

EVALUATION OF VITAMIN D, VITAMIN D BINDING PROTEIN GENE POLYMORPHISM WITH OXIDANT – ANTIOXIDANT PROFILES IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

PROCENA POVEZANOSTI VITAMINA D I POLIMORFIZMA GENA ZA VITAMIN D-VEZUJUĆI PROTEIN SA OKSIDANTNIM–ANTIOKSIDANTNIM PROFILIMA U HRONIČNOJ OPSTRUKTIVNOJ BOLESTI PLUĆA

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Summary

Background: The aim was to evaluate the association of plasma 25-hydroxyvitamin D (25-OHD) and vitamin D binding protein (VDBP or Gc-globin) gene polymorphism with oxidant–antioxidant profiles in patients with chronic obstructive pulmonary disease (COPD), and their role as biomarker risk factors in susceptibility and severity of COPD.

Methods: Eighty patients diagnosed with COPD (mild, moderate and severe according to lung function tests; FEV₁ 1%) and 80 healthy controls were included in the study. Serum nitric oxide (NO) and lipid peroxide (LP), plasma reduced glutathione (RGSH), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) activity, 25-OHD and VDBP polymorphism were analyzed in all subjects.

Results: COPD patients had significantly decreased serum NO, plasma SOD, RGSH, GSH-Px, CAT and 25-OHD versus controls, but had significantly increased serum LP. In COPD patients, 25-OHD levels were significantly lower (41.49 ± 13.65 ng/mL) versus controls, but more lower in severe COPD patients (30.54 ± 9.09 ng/mL; sensitivity 79.2%; specificity 73.2%, $p < 0.001$) versus mild and moderate COPD. VDBP genotypes frequencies were Gc1S-1S=23.8%, Gc1F-

Kratak sadržaj

Uvod: Cilj je bio da se utvrdi povezanost između 25-hidroksi-vitamina D (25-OHD) u plazmi i polimorfizma gena za vitamin D-vezujući protein (*vitamin D-binding protein*, VDBP ili Gc-globin) i oksidantnih–antioksidantnih profila kod pacijenata sa hroničnom opstruktivnom bolešću pluća (HOBP), kao i njihova uloga kao faktora rizika biomarkera za podložnost i ozbiljnost HOBP.

Metode: U ovu studiju je uključeno 80 pacijenata sa dijagnozom HOBP (blag, umeren i težak oblik na osnovu testova plućne funkcije; FEV₁ 1%) i 80 zdravih kontrolnih ispitanika. Kod svih ispitanika analizirani su nivoi azot-oksida (NO) i lipid-peroksida (LP) u serumu, redukovani glutation (RGSH), superoksid-dismutaza (SOD), glutation peroksidaza (GSH/Px) u plazmi, aktivnosti katalaze (CAT), 25-OHD i polimorfizam VDBP.

Rezultati: Oboleli od HOBP imali su značajno snižene nivoe NO u serumu, SOD, RGSH, GSH-Px, CAT i 25-OHD u plazmi u poređenju s kontrolom, ali značajno povišen serumski LP. Kod obolelih od HOBP, nivoi 25-OHD bili su značajno niži ($41,49 \pm 13,65$ ng/mL) u odnosu na kontrolu, ali još niži kod pacijenata sa teškim oblikom HOBP ($30,54 \pm 9,09$ ng/mL; osetljivost 79,2%; specifičnost 73,2%, $p < 0,001$) u poređenju s blagim ili umerenim oblicima

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1S=28.8%, Gc1F-1F=15%, Gc1S-2=20%, Gc1F-2=11.3% and Gc2-2=1.3%. Also, VDBP variants frequencies were Gc1S=48.1%, Gc1F=35% and Gc2=16.6%. However, Gc1F-1S genotypes and Gc1F variants were significantly higher than in controls (10%, 12.5%; $p=0.009$, $p=0.001$, respectively). Moreover, in severe COPD patients, Gc1F-1S genotype was significantly higher than in mild COPD (41.7% vs 31.3%, $p=0.04$).

Conclusions: COPD patients had significantly lower plasma 25-OHD and were associated with significantly higher VDBP Gc1F-1S genotypes and Gc1F variants frequencies than controls. Low vitamin D levels and VDBP polymorphism may be important as diagnostic risk factors in the susceptibility to and severity of COPD.

Keywords: vitamin D, COPD, VDBP polymorphism, lung function, smoking, oxidants–antioxidants

Introduction

Chronic obstructive pulmonary disease (COPD) is a lung disease associated with significant and progressive irreversible airflow obstruction (1). COPD patients become more susceptible to exacerbations induced by respiratory bacterial and viral infections (2). COPD is a major cause of chronic morbidity and mortality, and the World Health Organization predicts that COPD will become the third leading cause of death worldwide by 2020 (1).

Chronic tobacco smoking is the major risk factor for COPD (3). However, only 15–20% of heavy smokers develop symptomatic COPD (4). Cigarette smoking is also the major source of oxidants/reactive oxygen species (ROS) to the lungs that can lead to extensive tissue damage and disease exacerbation susceptibility (2–4).

Interestingly, epidemiological evidence suggests a role of vitamin D deficiency in COPD onset and progression (4). It has been suggested that low levels of 25-hydroxyvitamin D (25-OHD) do not indicate true vitamin D deficiency when levels of vitamin D binding protein (VDBP) also are low (5). The VDBP or human group-specific component (Gc) globulin is a 55 kDa protein secreted by the liver and has important physiological functions that include involvement in vitamin D transport and storage, scavenging of extracellular G-actin, enhancement of the chemotactic activity of C5a for recruitment of neutrophils in inflammation and macrophage activation factor (MAF) (4, 6, 7). Also, 1,25(OH)₂D also inhibits the expression of several matrix metalloproteinases (MMPs), which contribute to parenchymal destruction in COPD (4). Taken together, the direct impact of vitamin D deficiency on smoking-induced inflammation and the development of COPD remain unclear.

Also, epidemiological studies provide compelling evidence that genetic risk factors influence the development of COPD (4, 8). The genetic risk factors are generally related to protease-antiprotease interaction

HOBP. Učestalost genotipova VDBP bila je Gc1S-1S=23,8%, Gc1F-1S=28,8%, Gc1F-1F=15%, Gc1S-2=20%, Gc1F-2=11,3% i Gc2-2=1,3%. Takođe, učestalost varijanti VDBP bila je Gc1s=48,1%, Gc1F=35% i Gc2=16,6%. Međutim, genotipovi Gc1F-1S i varijante Gc1F bili su značajno viši nego kod kontrolne grupe (10%, odnosno 12,5%; $p=0,009$, odnosno $p=0,001$). Štaviše, kod pacijenata sa teškim oblikom HOBP, genotip Gc1F-1S bio je značajno češći nego u blagoj HOBP (41,7% prema 31,3%, $p=0,04$).

Zaključak: Oboleli od HOBP imali su značajno niže nivoe 25-OHD u plazmi i utvrđena je povezanost sa značajno većom učestalošću VDBP genotipova Gc1F-1S i varijanti Gc1F nego kod kontrolnih subjekata. Niski nivoi vitamina D i polimorfizam VDBP mogu biti važni kao dijagnostički faktori rizika u podložnosti i stepenu ozbiljnosti HOBP.

Ključne reči: vitamin D, HOBP, polimorfizam VDBP, plućna funkcija, pušenje, oksidanti–antioksidanti

(e.g., α -1 antitrypsin deficiency), oxidant-antioxidant effects, xenobiotic metabolism, and inflammation and immune response pathways, which trigger a series of events that damage the airways and terminal airspaces, leading to lung function decline and emphysema (3). One of the risk factors involved in inflammation and immune reaction is the gene encoding VDBP or Gc protein (4). The gene for VDBP is located on chromosome 4q11-q13 and two common substitutions in exon 11 result in three variants Gc2, Gc1S and Gc1F (not alleles, but haplotypes) composed of combinations of the single nucleotide polymorphisms (SNPs) at these loci. An individual may be homozygous or heterozygous for each variant, depending on the two haplotypes present (9). There are six genotypes that are combinations of the three variants, 1F-1F, 1F-1S, 1F-2, 1S-1S, 1S-2 and 2-2 (4). The levels of 25-OHD (6, 10) and VDBP gene (11) have been associated with COPD. However, evidence from epidemiological studies remains conflicting as different negative studies have been published showing absence of associations (12).

In this study, we investigated the blood level of vitamin D and VDBP gene polymorphism with oxidant-antioxidant profiles in the Egyptian COPD patients, and their association with the development and severity of COPD.

Material and Methods

Subjects

All subjects were collected from the Chest Department, Zagazig University, Faculty of Medicine, Egypt. We included 80 COPD patients from those regularly attending the Chest Clinic and 80 subjects of matched age (smokers and non-smokers, controls) were collected from non-chesty patients referred for clinical and functional assessment e.g. before abdominal, eye operations etc. and from people who work in this hospital. All control subjects had no historical,

clinical, or radiological data suggestive of chest problems and they had normal spirometry data, even the smokers, and pulmonary function tests (>90%).

Written informed consent was obtained from the study participants. The study was conducted in accordance to the ethical procedures and policies approved by the Ethical Committee of Zagazig University, Faculty of Medicine, Egypt.

In this study, all subjects were subjected to the following: full history taking, full clinical examination (general and local), plain chest X-ray (postero-anterior view), blood pictures, liver function tests, kidney function tests, random blood sugar and pulmonary function tests. Patients with COPD were assessed clinically for symptoms and signs (cough, wheezing, shortness of breath, and exercise intolerance), by chest radiology, and confirmed by spirometry.

Dynamic spirometry (Master Screen Pneumo, Erich Jaeger GmbH, Germany) was performed, with measurement of the forced expiratory volume 1 (FEV1) (% of predicted) and the forced vital capacity (FVC). The ratio FEV1/FVC is a measure of airflow obstruction. These measurements were performed according to the standards of the European Respiratory Society and the American Thoracic Society (13). The highest values of FEV1 of three forced expiratory maneuvers were used. The best FEV1, FVC, and FEV1/FVC values were selected for the analysis (FEV1/predicted FEV1%) as follows: mild > 80%; moderate 50–79%; severe 30–49%; and very severe < 30% (less than 50% but with respiratory failure). Very severe cases were not involved in this study (1, 14, 15). The exclusion criteria were as follows: (a) patients < 18 years, patients with renal or liver diseases, patients with diabetes mellitus or pregnant females; (b) intake of drugs that likely affect bone

metabolism (e.g. corticosteroids), and (c) intake of dietary supplements containing calcium or vitamin D. Classification of the severity of cigarette smokers was done according to the number of pack-years (P-Y): number of cigarettes smoked per day multiplied by the duration in years (Smoking Index) and divided by 20 (1 pack = 20 cigarettes) as follows: mild smokers (< 20 P-Y); moderate smokers (20–49 P-Y); and heavy smokers (> 49 P-Y) (10).

Methods

The venous blood samples (10 mL) were collected from all participants in early morning and divided into 2 parts. The first part (5 mL) was put in heparinized tubes for the determination of erythrocyte oxidant and antioxidants blood levels and DNA extraction. The second part (5 mL) was used to obtain serum for colorimetric assay of nitric oxide (NO, Abcam Scientific, USA) (16) and lipid peroxidation (LP). Among the secondary products of LP, generated as a result of further reactions (e.g., β -elimination and decomposition of polyunsaturated fatty acid derivatives) are aldehydes, mainly malondialdehyde (MDA) (MDA assay kit, Abcam Scientific, USA) (17). The plasma levels of reduced glutathione (RGS, Prinecton Biomedix Inc., USA) (18), glutathione peroxidase (GSH-Px, Cayman Chemical Company, USA) activity (19, 20), superoxide dismutase (SOD, Abcam Scientific, USA) activity (21), catalase activity (CAT, Sigma-Aldrich, USA) (22) were measured by a colorimetric assay according to the manufacturer's protocol as previously described.

The plasma 25-hydroxyvitamin D (25-OHD) was measured by Enzyme Linked Immunosorbent Assay (ELISA) (DiaSorin, Stillwater, Minnesota, USA) in all participants as previously described (23).

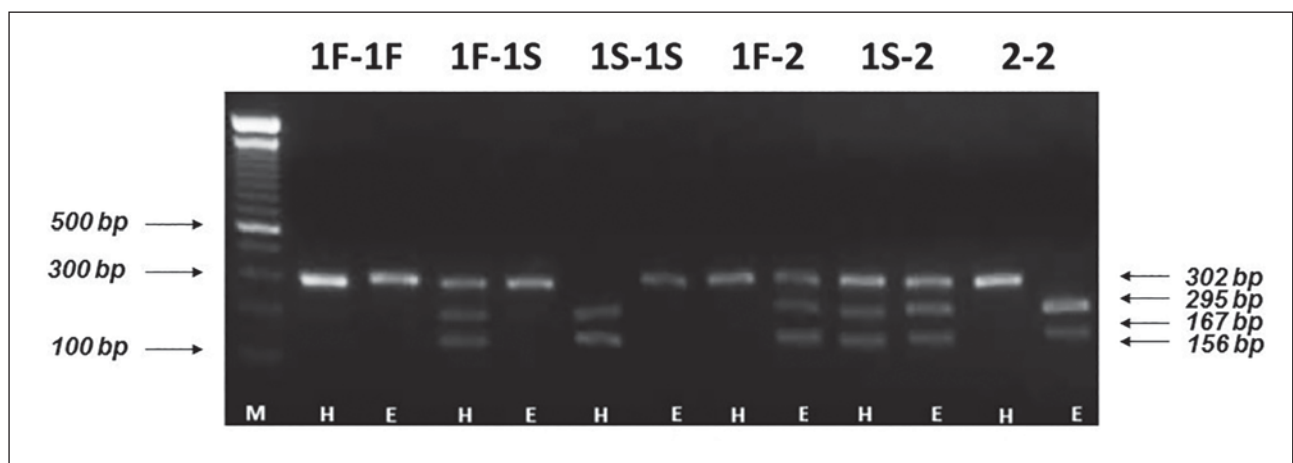


Figure 1 PCR gel shows the length of amplified PCR fragment analysis of Gc-globin genotypes. All six genotypes are shown. Hae III cuts the Gc-1S allele at the point (GGT, Gc-1S) (TGC, Gc-1F or Gc-2) into two bands of 295 bp and 167 bp, whereas Eco T14 I cuts Gc-2 allele into two bands of 302 bp and 156 bp at the point (AAG). The first bands in all six genotypes: digested with Hae III. The second bands in all six genotypes: digested with Eco T14 I. M: 100–1000 bp DNA ladder. 1F: Gc 1F, 1S: Gc 1S, 2: Gc 2.

DNA extraction and genotyping. Genomic DNA was extracted using a DNA extraction kit (Nucleon BACC2, Teqnel Life Sciences, UK) per the manufacturer's protocol. Primers for the polymerase chain reaction (PCR) were designed from the published VDBP gene sequence as previously described (24) and the VDBP gene was amplified using a commercially available PCR reaction master mix, according to the manufacturer's instructions (*Thermo Fisher Scientific Company*). The primer pairs used to amplify the VDBP gene were: upstream, 5'-TAATGAGCAAATGAAAGAAG-3'; downstream, 5'-AATCACAGTA-AAGAGGAGGT-3'. The cycling program involved preliminary denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 48 °C for 30 s, and elongation at 72 °C for 30 s, followed by a final elongation step at 72 °C for 7 min.

The PCR product was genotyped by restriction fragment length polymorphism (RFLP) analysis, involving overnight restriction enzyme digestion at 37 °C with the Eco T14 and HaeIII restriction enzymes (*Thermo Fisher Scientific Company*). HaeIII cuts the Gc-1S allele at the point (GGT, Gc-1S) (TGC, Gc-1F or Gc-2) into two bands of 295 bp and 167 bp, whereas Eco T14I cuts Gc-2 allele into two bands of 302 bp and 156 bp at the point (AAG). Therefore, the PCR product from Gc-1F homozygotes alone remains uncut by either of the enzymes. The digested fragments were resolved on 3% agarose gels, stained with ethidium bromide, and observed under ultraviolet light (*Figure 1*).

Statistical analysis

We analyzed all the results by IBM personal computer and statistical package SPSS version 11 (SPSS Inc., Chicago, IL, USA). Variables are presented as numbers, percentages (%), or mean \pm standard deviation (SD) as indicated. Student's t-test, Mann Whitney U test and Chi-Square (χ^2) test were used as indicated. Spearman correlation was used to correlate vitamin D level and different oxidants-antioxidants in COPD patients and controls. ROC (receiver operating characteristic) curve, sensitivity, specificity, and diagnostic accuracy were used to assess the diagnostic validity of vitamin D. Results are given as mean \pm SD, cut-off values per sample times (95% confidence interval, CI). Genotypes and alleles frequencies of VDBP were compared between COPD cases and controls using χ^2 test. P values <0.05 were considered statistically significant.

Results

All COPD patients (n=80) were 54.98 \pm 9.03 years, male/female ratio 58/22, number of cigarettes pack-years 46.4 \pm 1.7 P-Y and FEV1/FVC 59 \pm 3.7%.

The control patients (n=80) were 53.05 \pm 7.40 years old, male/female ratio 62/18. The number of cigarettes pack-years was 33.7 \pm 1.2 P-Y in smoker controls. Also, COPD patients were classified according to lung function tests as mild (n=16), moderate (n=40) and severe (n=24).

The laboratory data of all subjects such as serum NO and LP, plasma reduced GSH, SOD, GSH-Px, CAT activity and 25-OHD were analyzed in COPD, smoker, and non-smoker controls. COPD patients had significantly decreased 25-OHD, NO, SOD, RGSH, GSH-Px and CAT versus controls (smoker and non-smokers), but significantly increased plasma LP (*Table I*).

Plasma 25-OHD was significantly lower in COPD patients versus smoker and non-smoker controls (41.49 \pm 13.65 vs 53.85 \pm 13.68, 52.5 \pm 19.33 ng/mL, $p<0.001$, $p=0.001$ respectively) (*Table I*). Plasma 25-OHD was highly significantly lower in severe COPD patients versus smoker and non-smoker controls (30.54 \pm 9.09 ng/mL, $p=0.001$, $p=0.001$ respectively). Also, plasma 25-OHD was significantly lower in moderate COPD patients versus smoker and non-smoker controls (44.38 \pm 11.13 ng/mL, $p=0.029$, $p=0.049$ respectively). However, plasma 25-OHD was non-significantly lower in mild COPD patients versus smoker and non-smoker controls (49.81 \pm 15.37 ng/mL, $p>0.05$). Moreover, plasma 25-OHD was significantly lower in severe COPD patients versus mild and moderate COPD ($p<0.001$, $p<0.001$ respectively) (*Figure 2A*).

The odds ratio of COPD patients had higher risk with lower 25-OHD (45.5 ng/mL) versus controls (odds ratio = 0.72, 95% CI 0.65–0.80, $p<0.001$; sensitivity 70%; specificity 67.5 %. Also, odds ratio of severe COPD patients had higher risk with lower 25-OHD (38 ng/mL) versus mild and moderate COPD (odds ratio=0.86, 95% CI 0.76–0.95, $p<0.001$; sensitivity 79.2%; specificity 73.2 % (*Table II*, *Figure 2B*).

This study also assesses the prevalence of vitamin D in the pathogenesis of COPD through the oxidant-antioxidant role. Plasma 25-OHD was significantly correlated with lower serum NO ($r=0.38$, $p<0.001$), plasma SOD ($r=0.35$, $p<0.001$), RGSH ($r=0.30$, $p<0.001$), CAT ($r=0.19$, $p=0.04$) but with higher serum LP ($r=-0.31$, $p<0.001$) (*Table III*).

Our results on VDBP genotypes and variants (*Figure I*) in different groups were used to determine their susceptibility and severity of COPD progression (*Table IV–V*). In COPD patients, VDBP genotypes frequencies were Gc1S-1S=23.8%, Gc1F-1S=28.8%, Gc1F-1F=15%, Gc1S-2=20%, Gc1F-2=11.3% and Gc2-2=1.3%. Also, VDBP variants frequencies were Gc1S=48.1%, Gc1F=35% and Gc2=16.9%, but Gc1F-1S genotype and Gc1F variant were significantly higher than in controls (10%, 12.5%; $p=0.009$,

Table I Patient data, blood oxidant, antioxidant, 25-hydroxyvitamin D (25-OHD) levels in all subjects.

	COPD patients n=80	Smoker controls n=40	Non-smoker controls n=40	P value
Age (years) $\bar{x}\pm SD$ Range	54.98 \pm 9.03 35–72	52.05 \pm 6.48 43–65	55.70 \pm 8.52 37–67	0.11 ^a ; 0.73 ^b ; 0.09 ^c
Nitric oxide (NO) (μ mol/L) $\bar{x}\pm SD$ Range	7.43 \pm 0.73 6.1–9	17.66 \pm 0.98 15.9–19.1	19.19 \pm 2.28 16.1–23	<0.001 ^a ; <0.001 ^b ; <0.001 ^c
Lipid peroxide (LP) (mmol/L) $\bar{x}\pm SD$ Range	31.57 \pm 1.84 28–34	14.30 \pm 0.50 13.5–15.3	13.38 \pm 0.45 12.8–14	<0.001 ^a ; <0.001 ^b ; <0.001 ^c
Superoxide dismutase (SOD) (U/mL) $\bar{x}\pm SD$ Range	5.33 \pm 0.86 4–6.9	8.19 \pm 0.51 7.4–8.9	14.33 \pm 0.46 13.9–15.3	<0.001 ^a ; <0.001 ^b ; <0.001 ^c
Reduced glutathione (RGSH) (mg/dL) $\bar{x}\pm SD$ Range	8.08 \pm 0.53 7.3–8.9	22.32 \pm 0.66 21–23	24.17 \pm 0.56 23.6–25	<0.001 ^a ; <0.001 ^b ; <0.001 ^c
Glutathione peroxidase (GSH-Px) (nmol/min/mL) $\bar{x}\pm SD$ Range	11.98 \pm 1.01 10–13.1	12.11 \pm 0.91 10–13.1	12.14 \pm 0.92 10–13.1	0.50 ^a ; 0.41 ^b ; 0.89 ^c
Catalase (CAT) (μ mol/min/mL) $\bar{x}\pm SD$ Range	6.11 \pm 0.75 5.1–7.3	7.13 \pm 0.39 6.5–7.9	18.95 \pm 0.50 17.9–19.6	<0.001 ^a ; <0.001 ^b ; <0.001 ^c
25-hydroxyvitamin D (25-OHD) (ng/mL) $\bar{x}\pm SD$ Range	41.49 \pm 13.65 18–85	53.85 \pm 13.68 39–89	52.5 \pm 19.33 22–100	<0.001 ^a ; 0.001 ^b ; 0.97 ^c

COPD = chronic obstructive pulmonary disease, \bar{x} = mean, SD = standard deviation

a = COPD patients vs smoker controls, b = COPD patients vs non-smoker controls, c = smoker controls vs non-smoker controls

Table II ROC curve, sensitivity, specificity and accuracy of 25-hydroxyvitamin D (25-OHD) levels for determining susceptibility and severity of COPD among groups.

	25-hydroxyvitamin D (25-OHD) level	
	COPD patients versus controls	Severe COPD patients versus mild and moderate COPD
AUC	0.72	0.86
P value	<0.001	<0.001
95% CI	0.65–0.80	0.76–0.95
Cutoff point (ng/mL)	45.5	38.0
Sensitivity	70%	79.2%
Specificity	67.5%	73.2%
Accuracy	68.8%	75.0%

AUC = area under the curve, CI = confidence interval

Table III Spearman correlation was assessed between 25-hydroxyvitamin D (25-OHD) level and different oxidants and antioxidants in all subjects.

	25-hydroxyvitamin D (25-OHD) level	
	r	P value
NO (μ mol/L)	0.38	<0.001
LP (mmol/L)	–0.31	<0.001
SOD (U/mL)	0.35	<0.001
RGSH (mg/dL)	0.30	<0.001
GSH-Px (nmol/min/mL)	0.04	0.58
CAT (μ mol/min/mL)	0.19	0.04

COPD = chronic obstructive pulmonary disease; r = correlation coefficient

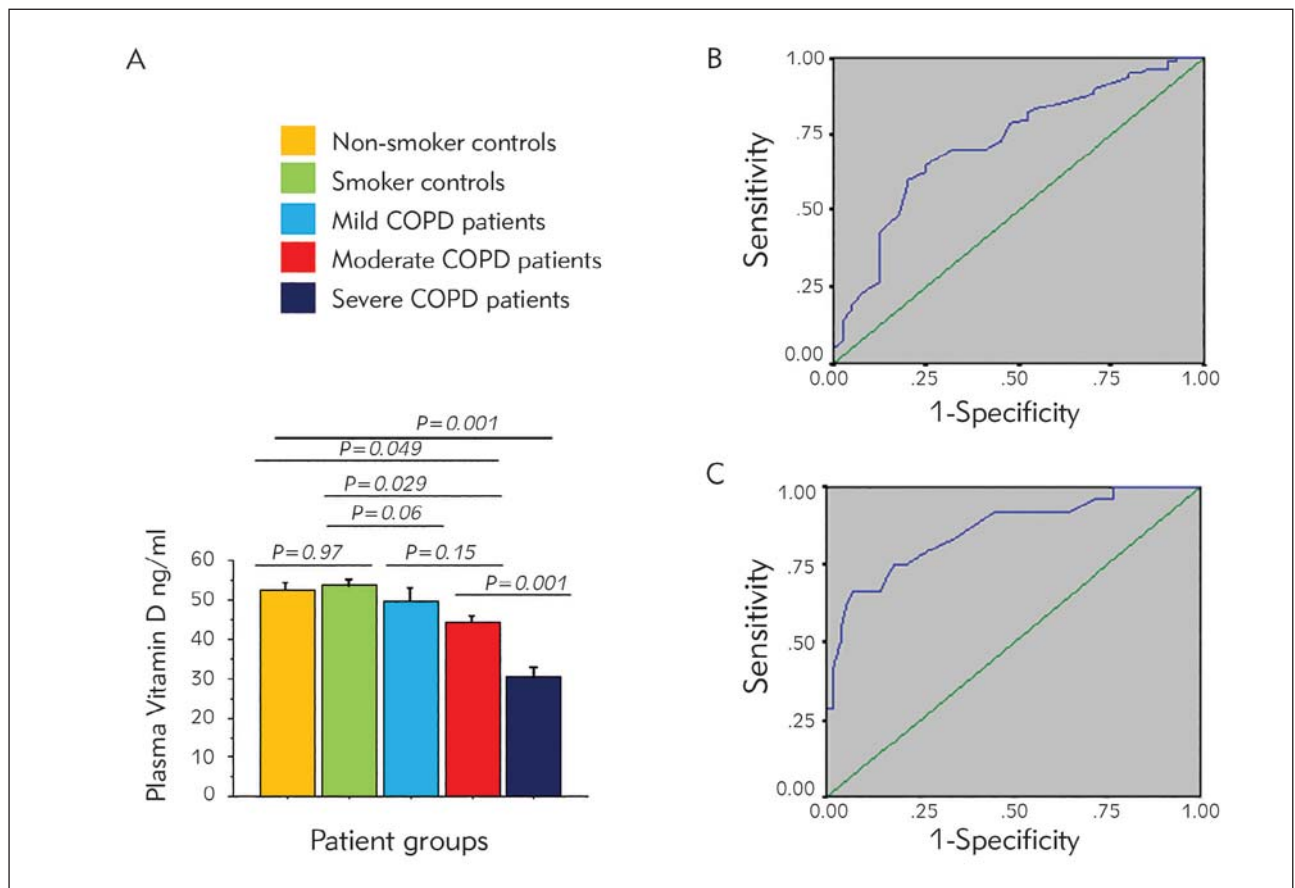


Figure 2 Plasma 25-hydroxyvitamin D (25-OHD) levels in different degree of COPD and control patients (A). ROC curves of concentration of 25-hydroxyvitamin D (25-OHD) in patients with COPD versus controls (B). ROC curves of concentration of 25-hydroxyvitamin D (25-OHD) in patients with severe COPD versus mild and moderate COPD (C).

Table IV Vitamin D-binding protein (VDBP or Gc protein) gene polymorphism in all subjects.

Genotypes	COPD patients	Smoker controls	Non-smoker controls	P-value
	n=80 n (%)	n=40 n (%)	n=40 n (%)	
1S-1S	19 (23.8)	8 (20.0)	20 (50.0)	0.64 ^a ; 0.004 ^b ; 0.005 ^c
1F-1S	23 (28.8)	16 (40.0)	4 (10.0)	0.21 ^a ; 0.02 ^b ; 0.002 ^c
1F-1F	12 (15.0)	4 (10.0)	2 (5.0)	0.45 ^a ; 0.11 ^b ; 0.40 ^c
1S-2	16 (20.0)	7 (17.5)	6 (15.0)	0.74 ^a ; 0.50 ^b ; 0.76 ^c
1F-2	9 (11.3)	4 (10.0)	2 (5.0)	0.84 ^a ; 0.26 ^b ; 0.40 ^c
2-2	1 (1.3)	1 (2.5)	6 (15.0)	0.61 ^a ; 0.002 ^b ; 0.047 ^c
Alleles				
1S	77 (48.1)	39 (48.8)	50 (62.5)	0.93 ^a ; 0.04 ^b ; 0.08 ^c
1F	56 (35.0)	28 (35.0)	10 (12.5)	1.0 ^a ; <0.001 ^b ; 0.001 ^c
2	27 (16.9)	13 (16.2)	20 (25.0)	0.90 ^a ; 0.13 ^b ; 0.17

COPD = chronic obstructive pulmonary disease
a = COPD patients vs smoker controls

b = COPD patients vs non-smoker controls
c = smoker controls vs non-smoker controls

Table V Vitamin D-binding protein (VDBP or Gc protein) gene polymorphism among the COPD patients.

Genotypes	COPD patients			P-value
	Mild n = 16 n (%)	Moderate n = 40 n (%)	Severe n = 24 n (%)	
1S-1S	1 (6.3)	10 (25.0)	8 (33.30)	0.11 ^a ; 0.04 ^b ; 0.47 ^c
1F-1S	2 (31.3)	11 (27.5)	10 (41.7)	0.23 ^a ; 0.048 ^b ; 0.24 ^c
1F-1F	1 (6.3)	10 (25.0)	1 (4.2)	0.11 ^a ; 0.77 ^b ; 0.03 ^c
1S-2	6 (37.5)	8 (20.0)	2 (8.3)	0.17 ^a ; 0.02 ^b ; 0.21 ^c
1F-2	6 (37.5)	1 (2.5)	2 (4.2)	<0.001 ^a ; 0.02 ^b ; 0.29 ^c
2-2	NA	NA	1 (4.2)	NA; 0.41 ^b ; 0.19 ^c
Alleles				
1S	10 (31.3)	39 (48.8)	28 (58.3)	0.09 ^a ; 0.02 ^b ; 0.29 ^c
1F	10 (31.3)	32 (40.0)	14 (29.2)	0.39 ^a ; 0.84 ^b ; 0.22 ^c
2	12 (37.5)	9 (11.3)	6 (12.5)	0.001 ^a ; 0.009 ^b ; 0.83 ^c

COPD = chronic obstructive pulmonary disease
a = mild COPD vs moderate COPD

b = mild COPD vs severe COPD
c = moderate COPD vs severe COPD

$p=0.001$, respectively) (Table IV). In severe COPD patients, Gc1F-1S genotype was significantly higher than in mild COPD (41.7% vs 31.3%, $p=0.04$) (Table V).

Discussion

Our results showed that patients with COPD manifested increased oxidative stress, as shown by the higher levels of LP products, accompanied by reduction in several endogenous enzymatic antioxidants in the blood, including SOD, CAT and GSH activity and reduction in NO. These results were in agreement with other studies (2, 3, 25). Smoking produces a shift in the normal balance between oxidants and antioxidants to impact oxidative stress both in the lungs and systemically (2–4, 25). Oxidants included in cigarette smoke can directly injure cells and tissues, inactivate defense mechanisms, and initiate inflammation, which further elevates oxidative stress (2–4, 25).

In this study, plasma 25-OHD was significantly lower in all COPD patients (41.5 ng/mL) versus controls; sensitivity 70%; specificity 67.5%, and much lower in severe COPD patients (30.5 ng/mL); sensitivity 79.2%; specificity 73.2%. Our results were in agreement with other studies (15, 26), but in contrast to some studies (5, 27). Recently, a meta-analysis study's results suggested that a low serum level of 25-OHD was not associated with COPD susceptibility, but a high

rate of 25-OHD deficiency was associated with COPD severity (1). In Egyptian patients and controls, vitamin D production from the sunlight usually occurs in the skin (Egypt is a hot country) in comparison to other countries such as Scandinavian countries (14). Also, serum levels of 25-OHD show seasonal variation. The prevalence of vitamin D deficiency is high during the winter months (28, 29). One limitation in the meta-analysis study showed that only one study contained African ethnicity (26), so this is considered the first study in Egypt.

In addition, vitamin D therapy prevents COPD exacerbation in patients with baseline serum 25-OHD concentrations of less than 20 ng/mL (12), but this is not consistent with our study (less than 30 ng/mL) and with another study (30). Lower vitamin D status in COPD may be due to diminished production of pre-vitamin D₃ associated with skin aging caused by smoking and limited ultraviolet band light (UVB) exposure (31). We clinically extend these data by showing that vitamin D deficiency accelerates lung disease progression upon smoking exposure (oxidants), and may have a role in the pathogenesis of COPD in smoker patients. Our results showed Spearman's correlation between plasma vitamin D level and oxidants-antioxidants (LP, SOD, reduced GSH and CAT) and NO. Our results are in agreement with another study that showed vitamin D deficiency is highly prevalent in the smoking population, and correlates with the severity of COPD (1, 15).

VDBP level had no effects on serum 25-OHD concentration (32). In general, VDBP levels were higher in COPD (33), but the effect of smoke exposure is uncertain (9). VDBP is an acute phase reactant and also interacts with a key mediator of lung damage in COPD – neutrophil elastase (NE) cleaves the VDBP-binding site on neutrophils, so that VDBP is released into the circulation (6, 7, 34). Furthermore, the ability of VDBP complexes to mediate neutrophil chemotaxis is prevented by NE inhibitors (7, 9, 34). However, the mechanism by which vitamin D affects the pathogenesis of COPD is unclear. Vitamin D can modulate the activity of various immune cells (35), inhibit inflammatory responses (36), and improve remodeling in airway smooth muscles (37). Therefore, disease susceptibility is controlled by inherited variations in genes involved in antiproteolysis, metabolism of toxic substances in the cigarette smoke and the efficiency of mucociliary clearance in the lung (4).

The potential association of polymorphisms in VDBP or Gc with the risk of COPD was investigated for the first time in an Egyptian population. Our results showed COPD was significantly higher in patients with Gc1F-1S (28.8%), Gc1S-1S (23.8%), Gc1S (48.1%) and Gc1F (35%) than in controls. In severe COPD patients, Gc1F-1S genotype frequency was significantly higher than in mild COPD (41.7% vs 31.3%). The Egyptian population in this study showed a similar distribution to the Korean and Japanese populations (11, 28, 38). The Gc1S variant has not been associated with COPD in any racial group (39). Gc1S variant and Gc1S-1S genotype were significantly more frequent in non-smoker controls. These results indicate that Gc1S variant in a homozygous state may be protective against COPD in smoking people. Therefore, the Gc1S variant may have a role in the detoxification of substances which are found in smoke. The Gc2 variant appears protective in Caucasians (8, 11, 39). It has also been shown that the genotypes which contain Gc2 variants (1F-2, 1S-2 and 2-2) had a protective effect (38, 40). Also, the genotype Gc2-2 was protective against COPD (11, 28). In American (41), Canadian (38) and Caucasian populations, Gc2 and Gc2-2 also showed as a protective factor for COPD (42). However, this observation has not been confirmed by a subsequent study (29). The Gc1F variant has been consistently associated with a range of COPD phenotypes in

Asian subjects (8, 29, 43). This result has been confirmed in the Japanese population, but results in Caucasians have been inconsistent (11, 38, 39). Gc1F and Gc1F-1F were significantly higher in COPD than in control in Japanese (8, 29) and Chinese populations (43).

Individuals who have one or two copies of variant 2 have been shown to be protected against COPD (11, 38, 40), whereas the 1F-1F genotype has been associated with increased risk (11, 29, 38, 40). Despite all these reports, several studies have failed to show an association between VDBP polymorphisms and COPD (12). The functional significance of this polymorphism is not yet completely understood, but may cause very different circulating free and hence intracellular levels of 25-OHD₃ (44).

We found that severe vitamin D deficiency predicted later decline in lung function in COPD patients, an important parameter of COPD disease progression. This may imply that the effects of vitamin D in COPD disease progression are too small to be clinically relevant, or that vitamin D deficiency is a later event rather than a potentially causal factor. COPD patients had significantly lower plasma 25-OHD and were associated with significantly higher frequencies of VDBP Gc1F-1S genotypes and Gc1F variants than controls. Plasma 25-OHD and VDBP polymorphism may be important as a diagnostic risk biomarker and vitamin D may be a useful therapy in COPD patients.

However, there are limitations to this study, because of the relatively small sample size and numbers of patients, especially Gc1S variant and genotype 1S-1S, 2-2, so additional studies with larger number of subjects are required in the future.

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Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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