1. Introduction

Clinically, AKI is defined as a catastrophic condition associated with various etiologies and pathogenic processes leading to suppressed kidney function (Hoste et al., 2018; Silver et al., 2018). There are fewer chances for complete recovery of kidney function and left such patients with high morbidity and mortality risk. The prevalence of AKI ranges from <1 to 66% (Hoste et al., 2018). Patients having vulnerability towards AKI [elderly patients, chronic kidney disease (CKD), heart failure or anemia] and those who already exist with predisposing factors (like sepsis, major surgery, or nephrotoxins) remain on the higher risk of developing AKI (Hoste et al., 2018). Among all, diabetes remains one of the major risk factors for AKI patients (Yu & Bonventre, 2018). Clinical studies revealed that type 1 diabetic hospitalized patients have greater chances of getting AKI episodes (Hertzberg et al., 2015; Takiyama & Haneda, 2014). Patschan and Muller have summarized that maintenance of blood glucose levels has significantly decreased the chance of AKI episodes in intensive care units (ICU) (Patschan & Müller, 2016). AKI has been designated with different terms depending upon the affected part of kidney tissue and the main categories are pre-renal, intrinsic, and post-renal (Makris & Spanou, 2016). Amongst, the assessment of intrinsic AKI (including IRI) is quite challenging because the major portion of the kidney (interstitium, tubules, glomeruli, renal blood vessels) got injured.

A big body of evidence suggested that genetic proclivity alone is inadequate to explain the complex pathogenesis of IRI-induced AKI. Similarly, it becomes highly essential to know the pathogenesis of AKI associated co-morbidities such as DM. Hence, this urges the need to focus on epigenetic mechanisms tangled in the development of the same (Guo et al., 2019). Epigenetics involves the study of inheritable changes in gene expression but does not include any alteration in the underlying DNA sequences (Fontecha-Barriuso et al., 2018). Histone PTMs such as histone H2A, H2B, H3, and H4, involving ubiquitination, methylation, acetylation, and phosphorylation at lysine residue play a crucial role in regulating gene transcription. Growing evidence has demonstrated the vital role of epigenetic regulation in inflammatory and apoptotic gene expression under AKI settings (Guo et al., 2019). Among all histone PTMs, histone acetylation has been studied quite extensively in AKI and a plethora of specific histone deacetylases (HDACs) inhibitors have

been utilized in the pathogenesis of AKI. Still, there is no permanent cure for the treatment of AKI. Here, the role of histone methylation came into the figure. As compared to histone acetylation, the role of histone methylation in AKI has not been much explored. Methylation of histone H3 lysine (H3KMe) is regulated by histone methyltransferases (HMTs) and is associated with either active or repressed gene transcription, determined by the position of lysine modified (Kouzarides, 2007). In general, histone H3K4Me, H3K36Me, and H3K79Me are linked with gene activation and transcriptional elongation. Whereas, H3K9Me and H3K27Me are associated with gene repression. HMTs like SET7/9, SET1 and MLL1-4 activate transcription via H3K4Me. However, HMTs like NSD1, SMYD2 and SET2 act via H3K36Me (Yu & Zhuang, 2019). Remaining, H3K79Me is regulated via a single HMT i.e. DOT1 and promotes active gene transcription. HMTs such as G9A, SET1/ESET and SUV39H1 promote H3K9Me, and PRC2 and EZH2 are specific for H3K27Me, and thus promotes repressor gene transcription (Portela & Esteller, 2010; Yu & Zhuang, 2019). A natural balance between active and repressive gene expression marks, if get disrupted, may lead to abnormal gene transcription and disease traits. One study demonstrated the upregulation of EZH2 along with H3K27Me3 in renal fibrosis of unilateral ureteral obstruction (UUO) mice and CKD patients, signifying its profibrotic functions. Pharmacological inhibition of EZH2 diminished the H3K27Me3 and attenuated renal fibrosis (Zhou et al., 2018). Another report suggested the role of H3K4Me3 in the induction of inflammatory factors (TNF-α & MCP-1) in various mouse models of AKI (Guo et al., 2019), but how the regulation of H3K4Me3 carried out under AKI is still remains a notable query. Therefore, the role of SET domain-containing lysine methyltransferase 7/9 (SET7/9), a histone methyltransferase (HMT) mediates active transcription through H3K4Me, arrived on the scene.

Recently, documented evidence has shown the pivotal role of HMTs SET7/9 in the progression of various kidney diseases including diabetic nephropathy (Goru et al., 2016; Reddy & Natarajan, 2015). Interestingly, Takemoto and his team have rediscovered a potent inhibitor of SET7/9-Cyproheptadine and has been successfully tested against human breast cancer cell lines (Takemoto et al., 2016). In diabetic nephropathy, increased H3K4me and SET7/9 leads to increased recruitment of MCP-1 and ECM-associated gene promoters in diabetic renal fibrosis (Chen et al., 2014a; Goru et al., 2016; Shuttleworth et

al., 2018; Sun et al., 2010). In I/R renal injury, increased transforming growth factor-beta (TGF-β) levels result in the upregulation of H3K4Me and its specific HMT-SET7/9, which was successfully suppressed by Apelin treatment and further protect the kidney from ischemic insult (Chen et al., 2015). SET7/9 also attains a crucial role in inflammation and diabetes, as evidenced by augmented NF-κB associated inflammatory gene expressions and SET7/9 recruitment in macrophages of diabetic mice (Li et al., 2008b). TNF-α induced recruitment of NF-κB p65 on inflammatory gene promoters was effectively reduced by targeted silencing of SET7/9 with siRNA (Li et al., 2008b). Therefore, these findings suggest the possible role of SET7/9 in diabetic kidney diseases. ***However, the epigenetic mechanism that involves histone methylation and SET7/9 in AKI and the effect of Cyproheptadine on these mechanisms still remain an enigma.***

Adverse renal outcomes in DM and AKI individually attributed towards the RAS driven activation of mitogen-active protein kinase (MAPK)-mediated apoptosis, NF-κB mediated inflammation, and redox imbalance promoting oxidative stress (Beker et al., 2018). In normal physiology, RAS is one of the crucial regulatory systems for blood pressure and fluid balance. As RAS has Janus's face, it is classified in its two major axes: conventional and non-conventional axis. The main effector of its conventional axis, angiotensin II (Ang II) is released by local and systemic RAS activation and in renal vasoconstriction as well as inflammation and tubular damage led to angiotensin II type1 receptor (AT1R) activation (Kwon et al., 2003; Malek & Nematbakhsh, 2015; Ruiz-Ortega et al., 2003). On the other hand, the non-conventional or protective axis of RAS, which exerts its vasodilatative effects in various tissues (heart, kidney, lung, blood vessels) (da Silveira et al., 2010; Ocaranza & Jalil, 2012; Ruiz-Ortega et al., 2003). Although, treatment with ACEi and ARBs cause vasodilation of the renal efferent arterioles and result in the reduction of glomerular filtration pressure. During hypovolemia, the reduced efferent vascular tone lowers glomerular filtration rate (GFR) and ultimately promoting AKI (Schoolwerth et al., 2001). However, the concern that the use of ARBs/ACEi leads to the precipitation of AKI is poorly substantiated, indicating the necessity for finding additional pathways within RAS as probable drug targets.

The auxiliary part of RAS; ACE2 is a mono-carboxypeptidase, and considered to be the negative regulator of RAS as it degrades Ang II to Ang (1-7) (Corvol et al., 2004). ACE2

is highly expressed within tubular epithelial cells, glomerular epithelial cells, and the renal vasculature. ACE2 activity has reported getting altered in numerous types of kidney injuries (Bae et al., 2017; Lv et al., 2017; Ross & Nangaku, 2017). Recently, studies have revealed the role of ACE2 in different animal models of AKI. Gupta *et al.* reported the downregulation of renal ACE2 mRNA levels in lipopolysaccharide-induced AKI (Gupta et al., 2007). Da Silveira *et al.* examined the renal profile of ACE2, Ang (1-7) and the MasR in renal I/R injury, and observed the diminished renal ACE2 activity and Ang (1-7) levels, along with increased MasR levels, illustrating the complexity and importance of ACE2-Ang (1-7)-MasR alliance in AKI (da Silveira et al., 2010). Moreover, Yang *et al.* have demonstrated the effect of imbalanced ACE/ACE2 in tourniquet-induced kidney injury, where the diminished ACE2 levels promulgate the disease along with increased free radicals and suppressed anti-oxidant levels (Yang et al., 2012). Along with the investigation of ACE2 regulation, a novel ACE2 activator-Dize, have also been studied in the AKI context. In gender-based I/R kidney injury, Malek *et al.* found the prominent reno-protective effect of Dize in male rats, as evidence by reduced kidney dysfunction biomarkers and maintained oxidant-antioxidant ratio (Malek & Nematbakhsh, 2014). Therefore, future studies are required to explore the mechanisms underlying these reno-protective effects of ACE2 in AKI settings.

Another interesting therapeutic target from the depressor arm is AT2R, which is a G-protein coupled receptor (Berry et al., 2001). R. Carey has done in-depth work on the depressor arm of RAS (especially AT2R) (Abadir et al., 2012; Carey, 2005; Carey, 2017a). He introduced a compiled report on the role of AT2R in maintaining blood pressure and kidney function (Carey, 2017b). AT2R mediates its action by inducing the release of kinins (bradykinin, kallikrein), cGMP, and nitric oxide (NO) levels (De Luca Jr et al., 2013) which facilitates the natriuresis and lowering blood pressure along with vasodilatory, anti-inflammatory, anti-oxidative stress responses (offsets the actions of AT1R) (Castoldi et al., 2014; Matavelli et al., 2011; Rompe et al., 2010; Villela et al., 2015). It is now widely recognized that AT2R has opposite actions compared to AT1R. Although, the importance and efficacy of this receptor are not much explored in the pathophysiology of AKI, yet one study characterized the folic acid nephropathy by up-regulation of AT2R and apoptotic signaling (Ruiz-Ortega et al., 2003), where AT2R re-expression along with AT1R

suggested the crucial role of AT2R in AKI. In the discovery of AT2R agonists, scientists have developed few peptides like CGP42112A or LP2 but possess no oral bioavailability and having low specificity for AT2R. In the year 2004, the first non-peptide, specific AT2R agonist, Compound 21 (C21) was developed which showed better oral availability (Wan et al., 2004) and tested against hypertension, heart failure, stroke, and diabetic nephropathy (Dai et al., 2016; Joseph et al., 2014; McCarthy et al., 2014; Pandey & Gaikwad, 2017a; Pandey & Gaikwad, 2017b). ***Hence, the involvement of AT2R and ACE2 in the pathogenesis of AKI is less explored. Therefore, the current study designed to delineate the role of AT2R and ACE2 activation in the development of IRI under normal and hyperglycemic condition***.

Under AKI settings, activation of RAS not only compromises the kidney functions but also has an immediate impact on distant organs (Bucsics & Krones, 2017; Panico et al., 2019). Remarkably, the clinical relevance proposed that all the organs have their own local RAS which remains compartmentalized from the circulation (Campbell, 2014). Following a similar approach, recent reports have demonstrated the activation of the pressor arm of the RAS (ACE, Ang II, and AT1R) in distant organs (Cao et al., 2017; Taskin & Guven, 2017). Nevertheless, the role of depressor arm of RAS [AT2R, Ang (1-7) and ACE2] in IRI-induced distant organ dysfunction remains elusive. ***Hence, the current study designed to evaluate the effect of AT2R and ACE2 activation on distant organ dysfunction.***