Neutrophils are important for innate immunity as well as to initiate an acute response to infection. During such a response, neutrophils are activated, move towards the site of inflammation and kill the invading microorganisms, such as bacteria and fungi by phagocytosis **(Nauseef *et al*, 2000)** and also by extracellular trap (NETs) formation **(Brinkmann *et al*, 2004)**. During phagocytosis, Neutrophils produce reactive oxygen species (ROS), including superoxide, hydrogen peroxide and hypochlorous acid and release cytotoxic granule components into pathogen-containing phagocytic vacuoles. Neutrophils ROS and granule components are highly effective at killing most human pathogens *in vitro*. Neutrophils eliminate the pathogens extracellularly via NETs formation. NETs can kill both Gram-positive, Gram-negative bacteria and fungi by high concentration of antimicrobial proteins and proteases. NETs contents are abundant *in vivo* at the site of infection and during acute inflammatory conditions **(Brinkmann *et al*, 2007)**. NETs formation is due to the generation of NADPH oxidase and myeloperoxidase (MPO) dependent ROS, which is distinct from apoptosis and necrosis. This was validated by studies on chronic granulomatous disease (CGD) patients and MPO deficient subjects where NETs were not formed due to nonfunctional NADPH oxidase **(Fuchs *et al*, 2007)** or absence of MPO derived radicals **(Metzler *et al*, 2010).**

Nitric oxide (NO) is a unique gaseous signaling molecule having very short half life. Reports in the literature suggest diverse functions of NO in regulating such as hematopoiesis **(Michurina *et al*, 2004)**, vascular hemostasis **(Dikshit M *et al*, 1993)**, smooth muscle relaxation, blood pressure regulation and peripheral immune response. It mediates action of growth factors and regulates the balance between proliferation and differentiation in diverse cell types **(Shami and Weinberg, 1996; Ishida *et al*, 1997; Enikolopov *et al*, 1999; Kumar *et al*, 2010)**. NO is synthesized from L-arginine in a reaction catalyzed by nitric oxide synthase (NOS) enzymes (EC1.14.13.39), a member of cytochrome P450 like reductase family. Three distinct isoforms of NOS have been identified, each is coded by a different set of genes. Human NOS genes are localized at different chromosomes - neuronal NOS (nNOS) at 12q24.2-12q24.3, inducible NOS (iNOS) at 17q11.2, while endothelial NOS (eNOS) is at 7q35-7q36 **(Forstermann and Kleinert, 1995)**. Among three NOS isoforms, iNOS is expressed mostly under inflammatory conditions whereas nNOS and eNOS are constitutively expressed. nNOS

was first reported in the mammalian brain, later on its presence was also reported in different cell types. nNOS derived NO plays several important roles in the brain, including in the regulation of synaptic signaling and plasticity where as eNOS derived NO is a critical regulator of cardiovascular homeostasis. Both eNOS and nNOS are calcium dependent and express constitutively. iNOS expresses only in the presence of stimuli generated by microbial products such as lipopolysaccharide (LPS) and/ or proinflammatory cytokines such as tumor necrosis factor-α (TNF- α) and interferon-γ (IFN- γ). iNOS enzyme activity is calcium-independent, and it can constantly produce high levels of NO for prolonged periods **(Alderton *et al,* 2001).**

Neutrophil generate NO at a rate of 10-100 nmoles/5min/10^6 cells, which is comparable to the production of NO by the endothelial cells **(Salvemini *et al*, 1989),** thus having a widespread impact on vascular homeostasis and warrants evaluation under diverse patho-physiological conditions. Ability of neutrophil to synthesize NO was first discovered by its ability to relax aortic rings **(Rimele *et al*, 1988)** and by the inhibition of platelet aggregation **(Dikshit *et al*, 1993)**. NO is a very important regulator of neutrophil functions and is involved in various physiological and pathological conditions. NO regulates neutrophil rolling, adhesion, migration and chemotaxis, aggregation, degranulation, free radical generation **(Sethi *et al*, 2000)** and apoptosis. **Junhai *et al*, 1997,** have demonstrated NO as a modulator of neutrophil migration to the infected sites. Infusion of NOS inhibitor (L-NAME) to LPS treated rats increased the greater neutrophil infiltration by upregulating ICAM-1 expression and treatment with NO donor prevented the neutrophil migration in presence of L-NAME and LPS. Further it has been reported that NO can modulate neutrophil migration by regulating microparticle formation **(Nolan *et al,* 2008)**. Incubation of unstimulated neutrophil with L-NAME increased the percentage of neutrophil migrating towards IL-8 which was reversed by the addition of L-arginine. Recently **Henric *et al*, 2010**, demonstrated the involvement of nNOS-derived NO in autocrine dendritic cell (DC) maturation and DC-mediated induction of T cell activation, hence it contributes to the fine-tuning of immune processes in health and disease.

Previous studies form this lab have shown significant alterations in the neutrophil nitrite content in the patients of Parkinson's disease (PD) **(Barthwal *et al*, 1999),**

schizophrenia (**Srivastava *et al*, 2001**), depression (**Srivastava *et al*, 2002**), as well as in experimental models of PD (**Barthwal *et al*, 2001; Singh *et al*, 2005; Shukla *et al*, 2006; Singh *et al*, 2007**). Studies on rat and human neutrophil have highlighted the importance of NO in the regulation of neutrophil derived free radical generation (**Seth *et al*, 1997; Sethi *et al*, 1999**) and NETs formation (**Patel *et al*, 2010**). Role of NO by modulating neutrophil functions of has thus important implications in various pathological conditions. Studies on NOS in the rat neutrophil from this lab demonstrated unique features of intracellular NOS distribution (**Saini *et al*, 2006**) and their functional and molecular characterization during maturation (**Kumar *et al*, 2010**).

NO is highly reactive and diffusible, NO production by NOS is therefore, under complex and tight control to dictate specificity to its signaling and to limit toxicity to other cellular components. Indeed, NO production from each of the three major NOS isoforms, nNOS, iNOS and eNOS are subject to a variety of transcriptional, translational, and post-translational controls. The post-translational controls, which include lipid modifications, phosphorylation events, trafficking and interactions with protein partners, serve to govern the timing, magnitude, and spatial distribution of NO release.

NOSs are associated with a vast array of interacting proteins. The activators of NOS are calmodulin, CAT1, dynamin-2, porin, protein Kinase B/Akt, Rac2, HSP90, NOSTRIN, PSD95 and PSD93, while caveolin1, caveolin3, bradikinin-B2, angiotensin-AT1 receptor, NOSIP, PIN, kalirin, NAP10, α-syntropin, capon, phosphofructokinase-M and EBP50 inhibit NOS activity. Some NOS interacting proteins such as caveolin-3, Hsp90, EBP-50, NOSIP caveolin-1, NOSTRIN also act as adaptors and traffickers.

Protein-protein interactions involving NOS represent an important and increasingly complex mechanism that regulates many cellular functions. The majority of these interactions have not yet been addressed to the neutrophil, where important functions as leukocyte rolling, adhesion, migration and chemotaxis, degranulation, free radical generation and NETs formation are modulated by NO itself. The challenging work for the future concerns the identification of NOS associated proteins to control NO production in human neutrophil. This will help to understand the specificity of NO actions in these cells. Previous report from this lab has helped to discern the sub cellular distribution of NOS and identified caveolin-1 as NOS interacting protein in rat

neutrophil. As the binding motifs for NOS-associated proteins are defined and functional proteomics gains more widespread use, new candidate proteins for NOS interaction and regulation need to be identified and tested.

The present study was undertaken to investigate the following:

1. Explore NO mediated free radical generation and NETs formation in human neutrophil.
2. NOS- interacting proteins in human neutrophil.