Chapter IV

Correlation of diagnostic parameters and risk factors of infectious and non-infectious diseases with the levels of elastase and its inhibitors
Introduction

Efficient and accurate diagnosis of diseases is of primary importance for clinical care, surveillance activities and outbreak control. Early laboratory confirmation and clinical diagnosis are important because some patients progress in a short period from mild disease to severe and at times to death. Therefore early diagnosis and early intervention can be sometimes life-saving. Diagnosis of a disease based only on clinical symptoms could be at times unreliable, as many diseases share common clinical symptoms (195, 196).

Clinical manifestations such as fever, respiratory symptoms, and chest x-rays are usually recommended for the diagnosis of pneumonia (197). On the other hand, viral culture, nucleic acid amplification, and serological assays have been used for the diagnosis of dengue (198). These physical and routine laboratory examinations are generally sufficient to identify diseases. However, there are no means for early prediction on the severity of these diseases as these diseases are known to turn severe without any warning signs leading to complications.

The diagnosis of diabetes mellitus depends solely on the demonstration of hyperglycemia. The recommended diagnostic criteria for diabetes mellitus are: classic symptoms of diabetes and random plasma glucose concentration ≥200mg/dl or FBS ≥126mg/dl and 2-h post load plasma glucose concentration ≥200mg/dl during the OGCT or HbA1C ≥6.5% (199). Symptoms of hyperglycemia may be relatively late development in the course of T2 diabetes delaying the diagnosis and thus approximately 30% of patients are presented with complications of diabetes such as retinopathy, nephropathy and neuromuscular disease at the clinical diagnosis of type 2 diabetes (200). Prolonged hyperglycemia has been shown to result in chronic inflammatory state ultimately leading
to multi organ dysfunction. Two predominant complications of diabetes are retinopathy and nephropathy as consequences of uncontrolled hyperglycemia. Studies have indicated that not all patients develop multiple complications but invariably develop one of the complications on account of prolonged hyperglycemic state. The assessment of renal function is used in the diagnosis of nephropathy and its severity. However there are no specific biomarkers for diabetic retinopathy which is diagnosed exclusively by fundoscopy (131).

Despite considerable research, identification of patients with severe form of PE continues to challenge clinicians. Progression from mild to severe on the disease spectrum may be gradual or rapid. Severity of PE is classified based on substantially increased BP and proteinuria (201). However, recent research have shown that waiting for proteinuria to present can result in delayed intervention or missed diagnosis, as not all women with PE will develop proteinuria. Hence, an early measurable indicator of the severity of PE is desirable. PE, characterized by enhanced inflammatory response is accompanied by increasing concentrations of pro-inflammatory cytokines, acute phase proteins and leukocyte activation (151). Studies have indicated elevated elastase levels in preeclamptic women (154).

Current diagnosis of stroke relies on clinical examination and is supplemented further with various neuroimaging techniques. However, interpretation of brain imaging appearances can be difficult, as computerized tomography (CT) is often normal after the onset of ischemia and may remain normal in patients with mild ischemic strokes. Achieving an accurate diagnosis quickly in patients with acute stroke is extremely important for prompt initiation of treatment. Thus, disease specific molecules to support clinical diagnosis, to identify patients at risk of disease and guide treatment and prognosis would be valuable to improve care of patients.
It is well known that simple laboratory investigations such as platelet, WBC, neutrophil counts, FBS, HbA1c, renal function parameters, BP, among others would aid in diagnosis of above mentioned diseases. However, a specific biological marker for the diagnosis of disease and assess treatment outcome is therefore vital and it might be appropriate to have some adjunct parameters reflective of diagnosis, progression, and severity of diseases for differential diagnosis. Since disease conditions considered in this study mimic inflammatory pattern, a systematic measurements of elastase and its endogenous inhibitors were relevant. Therefore, a correlative study was planned on the association of these molecules with basic diagnostic parameters of diseases under study to ascertain any association and to utilize the results for diagnosis, treatment and prognosis.

**Statistical Analysis**

Pearson's correlation coefficient was used to analyze the correlation between continuous variables. P value ≤0.05 was considered statistically significant and <0.001 as highly significant.

**Results**

Correlation studies were carried out with parameters which were significantly increased or decreased in comparison to the normal values.
In pneumonia, the base parameter neutrophil counts correlation with NE and α2-MG presented positive associations (Fig 11).

Correlation of platelet counts as base parameter with elastase and α1-AT in dengue patients revealed that elastase associated negatively and α1-AT correlated positively. While neutrophil counts with elastase correlated negatively (Fig 12).

Among non-infectious diseases, association studies of FBS with α1-AT in DN and DR indicated negative association. However the association was strongly negative with α1-AT in patients’ suffering from retinopathy (Fig 13).
When the levels of NE and its endogenous inhibitors were correlated with the severity parameter proteinuria of PE, a strong positive correlation was obtained with NE and α₂-MG. On the other hand, a strong negative association was indicated with α₁-AT (Fig 14).

![Fig 13: Linear correlation plots of FBS with α₁-AT: a) in DN r = -0.055, p=0.678; b) in DR r=-0.282*, p=0.029 (*correlation significant at p<0.05).](image)

![Fig 14: Linear correlation plots of proteinuria with elastase, α₁-AT, α₂-MG in severe PE patients: a) r = 0.766**, p=0.000; b) r= -0.682**, p=0.000; c) r=0.805**, p=0.000 (**correlation highly significant at p<0.01).](image)
Correlation analysis of SBP in stroke patients indicated a positive correlation with elastase and α₂-MG and negative association with α₁-AT (Fig 15).

**Discussion**

Correlation or association studies are conducted in many disease conditions to evolve newer diagnostic and therapeutic strategies. Since all the disease conditions studied had significantly altered levels of NE and its endogenous inhibitors, attempt was made to correlate levels of these molecules with the basic diagnostic and risk parameters of diseases under study to propose add on biomarkers which would aid in diagnosis, treatment and prognosis.

Pneumonia is infection of the lungs that can cause mild to severe illness. The difference of clinical course is associated with the virulence of etiologic agents and/or the host immune status. The circulating immune cells including neutrophils, lymphocytes, and monocytes could be involved in the pathogenesis of pneumonia. The pathogenesis of pneumonia in each etiologic agent may be different; in general, patients with typical bacterial pneumonia manifest more toxic clinical symptoms with leukocytosis,
neutrophilia and bacteremia (202). In pneumonia lesions, mainly activated neutrophils and mononuclear phagocytes are predominantly observed, and mediators such as proteolytic enzymes, oxygen radicals, and cytokines from these cells could be associated with host lung injury (203). Significant increases in plasma NE, IL-6 and IL-8 have been confirmed at infectious sites of pneumonia and IL-8 positively correlated with the number of neutrophils or NE (124). In a study on association of plasma NE with other inflammatory mediators and clinical features it has been shown that NE appears to play a critical role in severe pneumonia and determination of its concentration in blood could be a useful indicator of severity (187). In this study a significant elevation in elastase and α2-MG were observed and these parameters were correlated with neutrophil count. A positive correlation of neutrophil counts with elastase activity and α2-MG was observed. These observations could be suggestive of some significance as adjunct parameters for diagnosis of pneumonia.

Dengue virus infection symptoms vary from mild to severe; severe forms include DHF and DSS. Severe forms are characterized by hemodynamic disturbances, increased vascular permeability, hypovolemia, hypotension, and shock. Thrombocytopenia and platelet dysfunction are common features and are related to the clinical outcome (111, 204). Physiologically, platelets are involved in hemostasis, wound healing, and inflammation (111). Activated platelets release many cytokines and chemokines which in turn are involved in induction of immune cells such as neutrophils and monocytes to the site of injury (205). DENV infection activates platelets and induce their consumption due to ongoing disseminated intravascular coagulation, platelet destruction due to increased apoptosis and suppression of bone marrow causing platelets dysfunction and thrombocytopenia (116, 206, 207). Neutropenia observed in dengue patients here could be attributed to activated platelet-neutrophil aggregation followed by its destruction which is reflected in the elevated elastase levels observed in the study. There was also a
significant decrease in the levels of α1-AT in dengue patients. Correlation studies indicated negative correlation of platelets and neutrophils with elastase signifying the protective role of platelets and neutrophils against dengue pathogen and destructive effect of elastase leading to further complications. Juffrie et al also found negative correlation between platelet count and elastase (115). Incidentally, a significant decrease in the neutrophil counts was observed in DHF patients compared to DF patients. Neutropenia though not a characteristic feature in DHF patients needed to be evaluated with a larger sample size study as well as multicentric study. Thus, significantly elevated elastase activity in DHF patients with reduced neutrophil count implies that NE can be a relevant marker for severity in DENV infection. A positive association between platelet counts and α1-AT also indicates the destruction of α1-AT in DENV infection. Monitoring the plasma levels of these molecules thus could be of use for evaluation of severity of dengue infection.

One unique finding in this study is the differential levels of α1-AT and α2-MG in pneumonia and dengue. The levels of α2-MG increased in pneumonia with near normal α1-AT levels while the picture was reverse with α1-AT decrease with almost normal α2-MG in dengue. Are these observations presented in this study characteristic to bacterial infection (pneumonia) and viral infection (dengue) needed to be explored. If such consistencies are observed in different bacterial and viral infections, it would help in differential diagnosis of bacterial and viral diseases.

Diabetes is a chronic disease and is associated with multiple complications. The overall health condition of the diabetic patient largely depend upon the status of hyperglycemia, environmental factors and in some cases due to genetic factors. Results of first chapter showed that, there was a noticeable reduction in levels of α1-AT in diabetic complications, particularly in retinopathy patients and so correlation of this parameter
with FBS was made in DR and DN patients. A negative correlation was observed in both the groups. However, a significant negative correlation was observed in DR patients as compared to DN patients suggesting that α₁-AT levels in diabetes could be a predictive marker molecule for retinopathy. Recent experimental and clinical studies have shown protective effect of α₁-AT in diabetic retinopathy (31). The possible explanation for decrease in α₁-AT in retinopathy have been discussed in chapter III.

Preeclampsia is a progressive inflammatory disease characterized by elevation in levels of inflammatory mediators in maternal circulation (151). There are studies indicating significant increase in the activity of elastase in PE (154). However, a correlation of severity parameters with inflammatory parameters have not been reported. Though correlation of these molecules with both proteinuria and BP was carried out in this study, the most meaningful and relevant correlation was observed only with proteinuria and not with BP. The observation of significant increase in the levels of NE and α₂-MG in severe PE and their significant positive correlation with severity marker proteinuria makes the measurement of NE and α₂-MG as dependable parameters in the determination of severity of PE. The correlation was converse in the case of α₁-AT. Reduced levels of α₁-AT in PE group could be due to renal loss of this protein. Urinary estimation of α₁-AT would have explained this. The correlation analyses suggests that measurement of levels of NE and α₂-MG along with α₁-AT could strengthen the assessment of severity of PE.

Stroke is a leading cause of death and severe, long-term disability. Most of the first - time stroke patients have high BP. Hypertension acts as a major determinant of endothelial dysfunction and vascular damage, promoting inflammatory activation of endothelial cells and recruitment of inflammatory cells including neutrophils, into the ischemic brain tissue (208, 209). Results presented in chapter III showed significant
increase in the levels of NE and \( \alpha_2 \)-MG in stroke patients and so correlation of these parameters with SBP was made. The correlation study indicated positive association of elastase and \( \alpha_2 \)-MG with SBP. The positive association between SBP and elastase indeed indicates that stroke outcome is mediated by an inflammatory response. This observation also indicates possible role of these molecules in the development of complications of stroke.

**Conclusion**

The outcomes of correlation studies suggest that the measurement of NE, \( \alpha_1 \)-AT and \( \alpha_2 \)-MG could be of relevance in diagnosis of diseases in combination with other tests and clinical signs preferentially for differential diagnosis. These molecules are also relevant in determining the severity of dengue fever on account of increased NE in DHF with associated neutropenia. This study opens up the notion that in bacterial infection there is an increase in \( \alpha_2 \)-MG and in viral infection a decrease \( \alpha_1 \)-AT. Though it is premature to emphasis on this, the question whether these are characteristics of host response in bacterial and viral diseases or otherwise needed to be studied.

Similarly, measurement of NE, \( \alpha_1 \)-AT and \( \alpha_2 \)-MG in non-infectious diseases also make sense, as there were some relevant and significant correlations with complications of DM and severity of the disease. Measurement of \( \alpha_1 \)-AT levels in diabetic patients could be of relevance as a marker for retinopathy complications of diabetes. The characteristic feature in two disease conditions of PE and stroke, both attributed to hypertension are the increased levels of NE and \( \alpha_2 \)-MG but with a significant decrease in \( \alpha_1 \)-AT which is seen in severe PE and can be attributed to renal loss in PE associated with proteinuria. As, this is the first of its kind of study, more investigations are needed to take the results of this study to a logical end and in to clinical practice.