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Association of biomarkers of oxidative stress, stress glycaemia and glycated hemoglobin with acute coronary syndrome

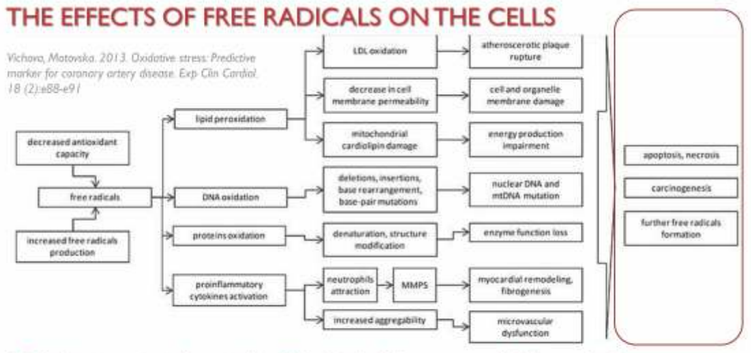
I declare no conflict of interest

"Oxidative stress is a situation where the steady-state ROS concentration is transiently or chronically enhanced, disturbing cellular metabolism and its regulation and damaging cellular constituents"

Lushchak, 2011

- ROS are produced mainly by mitochondrion under normal physiological conditions due to the partial reduction of molecular oxygen. ROS includes free radicals such as: superoxide (O2−), hydroxyl radical (OH*), hydrogen peroxide (H2O2), singlet oxygen (O_2) and peroxynitrite (ONOO−).
- Low levels of ROS production are essential for maintaining physiological functions: proliferation, host defense, signal transduction, and gene expression.
- Intracellular antioxidants including superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, also, other non-enzyme antioxidants (vitamin C, vitamin E, β-carotene, bilirubin, etc.) keep ROS at homeostatic levels.
- Oxidative stress occurs when the balance between ROS production and antioxidant defense capacity is disrupted in favor of the increased ROS production, leading to damage of the cell membrane, proteins, and DNA, contributing to muscle dysfunction, necrosis and apoptosis.

THE EFFECTS OF FREE RADICALS ON THE CELLS



Oxidative stress status is central to CV pathophysiology as an underlying mechanism common to all CV risk factors (DM, dyslipidemia, hypertension, obesity and smoking habit), while oxidative stress biomarkers are strongly associated with the presence and severity of CVD.

AIM OF THE STUDY

To anlyze the association between the OXIDATIVE STRESS, LIPIDEMIC and GLYCEMIC STATUS in patients with CORONARY ARTERY DISEASE.

METHODS

- Cross-sectional observational study performed at the University Clinic of Cardiology in Skopje, Macedonia.
- Study population: hospitalized and ambulatory treated patients with Acute Coronary Syndrome and Chronic Coronary Artery Disease, who gave their written informed consent to participate in the study.
- Study was approved by The Ethical Comity of the Medical Faculty.
- Analyzed variables: demographics, risk factors and co-morbidities, CAD characteristics (ACS versus HCAD), laboratory data: hemogram, lipoprotein profile, glycemic profile, biomarkers of myocardial injury, oxidative stress biomarkers: malondialdehyde (MDA) and hydro peroxide (HP), and antioxidant enzymes: superoxide dismutase (SOD), CATALASE and glutathione peroxidase (GPS).

Comparison was performed between:

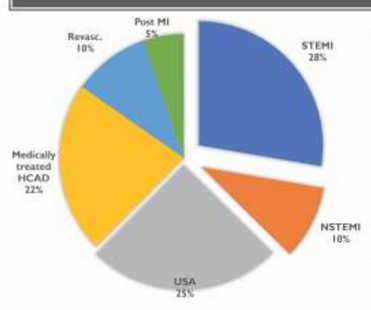
- CAD patients and healthy controls,
- Patients with acute coronary syndrome (ACS) versus chronic coronary artery disease (HCAD)

Statistical analyze: SPSS 17 software was used: descriptive and comparative analyze (Chi square, t-test), non-parametric (Mann-Whitney and Kolmogorov-Smirnov Test), correlations, and multivariate regression analyze. ROC curves were designed. Significance was determined at the level of 0,05.

RESULTS

GENERAL CHARACTERISTICS OF THE STUDY POPULATION

62,4% with ACS and 37,6% with HCAD



Comparative analyze of OS (oxidative stress) status was performed for:

1. Healthy controls versus CAD patients
2. ACS versus HCAD patients
3. Lipid and diabetes interplay with OS status

There was no statistically significant difference in the risk profile between the CAD groups, except for smoking status

Parameter	Total	ACS patients	HCAD patients	sig	Healthy controls
Gender	300 (100%)	187 (62,3%)	113 (37,7%)		30
• Female	106 (35,3%)	68 (36,4%)	38 (35,8%)		11 (36,6%)
• Male	194 (64,7%)	119 (63,6%)	75 (38,7%)	ns	19 (63,4%)
Age	62,9±11,2	62,1±12,2	64,3±9,0	ns	61,5±10,8
HTA	187 62,3%)	113 (60,4%)	74 (65,5%)	ns	13 (43,3%)
HLP	152 (50,7%)	101 (54,0%)	51 (45,1%)	ns	11 (36,6%)
DM	85 (28,3%)	55 (29,4%)	30 (26,5%)	ns	3 (10%)
Smoking					
• active	101 (33,7%)	82 (43,9%)	19 (16,8%)	0,000	20 (66,6%)
• former	70 (23,3%)	38 (20,3%)	32 (28,3%)		
Family history	89 (29,7%)	54 (28,9%)	35 (31,0%)	ns	6 (20%)
EF (%)	50,2±10,2	50,7±10,1	43,6±9,4	ns	59,4 ± 9,8
Hb (g/dl)	13,7±10,8	13,8±1,7	13,5±1,9	ns	14,0 ± 1,2
Myoglobin (ng/ml)	229,2 ± 368,8	268,9 ± 410,9	103,7±112,1	0,009	42,1 ± 8,3
Creatine kinase-MB, IU/l	46,4 ± 67,5	60,4 ± 81,1	23,3 ±19,2	0,000	20 ± 8
Creatine kinase, IU/l	391,5 ± 690,6	529,4 ± 842,3	163,2 ±103,5	0,000	94 ±11
Lactate dehydrogenase, IU/l	483,0 ± 517,8	582,8 ± 603,2	317,8 ± 259,7	0,000	121,3 ± 12,6
hsTroponin (I/T) pg/ml	197,04 ± 365,05	243,0 ± 394,7	13,1 ± 38,6	0,001	16,3 ± 8,4

Parameter	Total	ACS patients	HCAD patients	sig	Healthy controls
Glycemic status					
Stress GI	10,3 ± 6,5	10,5 ± 5,6	5,6 ± 2,1	0,001	5,8 ± 2,1
HbA1c	6,5 ± 1,5	6,5 ± 1,6	6,4±0,8	na	
DM category					
• Non DM	33 (20,0%)	33 (24,4%)			
• Pre-DM	38 (23,1%)	38 (28,1%)			
• New-DM	9 (5,4%)	9 (6,7%)			
• Known regulated DM	44 (26,7%)	24 (17,8%)	20 (12,3%)	0,000	
• Known-unregulated DM	41 (24,8%)	31 (22,9%)	10 (6,1%)		
• Total	165 (100%)	135 (100%)	30 (18,4%)		
Lipidemic status					
TG mmol/L	1,7 ± 6,5	1,8 ± 2,0	1,6 ± 0,9	ns,	2,0 ± 0,9
C mmol/L	5,5 ± 1,8	5,6 ± 1,8	5,2 ± 1,7	0,042	5,8 ± 1,2
LDL-C mmol/L	3,3 ± 1,4	3,5 ± 1,4	2,9 ± 1,2	0,001	3,6 ± 1,6
NO pts LDL-C >1,8mmol/L	266 (88,7%)	172 (92%)	94 (83,2%)	0,017	OR 1,6 (CI 1,1-2,2); p-0,023
HDL-C mmol/L	1,5 ± 0,8	1,5 ± 0,8	1,6 ± 0,8	ns	1,1 ± 0,9
ApoA1	1,6 ± 0,4	1,5 ± 0,4	1,7 ± 0,4	ns	1,5 ± 0,9
ApoB	1,1 ± 0,5	1,1 ± 0,4	0,9 ± 0,4	0,001	1,1 ± 0,8
Lp(a)	37,6 ± 38,7	36,0 ± 37,5	42,8 ± 42,5	ns	29,6 ± 22,1
NO pts Lp(a) >30mg/dl	42%	41,3%	44,2%	ns	

Mean values of ROS and ANTIOXIDANTS in CAD patients and healthy controls

PATIENTS SUBGROUPS	NO	ROS		ANTIOXIDANS		
		MDA (nm/ml)	HP (CARR U)	SOD (U/ml)	CAT (KU/L)	GPx (U/ml)
CAD	300	34,1±9,1	282,7±73,9	131,7±113,0	64,6±38,1	6,4±6,0
Healy volunteers	30	22,2±6,7	240,5 ±62,2	358,7±180,9	99,1±36,7	7,0±5,5
Sig		<0,00001	<0,00185	<0,00001	<0,000013	ns
ACS	187	34,3±9,9	285,6±76,3	118,8±106,9	63,2±39,5	6,5±6,9
HCAD	113	33,9±7,6	278,1±72,1	129,2±107,9	66,8±36,8	6,3±4,9
Sig		ns	ns	ns	ns	ns
NP (Mann-Whitney and Kolmogorov-Smirnov Test)		ns	ns	ns	ns	ns

Patients with CAD had significantly higher levels of ROS and lower levels of anti-oxidative biomarkers, as compared with healthy volunteers, however, no statistically significant difference was observed between ACS and HCAD patients.

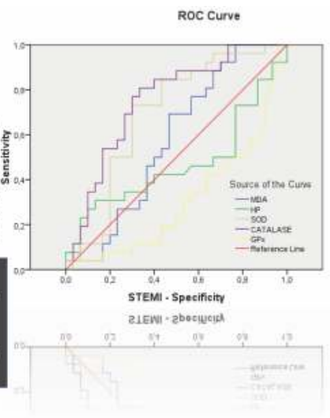
Mean values of ROS and ANTIOXIDANTS in ACS patients

PATIENTS SUBGROUPS	NO	ROS		ANTIOXIDANS		
		MDA (nm/ml)	HP (CARR U)	SOD (U/ml)	CAT (KU/L)	GPx (U/ml)
STEMI	84	34,6±9,7	278,8±68,9	106,8±91,1	67,0±37,1	4,7±5,7
NSTEMI	22	30,2±6,1	307,3±73,4	82,8±79,0	63,6±31,8	7,9±5,4
USA	81	34,9±10,9	286,1±77,1	140,4±123,5	59,3±39,5	7,9±8,8
Total	187	34,3±9,9	285,6±76,3	118,8±106,9	63,2±39,5	6,5±6,9
Sig (ANOVA)		0,0441	ns	0,034	ns	0,0314
Post Hock				2 vs 3 0,51		
NP Kruskal Wallis Test		0,050	ns	0,050	ns	0,033

In patients with ACS statistically significant differences were found for MDA-malonic dialdehyde and antioxidant enzymes superoxide dismutase (SOD), being lowest in NSTEMI patients, and GPx-glutathione-dependent peroxidase lowest in STEMI patients.

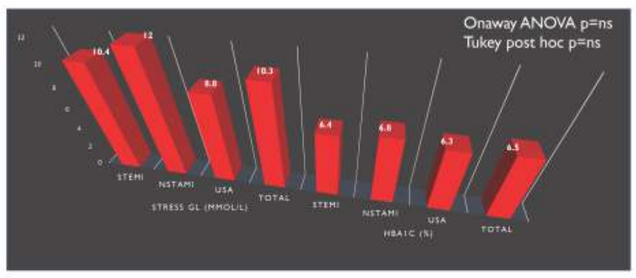
ROS and antioxidant activity in STEMI patients

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% CI Lower Bound	Upper Bound
MDA	,574	,078	,341	,422	,726
HP	,469	,082	,687	,309	,629
SOD	**,694**	**,072**	**,013**	,552	,836
CATALASE	**,751**	**,066**	**,001**	,622	,881
GPx	**,306**	**,071**	**,013**	,167	,446



The performance of classification of ROS and antioxidants for STEMI patients demonstrated statistically significant high performance, with area under the curve ,694; p=0,013, and ,751; p=0,001 respectively for superoxide dismutase and catalase surprisingly high values, and as expected low values for glutathione peroxidase ,306; p=0,013.

Glycemic status in ASC patients



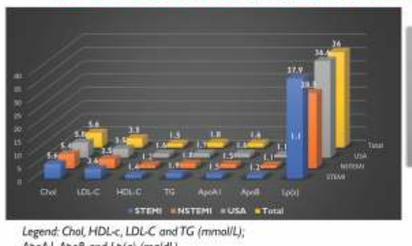
Spearman's rho		HbA1c (%)	MDA	HP	SOD	CATALASE	GPx
Stress GI (mmol/L)	Correl. Coeff.	,600**	-,134	,114	-,089)	-,077)	,101
	Sig. (2-tailed)	,000	,152	,225	,349	,431	,494
HbA1c (%)	Correl. Coeff.		-,114)	-,047)	,079	,061	,216
	Sig. (2-tailed)		,223	,617	,401	,538	,140

Mean values of ROS and ANTIOXIDANTS in ACS patients -diabetic interplay-

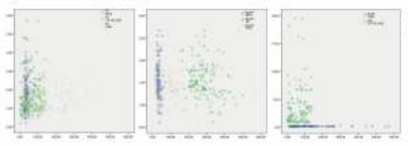
PATIENTS SUBGROUPS	ROS	ANTIOXIDANS			
	MDA (nm/ml)	HP (CARR U)	SOD (U/ml)	CAT (KU/L)	GPx (U/ml)
Total	34,0±11,3	282,4±67,6	111,8±107,4	66,3±39,7	6,4±6,7
Non-DM pts. (0)	33,6±7,6	289,4±66,2	88,6±94,2	56,6±42,2	5,6±6,1
New-DM pts. (1)	36,7±14,9	280,9±73,9	125,1±97,6	72,8±37,1	6,4±7,6
Known-DM pts. (2)	30,8±7,6	277,9±61,5	114,8±128,1	66,4±40,4	7,6±6,3
Sig (ANOVA)	0,057	ns	ns	ns	ns
Tukey HSD post hoc test	1 vs 2 0,045				
NPar Tests (Kruskal-Wallis Test)	0,023	ns	0,043	ns	ns

When compared patients with known, versus newly-diagnosed DM, MDA-malonic dialdehyde, which is considered a marker of lipid peroxidation was significantly higher in patients with newly-diagnosed DM.

LP and ROS / antioxidative activity interplay in ACS patients



Legend: Chol, HDL-c, LDL-C and TG (mmol/L); ApoA1, ApoB and Lp(a) (mg/dL)



		MDA	HP	SOD	CAT,	GPx
TG	Correl. Coef.	,159*	-,027	,138	,192*	-,092
	Sig. (2-tailed)	,030	,712	,062	,011	,478
ApoA1	Correl. Coef.	-,198*	-,247**	-,212*	-,083	,157
	Sig. (2-tailed)	,020	,004	,013	,355	,232
Lp(a)	Correl. Coef.	-,025)	,109	,129	,201*	-,153
	Sig. (2-tailed)	,768	,202	,134	,024	,244

INDEPENDENT ASSOCIATIONS identified for OXIDATIVE STRESS biomarkers and gender (female-HP beta ,463, p0,000); DM (MDA beta -,181; p 0,052); C and LDL-C-(GPx beta -1,272; p 0,018).

CONCLUSIONS

- CAD patients demonstrated pronounced oxidative stress when compared to healthy controls, however our results were inconclusive with respect to identifying specific oxidative stress biomarkers for different levels of CAD activity.
- Diabetes and hyperlipidemia are linked to the oxidative stress status.
- However, the key question remains, we still don't have data to support routine measurement of biomarkers of oxidative stress neither in diagnostic purposes, or as therapy guiding.