2. REVIEW OF LITERATURE

2.1 Cardiometabolic disorders

The Cardiometabolic Disorders (CMets), is a state of metabolic and vascular deregulation that is associated with high blood pressure, obesity, insulin resistance, and hyperlipidemia as primary causative factors with profound presence of hypercoagulation and elevated inflammatory response (65). The last decades have witnessed growing interest in MetS has due to its elevated prevalence in the general population. The prevalence of CMets is increasingdrastically due to dynamic socioeconomic development, increased urbanization and reduced physical activities. Prevalence of CMets is difficult to determine as it is clustering of myriad components, combination of different components produce number of possibilities to diagnose however according to survey, it is believed that at least 25% of the world population has MetS (66), and the proportion of individuals with MetS is projected to increased significantly combining the occurrence of hypertension, central obesity and type II diabetes (67). The unattended metabolic dysfunction predisposes the patients for development of atherosclerotic cardiovascular diseases such as stroke and myocardial ischemia, cardiovascular diseases, type II diabetes, nonalcoholic fatty liver disease and chronic kidney disease (CKD) (68), (69). The presence of metabolic syndrome and associated disorders greatly affect the quality of life and increase the financial burden and hospitalization.

2.1.1 History and epidemiology of CMets

In 1998, The WHO has framed its definition of CMets, which corporate diabetes mellitus and insulin resistance as necessary complications combining with two other metabolic abnormalities such as “obesity, elevated blood pressure and TG level, depleted HDL-C level, or micro-albuminuria” (70) simultaneously, EGIR also presented their definition for CMets in 1999, by incorporating different primary risk factor that were recognized by WHO. According to EGIR, obesity, later revised as “central obesity” was identified as the potential risk factor.

According to the criteria presented by “National Cholesterol Education Program Adult Treatment Panel III” (NCEP-ATP III) in early 2000, presence of any three metabolic complication from the groups of symptoms [central obesity, systemic arterial hypertension and insulin resistance with hyperglycemia, elevated triglyceride and apolipoprotein B and low level of High Density Lipoprotein (HDL),] would be diagnose as the metabolic

disorders (71). Guidelines proposed by NCEP-ATP III are widely accepted guidelines for diagnosis of Cardiometabolic disorders in clinical In 1998, The WHO has framed its definition of CMets, which corporate diabetes mellitus and insulin resistance as necessary complications combining with two other metabolic abnormalities such as "obesity, elevated blood pressure and TG level, depleted HDL-C level, or micro-albuminuria" simultaneously, EGIR also presented their definition for CMets in 1999, by incorporating different primary risk factor that were recognised by WHO. According to EGIR, obesity, later revised as "central obesity" was identified as the potential risk factor.

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2.1.2 Major components of Cardiometabolic disorders

As mentioned above, cardiometabolic disorders represent the state of pathological and metabolic alterations characterized by compromised insulin sensitivity, hypertension, abdominal adiposity in conjunction with by prothrombotic and proinflammatory stage(46). It is very important to note these primary factors of CMets either act alone and most often in combination with other metabolic alteration to initiate the vicious loop of physiological disturbances. A very unique feature about these phenotypes is; they are interconnected by cause-effect relationship. i.e. Hyperinsulinemia is responsible for hypertension via over activation of SNS and RAAS and vice versa effect is also observed where elevated angiotensin levels(72) and SNS activity (71)which lead to inhibition of insulin actions and altered skeletal glucose uptake. Owing to the close association of different metabolic culprits, identification of root cause and subsequent consequences are difficult to predict and analysed. Due to intricate association with each other, Disturbances in function of one component of CMets set off the loop of metabolic and cardiac consequences which significantly poses threat for the development of ASCVDs and CKD.

In general, Unger et al. defined this phenomenon as "a failure of the system of intracellular lipid homeostasis which prevents lipotoxicity in organs of over nourished individuals" (73).

2.2 Role of Hypertension as the initiator of CMets

The prevalent feature of CMets, is elevated blood pressure and its presence is found to be around more 2/3rd of diagnosed population. Such prominent presence of hypertension makes it an important diagnostic as well as therapeutic target for the early detection and management in CMets.

Akintunde et al. reported that among newly diagnosed non-diabetic hypertensive patients, atleast 33% of patients have Mets components. Prevalence was observed to even higher in patients with uncontrolled hypertension than controlled hypertension (74). On the contrary, efficient control of blood pressure substantially decreases the risk of coronary events in patients with CMets(75). Another striking observation suggested that about half of hypertensive patients exhibited insulin-resistant which further adds the complexity(76). Moreover, presence of hypertriglyceridemia and depleted HDL level are likely to be correlated with inferior outcome in response to antihypertensives in hypertensive patients with MetS. Instead of the intricate relationship between Mets and hypertension, the elucidation of the mechanism for such phenotype is complex to understand. As a major culprit, visceral adipocity potentiates the release of cytokines such as leptin, TNF-α, IL-6 and Angiotensin that further drives this vicious loop which eventually leads to uncontrolled hypertension with marked metabolic abnormalities.

2.2.1 Hemostatic control of blood pressure and hypertension

Blood pressure can be best fundamentally understood by the definition “pressure exerted by the flow of blood upon the walls of capillaries”. The generation of pressure as the heart contracts against the resistance offered by blood vessels can be summarised by Ohm’s law. as “the product of the cardiac output, heart rate and systemic vascular resistance” (77). In this context, blood pressure is highest in the large arteries gradually falls down to zero as it reaches the larger veins and vena cava. The normal value of BP has been recognised as 120/80 mm Hg as systolic/ diastolic pressure. This pressure gradient is prerequisite for maintaining a sufficient perfusion rate and supply of nutrients to all the parts of the body. Regulation of blood pressure is performed by continuum working of several physiological, endocrine and neurohormonal mechanism which regulate short term to chronic pressure changes (10). Among them, RAAS, sympathetic system, baroreflex system, myocardium and vagal responses play are the major regulators. Despite of such a strict and intricate assembly of factors regulating the normal blood pressure, individuals often tend to escape

these control mechanisms and develop a condition of higher blood pressure termed as 'systemic hypertension'. Hypertension typically develops with an increase in systemic vascular resistance with or without increase in cardiac output (78)

2.3 Role of RAAS in hypertension and Mets

The prominent role of renin-angiotensin-aldosterone system in the physiological control of systemic blood pressure and balance of electrolytes has been studied and well documented. In addition, research conducted in the last decade has provides the proof of presence of RAAS in a wide variety of tissues. The role tissue specific RAAS has been explored for adrenal glands, kidneys, brain, heart, or blood vessels; this suggests the profound role RAAS in the regulation of several function in different tissues(79). Local RAAS has also been significantly involved in structural and functional physiological and pathological changes that can occur in different organs, by altering growth, gene expression and the inflammatory response(80).

Activation of the renin-angiotensin system (RAS) also serves as a trigger for activation neurohumoral pathway contributing to the development of MetS. Angiotensin II (Ang II), formed as a result of action of angiotensin-converting enzyme by forming Ang I to active Ang II. Obesity and insulin resistance are associated with increased production of Ang II(81). Ang II activates AT_1 receptor and subsequently activates nicotinamide adenine dinucleotide phosphate oxidase leading to the generation of reactive oxygen species (ROS) (82). Generation of highly reactive free radical produce plethora of effects including oxidation of LDL, endothelial damage, promotes platelet aggregation, enhanced expression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and LOX-1 on the endothelium and vascular smooth muscle cells (83). RAAS, ROS, and LOX-1 participated in the interrelated positive feedback loop that initiates a vicious cycle of inflammation, endothelial damage, and fibroblast proliferation that contributes to the development of hypertension, dyslipidemia, diabetes, cardiac hypertrophy, and CVD (84).

2.3.1 Renin Angiotensin Aldosterone System (RAAS): activation, signalling and receptor function

RAAS plays a pivotal role in the regulation of physiological processes in the body related to CVS. It is a hierarchical enzymatic signalling that starts with the proteolytic breakdown of angiotensinogen to angiotensin I by renin enzyme secreted by juxtaglomerular (JG) cells

in kidney(85). However, produced decapeptide is inactive and it is further cleaved by Angiotensin Converting Enzyme (ACE) secreted predominantly by lung and liver tissue to form potent octapeptide Ang II. Alternatively, a recently identified isoform of ACE enzyme ACE-2, cleaves one amino acid from either Ang I or Ang II, to form Ang 1-7 which has vasodilatory action contrary to AngII. Hence, the balance between ACE and ACE2 is an important factor for the regulation of Ang II levels (86).

Ang II exerts its main physiological response by binding with angiotensin type 1 receptor (AT_1R) and angiotensin type 2 receptor (AT_2R) in the vasculature, kidney, skeletal muscle, heart, adipocytes, pancreas, and adrenal tissues (87). AT_1R mediated Ang II effects include: vasoconstriction of the arterioles and sodium retention in the renal proximal tubules while vasodilation, anti-proliferative effects and anti-inflammatory effects are elicited by AT_2 receptor(88).

2.3.1.1 The Angiotensin receptor type 1 (AT_1 receptor)

The octapeptide angiotensin II (Ang II) mediates its effects through the several angiotensin receptors such as Angiotensin receptor type 1 to type 4 in mammalian cells. Two types of receptors are widely studied for their physiological process and as therapeutic target. Subtypes receptors have been cloned and characterized: angiotensin receptor type 1, AT_1 receptor and angiotensin receptor type 2, AT_2 receptor (89). The remaining two receptor family, AT_3 and AT_4 receptor's structure and functions are not fully elucidated and their potential as a therapeutic target is under investigation. therapeutic utilization in under study (90). All the subtypes, except AT_4, are known to be the binding site for angiotensin (91). The AT_1 receptors are structurally Protein coupled Receptor and consist classical seven transmembrane helices as other GPCR. The extracellular loops and the transmembrane domain (TMD) acts as a ligand binding domain for Ang II. This receptor holds paramount importance and recognized by its ability to bind antagonistic ligands collectively known as "sartans", however, allosteric biding of drug is observed within the receptor (92), (93). AT_1 receptors are primarily expressed in the liver, adrenal glands, kidney, vascular smooth muscles and lungs. Acutely increased levels of ang II are known to activate AT_1R, however, chronic exposure to Ang II may lead to downregulation of the receptor via receptor desensitization (94). While insulin and LDL are known to upregulate AT_1R (95) However, downregulation of receptors was observed with following estrogen, EGF and PDGF release. (96).The effects of ang II elicited in the target tissues are very acute and receptors

are endocytosed within 10 minutes after activation. They are devoid of intrinsic kinase activity like receptor tyrosine kinases.

2.3.1.2 Signaling pathways

The AT_1R belonging to the superfamily of GPCR, is coupled to the $G_{q/11}$ protein for effecting any downstream signal. Activation of $G_{q/11}$ protein upon binding of Ang II, in turn activates second messenger systems like the IP_3/DAG pathway activated through phospholipase Cβ (97). IP_3 and DAG are formed upon cleavage of PIP_2 by phospholipase Cβ. IP_3 subsequently binds with IP_3 receptor on sarcoplasmic reticulum and caused release of massive release of calcium. This calcium forms a complex with calmodulin to activate MLCK which phosphorylates the light chain of myosin to enhance its interactions with actin filaments This effect leads to smooth muscle contraction particularly in vascular smooth muscle cells (98). In the parallel arm, DAG activates protein kinase C (PKC), which phosphorylates the Na^+/H^+ pump and increases the cellular pH during contraction phase which helps in sustaining contraction of cells. Thus, by activating the Na^+/H^+ pump, AT_1R activation ultimately modulates VSMC contraction and growth. DAG also contributes in the Ras/Raf/MEK/ERK pathway and regulation the protein function via phosphorylation and cause activation or inactivation of targeted protein (99). Agonist binding at AT_1R leads to activation of PLD which is responsible for hydrolysis of phosphatidylcholine to choline and phosphatidic acid. Phosphatidic acid is rapidly converted to DAG leading to activation of PKC and subsequent effects mediated through DAG (Alexander *et al*, 1985; Griendling *et al*, 1989). The AT_1R also leads to phosphorylation and activation of PLA_2, which forms arachidonic acid. Function of metabolites of arachidonic is to regulate vascular tone and NAD(P)H oxidation of VSMCs. AT_1R activation also mediates stimulation of growth and migration related signaling, through a myriad of downstream proteins, which is independent from its mechanism related smooth muscle contraction (100).

2.4 Autonomic Nervous System and blood pressure control

It has been well understood that autonomic nervous system (ANS) plays pivotal role in modulating in maintenance of blood pressure and cardiovascular functions, both at rest and in response to environmental stimuli. It is performed by two arms of ANS.

1) Parasympathetic Nervous System 2) Sympathetic Nervous system. Imbalance between two components of ANS can lead to initiation and progression of cardiovascular disease ranging from mild hypertension, cardiac hypertrophy to stroke and chronic kidney disease. Hypertension stems from the imbalance of sympathetic and parasympathetic control is one of the most widely approved hypotheses of hypertension and extensively explore in cardiovascular research. Animal models have shown both an increased sympathetic nerve activity and a reduction of vagal cardiac tone are associated with hypertensive changes(101).

The autonomic nervous system and its sympathetic arm play important roles in the regulation of blood pressure (102), (103). Evidences collected in the past few years have strengthened the concept that the SNS plays a primary role in the development and progression of the hypertensive state, starting from the early stage, and in the hypertension-related cardiovascular diseases(104).

2.4.1 Autonomic Dysfunction in Early Hypertensive Phases

2.4.1.1 Parasympathetic nervous system alteration in hypertension

Further evidences for a causative or co-causative involvement of autonomic dysregulation in hypertension stems from the multiple reports showing that young hypertensive patient and those in the initial stages of hypertension also attributed increased sympathetic and a reduced cardiac vagal response. The elevations in heart rate depended on a reduced vagal inhibitory response to the sinus node. Because of the intravenous administration of atropine (which selectively blocks the effect of the vagal neurotransmitter acetylcholine on muscarinic receptors) abolish the parasympathetic response normalise the heart rate and blood pressure to the normal values. Additional studies have confirmed the observations, by reduced tonic vagal cardiac inhibition in subsequent studies; in which atropine treatment produced a lower increase in heart rate in young borderline hypertensives than in age-matched controls (105).

2.4.1.2 Sympathetic nervous system association with hypertension and CMets

Early hypertension is characterised by elevated sympathetic activity and heightened cardiovascular effects in addition to the reduced parasympathetic functions. Observations have been made regarding borderline hypertensive people who received an intravenous injection of the beta-blocker propranolol experienced a greater reduction in heart rate than

did controls. This finding supported the theory that people with initial hypertension have higher sympathetic tone than control groups. Finally, microneurographic recordings enable to study the sympathetic nerve activity and it is reported that efferent postganglionic sympathetic nerve fibers in early hypertension showed increase sympathetic outflow. These results provide direct correlation of overwhelming response of SNS in hypertension (101). Intriguingly, borderline hypertension has increased expression of adrenergic receptors at both the cardiac and vascular sites who showed elevated pressor response to intravenous catecholamine administration These observations led to the realization that that early hypertension is associated with peripheral and central adrenergic drive. However, prolonged activation adrenergic receptor leads to downregulation of receptor function as a counter regulatory mechanism.

Sympathetic neural activity has been determined in several established hypertensive stages by a variety of techniques. Heightened activity of SNS was found in both hypertensive males and females, although in females had pronounced effect only in more advanced stages of the disease(106),(107). This could be due to protection offered by estrogen and other female sex hormones. Hypertensive patients regardless of their age exhibited chronic sympathetic nerve discharge. Similar phenomena was observed for pregnancy induced hypertension as well (108). The same has been found in patients with both high blood pressure and metabolic risk factors, such as obesity, metabolic syndrome, or diabetes mellitus(109)**,**(110). These observations have led to the conclusion that in hypertension, sympathetic hyperactivity is a generalized response, irrespective of the myriad clinical aspects that accompany a high blood pressure state.

Clinical trials have shown that sympathetic stimulation is significantly enhanced in obese hypertensives than in lean ones (109) possibly because obesity is frequently accompanied by an insulin-resistant state that elevates the level of circulating insulin resulted into sympatho-excitation. Owing to the close association of obesity, metabolic syndrome, and diabetes mellitus with hypertension, it is to have in general an even greater degree of sympathetic activity than shown in studies on more selected groups of patients.

2.5 Cross talks between RAAS and the α-Adrenergic system

The concept of receptor and signal transduction has been demonstrated in pharmacology and biochemistry. However, it is being extensively acknowledged that these systems do not operate individually but rather operate in tandem to maintain the physiological function as

well as in pathological development of disease. Receptor cross talk can be simply defined as “biological talk between two signal transduction pathways in which response of one system affects another via compensatory or inhibitory pathway”. One of major cross talk exists between sympathetic nervous system and RAAS system. The signal transduction is regulated by involvement of numerous neurotransmitters, multiple second messengers. Cross talk of RAAS and SNS is widely appreciated for its maintenance of blood pressure. Upon reduced perfusate kidney pressure, release of renin is accompanied by increase sympathetic nerve activity while potentiation in sympathetic neurotransmitter outflow promotes the action of Ang II (111). Moreover, ample of evidences suggested that receptor cross talk of these two systems is critically involved in pathogenesis of hypertension and other cardiovascular disease in animals and human. (112),(113), (114). This receptor cross talk generally maintained in a very strict manner however, deregulation one system can provide signals to other system resulted into increment of blood pressure and related pathological situations. Along with their function in normal physiological process, cross talk of receptor provides attractive therapeutic target to efficiently regulate the consequences. In such consequences, it becomes imperative that the entire compensatory response should be put on hold to prevent the damage inflicted by over- activated systems. Van Zwieten and coworkers suggested that chronic activation of the sympathetic nervous system and RAAS produce harmful responses for the cardiovascular system. In the development of congestive heart failure (CHF) both systems produced hypertrophic response which is beneficial initially however persistent activation of these connected response soon cause detrimental effects to heart(115).

Clinical study conducted by Lang *et al.* revealed increased sodium reabsorption in the proximal tubule and distal segment of the nephrons upon AngII infusion and thus produced anti-natriuretic action. However, when non-depressor doses of prazosin were given along with Ang II, the response of vasoconstrictive peptide is diminished. This study provides strongconnection lies between α_1 adrenoceptors and Ang II (116).

Farivar *et al* evaluated the effects of losartan, AT_1 receptor antagonist in phenylephrine mediated fibrosis. Phenylephrine is known to mediate fibroproliferative responses in cardiac fibroblasts and these effects are mediated by α_1 adrenoceptors. It was hypothesized that prazosin might show beneficial effects. Administration of prazosin resulted into no sign of hypertrophic response in the heart of rats. Surprisingly, animals treated with losartan also revealed protection against fibro-proliferative changes. The results of this study

suggest that AT_1 receptors are also involved in the fibro-proliferative responses to phenylephrine (117).

Li *et al*. studied the effects of ang II stimulation on α_1 adrenoceptor subtype expression in ventricular myocytes, he found out that only one receptor mRNA was found to be downregulated and this effect was mediated through the AT_1 while other two isoform α_{1B}/ α_{1D} receptors were unaffected. Studies with transcription inhibitor also did not cause change in expression level and it was believed to be mediate by Ang II induced reduced stability of receptor mRNA (118).

Abdullah *et al.* studied the effects of carvedilol in intact rats and its response to ang II. The study involved injection of ang II in rats evaluated in two groups, one with treatment with carvedilol and other is without treatment. This adrenergic blockade did show an inhibitory effect on the vascular responses of ang II suggesting the interactions between the adrenergic system and RAAS in normotensive animals.

Barrett-O'Keefe *et al*. analysed the effects of age related cross-talks between ang II and α_1 adrenoceptor mediated vasoconstriction. The study was carried out with a notion that elderly are more responsive to dose of Ang II owing cross talk of α_1 adrenoceptors and simultaneously this effect may be diminished in the presence of α_1 antagonism (119). The results of the study demonstrated that enhanced response to ang II mediated vasoconstriction proceed in part by, potentiation of α_1 adrenoceptor mediated vasoconstriction caused by ang II. They also suggested that this observation can be translated clinically to design appropriate therapy for patients of hypertension and heart failure.

Vittorio *et al***.** retrospectively reviewed the effect of potential interactions observed between the adrenergic system and RAAS in major clinical trials like Val-HeFT and CHARM-added. The authors suggest that α_1 adrenoceptor and AT_1 receptor cross talk occur at two levels: at the molecular receptor and the second messenger levels. Heterodimerization between the α_{1D} adrenoceptor and AT_1R has been noted in preeclamptic pregnant rats. Further, since both receptors are coupled to Gq subunit, the following receptor signalling produced similar regulation. (114)

2.6 Insulin resistance: Major culprit for Cardiometabolic disorder

2.6.1 Insulin signalling

Insulin binds to insulin receptor IR, which comprises the two α and two β subunits. Insulin, insulin growth factor-1 (IGF-1), and epidermal growth factor (EGF) binds with α subunit of insulin receptor while β subunit is located extracellularly, and in transmembrane, and cytosolic region. The cytosolic part of the β subunit has tyrosine kinase activity, which undergoes conformational changes and auto phosphorylated after insulin binding to the α subunit. Activated IR phosphorylates also number of proteins on tyrosine residues, for example, insulin receptor substrate (IRS), Shc proteins, or Gap-1 (120). There are three isoform of IRS (IRS-1, -2, and -4) were identified to play a unique role, depending on cell type and metabolic state. Moreover, those two insulin receptor substrates show different kinetics, compartment distribution, and substrate interactions (IRS-1 is a transmembrane protein, while IRS-2 is primarily found in the cytoplasm) (121). IRS-1 is crucial for the mechanisms of insulin secretion in skeletal muscle (122). IRS-2 plays crucial role for the development of pancreatic β cells and insulin action in liver. Growth retardation especially in skeletal muscle and liver found in IRS-1 knockout mice (123). IRS-1 knockout mice exhibited symptoms of the metabolic syndrome (hypertension and hypertriglyceridemia) and also developed insulin resistance and hyperinsulinemia rather than diabetes (123). IRS-2 knockout animal exhibited insulin resistance with fasting hyperglycemia, due to lack of insulin production, that leads to development of diabetes, which was more severe than lack of IRS-1 (123). For the insulin response, IRS tyrosine phosphorylation is necessary. Moreover, phosphorylation of IRS intensifies or diminishes insulin action (124)

2.6.2 Insulin resistance and Renin Angiotensin Aldosterone System

Insulin and Ang II are two important hormones in the control of metabolic and hemodynamic homeostasis, respectively. It is reported that increased Ang II level caused altered binding with insulin receptor via phosphorylation of Ser312 and Ser316 in human umbilical cell culture (125) and it also caused reduced aortic response via serine phosphorylation at multiple second messenger system. Recent studies have suggested that the signal transduction pathways of insulin and Ang II share a number of downstream effectors and cross-talk at multiple levels (125).

One of the main contributors to insulin resistance and a major risk factor for the emergence of cardiovascular complication and type 2 diabetes is obesity (126). Numerous studies indicate that obesity is linked to a systemic chronic inflammatory response that is defined by altered production of proinflammatory cytokines and activation of inflammatory signaling pathways in adipose tissue (127) Adipose tissue is now recognized as an active endocrine and paracrine organ that actively secretes and creates a large variety of cytokines and bioactive mediators known as adipocytokines that control insulin signaling (128). Pre-adipocytes (undifferentiated adipocytes) are primarily responsible for producing inflammatory cytokines or diabetogenic adipokines, whereas differentiated adipocytes are responsible for producing antidiabetic effect of adipocytokines like adiponectin.

All RAS constituents, including angiotensinogen, ACE, AT_1 and AT_2 receptors, are expressed by adipocytes (129). Evidence from experimental research demonstrates that Ang II reduces human adipocytes ability to differentiate into adipocytes via the AT_1 receptor, which results in increased release of inflammatory cytokines or adipokines that promote diabetes and inhibit insulin signalling (130) The recruitment and differentiation of pre-adipocytes as well as the increased production of small insulin-sensitive adipocytes have been reported to be enhanced by RAAS blockage, which can lead to increase insulin sensitivity. Lee et al. demonstrated that therapy with an ARB enhanced adipocytes differentiation and suppressed the inflammatory process in adipose tissue in the OLEFT rat, an animal model of T2DM. This was accompanied by an increase in adiponectin and reduction in NF-kappaB, PAI-1, and MCI-1 (131).

Another facet of insulin resistant induced by overwhelming RAAS response is mediated by endogenous mineralocorticoid, aldosterone. Overactivity of AT_1 receptor and subsequent responses along with aldosterone release propagate the detrimental effect of insulin. Clinical and preclinical evidences highlighted the important role of aldosterone in development reduced insulin sensitivity, myocardial fibrosis and increased inflammatory response. Both experimental and clinical studies also implicate aldosterone in the development of insulin resistance, hypertension, endothelial dysfunction, cardiovascular tissue fibrosis, remodeling, inflammation and oxidative stress (72).

Several lines of clinical observations emphasize the role of RAAS in the development of insulin resistance and type 2 diabetes mellitus (DM) in humans. Studies have reported the superior therapeutic effect in terms improvement of insulin resistance that ARBs and ACEs

inhibitors treated patients marked increase in insulin sensitivity compared to the treatment of other antihypertensive drugs (132), (133).

2.6.3 Insulin resistance and Sympathetic nervous system

As discussed earlier, the sympathetic nervous system (SNS) plays a profound role in the regulatory mechanisms of blood pressure, sodium balance and maintenance of homeostatic state. Apart from its involvement in physiology of blood pressure, it is also largely involved in daily energy expenditure through the regulation of resting metabolic rate and thermogenesis in response to physiological stimuli, changing energy states, food intake, carbohydrate consumption and hyperinsulinemia. Furthermore, catabolic responses can be elicited by the activation of sympathetic nerves in major organs like liver, pancreas, adipose tissue. (i.e., glycogenolysis and lipolysis) (70).

Over activation of SNS is strongly in correlation with major components of cardiometabolic disorders, namely obesity and hypertension (71). In turn, profound activation of this system produced detrimental effects in myocardial hypertrophy, arterial remodeling and stiffness,and endothelial dysfunction on the cardiovascular system (72). It is concluded from the isolated sympathetic nerve studies or by *in-vivo* experiment that stimulation of sympathetic activity promotes systemic and regional norepinephrine spillover and produced tachycardia. This condition has been associated with hypertension, obesity, and insulin resistance (73). Furthermore, it has been shown that high levels of fasting insulin, a marker of insulin sensitivity is associated imbalance of sympatho-vagal balance in heart (74).

Close association among obesity, MS and the development of cardiovascular risk factors, it is of paramount importance to understand physiology involved behind such dysfunctions. There are two lines of evidences explaining the role of SNS in obesity-induced hypertension: (i) Elevated sympathetic outflow in obese patients compares to normal individual and (ii) Control of hypertension associated with presence of obesity through pharmacological modulation via adrenergic blockade (134); (135); (136). Overwhelming sympathetic activity in obesity point out that obesity compromised the normal renal- pressure natriuresis, increases renal tubular, sodium reabsorption and causes hypertension (137); (136). This response is potentiated by subsequent activation of RAAS which furthersynergistically produces metabolic complications.

The SNS disturbances are closely related to all the main features of MS. Although SNS participation in the pathophysiology of MS is clear, it is difficult to determine whether the metabolic changes are responsible for sympathetic disturbances or vice versa.

2.7 Endothelial dysfunction and Vascular reactivity

NO is the one of most potent vasodilators involved in the maintenance of vasodilation in body to ensure proper blood supply without implicating excessive pressure in arteries. NO, apart from its prominent action, is also critically involve in platelet aggregation after vascular injury(138). NO is released from the endothelium upon the detection of increased blood pressure, excessive arterial stretch and shear stress due to resistance to flow. In case of human hypertension and associated metabolic abnormalities leads to damage in macro- and micro-vessels and highly perfused organs such as heart and kidney undergo endothelial damage (139). Impaired insulin sensitivity in metabolic disorders also leads to impairment of PI3/akt signaling leads to inhibition of nitric oxide synthesis. Damage arteries undergo prothrombotic response evident by accumulation of platelet and formation thrombus (140).Overwhelming vascular reactivity is one of the facets of hypertension and vascular dysfunction. It has been observed that hypertensive patients exhibit a higher vasoconstrictor tone in response to norepinephrine and other vasoconstrictive agents (141), (142). In healthy individual persistent high levels of circulating norepinephrine cause down regulation of noradrenergic receptors but this compensatory response in hampered in hypertensive states which cause an increase in peripheral resistance and rise in blood pressure(143). Progeny which are normotensive but are from hypertensive parents also show an increased sensitivity to the actions of norepinephrine. This suggests that vascular reactivity may be inheritable. Since offspring's from parents without a history of hypertension do not show enhanced vascular reactivity, it may be concluded that hypersensitivity is of genetic origin and merely not a result of elevated blood pressure (144)

2.8 Dyslipidemias in the Metabolic Syndrome

Dyslipidemia is considered as an imbalance of bad cholesterol i.e LDL and good cholesterol, i.e HDL. It is observed that the earlier type of lipoprotein is increase in CMets an initiated atherogenic response, while HDL particles provide protection against such ramifications by clearing the LDL particles from the blood stream (145). Abnormal lipid levels frequently occur as consequences of reduced insulin sensitivity. Stage of

hyperinsulinemia with reduced sensitivity, “Insulin resistance” elevated the hepatic TG concentration. Subsequence release of TG particles could decrease the level of HDL cholesterol and stimulate the level of small and denser particles of LDL cholesterol. The increment in the concentration of small and denser atherogenic LDL cholesterol particles could potentiate the accumulation of triglyceride in the vessels which cause atherogenic complications. (146)

2.9 Management of hypertension and cardiometabolic disorders

CMetS is closely associated with an increased risk of atherosclerotic and non-atherosclerotic CVD. It is still not clear that the risk is a sum total of its individual components or constellations of pathological symptoms the induces synergistic risk (147). Recent observation made by Motillo and colleagues indicated that underlying MetS increases the risk of CVD outcomes by 2-fold and boosts all-cause mortality by 1.5 times. (148). These data are representative of threat poses by METs and its ramifications if it is not controlled. Management of MetS involves a dual approach that combines lifestyle changes and pharmacological interventions to mitigate the risk of CVD.

2.9.1 Lifestyle modification

As described earlier, chronic imbalance between calorie consumption and metabolic requirements leads to increased central adiposity and development of Metabolic disorder. Adopting healthy lifestyle is imperative for the control of metabolic disturbances, in addition, lifestyle modifications hold major importance considering the sedentary lifestyle in the recent times. Weight reduction and maintenance of body mass index are very important management strategies. The goal of therapy is to reduce 7–10% baseline body weight over a period of one year with strict calorie intake not more than 1000kcal/day. Dietary modifications also displayed significant role and regulate other MetS components: Limited intake of atherogenic fats, cholesterol, sodium, and plant sugars is found to be beneficial in hyperlipidemia, reduction in blood pressure and improved insulin sensitivity with controlled glucose level. Surgical intervention such as bariatric surgery has displayed encouraging results in severe obese patients. High intensity exercise increases calorie consumption, aiding weight loss and reducing overall CVD risk: around 30–60 min of moderate intensity exercise and conscious efforts to alter a sedentary lifestyle can be beneficial for the management of MetS(147).

2.9.2 Pharmacological approaches

Along with modifying the underlying risk factors, pharmacotherapy is another option for the prevention of CVD. Management of cardiometabolic disorders includes prescribing of individual drug for a particular component of CMets. Treatments often comprise of cocktailof anti-hypertensive (Angiotensin receptor blockers like telmisartan, losartan) and anti- diabetic (Metformin, glimepiride, sitagliptin etc.). These drugs are often combined with aspirin and clopidogrel to offer protection against possible thrombotic events (149). Lackof specific drug therapy for MetS and associated co-morbidities required lifelong use of multiple medications, which is troublesome for the patients due to concerns of polypharmacy and reduced compliance.

2.9.2.1 Network Pharmacology: Polypill and combination therapy approach

To address issue at hand, it was envisaged that a parallel targeting of associated pathways by combining multiple therapeutic mechanisms would be an attractive strategy for the management of multifactorial disorders (150), (151).

The concept of combination therapy initially involved polypharmacy where the patient was prescribed with two or more pills. This approach simultaneously leads to patient non-compliance, multiple side effects, and increase the cost of therapy. This problem was partially solved by the invention of fixed-dose combinations (FDCs). FDCs involve formulation of two-or more pharmaceutically compatible drugs or biological into a single formulation at required doses so that dosing regimen may be simplified, and the pill burden can be reduced. This improves patient compliance especially in treatment of chronic disease such hypertension and other CVDs. It also improves adherence in geriatric class of patients in whom swallowing pills is a common problem. Fixed dose combinations have served well with its distinct advantages over polypill approach for very long time (152). FDC should have following characteristics that 1) drugs in the combination should act by different mechanisms 2) The pharmacokinetics must not be widely different. 3) The combination should not have supra-additive toxicity of the ingredients. However, the concept of FDCs also has its own limitations. Dosage alteration of one drug is also cause change in the dose of other drug as well as Different pharmacokinetics of profile constituents pose the problem of frequency of administration of the formulation (153) The alternative to overcome the obstacles poses by FDC is development of a new chemical entity that simultaneously affects multiple pharmacological targets (154).

2.10 The concept of Designed Multiple Ligands

Classical drug development process was focused on the one drug-one-target-one-disease paradigm. It was believed that desired therapeutic action can be produced through selective modulation of a particular target, and this would also prevent binding of drugs to off-target. Following this notion, vast number of selective drugs have been discovered, explored and utilised in the management of treating certain diseases (40). However, complex diseases, also recognised as "multifactorial diseases", are posing significant challenges because they involve complex interplay of multiple target proteins and/or signaling pathways. These complex interactions of multiple signaling pathways have been explored in the pathogenesis of cancers and inflammation (155), neurodegenerative, cardiovascular, pain disorders as well communicable disease (156), (157), (158),(159). The medical professionals were not too late in realizing this intrinsic biological talk between multiple pathways and this machinery was identified to be the reason for the failure of different compounds in the clinic (150). Additionally with advanced understanding of the etiopathologies of different diseases, it was realized that many diseases stem from of multifactorial etiology ultimately manifesting the symptoms and for such complex diseases, targeting single protein might be inadequate to achieve satisfactory therapeutic outcome.

Morphy *et al.*, first coin the idea of the deliberate designing of compounds that modulate two- or more targets what he termed as "Designed Multiple ligands". It was suggested that single chemical entities that target multiple factors can certainly provide superior efficacy coupled with desirable ADME profile and minimal side effects.

Earlier drugs which are identified as multitargeted ligands were not intentionally designed instead their multiple effects were invented either by retrospective or serendipitous observations. The high therapeutic efficacy of some of the best known drugs such as aspirin, paracetamol, metformin, are ascribed to their pleiotropic activities on complementary multiple targets (160), (161). Labetalol and carvedilol are the cardioprotective agents with a dual antagonistic activity on adrenergic α_1 and β pan receptors (162).

This remains true for efficacious control of hypertension and metabolic disorders as well where several factors are acting in concert leading to multiple metabolic abnormalities accompanied with vascular dysfunction. Hence combination therapy is usually preferred by clinicians over monodrug therapy for the management of hypertension and metabolic

disorders (163). Major clinical trials have also outlined the importance of modulating more than one target to achieve optimal blood pressure (164). The advantages of such a therapy would be increased efficacy in such a way that clinical end points can be achieved more easily within a predicted time frame and a reduction in dose, while avoiding drug interactions and adverse events.

The major challenge faced during designing such compounds is, selection of appropriate targets. It is observed that target located on the same signal transduction pathway afford additive action while targeting different proteins located on separate receptor signalling provide synergism in biological response (165) However, it is not as straightforward as it seems. Another significant obstacle remains is the accomplishing a balanced modulation of target under interest (166), (167). Over inhibition or stimulation of one particular target can compromise the goal and produce untoward actions. Designing of MTDL involved utilization of mainly Pharmacophore combination approach. In this approach, targets for desired actions are identified, pharmacophores are addressed from selective ligands acting on individual targets are joined by cleavable or non-cleavable linkers (168).

The systematic identification of synergistic target combinations is well defined and explored for their potential in the treatment of cancer and antibiotics, however it is still in its infancy in the field of metabolic diseases (169). Recent advancement in computational chemistry and intense efforts to MTDL resulted in approval of several dual PPARα/γ agonist (Saroglitazar, developed by Zydus Cadila) for hyperlipidemia with diabetes (84).

2.10.1 Dual blockers involving AT_1R or α_1-adreno receptor blockade

- ***α_1 and β receptor blockers***

A prominent reduction in peripheral resistance and cardiac output, two major physiological changes involved in hypertension could displayed substantial advantages. Labetalol and carvedilol are the prototype examples of this strategy and have been used in the management of hypertension with improved outcome in clinical endpoints (170). Reversal of endothelial damage with improved ejection fraction in elderly patients is also observed with carvedilol therapy (171).

- ***α_1 and calcium channel blockers***

Though none of the agents of this class is in clinical practice, however in search of multitargeted ligands, Iwaki et al. had discovered the S-2150 excreted very potent

vasorelaxant activity. *In-vivo* experiments also supplement the data observed in tissue study by exhibiting hypotensive effect in different models of hypertension including the SHRs and two-kidney-one-clip rats (172).

- ***Dual inhibition of AT_1R and ET receptor***

Standard AT_1 antagonist losartan in combination with non-selective endothelial receptor antagonists exhibited additive response for blood pressure reduction compared to individual therapy (173). This led to an idea of developing AT_1R and ET dual receptor antagonists. In attempt to achieve the same, investigational molecule PS433540 was explored dual receptor antagonism and receptor inhibition studies revealed IC_{50} values of nanomolar range (0.8 nM for AT_1 and 9.3 nM for ET_A). Subsequent clinical investigations also reported that potent antagonist is efficacious, safe with well tolerated profile and has currently advanced into last stage of clinical trials. Another molecule is BMS346567, which also shows good binding affinities for both the receptors (2 nM for AT_1 and 14 nM for ET_A) (37).

- ***Dual AT_1R blockade and PPARγ agonism***

Some AT_1R blockers are known to possess partial agonism of peroxisome proliferator-activated receptor gamma (PPARγ) receptor. It has been reported that telmisartan inhibits AT_1 receptor gene expression through PPARγ activation. Research also reported the capacity of telmisartan induce adipocytes differentiation by PPARγ showed intriguing results and provide encouragement of development of similar agents (174). The dual inhibition of angiotensin II function by telmisartan-AT_1 receptor blockade and downregulation would contribute to more complete inhibition of the RAAS. This additional property has been associated with wide use in patients with hypertension and diabetes. Two more molecules azilsartan and PF-03838135 are reported to possess AT_1 receptor antagonism and a partial agonism of PPARγ (175).

- ***Dual AT_1R and calcium channel blockade***

Dihydropyridines is extensively utilised scaffold for the identification of calcium channel blockers. Hadizadeh et al. developed novel compounds exhibiting dual antagonism by connecting the imidazole nucleus of losartan to the dihydropyridine rings. Two potential compounds were identified by screening on rat aorta and showed 10^3 fold antagonism

offered by losartan alone (176). This observation led to the following investigation of identifying the compounds which target two major systems involved in CVDs.

- ***Dual ACE and DPP4 modulation***

DPP4 inhibitors "also called Gliptins" are extensively utilised drugs in clinical setting with improved therapeutic outcome. These molecules act via insulin independent mechanism, and they inhibit dipeptidyl peptidase IV hormone in intestine which leads to increase action of GLP-1 peptide. Thus, it ultimately promotes glucose uptake and reduced glycemic level. Similarly, Angiotensin converting enzymes inhibitors are the first choice of drug for hypertension and congestive heart failure. Wang et al. reported the discovery of an egg protein hydrolysate, showing DDP4- and ACE-inhibitory activity (177) *in- silico* analysis to identify the protein responsible for dual action and NWT-03 was identified as the promising compound. Subsequent *in-vitro* assayed showed inhibition on ACE (IC_{50}= 0.07 mg/ml) and DPP4 (IC_{50}= 0.9 mg/ml) by enzyme inhibition studies.

- ***Dual targeting of DPP4 and GPR119***

As described above, inhibition of DPP4 enzyme leads to potentiating of GLP-1 peptide in plasma and subsequent secretion of insulin in response to elevated blood glucose levels. GPR-119, a receptor belonging to the family of GPCR produced its action via two ways. Firstly, increased glucose-dependent insulin secretion and secondly, by increased release of GLP-1 in intestinal L-cells. (178) Hence, GPR119 agonists are expected to play key role in improving glucose homeostasis in patients with T2DM. The first dual DPP4/GPR119 modulator was introduced in 2016 by Li et al. by utilizing pharmacophore approach. Analysis of important structural features of linagliptin (xanthine scaffold) and the three GPR119 agonists AR231453, APD597, and PSN632408 possessing 4-piperidine moiety are developed. A systematic optimization of library of compounds showed compound (21) showed dual activity at the target of interest. (EC_{50}: 0.95 μM, 81.5% activity of AR231453 at 1 μM) and inhibit DPP4 (IC_{50}: 0.22 μM) in vitro.

2.11 Hypercoagulation and cardiometabolic disorders

As mentioned in earlier section, apart from metabolic and hemodynamic abnormalities, elevated prothrombotic response is consistent with CMets characterised by imbalance of coagulation and fibrinolytic process. i.e. enhanced thrombin generation and platelet

activation and decreased fibrinolysis (179), (180) which ultimately contribute to atherogenesis and acute atherothrombotic episodes. Hypercoagulability can result from a variety of inherited and, more commonly, acquired conditions. Many lines of evidence suggest a strong correlation between metabolic disorders and hemodynamic such as obesity, dyslipidemia, diabetes, hypertension, and cardiovascular (CV) diseases (CVD), with endothelial dysfunction as the initial step toward atherothrombosis. Moreover, the imbalance of haemostatic function of coagulation cascade is impaired in subjects with central obesity includes alterations of both intrinsic and extrinsic pathways with increased levels of factor VIII (FVIII) and von Willebrand factor (vWF), TF, FVII, and fibrinogen (180), (181)

2.11.1 Haemostasis of blood flow and Blood clotting cascade

Maintenance of adequate blood flow results from the delicate balance between haemostasis and fibrinolytic processes. Haemostasis is maintained by tight regulated interplay between the vessel wall (vasoconstriction), platelet aggregation, and coagulation, and series of sequential proteolytic reactions performed by serine proteases under different pathways. Abnormal activity of these coagulation factors has been related to multiple conditions, such as bleeding and thrombosis, Alzheimer's disease, sepsis, multiple sclerosis, and COVID-19(182), (183), (184).

- **Pathways of blood coagulation**

The coagulation pathway is a series of hierarchical events that leads to haemostasis and prevent excessive loss of blood due to injury or hemorrhagic condition. The intricate pathway allows for rapid healing and prevention of spontaneous bleeding. Two paths, intrinsic and extrinsic, originate separately but converged at a specific point, leading to fibrin activation and subsequent formation of hemostatic plug. The purpose is to ultimately stabilize the platelet plug with a fibrin mesh (185). Upon injury to the blood vessel, primary haemostasis attracts accumulation of platelets at the injury site to form a loose plug. Secondary haemostasis then proceeds by two main coagulation pathways, intrinsic and extrinsic, both converge at one point and collectively run as common pathways. The common pathway ultimately converts inactive fibrinogen into active fibrin. These fibrin subunits possess binding affinity for each other by specialised receptor located on the

platelet surface (gpIIb/IIIa) from fibrin strands that bind the platelets together, stabilizing the platelet plug (186), (187).

- **Mechanism of blood clotting**

The coagulation cascade for haemostasis is a series of intricate steps that are performed by a series of clotting factors. The intrinsic pathway constitutes "*fibrinogen* (FI), *prothrombin* (FII), *Christmas factor* (IX), *Stuart-Prower factor* (FX), *plasma thromboplastin* (XI), and *Hageman factor* (XII)". The extrinsic pathway comprises of "factors I, II, VII, and X". Factor VII is known as *stable factor*. The common pathway comprises of factors I, II, V, VIII, X. These factors circulate in blood steam as zymogens (Inactivated enzyme)and are activated into serine proteases. This in turn starts the domino process leading to activation of the next zymogen into active enzyme which ultimately activates fibrinogen. The intrinsic pathway is activated by exposed collagen of endothelial cells, while activation of extrinsic pathway is initiated by release of tissue factor (TF) by endothelial cells after aninsult to blood vessel (188).

- **Intrinsic Pathway**

This pathway is the longer pathway of secondary hemostasis. It begins with the activation of Factor XII (a zymogen, inactivated serine protease) which is converted into Factor XIIA (activated serine protease) after exposure to endothelial collagen. Endothelial collagen is only exposed when endothelial damage occurs. Factor XIIA acts as a catalyst and facilitatesthe conversion of activate factor XI to Factor XIA. Factor XIA then goes on to activate factor IX to factor IXA. Factor IXA later served as a catalyst for turning factor X into factorXa. In such domino process, activation each factor resulted to activation of next factor in cascade (189).

- **Extrinsic Pathway**

The extrinsic pathway is the shorter pathway of secondary haemostasis. And it is activated only after damage is inflicted on the vessels. After injury, the endothelial cells release tissue factor, which is responsible for activation factor VII to factor VIIa. Factor VIIa later activatesfactor X into factor Xa. This is the convergence point where intrinsic and extrinsic pathway interact and FXa is critically located at this station revealing importance in the cascade (190).The extrinsic pathway is clinically measured as the prothrombin time (PT).

- **Common Pathway**

This pathway begins at factor X which is activated to factor Xa. The process of activating factor Xa is a complex reaction initiated by formation of tenase complex. It cleaves s factor X into factor Xa, active form of factor Xa. Also, factor Xa requires presence of factor V as a cofactor to cut prothrombin into thrombin. Factor IIa, an activated thrombin catalyzes ultimate conversion of fibrinogen into fibrin. Fibrin subunits adhere to each other to form fibrin strands, later factor XIII acts on fibrin strands to form a fibrin mesh. (188)

2.12 Role of Factor Xa in haemostasis and pathophysiology of CVD

2.12.1 Structure and activation of Factor Xa

Factor X belongs to the serine protease family of enzymes. The gene coding for factor X issituated on chromosome 13 at position q34, adjacent to the gene code for factor VII. It has a molecular weight of ~27 kb and possesses seven introns and eight exons in its structure. Exon-I denotes the signal peptide while exon II, the propeptide/Gla domain, exon III the C-terminal part of the Gla domain and the aromatic amino acid stack, exons IV and V the EGF-like. Factor X is primarily synthesized in the liver but mRNA and/or protein for the enzyme has been identified in many tissues as well.

The activated isoform of factor X is, Factor Xa which is an arginine specific serine protease mimicking trypsin but exhibited meagre substrate specificity. The interactions of FXa with phospholipid membrane components is initiated by binding of 8-10 calcium ion with N-terminal γ-carboxyglutamic acid (Gla) domain. (average $K_d \sim 0.5$ mM) (191). In the presence of calcium ions, factor Xa and FVa associate together to form a phospholipid-bound complex. This 'prothrombinase' complex has ~300000-fold potency to activate prothrombin than FXa alone. Studies have reported phospholipid bound complex reduce k_m and increase V_{max}

Conversion of inactive factor X to active form proceeds by two main pathways. 1) Extrinsic pathway in which Factor X is activated by VII/VIIa in complex coupled with a non-enzymatic membrane-bound cofactor, tissue factor (TF). Extrinsic pathway and is responsible for the initiation of coagulation, occur mainly on the surface of damaged endothelial cells and macrophages and possibly on platelets. Furthermore, factor X is activated by a membrane-bound 'tenase' complex containing factor IXa, VIIIa with calcium ions. This activation pathway leads to pronounced activation of enzyme at rate of $\sim 10^6$-fold more rapidly than factor IXa alone. This pathway is recognized as "Intrinsic

Pathway" which amplify the the actions in coagulation cascade.(191). *De-novo* inactivation of free factor Xa in the bloodstream is done by serine protease inhibitors such as antithrombin and α_1-protease inhibitor (192).

2.12.2 Pleotropic effect of Factor Xa and thrombin via Plasminogen Activated Receptors (PARs)

The PAR family comprises four isoforms (PAR1 to PAR4) with a proteolytic cleavage-based activation mechanism. (61). Except for PAR2, other subfamily of response to only thrombin while PAR2 is exclusively responsive to FXa. The FXa receptors PAR-1 and 2 are almost ubiquitously expressed, and FXa signaling has been observed constitutively in different cell types, including endothelial and epithelial cells, Blood cells (leukocytes), fibroblasts and VSMCs, neurons and tubular cells. The different organs and the constituting cell types in which FXa signaling has been observed owing to specific differences in the amino acid sequences of these receptors. They are also found to be expressed in cancer cells with involvement important pathological condition such as atherosclerosis, fibrotic lung disease, arthritis. (193), (194). In addition, indirect activation of PAR2 via thrombin-cleaved PAR1 tethered ligand to transactivate PAR2 is also reported, which, in turn, modulate hyperplastic response to arterial damage (195). Factor Xa-initiated cellular responses occur via PAR1 and/or PAR2 cleavage, and these responses are dependent on the mostly expression of receptor on specific cell types, ligand concentration and solubility as well as its relationship with other coagulation factors. The proteolytic cleavageof PARs by thrombin or factor Xa results in the activation of a canonical G-protein pathway and which produces activation of downstream signalling pathways that initiate multiple transcription-regulated, cell-specific events. FXa induces hydrolysis of phosphoinositide leading to inositol and DAG activation causing calcium oscillation. FXa also modulate the phosphorylation of mitogen-activated protein kinases (MAPKs), specifically extracellular-signal related kinase (ERK) and c-Jun N-terminal kinase (JNK) pathways, leading to initiation of different transcriptional programs which are key regulator of cytokine production, fibroblast growth and proliferation. Factor Xa-induced cellular responses are dependent on this extravascular presence of the factor due to vascular injury, macrophage-mediated binding and migration into sites of ischemia or inflammation, and tissue ectopic expression of factor Xa, which may occur in both normal and distinct pathophysiological conditions, including tumor and fibrotic tissues or even in atherosclerotic tissues (196).

Pleotropic effect of FXa is extensively studied in pathogenesis if fibrosis, cancer, airway remodelling and aortic stenosis and cancer for the development of therapeutic strategies (197), (198).

2.12.3 FXa: promising target for anticoagulant therapy

The coagulation cascades advance in an amplified manner and factor X is located at the critical juncture and controls thrombin generation. The activation of one molecule of FX resultsin the generation of 1000 molecules of FIIa. On a molar basis, activated FX is more thrombogenic than thrombin (199). Factor Xa is the prime component of the prothrombinase complex which converts large amounts of prothrombin—the "thrombin burst". Each molecule of Factor Xa can generate 1000 molecules of thrombin. This large burst of thrombin is responsible for fibrin polymerization to form a thrombus.

Clinical evidence also suggested the therapeutic efficacy over thrombin and LMWH with better control of mortality and morbidity with significant reduction in bleeding risk. Rivaroxaban, a selective, oral, direct, FXa inhibitor developed by Bayer HealthCare, has been studied in two orthopedic dose-ranging phase IIb clinical studies in over 1300 patients. Rivaroxaban at all the intended doses resulted in similar or lower rates of VTE compared to enoxaparin arm, and no dose exhibited higher rate of major bleeding risk than its counterpart (200).

Apixaban (Bristol-Myers-Squibb), another selective, oral, direct, factor Xa inhibitor studied in a phase IIb orthopedic dose-ranging study compared with enoxaparin and warfarin, observed to lower estimate for VTE/death at each dose level. Both rivaroxaban and apixaban had a favorable bleeding risk profile compared with enoxaparin at effective doses (201).

2.12.4 FXa vs. FIIa inhibition - Which one is better?

FXa and FIIa (Thrombin), both provide potential target sites to modulate hypercoagulation. Literature has reported that there are obvious advantages over vitamin K antagonist. However, when evaluated considering physiological function and results from clinical efficacy, FXa inhibitor treatment has an edge over thrombin inhibition. Hoffman et al suggested that even as we adapt our thinking depend on the location and sequence of coagulation factor interactions as the concept of amplification still holds true, consistent with the theory that upstream inhibition in the sequence of coagulation factor has greater

antithrombotic potential (202). Compared to thrombin, role of FXa is exclusively involved in coagulation cascade and haemostatic blood flow with very limited function outside such as proinflammatory and proliferative effect whereas thrombin participates in different physiological process outside of coagulation process(203),(204), perhaps of greatest importance are prothrombotic role in platelet activation, thrombin's influence on positive feedback of coagulation factors earlier in the coagulation scheme, and thrombin's paradoxical effect such as antithrombotic action via activation of protein C and thrombin activated fibrinolysis inhibitor provides sufficient evidences for the superiority of FXa targeting (205).

Thrombin is the principal activator of recruiting platelets to sites of injury via PAR1/4 activation (206) whereas FXa has no effect on platelet activation .Thrombin plays a positive role in the feedback activation of FV, VIII and XI, further advancing coagulation, amplification and propagation and important component in the protein C regulatory pathway promotes prothrombotic activities. (205). Selective FXa inhibitors compared with direct thrombin inhibitors have been shown to decrease endogenous thrombin potential and prolong the lag phase in a dose-dependent manner (207). There is lack of head-to-head comparison of FXa inhibitors with FIIA inhibitors, however clinical trials conducted for ximelagatran at the intended doses which were later modified, and dabigatran showed narrower safety and efficacy window compared to that of direct FXa inhibitors (208 {Eriksson, 2002 #116)

2.13 Management of hypercoagulable events

2.13.1 Anticoagulants: Utilization and drawbacks of current therapy

Anticoagulants are used in thrombotic event prevention and reoccurrence in many cardiovascular diseases, pulmonary embolism, myocardial ischemic injury, percutaneous coronary intervention, venous and arterial thromboembolism, stroke prevention in atrial fibrillation, treatment and secondary prevention of acute coronary syndrome (209), (210). Vitamin K antagonist, warfarin and low molecular weight heparin and hirudin analogues (Argatroban) are extensively during hospitalization while FXa inhibitors (Apixaban, Betrixaban, Rivaroxaban) and thrombin inhibitor (Dabigatran) are used as maintenance therapy in post hospitalization period. Anticoagulants are often combined with antiplatelet agents such as P2Y12 inhibitor, clopidogrel. (211). They are widely utilised for the

prevention hypercoagulability and risk of pulmonary embolism during outbreak of COVID 19 (212).

Vitamin K antagonists (VKAs) such as warfarin, heparin analogues such as Low molecular weight heparin (LMWH), Unfractionated heparin (UFH) have been the mainstay of oral anticoagulant therapy. Despite their efficacy in clinical setting, they pose certain significant disadvantages in patient care and monitoring. (213). The VKAs possess substantial threatsthat predispose patients to severe adverse events, and it is associated with impaired quality of life, and restrict their use for well documented indications. For example, warfarin is coupled with numerous drug and food interactions, an unpredictable pharmacokinetic (PK) and pharmacodynamic (PD) relationship, and considerable intra- and interpatient variability in drug response due pharmacogenomic variation of CYP isoenzyme. These irregularities cause overdosing of drug which expose patient to significant risk of bleedingand hemorrhage. (214).

While use of UFH and LMWH and other parental drugs are associated with concerns regarding their administration route and expected side effects. Agents for acute anticoagulation therapy involve UFH and LMWHs which indirectly inhibit FXa, direct inhibitor of FXa fondaparinux, and the direct thrombin inhibitors (DTIs) argatroban, bivalirudin, and hirudin. These anticoagulants require parenteral administration, which makes their use outside the hospital troublesome and frequent administration is also associated with injection-site hematomas. UFH and, to a lesser extent, LMWHs carry the risk of thrombocytopenia, and produce immunogenic responses as they are derived from animal tissue (59).

2.13.2 Novel Anticoagulant drugs

A wide range of armamentariums of new anticoagulants, both parenteral and oral, are in various stages of development. The major difference in many of these new agents is their mechanism of action, with targeted specificity and with particular neutralization of a specific coagulation factor, rather than the broad effect compared to VKAs or heparin-related compounds which poses non-selective action on multiple coagulation factors in blood clotting cascade.

Recently, targeting FXa and FIIa seems to be very attractive sites for the control of hypercoagulability. Inhibitors of the activated forms of factor (F) IIa and FXa are fertile areas of exploration and are further advanced in development than most other candidate

drugs. With the early results of clinical phase studies, there is a debate over superiority of inhibition of one coagulation factor over other for efficacy and safety. Some of the theoretical and observational data indicate the benefits of FXa inhibition compared with FIIa inhibitionwhich are backed by the clinical evidences (215).