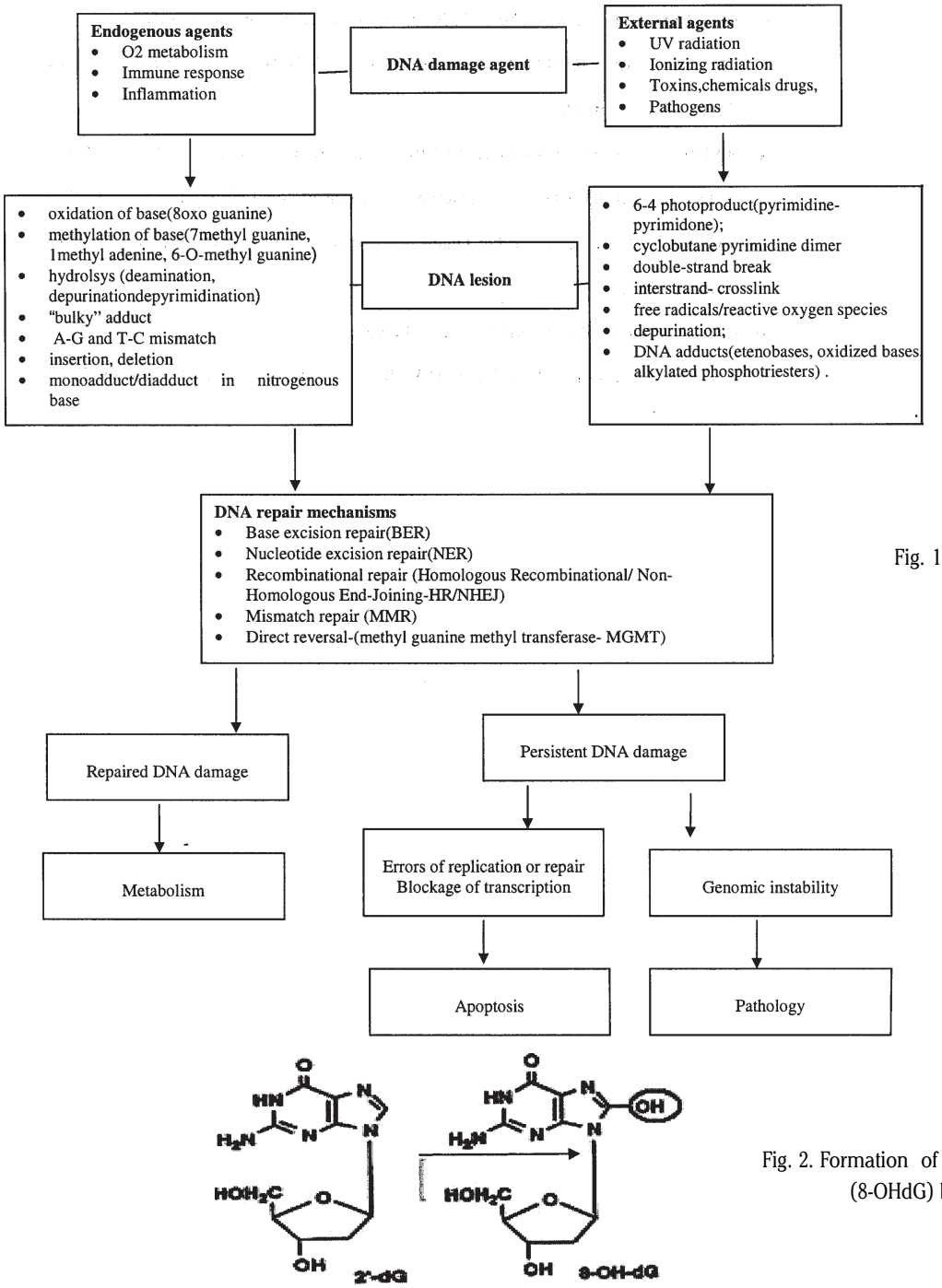

Fig. 1.Global response in DNA damage

Fig. 2. Formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) by oxygen radicals.

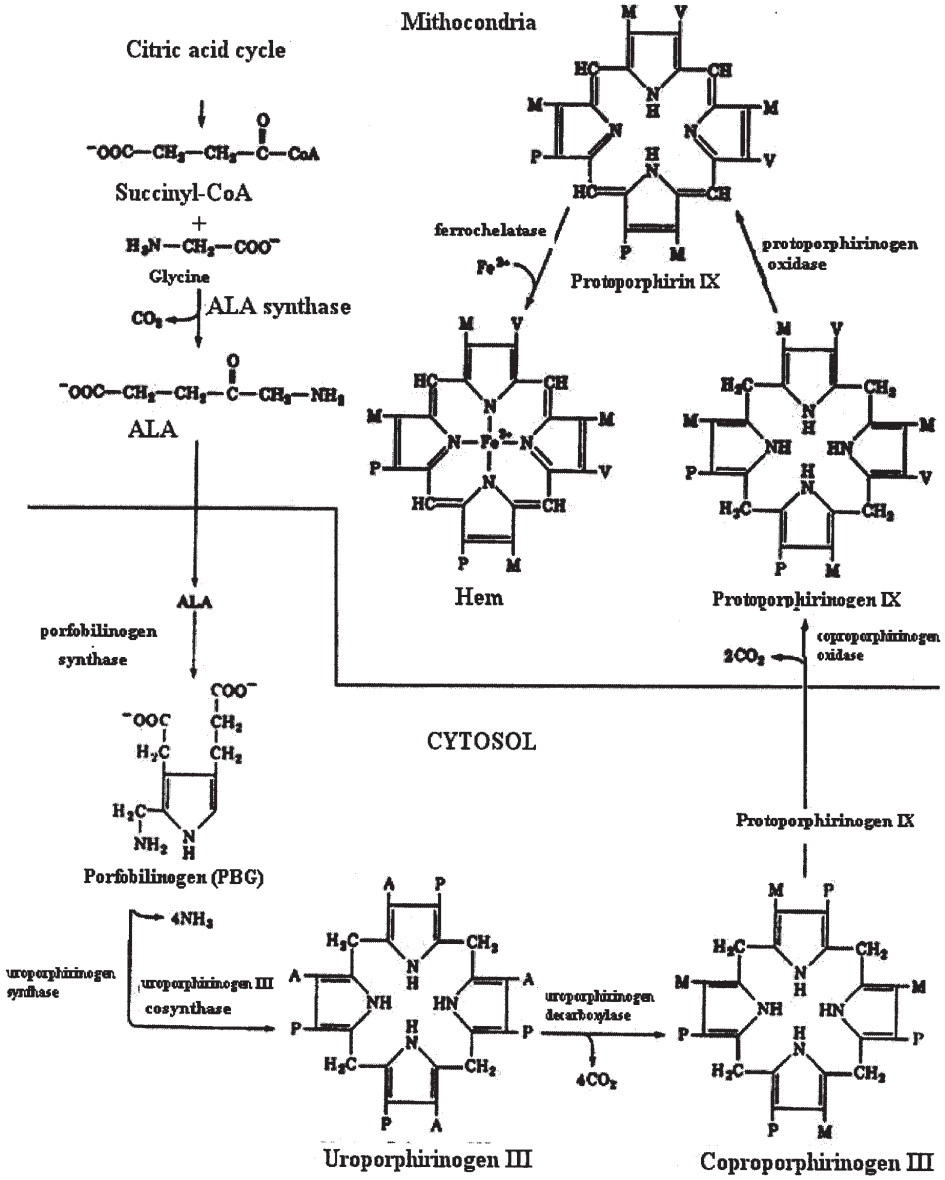

Fig. 3. The heme biosynthetic pathway

A photosensitive acquired metabolic disorder with autosomal dominant transmission is represented by porphyria cutaneatarda (PCT) [5, 11, 13, 15, 16].

Heme biosynthesis includes a sequence of reactions with well defined intermediate compounds: delta aminolevulinic acid (ALA), porfobilinogen (PBG), uroporphyirinogen III coproporphyrinogen III, protoporphyrinogen IX, protoporphyrin IX, heme (fig. 3) [5, 13].

These metabolic sequences are catalyzed by: coproporphyrinogen oxidase (EC number 1.3.3.3), protoporphyrinogen oxidase (EC number 1.3.3.4), oxygen-independent coproporphyrinogen-III oxidase (EC number 1.3.99.22), aminolevulinate synthase (EC number 2.3.1.37), porphobilinogen deaminase (EC number 2.5.1.61), uroporphyrinogen Decarboxylase (EC number 4.1.1.37), aminolevulinate dehydratase (EC number 4.2.1.24), uroporphyrinogen -III synthase (EC number 4.2.1.75), holocytochrome-c synthase (EC number 4.4.1.17), ferrochelatase (EC number 4.99.1.1), cytochrome c (EC number none).

Assuming that excessive sun exposure may be associated with oxidative degradation of DNA, the authors investigated the possible association between iron overloadand serum levels of 8-hydroxy-2'-deoxiguanosine in porphyria cutanea tarda patients.

Experimental part

Materials and methods

Selection of patients.The authors excluded from the study patients with: alcohol poisoning, halogenate-dorganic compounds or heavy metals, cirrhosis, fatty degeneration of liver, hematologic disorders, infectious diseases, metabolic syndrome, hemosiderosis, hereditary hyperbilirubinemia, malignancy, myocardial infarction, adverse drug reactions, hereditary tyrosinemia, acute-porphyria, treatment with phenothiazines.

Inclusion criteria.50 adult males diagnosedwith naive-porphyria cutaneatarda, (age=56.8±8.6 years, BMI=22.1±1.7kg/m) and 50 healthy men (age =53,6±9.3years, BMI=23.1±1.2kg/m) were included in the study. The diagnose of porphyriacutaneatarda was made by clinical examination and laboratory determinations.

Laboratory determinations:

- Full blood count was performed automatically with Pentra C60 (ABX, France).
- Determination of liver function tests, glucose, kidney tests, lipid profile and iron was made by photometric methods (HumaStar 300, GmbH, Germany).

Variable	PCT (n=50)	Control (n=50)
Whole blood		
Hemoglobin(mg/dl)	17.9±2.7(1)	14.3±2.1
Hematocrit(%)	58.1±8.1(1)	41.5±1.4
Serum		
AST(U/L)	87.3±22.1(1)	21.3±5.2
ALT(U/L)	79.4±19.3(1)	18.4±4.1
GGT(U/L)	69.4±8.5(1)	19.5±4.3
BChE(U/L)	3411±876(1)	5912±702
Albumin(g/dl)	3.4±0.5(1)	4.1±0.2
CRP(mg/dl)	1.1±0.9(1)	0.19±0.19
Iron (ug/dl)	204±61(1)	117±38
Transferrin(mg/dl)	186±79(1)	274±86
Ferritin(ng/ml)	304±109(1)	64±48
8OHdG(ng/ml)	34.2±11.3(1)	5.1±3.8
Urine		
Coproporphyrin (ug/24h)	466±202(1)	18±18
Uroporphyirin (ug/24h)	735±311(1)	4.3±4.3
ALA(mg/24h)	4.8±2.3	0.2±0.2
PBG(mg/24h)	1.2±1.2	0.4±0.4

Table 1
BIOLOGICAL CHARACTERISTICS OF PARTICIPANTS IN THE STUDY

1 = p <0,05 statistically significant variation between phorphyria cutanea tarda versus control
n = number of patients, PCT=pophyiria cutanea tarda,AST = aspartate aminotransferase, ALT = alanine aminotransferase, GGT = gamma-glutamyltransferase, BChE=Butyrylcholine esterase, CRP = C-reactive protein, 8-OHdG = 8-hydroxy-2'-deoxyguanosine, ALA=Aminolevulinic acid, PBG=Porphobilinogen

- Ferritin, transferrin, and C-reactive protein were determined by immunoturbidimetric method (HumaStar 300, GmbH, Germany).
- 8-hydroxy-2'-deoxyguanosine serum (8OHdG) determination was done by ELISA method (TECAN, GmbH, Austria).
- Coproporphyrins and urinary uroporphyrins dosage was done by chromatographic and spectrophotometric methods (396-410nm).
- Aminolevulinic acid (ALA) and porphobilinogen in urine(PBG) determination were done by column chromatography with ion exchange resins and spectrophotometry (555nm).

Biological samples
- venous blood collected in anticoagulant vacutainer K3EDTA to determine haematological parameters;
- venous blood collected a jeun in vacutainer without anticoagulant with/without gel separator for serum determinations.
- urine collected within 24 h, stored at 2-8°C and kept in the dark to determineCoproporphyrins uroporphyrins, aminolevuluinic acid and porphobilinogen

Statistical analysis of data
The clinical and laboratory quantitative data were expressed by mean and standard deviation. The correlation between the phenomena was expressed by the correlation coefficient r. The value threshold for the statistical significance was 0.05. The processing of data was performed using SPSS software.
The study was approved by the Hospital Committee of Ethics. All patients consented for the use of their biological samples in research and for teaching.

Results and discussions
PCT occurs especially in adult hood and affects usually males. PCT is triggered by liver disorders, resulting in elevated aminotransferase and gamma glutamyl-transferase and low levels of butyrilcholinesterase (table 1).
The diagnosis of PCT was based on the following laboratory investigations:
- significantly increased serum levels of iron in PCT compared to controls ($p<0.05$);
-increased values of Coproporphyrins and urinary uroporphyrinsin PCT compared to controls ($p<0.05$) (table 1).
Level of aminolevulinic acid (ALA) and porphobilinogen (PBG) in urine collected in 24 hshowed no statistically significant differences between patients with PCT and control ($p>0.05$) (table 1). These biochemical abnormalities recorded in patients with PCT were caused by a moderate reduction of the uroporfirinogen decarboxylase [8, 11, 13, 15, 16].
Iron overload evidenced in patients with PCT was confirmed in this study also by other results (table 1):
-increased concentration of hemoglobin in PCT patients compared to control ($p<0.05$);
-significantly increased serum levels of ferritin in PCT compared to control ($p<0.05$);
-significantly lower serum of transferrin in PCT compared to controls ($p<0.05$).
Under physiological conditions most of body iron is found in compounds with heme [5, 8, 11, 15, 16]. Non hemin iron is stored as ferritin or hemosiderin in macrophages and hepatocyte. A very small amount of circulating iron is bound to transferr in. Alteration of hepcidin regulating mechanism (protein with negative effects on serum iron) or of signaling molecules involved inregulation of hepcidin synthesis plays an important role

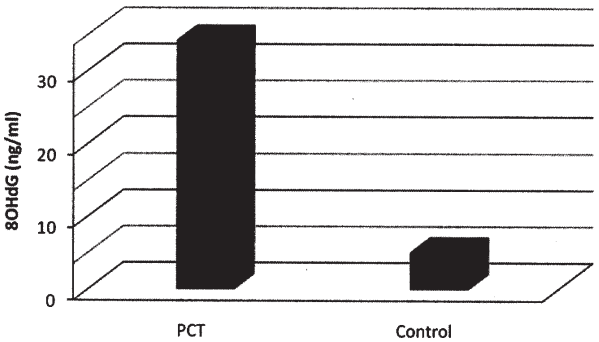

Fig. 4. Graphical representation of 8-hydroxy-2'-deoxyguanosine (8-OHdG-ng/ml)in patients with porphyria cutanea tarda(PCT) and control group ($p<0.05$)

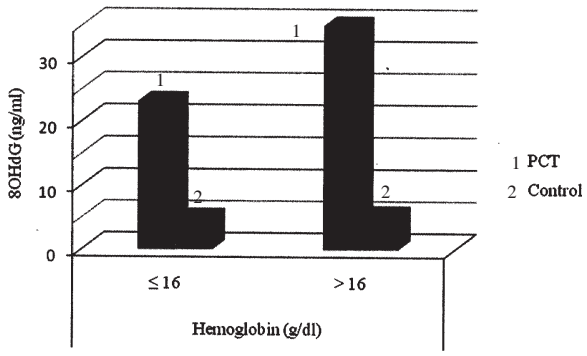

Fig. 5. Graphical representation of 8-hydroxy-2'-deoxyguanosine (8-OHdG-ng/ml) in relattion with hemoglobin(g/dL) in patients with porphyria cutanea tarda and control group ($p < 0.05$)

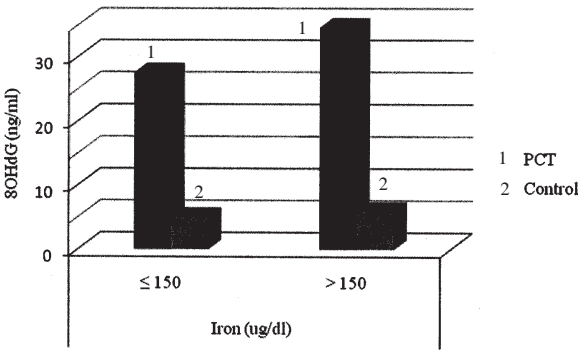

Fig. 6. Graphical representation of 8-hydroxy-2'-deoxyguanosine (8-OHdG-ng/ml) in relattion with to iron(ug/dl)in patients with porphyria cutanea tarda and in the control group ($p < 0.05$)

Study group	Variable	Hemoglobin	
		≤ 16	> 16
PCT	n	6	44
	8OHdG	23.1±3.4	35.6±14.7
	r	0.76	0.89
	p	0.00	0.00
Control	n	46	4
	8OHdG	5.0±3.6	5.4±2.2
	r	0.09	0.19
	p	0.63	0.05

Table 2
VARIATIONS OF THE 8-OHdG (ng/mL) AND HEMOGLOBIN (g/dL) IN THE STUDY PARTICIPANTS

n= number of patients; 8-OHdG=8-hidroxi-2'-deoxiguanosine; PCT-porphyria cutanea tarda; r=correlation index; p=statistical significance

Study group	Variable	Iron	
		≤ 150	> 150
PCT	n	2	48
	8OHdG	27.5±3.1	34.6±10.7
	r	0.02	0.88
	p	1.00	0.00
Control	n	47	3
	8OHdG	5.0±3.9	5.9± 0.9
	r	0.04	0.16
	p	1.00	0.02

Table 3
VARIATIONS OF THE 8-OHdG (ng/mL) AND IRON(ug/dL) IN THE STUDY PARTICIPANTS

n= number of patients; 8-OHdG=8-hydroy-2'-deoxiguanosine; PCT-porphyria cutanea tarda r=correlation index; p=statistical significance

Study group	Variable	Ferritin	
		≤ 100	> 100
PCT	n	5	45
	8OHdG	24.3±6.1	35.2±9.7
	r	0.73	0.92
	p	0.01	0.00
Control	n	46	4
	8OHdG	5.0±3.7	5.7± 2.2
	r	0.04	0.17
	p	0.91	0.26

Table 4
VARIATIONS OF THE 8-OHdG (ng/mL) AND FERRITIN (ng/dL) IN THE STUDY PARTICIPANTS

n= number of patients; ; 8-OHdG=8-hydroxy-2'-deoxiguanosine; PCT-porphyria cutanea tarda; r=correlation index; p=statistical significance

Study group	Variable	Transferrin	
		≤ 200	> 200
PCT	n	41	9
	8OHdG	35.5±12.2	29.1±7.4
	r	0.66	0.41
	p	0.00	0.03
Control	n	4	46
	8OHdG	5.4±2.3	5.0± 3.2
	r	0.27	0.16
	p	0.04	0.25

Table 5
VARIATIONS OF THE 8-OHdG (ng/mL) AND TRANSFERRIN (mg/dL) IN THE STUDY PARTICIPANTS

n= number of patients; 8-OHdG=8-hydroxy-2'-deoxiguanosine; PCT-porphyiria cutanea tarda;r=correlation index; p=statistical significance

in pathogenesis of diseases caused by disorder of iron metabolism.

Cutaneous manifestations in patients with PCT increased after exposure to sunlight. This finding is justified experimentally by obtaining significantly higher serum concentrations of 8OHdG in PCT patients compared to controls (fig. 4).

Another important aspect obtained in this study is the significant positive correlation, registered in PCT patients between serum levels of 8OHdG and hemoglobin concentration (fig. 5), between serum levels of 8OHdG and sideremia (fig. 6), between serum levels of 8OHdG and ferritin (fig. 7). A negative relationship with statistical significancewas obtained between serum levels of 8OHdG and transferrin in patients with PCT (fig. 8). In control group, total body iron content showed no association with serum levels of 8OHdG. In PCT patients, the association between total body iron content and serum levels of 8OHdG is even

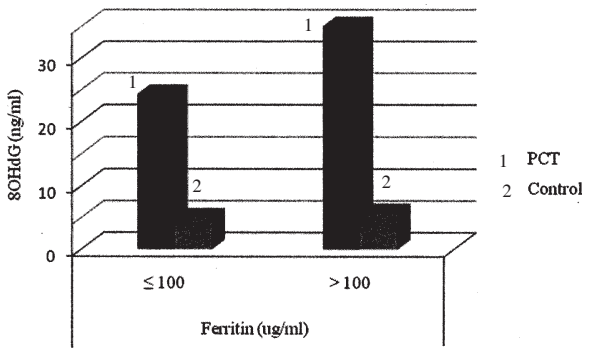

Fig. 7. Graphical representation of 8-hydroxy-2'-deoxyguanosine (8-OHdG-ng/ml) in relattion with to ferritin(ng/ml)in patients with porphyria cutanea tarda and control group ($p < 0.05$)

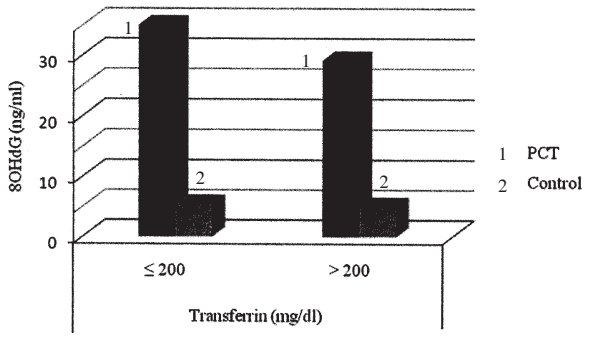

Fig. 8. Graphical representation of 8-hydroxy-2'-deoxyguanosine (8-OHdG-ng/ml) in relattion with to transferin(mg/dl) patients with porphyria cutanea tarda and control group ($p < 0.05$)

stronger when metabolic abnormalities are more pronounced (tables 2-5).

Although it is accepted that inactivation of uroporfirinogen decarboxylase is the main patho-physiological cause of PCT, the results obtained in this study supported the involvement of oxidative DNA modifications in disease pathogenesis. These results could be explained, in the author conception, in that patients with iron overload could promote inflammation, oxidative stress, mitochondrial dysfunction, impaired synthesis of hepcidin. As previously reported, the authors assume that oxidative DNA lesions associated with cellular redox imbalance, could be central element for the development of hepatocellular carcinoma in patients with PCT [8]. Production of oxidative lesions in DNA caused by reactive oxygen species, in relation with genetic and nongenetic factors, could induce an exacerbation of metabolic deficits, resulted in a decrease in uroporphirinogen conversion in coproporphirinogen.

Conclusions

This study demonstrated that 8OhdG-a modified gene product was correlated with altered iron stores in the body and hence with severity of PCT. Iron accelerates the process of malignant transformation by DNA damage-dependent oxidative reactions.

The association between iron overload, oxidative DNA adduct could be regarded as a new risk factor in a number of diseases mediated by oxidative stress. PCT patients with increased risk for developing hepatocellular carcinoma can be identified by increased serum levels of 8OHdG and ferritin.

Therapeutic methods of decreasing the iron content in organism (therapeutic phlebotomy, iron chelators use), diet, genetic manipulations, reducing sun exposure could have a decisive role in reducing the risk of carcinogenesis.

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