SHORT COMMUNICATION

ABERRANT PROTEIN S-NITROSYLATION; A NEW PERSPECTIVE IN HYPERTENSIVE AND DIABETIC HYPERTENSIVE DISORDERS

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ABSTRACT

Background: Diabetes and hypertension frequently coexist, leading to additive increases in the risk of life-threatening cardiovascular events. Large scale proteomic studies implicate the role of aberrant protein expression in these groups' specifically post translational modifications. Protein S-nitrosylation conveys a large part of the ubiquitous effect on cellular signal transduction, accumulating evidence indicates important roles in normal physiology. Dysregulated S-nitrosylation has been implicated as a cause or consequence of a broad range of diseases, including asthma, cystic fibrosis, Parkinson disease, heart failure, and stroke. The purpose of study is to identify molecular changes and potential alterations in expression of specific aberrant s-nitrosylation in hypertensive and diabetic hypertensive patients. We aimed to identify such blood biomarkers and potential drug targets which can provide insight into the underlying molecular mechanisms, associated with its pathology.

Methods: Proteome mapping of hypertensive, diabetic hypertensive serum samples was conducted to get the expression of aberrant nitrosylated proteins. Serum samples (n=15 from each group) by using sodium dodecyl sulphate polyacrylamide gel electrophoresis coupled with immunoblot by using anti S-nitrosylated antibody followed by imaging and statistical analysis by Quantity-One software (BioRad).

Results: We have identified in total fifteen nitrosylated protein components with altered expression among the studied groups. The 177.8KDa, 119KDa, 74.02KDa, 61.5KDa, 52.3KDa protein, and 24.93KDa proteins are showing hyper-nitrosylation in diabetic hypertensive serum samples. However, he 119KDa, 74.02KDa and 61.5KDa protein components showed hyper-nitrosylation in hypertensive serum samples as compared to normal controls while rest of the proteins component were found hypo-nitrosylated.

Conclusion: The characterization of aberrantly expressed nitrosylated proteins globally and their association with disease associated pathways probably are playing modulatory roles in the pathophysiology of the disease, following post-translational modifications.

KEY WORDS: S-nitrosylation; proteomics; Diabetic hypertensive ; Hypertension; Post-translational modification.

INTRODUCTION

Hypertension is a major public health problem with increased prevalence throughout the world and the number of individuals suffering from hypertension is increasing day by day[1]. Hypertension is a complicated disorder resulting from genetic and environmental factors[2]. According to Pakistan national health survey report 2013, high blood pressure affects 18 percent of adults over 15 years of age and 33 percent of adults above 45 years. However, only 50 percent of those affected are diagnosed and more than 70 percent of all hypertensive patients in the country are unaware of the disease. Patients with undiagnosed hypertension may have risk for serious health problem i.e. cardiovascular disorders, heart failure, renal problems, diabetic complications, strokes etc[3].

Diabetes Mellitus (DM) is a leading cause of morbidity and mortality throughout the world [4]. It is a major cause of heart disease and stroke among adults

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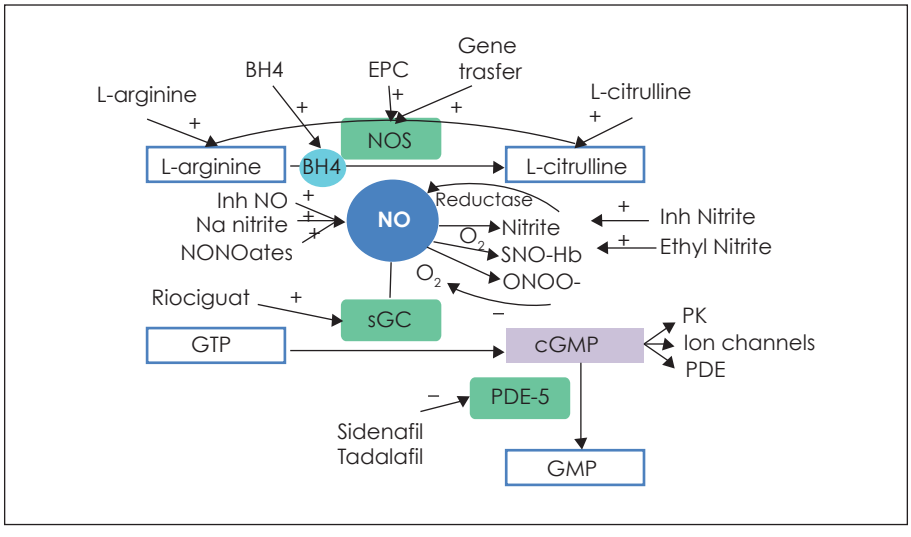
and is the leading cause of nontraumatic lower-extremity amputations, blindness, and kidney failure. Pakistan ranks at number six in terms of number of people with diabetes worldwide. It was estimated that in 2000 there were 5.2 million diabetic patients and this will rise to 13.9 million by 2020, leading Pakistan to 4th most prevalent country [5].

Hypertension is a risk factor for diabetic patients, it is reported in over two-third patients with diabetes mellitus, and its development is associated with the hyperglycemia. The patients of hypertension with diabetes are at high risk for cardiovascular diseases. People with controlled diabetes have a similar cardiovascular risk as to patients without diabetes but with hypertension[6].

Protein S-nitrosylation Nitric oxide is a small highly reactive gaseous molecule. It is endogenously synthesized, diffusible, lipophilic gas that is produced by a group of enzymes known as nitric oxide synthases (NOS). Its role is to convert the amino acid L-arginine to L-citrulline and NO and plays a key role as a biological messenger[7]. It is unstable in nature and not only reacts with metal ions but also can react with superoxide and molecular oxygen, and form peroxynitrite and dinitrogen trioxide N_2O_3 (or higher oxides like NO_2). Moreover, adding or removing one electron from the antibonding highest occupied molecular orbital by reducing or oxidizing chemicals yields nitroxyl anion (NO^-) and nitrosonium cation (NO^+). Collectively, these species are referred to as reactive nitrogen species (RNS) each having distinct chemical properties leading to numerous reactions with biological molecules like lipids, carbohydrates, nucleic acids, and proteins.

Reactive Nitrogen Species are important redox-signaling molecules in the cell that bind covalently to target proteins[8]. There are three important NO-dependent modifications: metal nitrosylation, tyrosine nitration, and cysteine S-nitrosylation. The binding of nitric oxide to the protein cysteine residue can also modify the activity of many proteins, and the reaction is termed as, S-nitrosylation[9]. S-nitrosylation is a supplementary post translational modification process, facilitated through nitric oxide and reactive nitrogen species[10].

Protein S-nitrosylation in disease state: In mammalian cells, L -Arginine dependent nitric oxide (NO) synthases are the major source of endogenous NO[11]. NO is involved in a variety of cellular signal transduction pathways, through protein S-nitrosylation, pointing to the possibility that dysregulated S-nitrosylation could contribute to pathophysiological characteristic of a wide range of disease states [12]. Hypo- or hyper-S-nitrosylation of protein (which result in alterations in protein function) are directly concerned with symptomatology of increasing number of human diseases, including prominently disorders of cardiovascular, musculoskeletal and nervous systems [13].



NO synthesis and signaling pathways
(Adapted from J. Pulmonary Circulation.2013 3; (20):30)

Dysregulated Snitrosylation has been implicated as a cause or consequence of a broad range of diseases, including asthma, cystic fibrosis, Parkinson disease, heart failure, and stroke, and the role of nitrosylases and denitrosylases in governing levels of S-nitrosylation under both physiological and pathophysiological conditions is increasingly appreciated [14]. It is apparent that protein S-nitrosylation is spatially and temporally regulated, not only by the direct interaction or compartmentation of SNO target with

NO synthases, but also by the enzymes that remove SNO from glutathione (e.g.GSNOR) and proteins (e.g. thioredoxin) [15].

METHODS

The present study was aimed to identify the aberrant protein s-nitrosylation in hypertensive and diabetic hypertensive patients. This section includes the analytical procedures carried out in this study. Control samples with no known clinical complications, Diabetic hypertensive and Hypertensive samples were collected from different hospitals in Karachi following strict inclusion/exclusion criteria. Samples were stored at -80°C until used for proteomic analysis. Total protein was quantified by using Bradford assay. After dilution, the serum samples (n=15 from each group) were subjected to SDS PAGE (10%) and the protein profile obtained thus showed approximately 13 -16 bands of proteins ranging from 11 to 350 kDa in normal, diabetic hypertensive and hypertensive subjects. Molecular weight determination and semi quantitative analysis was carried out using "Quantity One" (Bio Rad) software.After SDS PAGE gel analysis, western blot was carried out to find out s-nitrosylated serum proteins in diabetic hypertensive and hypertensive serum samples, and the gel was analyzed. The protein profile we obtained contain 12 -15 bands of protein components, ranging from 15kDa to 245kDa in Normal, Diabetic hypertensive and Hypertensive subjects serum samples.

RESULT

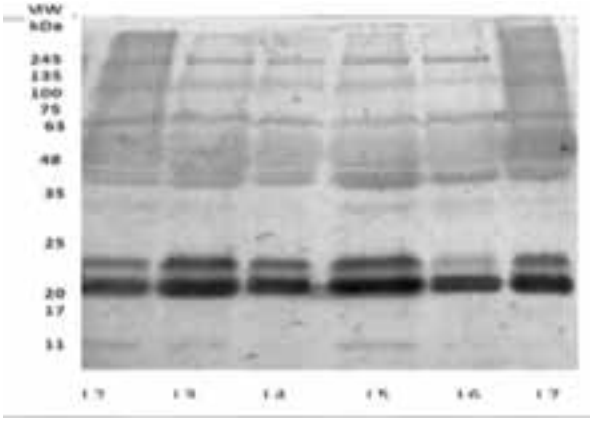


Figure: 1 Western blot (10% gel) of normotensive, hypertensive and diabetic hypertensive serum samples analyzed by quantity one software.

We found down regulation of 250kDa and 66kDa, protein components in diabetic hypertensive subjects by protein profiling on 10% SDS-PAGE. The down regulated 66kDa protein probably albumin and its down regulation is associated with impaired kidney function16. 20kDa protein possibly superoxide dismutase (SOD) which is down regulated in diabetic hypertensive sample indicate increased oxidative stress. As a consequence, SOD serves as an anti-oxidant but it's down regulation shows that there is increase oxidative stress in diabetic hypertension. From western blotting we have identified in total fifteen nitrosylated protein components with altered expression among the studied groups. The 177.8KDa, 119KDa, 74.02KDa, 61.5KDa, 52.3KDa protein, and 24.93KDa proteins are hyper-nitrosylated in diabetic hypertensive s. The 119KDa, 74.02KDa and 61.5KDa proteins are hyper-nitrosylated in hypertensive patients as compared to normal controls, while the remaining protein components were hypo-nitrosylated.

DISCUSSION

Hyperglycemia plays an important role in the decreased NO production in type 2 diabetes as high glucose inhibits endothelial NOS activity, through a protein kinase C–associated mechanism [17]. Moreover, high glucose and/or the associated advanced glycosylation end products decreased NOS expression. S-nitrosylation also inhibits the activity of protein kinase C as well as other serine/threonine kinases, including IκB kinase and the insulin receptor kinase [18]. An impaired NO generation in type 2 diabetes may be another feature of insulin resistance [19]. Regarding hypertension, endothelial cells play a major role in arterial relaxation. Endothelial cells release a factor NO by the eNOS that causes vascular relaxation [20]. The NO is rapidly degraded (the half-life of NO is only of few second) into superoxide anion by the oxygen derived free radical. These superoxide anions thus modify the endothelial function and can also act as a vasoconstrictor. In addition, nitric oxide synthase (NOS), and in particular the endothelial isoform of NOS (eNOS) is now recognized as an important source of superoxide [21]. As a result of these reactions, eNOS may become a peroxynitrite generator that leads to dramatic increase in oxidative stress. A decrease in bioavailability of NO and increase in oxidative stress are present in hypertension [22]. The renin enzyme circulates in the blood stream and breaks down (hydrolyzes) angiotensinogen secreted from the liver into the peptide angiotensin I. Angiotensin I is further cleaved in the lungs by endothelial-bound angiotensin-converting enzyme (ACE) into angiotensin II [23].Ang II levels increase in hypertension which leads to increase level of ROS and decrease level of NO subsequently decrease the level of S-nitrosylation in hypertension. Recent study suggest that Angiotensin II treatment resulted in inactivation of thioredoxin reductase and increased S-nitrosylation, indicating that S-nitrosylation may provide a critical mechanism in hypertension associated with abnormal vascular reactivity[17].

CONCLUSION

We propose that s-nitrosylation is decrease in hyper-

tension and slightly increase in diabetic hypertension because insulin resistant diabetes mellitus leads to increase in NO production which results in increased s-nitrosylation. Moreover, angiotensin II which has a major role in production of hypertension is associated with decreased NO or S nitrosylation and the characterization of aberrantly expressed nitrosylated protein components, and their association with disease pathways probably is a result of their modulatory roles following post-translational modifications. Further studies are required to completely characterize the proteins involved and confirm the present findings.

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