Chapter I

Introduction
Infectious diseases, also known as contagious or communicable diseases, are diseases that are caused by infectious agents such as bacteria, viruses, fungi or parasites those are passed directly or indirectly, from one person to another. Infectious diseases remain a leading cause of morbidity and mortality worldwide with HIV, tuberculosis and malaria estimated to cause 10% of all deaths each year and account for nearly half of India's disease burden (1). Despite the commendable successes in control afforded by improved sanitation, immunization, and antimicrobial therapy, the infectious diseases continue to be a common and significant problem of modern medicine. There is a continuous emergence of new infectious diseases as demonstrated by the SARS epidemic in 2003, the swine flu pandemic in 2009, MERS CoV in 2013 and Zika in 2016. In the recent past, India has witnessed many outbreaks of emerging infections. Human pathogens emerge and re-emerge due to interaction of multiple complex factors between the host and the pathogen, each driven by the need to secure the success of the species in changing environments (2).

Infectious diseases comprise clinically evident illness resulting from the infection, presence and growth of pathogenic biological agents in host tissue. In all of these cases the pathogen must have the ability to recognize, become associated with, exploit the nutrient reserve and combat the defense responses of its specific hosts. To accomplish these tasks, pathogens use an extensive battery of virulence and related factors. The infectious diseases are usually characterized by the major organ system involved. Thus infections can be classified as respiratory infections, gastrointestinal infections, genitourinary infections, nervous system infections, skin and soft tissue infections, bone and joint infections, cardiovascular infections and generalized infections (3).
In contrast to infectious diseases, non-infectious diseases or non-communicable diseases (NCDs) are those that are not caused by a pathogen and are not contagious. They are of long duration and generally progress slowly. These diseases are primarily caused by four major modifiable risk factors that include unhealthy diets, physical inactivity, the harmful use of alcohol and tobacco use. For example, unhealthy diets may show up in individuals as raised blood pressure, increased blood glucose, elevated blood lipids and obesity which can lead to cardiovascular diseases and other microvascular diseases. The main types of NCDs are cardiovascular diseases (like heart attacks and stroke), cancers, chronic respiratory diseases (such as chronic obstructive pulmonary disease and asthma) and diabetes mellitus which are responsible for almost 70% of all deaths worldwide.

Inflammation is a physiological, protective immune response of the host to any injury or infection. It is one of the most important host defense mechanisms to resolve the initial injury, remove debris, initiate tissue repair and regeneration, suppress and prevent spread of an infection. It comprises a series of changes in the terminal vascular bed, in blood and in connective tissues to eliminate the offending irritant and repair the damaged tissue. However, inflammatory response sometimes results in further injury and organ dysfunction. The process of inflammation is characterized by endothelial cell activation, leukocyte recruitment and activation, vasodilation and increased vascular permeability (4, 5, 6). It involves cellular / tissue, humoral and chemical (cytokines) participants.

Neutrophils are the first white blood cells to migrate towards the infected or damaged site. They participate in the innate immune response at the early stage of inflammation and migrate to areas of inflammation within minutes. Neutrophil recruitment is initiated by changes on the surface of endothelium that result from stimulation by inflammatory mediators including histamine, cysteinyl-leukotrienes and cytokines [tumor necrosis factor- α (TNF-α), IL-1β and IL-6]. These inflammatory
mediators are released from tissue resident sentinel leukocytes when they come into contact with pathogens. Activation of endothelial cells leads to increased expression of adhesion molecules, such as selectins and integrin ligands, and presentation of chemokines upon their surfaces, which participate in the neutrophil adhesion to endothelial cells. This step is followed by transmigration and extravasation of neutrophils from circulation to sites of inflammation or infection (7, 8). Although neutrophils are major effectors of acute inflammation, several recent evidence indicate that they also contribute to chronic inflammatory conditions and adaptive immune responses (7).

Once at the site of infection, neutrophils encounter microorganisms and phagocytose them using both oxidative and non-oxidative mechanisms. After they are encapsulated in phagosomes, neutrophils mediate direct killing of the pathogens by using reactive oxygen species (ROS) generated by membrane-bound NADPH oxidase system (oxidative mechanism) or antimicrobial proteases and peptides (non-oxidative mechanism). These antimicrobial proteins are released from the neutrophil granules either into phagosomes or into the extracellular environment, thus acting on either intra- or extracellular pathogens, respectively (9, 10). Neutrophils can also eliminate extracellular microorganisms by releasing neutrophil extracellular traps (NETs). NETs are composed of decondensed chromatin to which histones, proteins (for example lactoferrin and cathepsins) and enzymes (for example myeloperoxidase and neutrophil elastase) released from neutrophil granules are attached. NETs immobilize pathogens, thus preventing them from spreading and they are also thought to directly kill pathogens by means of antimicrobial histones and proteases such as elastase (11, 12).

The non-oxidative arm of antimicrobial action is mediated by the release of several potent cytotoxic proteins and proteases. These molecules are stored in the cytoplasmic granules of neutrophils. Neutrophils carry four different kinds of granules
and each are able to release up to forty varieties of molecules (13, 14). These granules undergo differential exocytosis following activation of neutrophils upon exposure to various inflammatory mediators such as cytokines and chemokines and lead to release of variety of proteins (15, 16). Proteases secreted by the azurophil granules and particularly the human neutrophil elastase (HNE) are the most deleterious molecules, if not properly controlled they could cause severe damage to healthy tissue. The term elastase refers to a group of enzymes capable of release of soluble peptides from insoluble elastin by proteolysis (17).

HNE, a serine protease exhibits number of biological effects. In addition to its intra-and extracellular effects mediating host defense against infection, NE has been associated with non-infectious, inflammatory process regulation. It functions as a negative regulator of inflammation by degrading various pro-inflammatory cytokines such as IL-6, IL-1 and TNF-α (16, 18). This inactivation might limit further activation of neutrophils and dampen the inflammatory process (15). In vitro studies have suggested that HNE may also play a role in neutrophil adhesion. It can cleave cell-bound intercellular adhesion molecule-1 (ICAM-1), required for the adhesion of neutrophils to the endothelial surface and decreasing neutrophil adhesion and migration (19). Even though elastase has these positive attributes, this enzyme also poses a significant challenge to the body because it has been shown to have potentially pro-inflammatory effects (16, 18, 20). For example, it can enhance neutrophil migration by inducing the secretion of pro-inflammatory cytokines granulocyte macrophage-colony stimulating factor (GM-CSF), IL-6, and IL-8 from epithelial cells enhancing the inflammatory responses (18). HNE cleaves α1-antiprotease inhibitor, generating a fragment that is chemotactic for neutrophils (21). HNE is a proteolytic enzyme that digests virtually every type of tissue matrix proteins such as insoluble elastin, collagen, fibronectin, proteoglycan
and cadherins (16, 22). Elastase is the only protease capable of degrading the mature elastin that imparts elastic recoil to tissues. Uncontrolled elastase activity results in excessive degradation of the elastin network and thus is implicated in various infectious as well as non-infectious diseases. High concentrations of neutrophil elastase, component of NETs, can cause degradation of the wound matrix and delay healing (23).

As mentioned above, elastase could be potentially damaging when over expressed or when present in high concentrations as uncontrolled secretion can trigger off destructive processes associated with various chronic diseases. Hence, in vivo the proteolytic activity of NE, released to the extra cellular region is well regulated and controlled by a number of potent endogenous macromolecular antiproteases, such as alpha1-proteinase inhibitor or alpha1-antitrypsin (α1-PI/α1-AT), α2-macroglobulin (α2-MG) and secretory leukoproteinase inhibitor (SLPI). The inhibition of elastase activity by these endogenous inhibitors represents a major mechanism limiting host tissue destruction. These inhibitors rapidly and irreversibly interact with the free enzyme, resulting in formation of a stable protease inhibitor complex that is incapable of showing enzymatic activity (24, 25). The ability to evade these antiproteinases is key to the success of NE in the extracellular environment. In inflammatory states where large numbers of polymorphonuclear neutrophils are infiltrated and activated, enormous amount of elastase is released. It is known that during infection neutrophils release ROS such as superoxides, H₂O₂ and hydroxyl radicals (25). These have been shown to inactivate proteins including the endogenous protease inhibitors. Thus, during infection or inflammation the possibility of enhanced NE activity is very high resulting in excessive proteolysis of extracellular matrix proteins causing crucial tissue damage and progress of disease. Physiological balance between elastase-antielastase factors is essential to maintain normal integrity of tissues and an imbalance has been implicated in the
pathogenesis of several acute and chronic inflammatory diseases. Thus, study of the role of elastase and its endogenous inhibitors is still an important area of investigation as both these factors play important roles in the disease progression or its prevention.

HNE was first recognized as protein-degrading enzyme but have now proven to be multifunctional protease participating in a variety of pathophysiological processes. Thus, there has been a significant interest in investigating the properties of elastase and its inhibitors in the recent years. Neutrophil proteases, particularly NE have long been therapeutic targets. Designing a specific inhibitor capable of targeting and inhibiting elastase would probably help reduce progression of inflammation. The available data from experimental and clinical studies suggest that inhibition of NE using therapeutic inhibitors would suppress or attenuate deleterious effects of inflammation including lung diseases (6). At present, a number of natural and synthetic inhibitors of elastase have been used in the treatment of HNE-related diseases (16).

α1-AT is the major circulating serine protease inhibitor which exhibits diverse roles in living cells. It is a potent inhibitor of multiple serine proteases with high activity against neutrophil serine proteases, neutrophil elastase and proteinase 3. In addition to its anti-protease activity, α1-AT expresses anti-inflammatory, antiapoptotic, immunomodulatory and antimicrobial effects (26, 27, 28). These multiple functions highlight the importance of this inhibitor in health and diseases. α1-AT deficiency (AATD), an inherited disease, is associated with excessive proteolytic activity and is well established in the development of pulmonary emphysema, Chronic Obstructive Pulmonary Disease (COPD), acute lung injury, cystic fibrosis and acute respiratory distress syndrome (ARDS) (29). The intravenous or intratracheal aerosol administration of recombinant or purified, plasma derived α1-AT is a standard therapy used for individuals with AATD and pulmonary emphysema (30). Recent studies suggest that, α1-
AT therapy could be beneficial in patients with type 1 and type 2 diabetes, acute myocardial infarction, stroke, multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease and transplant rejection (27) and is currently under evaluation in multiple clinical trials to assess its efficacy in maintaining pancreatic beta cell reserve and glycemic control in type 1 diabetes patients. Various research groups working on experimental diabetes in animal models and retinal pericyte cell cultures have demonstrated potential, protective role of $\alpha_1$-AT on retinal vasculature and it was also suggested that early use of $\alpha_1$-AT therapy may be an effective strategy to prevent or hinder the progression of diabetic retinopathy (31). $\alpha_2$-MG, a major antiprotease within the circulation is able to inactivate variety of proteinases and its increased or decreased levels are implicated in the pathogenesis of various inflammatory disease conditions such as nephrotic syndrome, type 2 diabetes, stroke, preeclampsia, atherosclerosis and cancer (32, 33, 34, 35).

In view of the profound and cardinal roles that NE and its endogenous inhibitors could play in various infectious and non-infectious diseases, they might be of use as add on biomarkers in various disease conditions which could support diagnosis, prognosis and treatment. Research in this area is likely to yield insights that will contribute to our understanding on the role of these multifunctional molecules in health and disease.