IJBPAS, April, 2021, 10(4): 1362-1374

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

UP-REGULATION OF PROPHETIC VARIABLE OF MEDICAL IMPORTANCE IN PATIENTS OF ACUTE CORONARY SYNDROME (ACS)

MALIK A[3], KHOKHAR MAS[2], ANWAR MZ[2], RASOOL R[1], KHAWAJA AI[1] AND MALIK A[1*]

1: Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore-Pakistan

2: Major Shabbir Sharif Shaheed Hospital, Kunjah-Gujrat Pakistan

3: Department of biochemistry, CMH Lahore Medical College, Lahore-Pakistan

***Corresponding Author: Dr. Arif Malik (Ph.D): E Mail: arifuaf@yahoo.com; Cell: 0321-8448196; Tel: +92 42-7515460-7, Fax: +92-42-7515519**

Received 3rd June 2020; Revised 8th July 2020; Accepted 8th Aug. 2020; Available online 1st April 2021

https://doi.org/10.31032/IJBPAS/2021/10.4.5463

ABSTRACT

BACKGROUND: Patient approaching hospital with the chest pain or other acute coronary syndrome signs or symptoms evaluation is time taking, pricey, complex and challenging. Raised biomarkers of necrosis including oxidative stress, cytokines, cell adhesive molecules, biomarker of destabilization of formed plaque and rupture, ischemia biomarkers and myocardial stretch biomarker endow initial outlook of patient's overall risk and also be familiar with high risk patients of adverse events.

METHODOLOGY: Different biomarkers of AGEs, AOPPs, lipid biomarkers, and inflammatory markers among 132 control and acute coronary syndrome patient's serum samples via different lab tests and ELIZA kits were assessed. The results were analyzed by independent t-test in SPSS version 21.

RESULTS: Significantly increasing trends of MDA (0.911±0.033 nmol/ml vs. 4.19±1.09 nmol/ml), NO (20.59±1.89 ng/ml vs. 81.25±5.19ng/ml), AGEs (0.61±0.014 ng/ml vs. 4.19±0.48 ng/ml) and AOPPs (95.65±5.19ng/ml vs. 133.26±10.32ng/ml) was depicted in patients of ACS than controls. Results on the subject of lipid profile biomarkers TCh (4.59±0.59 mmol/L vs. 9.65±2.16 Mmol/L), triglycerides (1.08±0.017 mmol/L vs. 5.19±1.48 mmol/L), and LDL (2.18±0.008 mmol/L vs. 3.29±0.011 mmol/L) showed significantly increasing pattern with reduction in HDL (1.79±0.018 mmol/L vs. 0.985±0.008 mmol/L) levels in ACS patients. The scrutiny of inflammatory biomarkers including IL-1α (8.19±1.99 pg/ml vs. 3.99±0.32 pg/ml) and TNF-α (41.58±6.35 pg/ml vs. 21.25±4.29 pg/ml) portrayed augmented picture in disease patients in contrast to healthy controls'. The study also reveals that MMP-11 (96.35±5.18ng/ml vs. 41.59±5.26ng/ml) level was increased in acute coronary syndrome patients as compared to normal individuals.

CONCLUSION: This study corroborates that activation and progression of local and systemic inflammation as the result of elevated inflammatory markers (IL-1α, TNF-a and MMP-11) and oxidative markers especially AGEs, AOPPs, NO and MDA may consider to have a supportive pathogenic role in acute coronary syndrome and could identify high-risk patients with subsequent increase ischemic events and vulnerable plaque destabilization.

Keywords: Acute coronary syndrome, advanced glycation end products, advanced oxidation protein products, matrix metalloproteinase

INTRODUCTION

Cardiovascular diseases are the foremost cause of morbidity and fatality in all over the world accounting one third of all deaths **[1]**. Acute coronary syndrome (ACS) has clinical presentation of unstable angina and commonly related to MI despite of CAD **[2]**. Unstable angina and myocardial infarction are pathophysiological events precipitating acute coronary syndrome is considered to be thrombosis overlay on atherosclerotic plaque. Atherosclerotic plaque as long as significantly reducing lumen area may not impair the blood flow so acute coronary syndrome is not generally seeming to come out of a clear sky. STEMI resulted from the intra-luminal formed thrombus leading to complete occlusion of blood vessels. ACS is caused by four different pathological entities including rupture of atherosclerotic plaque followed by endothelial erosion which leads to exposing calcium nodules and thrombogenic molecules result in intra-plaque haemorrhage arising from plaque microvasculature itself **[3,4]**. In western countries, Non- STEMI and unstable angina accounting worldwide 2.5 million hospital entries are main cause of morbidity and mortality. Its prognosis is worse than that of chronic stable angina. 5-10%patients after acute episode may affect by hospital death and recurrent myocardial infarction **[5]**. Older age, previous cardiovascular disease, hypertension, smoking, diabetes, gender, increased cholesterol, and family history are common risk factors of acute coronary syndrome. It can also associate along with valvular disease, cardiomyopathies and arrhythmias **[6,7]**.

Inflammation and oxidative stress are involved in the pathogenesis of CAD destabilization leading to acute coronary syndrome. Neutrophils and macrophages infiltration participate in transforming stable plaque to unstable abrasions within a fibrous cap. Neutrophils and macrophage are found in high concentration in lesions of unstable angina and AMI patients in comparison to stable coronary disease patients **[8,9]**. Respiratory chain, NADPH oxidase, xanthine oxidase, inflammatory cells, cyclo-oxygenase and mitochondria are various sources for generation of free radicals **[4]**. ROS production damages to a variety of cellular organelles including mitochondria, endoplasmic reticulum, proteins, DNA and lipids that trigger signaling pathways leading to cell apoptosis **[10, 11, 12]**. Current researches have showed that raised biomarkers upstream from necrosis such as cellular adhesion molecules, inflammatory cytokines, acute phase reactants, biomarkers of destabilization of plaque and rupture, ischaemic biomarkers, and myocardial stretch biomarkers may provide initial estimation of general risk and help in predicting higher risk patients with adverse events. Acutely after acute coronary syndrome event, increased atherogenic lipoproteins such as triglycerides and low-density lipoproteins levels along with the reduction of HDL levels are prophetic of cardiovascular events **[13]**. Vascular inflammation and RAAS blocking is facilitated by inflammatory biomarkers including ICAM-1, VCAM-1, TNF-α, IL-6 and hs-CRP **[14]** and their fluctuation levels can help in evaluating cardiovascular risks **[15]**. Advanced oxidation protein products (AOPPs) and advanced glycation end-products (AGEs) formed from oxidative stress protein modification, glycation may alter the structure and functionality of CVS such as hardening of vessels, reduced central compliance of arteries, anomalies of myocardium, atherosclerotic lesions and endothelial dysfunction that aggravated in the presence of diabetes, hypertension and renal disease **[16]**. Raised receptor activity mediates pro-inflammatory responses **[17,**

18] and causes oxidative stress by ROS production that may contribute in causing cardiovascular diseases **[19,20]**.

MATERIALS AND METHODS METHODOLOGY

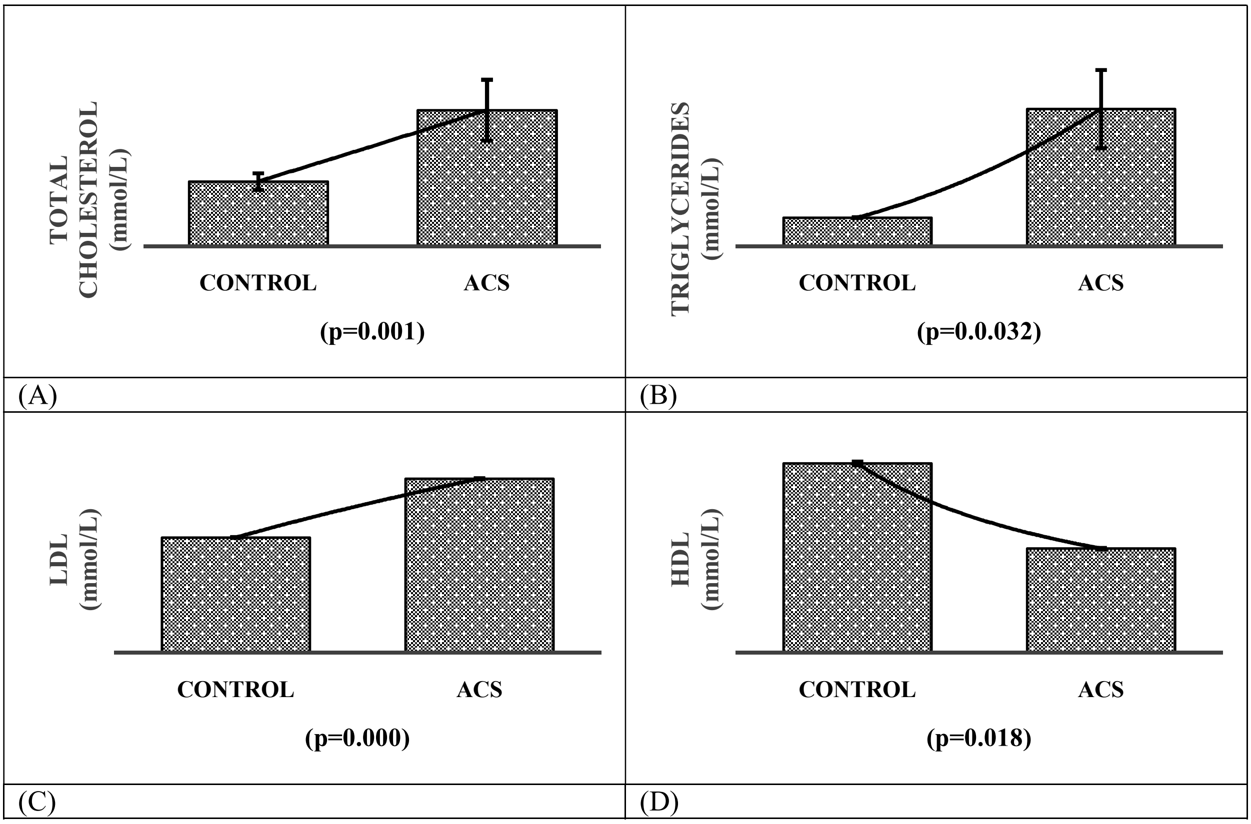
The study was conducted to evaluate the main mechanism involved in AGEs, AOPPs, lipid profile, oxidative stress along with inflammatory biochemical markers in the development of acute coronary syndrome (ACS). In present study, seventy-six patients after well-versed consent were screened from University of Lahore, teaching Hospital, Lahore. The control group includes fifty-six apparently healthy individuals. The experimental protocols were permitted by Research Ethical Committee of institute of molecular biology and biotechnology, at University of Lahore. Five ml of anti-cubital vein blood sample was drawn from every participant and collected in sterile test tubes which are centrifuged within one hour of collection of sample after which the serum was separated and stored at -70°C until further analysis. The diagnosis of acute coronary syndrome was based on following criteria 1) 20-70years aged patients were included in study 2) All patients of unstable angina, STEMI and non-STEMI were included**.** The subjects with drugs history (including alcohol and cigarette), pre-diagnosis medications (e.g. antiparkinsonian/antipsychotic), were excluded from this study. Following variables were performed by the technique of: Malondialdehyde **[21]**, NO **[22]**, AOPPs **[23]**, AGEs **[23]**, lipid profile (TCH, triglycerides, LDL-C, HDL-C) is determined by Commercial Human Diagnostics Kits. MMP-11, IL-1α and TNF-α were analyzed with their respective ELIZA kits.

STATISTICAL ANALYSIS

The study was prospective case control and the data was statistically analyzed by independent T-test using SPSS version 21 and expressed as mean±SD. $P \leq 0.05$ is cut point to be statistically significant.

RESULTS

Serum samples of fifty-six healthy and seventy-six ACS patients were evaluated in the study on bases of their Lipid biomarkers, circulating oxidative stress markers, and inflammatory biochemical markers. Data analysis in regarding oxidative stress biochemical markers MDA (0.911±0.033nmol/L vs. 4.19±1.09nmol/L), NO (20.59±1.89 ng/ml vs. 81.25±5.19 ng/ml), AGEs (0.61±0.014 ng/ml vs. 4.19±0.48 ng/ml) and AOPPs (95.65±5.19 ng/ml vs. 133.26±10.32 ng/ml) shows highly significant pattern between and within the groups. Data regarding pro-inflammatory biomarkers concluded that statistically significant (p=0.001 and 0.008 respectively) increasing trends of IL-1α (3.99±0.32 pg/ml vs. 8.19±1.99 pg/ml), TNF-α (21.25±4.29 pg/ml vs. 41.58±6.35 pg/ml) were observed acute coronary syndrome patients than normal subjects. The results of lipid profile concluded that total cholesterol, triglycerides and low density lipoproteins were significantly higher in ACS patients than normal subjects while mean HDL {1.79 mmol/L (normal) vs. 0.985±0.008 mmol/L (ACS)} in ACS subjects was lower and significant than control group. The mean value of MMP-11 in ACS patients was 96.35±5.18 ng/ml while in control group was 41.59±5.26 ng/ml and also found to be statistically significant (p=0.001).



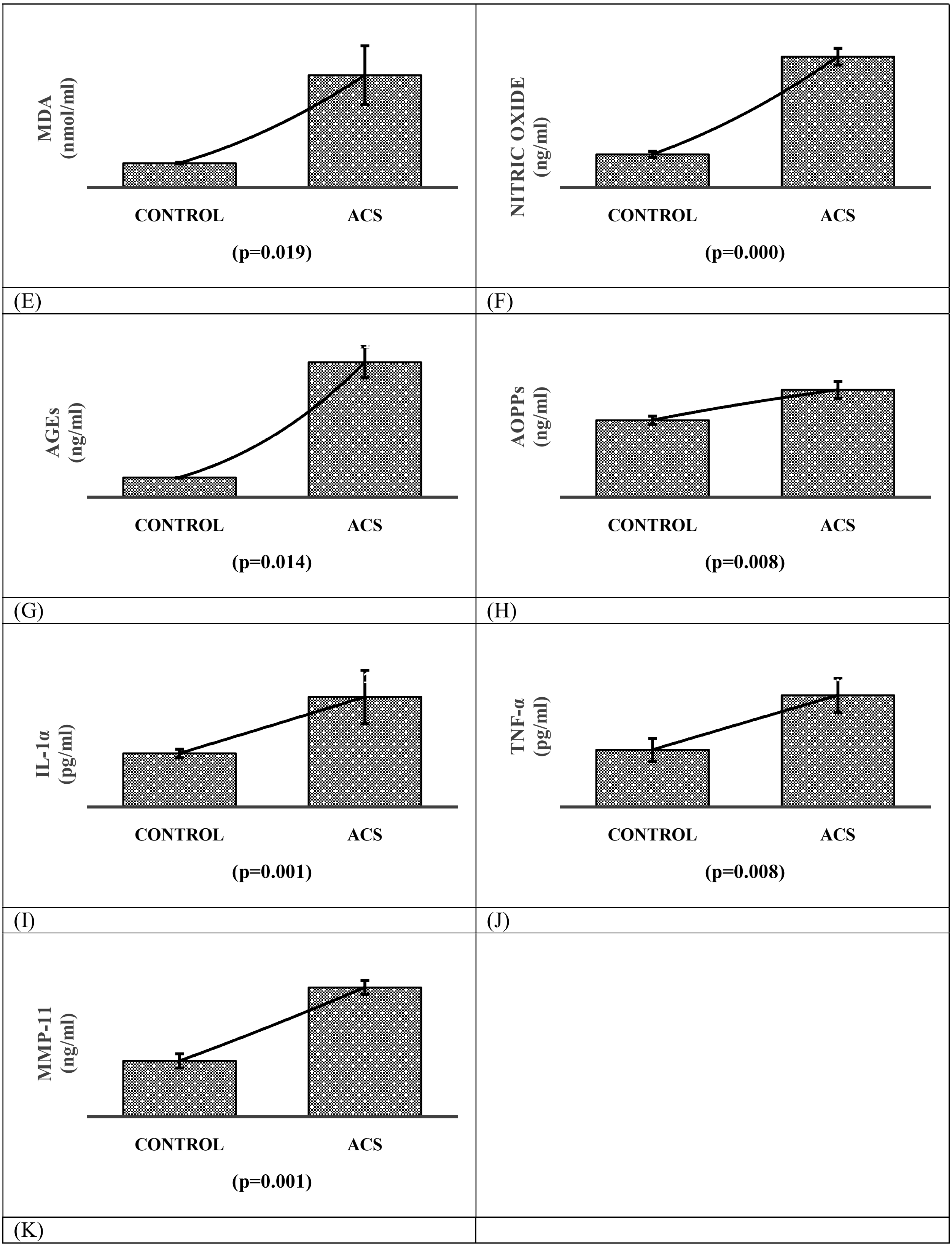


Figure 1: Up-Regulation of Prophetic Variable of Medical Importance in Patients of Acute Coronary Syndrome (ACS)

DISCUSSION

Atherosclerosis leading to acute coronary syndrome is regard as immune-inflammatory event tempted by vascular intimal wall impairment. The pathogenicity of atherosclerosis is dependent on various diverse factors initiated endothelial dysfunction provoked by dyslipidaemia and activation of cellular and hormonal components of inflammation resulting in generation of stress condition by the production of free oxidant radicals. The association among oxidative stress and pathophysiology of ACS is extremely feasible. Therefore, the study was deliberated to evaluate the role of circulatory oxidative markers, and inflammatory and lipid profile changes in the development and progression of acute coronary syndrome.

Modification of LDL as the result of oxidative stress is central paradigm of plaque destabilization. The current study showed that raised triglycerides, LDL and cholesterol levels along with decrease in HDL levels in acute coronary syndrome patients as compared to controls. These results are in agreement with the results of Serdar *et al.* (2007) and Gururajan *et al.* (2010) which showed significantly higher total cholesterol, LDL and tri-acyl glycerol and lower levels of HDL in ACS patients **[24, 25]**. The modified OxLDL cause the activation and migration of monocytes and increase VSMCs. These recruited cells generate numerous cytokines, chemokines and adhesion molecules (ICAM, VCAM) which prompt pro-inflammatory mediators' i.e. (TNF-α, IL-1α) bio-synthesis. AGEs and AOPPs are recently emerged as the chief prognostic oxidative markers in inducing cardiovascular events leading to acute coronary syndrome. Advanced oxidation protein products (AOPPs) acts as inflammatory mediators originate by action of free radicals on proteins triggering oxidative ignition of neutrophils, monocytes resulting in upregulation and activation of excessive dendritic cells may account for immune disorders in atherosclerosis **[26]**. In this study, it is evident a significantly increase in AOPPs level in case of acute coronary syndrome as compared to normal individuals because of excessive generation of hypochlorite ions in diseased stress condition which react with albumin protein to transformed it in AOPPs. Our results are concurrent with the studies of Skvarilova *et al.* (2005) and Zhou et al. (2012) that demonstrated increased AOPPs in patients of ACS **[27, 28]**. AGEs bind to receptor of advanced glycation end product (RAGE) to aggravate the ROS production by triggering NADPH oxidase. RAGE is multi-ligand receptors that interact with various ligands to mediate pro-inflammatory response **[17]**. Advanced glycation end-products are formed via non-enzymatic glycation of lipids, proteins and nucleic acid alter gene expression, migration of cells, proliferation and signalling pathway activation by binding to RAGE and have a significant role in atherosclerosis and vascular complication **[29, 30, 31]**. The current study reported significantly elevated levels of AGEs in acute coronary syndrome patient as compared to healthy group. Our finding showed similarity with Raposeiras-Roubin *et al.* (2015) which showed the association of AGEs with ACS. Raised levels of interleukin-6 and Tumor necrosis factor-α (TNF-α) have been reported in ACS patients than normal' because human atherosclerotic lesions express pro-inflammatory markers i.e. IL-1α, TNF-a which stimulate smooth muscle cells to produce IL-6 to initiate process of inflammation and cause liberation of C- reactive proteins from liver **[32, 33]**. These results are comparable with other studies **[34, 35]**.

Our finding showed significantly increased NO levels in ACS patients as compared to control group because of uncoupling of nitric oxide synthase, induction of iNOS upregulation and production reactive NO intermediates that leads to excessive production of NO that may react with superoxide free radicals to generate peroxynitrite ions (ONOO-) which trigger lipid peroxidation and cause protein damage **[4]**. Atherosclerotic lesions leading to acute coronary syndrome also affect the composition and concentration of lipid and lipoprotein in blood plasma. Lipid peroxidation, secondary by-product Malondialdehyde (MDA) can augment ROS production. The TBARS is the indication of oxidative stress in a variety of cardiovascular disease models **[36, 38-50]**. In present study, levels of MDA are high than normal individuals due to lofty ROS produced OxLDL particles of cholesterol linked with the findings of Arab *et al.,* (2011) MDA levels were observes elevated in CAD patient in comparison with controls **[37]**.

CONCLUSION

Acute coronary syndrome including unstable angina, STEMI and non-STEMI is originated from plaque destabilization and thrombus formation which in turn represents a heightened state of oxidative stress and inflammation and engross protein and lipid functional oxidative modifications within the vessel walls that lead to endothelial dysfunction leading to atherosclerosis. As oxidative and inflammatory markers act as integrators of various processes that trigger cardiovascular pathobiology, so their identification along with lipid profile analysis provide valuable information regarding the intensity and severity of disease circumstances.

ACKNOWLEDGEMENTS

Authors are highly thankful and pay their gratitude to Mr. Awais Raoof, Chairman BOG, The University of Lahore for supporting and providing financial assistance for the current project.

CONFLICT OF INTEREST

Authors declare no conflict of interests.

REFERENCES

[1] Benjamin EJ, Virani SS, Callaway CW, Chamberlain AM, Chang AR, Cheng S, Chiuve SE, Cushman M, Delling FN, Deo R, de Ferranti SD. Heart disease and stroke statistics—2018 update: a report from the American Heart Association. Circulation. 2018; 135(10): 146-603.

[2] Lippi G, Sanchis-Gomar F, Cervellin G. Chest pain, dyspnea and other symptoms in patients with type 1 and 2 myocardial infarction. A literature review. International Journal of Cardiology. 2016; 215:20-2.

[3] Libby P, Theroux P. Pathophysiology of coronary artery disease. Circulation. 2005;111(25):3481-8.

[4] Förstermann U, Xia N, and Li H. Roles of Vascular Oxidative Stress and Nitric Oxide in the Pathogenesis of Atherosclerosis. Circulation research, 2017;120(4) :713-735.

[5] Grech ED, Ramsdale DR 2003. Acute coronary syndrome: unstable angina and non-ST segment elevation myocardial infarction. BMJ. 2003; 326:1259.

[6] Sarkees ML, Bavry AA. Acute coronary syndrome (unstable angina and non-ST elevation MI). BMJ clinical evidence. 2009;2009.

[7] Khatri P, Simkhada R. Study on conventional risk factors in acute coronary syndrome. Journal of Universal College of Medical Sciences. 2015;3(2):1-4.

[8] Naruko T, Ueda M, Haze K, van der Wal AC, van der Loos CM, Itoh A, Komatsu R, Ikura Y, Ogami M, Shimada Y, Ehara S. Neutrophil infiltration of culprit lesions in acute coronary syndromes. Circulation. 2002;106(23):2894-900.

[9] Bobryshev YV, Ivanova EA, Chistiakov DA, Nikiforov NG, Orekhov AN. Macrophages and their role in atherosclerosis: pathophysiology and transcriptome analysis. BioMed research international. 2016;2016.

[10] Ott M, Gogvadze V, Orrenius S, Zhivotovsky B. Mitochondria, oxidative stress and cell death. Apoptosis. 2007;12(5):913-22.

[11] Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative medicine and cellular longevity. 2014;2014.

[12] Apostolova N, Victor VM. Molecular strategies for targeting antioxidants to mitochondria: therapeutic implications. Antioxidants & redox signaling. 2015;22(8):686-729.

[13] Rosenson RS, Brewer HB, Rader DJ. 2014. Lipoproteins as Biomarkers and Therapeutic Targets in the Setting of Acute Coronary Syndrome. Circ Res. 2014; 114: 1880-1889.

[14] Pacurari M, Kafoury R, Tchounwou PB, Ndebele K. The renin-angiotensin-aldosterone system in vascular inflammation and remodeling. International Journal of Inflammation. 2014.

[15] Nibbs RJB, Graham GJ. Immune regulation by atypical chemokine receptors.Nature Reviews Immunology. 2013; 13(11) :815–829.

[16] Peppa M, Uribarri J, Vlassara H. The role of advanced glycation end products in the development of atherosclerosis. Curr Diab Rep. 2004; 4(1):31-6.

[17] Wautier MP, Guillausseau PJ, Wautier JL. Activation of the receptor for advanced glycation end products and consequences on health. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2016.

[18] Rojas A, Delgado-López F, González I, Pérez-Castro R, Romero J, Rojas I. 2013. The receptor for advanced glycation end-products: a complex signaling scenario for a promiscuous receptor. Cellular signalling. 2013; 25(3): 609-614.

[19] Chen J, Song M, Yu S. Advanced glycation endproducts alter functions and promote apoptosis in endothelial progenitor cells through receptor for advanced glycation endproducts mediate overpression of cell oxidant stress. Molecular and Cellular Biochemistry. 2010; 335(1-2) :137-146.

[20] Villegas-Rodríguez ME, Uribarri J, Solorio-Meza SE, Fajardo-Araujo ME, Cai W, Torres-Graciano S, Rangel-Salazar R, Wrobel K, Garay-Sevilla ME. The AGE-RAGE Axis and Its Relationship to Markers of Cardiovascular Disease in Newly Diagnosed Diabetic Patients. PloS one. 2016; 11(7) :0159175.

[21] Ohkawa H, Ohishi N, Yagi KK. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. J Anal Biochem. 1979; 95 :351-358.

[22] Moshage H, Kok B, Huizenga JR, Jansen PL. 1995. Nitrite and nitrate determinations in plasma: a critical evaluation. Clin Chem. 1995; 41:892-896.

[23] Kalousova M, Skrha J, Zima T. 2002. Advanced glycation end-products and advanced oxidation protein products in patients with diabetes mellitus. Physiol Res. 2002; 51(6):597-604.

[24] Serdar Z, Serdar A, Altin A, Eryilmaz U, Albayrak S. Th e relation between oxidant and antioxidant parameters and severity of acute coronary sydromes. Acta Cardiol. 2007; 62(4): 373-380.

[25] Gururajan P, Gurumurthy P, Nayar P, Srinivasa Nageswara Rao G, Babu S, Cherian KM. 2010. Heart fatty acid binding protein (H-FABP) as a diagnostic biomarker in patients with acute coronary syndrome. Heart Lung Circ. 2010; 19(11): 660-664.

[26] Ou H, Huang Z, Mo Z, Xiao J. 2017. The Characteristics and Roles of Advanced Oxidation Protein Products in Atherosclerosis. Cardiovascular Toxicology. 2017; 17(1):1-12.

[27] Skvarilova M, Bulava A, Stejskal D, Adamovska S, Bartek J. Increased level of advanced oxidation products (AOPP) as a marker of oxidative stress in patients with acute coronary syndrome. Biomed papers. 2005; 149(1): 83-87.

[28] Zhou Q, Wu S, Jiang J, Tian J, Chen J, Yu X, Chen P, Mei C, Xiong F., Shi W, Zhou W. Accumulation of circulating advanced oxidation protein products is an independent risk factor for ischaemic heart disease in maintenance haemodialysis patients. Nephrology. 2012; 17(7): 642-649.

[29] Schmidt AM, Yan SD, Yan SF, Stern DM. 2001. The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. J Clin Invest. 2001; 108: 949-955.

[30] Jensen LJ, Flyvbjerg A, Bjerre M. Soluble receptor for advanced glycation end product: a biomarker for acute coronary syndrome. BioMed research international. 2015;2015.

[31] Raposeiras-Roubín S, Rodiño-Janeiro BK, Paradela-Dobarro B, Grigorian-Shamagian L, García-Acuña JM, Aguiar-Souto P, Jacquet-Hervet M, Reino-Maceiras MV, González-Juanatey JR, Álvarez E. Fluorescent advanced glycation end products and their soluble receptor: the birth of new plasmatic biomarkers for risk stratification of acute coronary syndrome. PLoS One. 2013; 8(9):e74302.

[32] Packard RR, Libby P. Inflammation in atherosclerosis: from vascular biology to biomarker discovery and risk prediction. Clinical chemistry. 2008;54(1):24-38.

[33] Vitulli P, Tanzilli G, Marullo AG, Peruzzi M, Zoccai GB. Inflammation and oxidative stress in acute coronary syndromes: a continuum from plaque vulnerability to thrombus formation. Enliven Clin Cardiol Res. 2014;1(1):1-7.

[34] Liuzzo G, Biasucci LM, Gallimore JR, Grillo RL, Rebuzzi AG, Pepys MB. The prognostic value of C-reactive protein and serum amyloid a protein in severe unstable angina. N Engl J Med. 1996; 331: 417-424.

[35] Shimada K. 2009. “Immune System And Atherosclerotic Disease: Heterogeneity Of Leukocyte Subsets Participating In The Pathogenesis Of Atherosclerosis,” Circulation Journal. 2009; 73(6): 994-1001.

[36] Van Dam PS, Van Asbeck BS, Van Oirschot JF, Biessels GJ, Hamers FP, Marx JJ. Glutathione and α-lipoate in diabetic rats: nerve function, blood flow and oxidative state. European journal of clinical investigation. 2001;31(5):417-24.

[37] Arab S, Khazaai H, Hambali Z, Ahmad Z. Homocysteine and malondialdeyde (MDA) levels associated with the occurrence of cardiovascular disease (CVD) in chronic renal failure (CRF) in Malaysia. Global Journal of Health Science. 2011;3(1):119-127.

[38] Hafeez M, Yasin T, Safdar U, Waquar S, Rana M, Malik A. An evidence based assessment of most common risk factors of myocardial infraction: analysis from a local population. Biol Clin Sci Res J 2020(1):e044.

[39] Khalil R, Ali Q, Hafeez M, Malik A. Phenolic acid profiling by rp-hplc: evaluation of antibacterial and anticancer activities of *Conocarpus erectus* plant extracts. Biol Clin Sci Res J 2020(1):e010.

[40] Hameed B, Ali Q, Hafeez MM, Malik A. Antibacterial and antifungal activity of fruit, seed and root extracts of Citrullus colocynthis plant. Biol Clin Sci Res J. 2020;33.

[41] Ali J, Ali Q, Hafeez MM, Malik A. Clinical features, diagnosis and treatment of COVID-19. Biol Clin Sci Res J. 2020;2020:e032.

[42] Siddique A, Fateh A, Idrees N, Hafeez MM, Ali Q, Malik A. The epidemics of COVID-19. Biol Clin Sci Res J. 2020:e030.

[43] Rashid M, Kari M, Rashid R, Rana M, Amjad A, Hafeez M. Uterine artery doppler indices as predictive measures for the pre-eclampsia and intrauterine growth restriction. Biol Clin Sci Res J; 2020(1):e023.

[44] Omer M, Malik S, Anjum M, Riaz A, Ali R. Diagnostic accuracy of ultrasound in detecting meniscal tears taking magnetic resonance imaging as gold standard. Biol Clin Sci Res J 2020(1):e040.

[45] Ali Q, Khalil R, Nadeem M, Hafeez, MM, Malik, A. Antibacterial, antioxidant activities and association among plant growth related traits of *Lepidium draba*. Biol Clin Sci Res J. 2020:011.

[46] Khalid A, Anjum M, Daraaz U, Hussain K, Omer M. Predictive accuracy of cervical length in mid trimester on transabdominal ultrasound for cesarean section. Biol Clin Sci Res J 2020(1):e043.

[47] Chudhary H, Amin A, Malik M, Hafeez M, Rana M, Malik A. Risk assessment of non-conventional contributory factors in onset of diabetes mellitus type II. Biol Clin Sci Res J 2020(1):e036.

[48] Ali S, Gillani S, Afzal M, Parveen K. Assessment of nurses management skills for critically ill patients. Biol Clin Sci Res J 2020(1):e013.

[49] Tabassum S, Bibi T, Tariq F, Tariq S, Raza S, Hafeez M, Rana M. Unusual leukemoid reaction in a covid-19 patient: a case report. Biol Clin Sci Res J 2020(1):e034.

[50] Mady A, Ramdan O, Al Yousef R, Ishag A, Bakirova G, Kuhail A, Shahzad S, El-Etreby W, Mumtaz S, Almozainy S, Palacio K, Aldamahshi D, Alcazar A, Alodat M, Abdelrahman B, Harthy A. COVID 19 critical care training surge experience for physicians in riyadh health cluster one, Saudi Arabia. Biol Clin Sci Res J 2020(1):e041.