INTRODUCTION

Literally the term Epigenetic means the mechanism on top of genetic ***(Riya R. Kanherkar et al., 2014)***. Epigenetics refers to a heritable phenotypic changes caused by mechanisms unrelated to the DNA sequence of the individual ***(Silvia Gravina et al., 2010)***.

Three main mechanisms are under scrutiny, these include DNA methylation, histone modification and RNA silencing ***(Riya R. Kanherkar et al., 2014)***.

DNA methylation refers to the covalent binding of methyl group in position number 5 in the pyrimidine ring of cytosine ***(Ziller et al., 2013)***.

DNA methylation occurs under the control of an enzyme called DNA methyltransferase. It is refered to as Dnmt. There are three types of Dnmt ***(Singh et al., 2012)***.

The binding of methyl group into cytosine in the DNA prevents the latter binding to the transcription proteins, leading to prevention of gene expression. Mammals, birds, reptiles, fish and some fungi, all use DNA methylation as a mechanism for control of gene expression ***(Craig A. Cooney 2007)***.

Aberrant DNA methylation have been implicated in aging ***(Silvia Gravina et al., 2010)***, carcinogenesis; contrary to the belief that cancer is a genetic diseases, accumulating evidence refers to the role of epigenetics in the development of cancer ***(Riya R. Kanherkar et al., 2014 and Coppede 2014).***

Another role for aberrant DNA methylation is mentioned in the development of menopause ***(Kosa et al., 2009)***, atherosclerosis; hypermethylation has been observed in patients with coronary artery disease ***(Byrneet al., 2014).*** Another clue to its contribution to oxidative stress and inflammation has emerged and is currently under investigation to be modified as a therapeutic option to prevent endothelial dysfunction and vascular complications in diabetic people ***(Paneni et al., 2013).***

Alzheimer disease pathogenesis has been linked to hypermethylation of certain genes in the brain together with hypomethylation in the entorhinal regions of the cerebral cortex ***(Silva et al., 2008, Mastroeni et al., 2008).***

Accumulating evidence refers to the role of protein pound uremic toxins in the development of uremic complications ***(Toshimitsu Niwa 2013)***. Of these toxins indoxyl sulfate had gain interest for being not only responsible for cardiovascular complications, but also it is a nephrotoxic. It acts through specific molecular pathways to induce tubular atrophy, glomerular sclerosis and nephron loss ***(Toshimitsu Niwa 2010)***.

Indoxyl sulfate is derived from the metabolism of Tryptophan by tryptophanase derived from the intestinal bacteria *Escherichia coli*. The derived indole is absorbed into the blood from the intestine and then metabolized in the liver into indoxyl sulfate ***(Toshimitsu Niwa, 2011).***

AIM OF THE STUDY

To compare the effect of Hemodiafiltration versus Hemodialysis as regard DNA methylation and Indoxyl Sulfate removal.

Chapter 1
INTRODUCTION ON EPIGENETICS

Epigenetics; it can be defined as a set of heritable extra DNA modifications which can lead to phenotypic changes in the individuals. As from the previous statement, these changes are not accompanied by corresponding DNA sequences. Epigenetics can be consequences of environmental stressors; hence it can be used to explain how the environment can promote evolution ***(Katalin, 2014).*** In other words it can be considered as a mirror to our environment ***(Craig Cooney 2007).***

The mechanisms through which epigenetics can exert its effects has been long identified as four separate mechanisms; 1- histone post translational modification 2- DNA cytosine methylation, 3- non coding RNA sequences and 4- chromatin remodeling ***(Massimo Romani et al., 2015)***. These four mechanisms comprise a superior mechanism that control gene expression, hence the name epigenetic ***(Marpadga et al., 2015)***.

Mechanisms of epigenetics

1- Histone modification.

The human DNA double helix is naturally folded upon specific proteins through specific architecture in order to be fit for nuclear residence. Normally the DNA double helix is folded

around a basic protein called Histone. This structure is called *chromatin.* Every 147 base pair being folded around Histone is termed *nucleosome.* The latter is a compact protein. DNA while encased in this compact matter is not subjected to the transcription process ***(Yi-An Ko et al., 2013)***.

The nucleosome is a globular protein consists of eight different proteins (two copies of each protein H2A, H2B, H3, and H4). The structure of double stranded DNA arranged on a row of nucleosomes is called beads on a string ***(Friedman and Rando 2015)***.

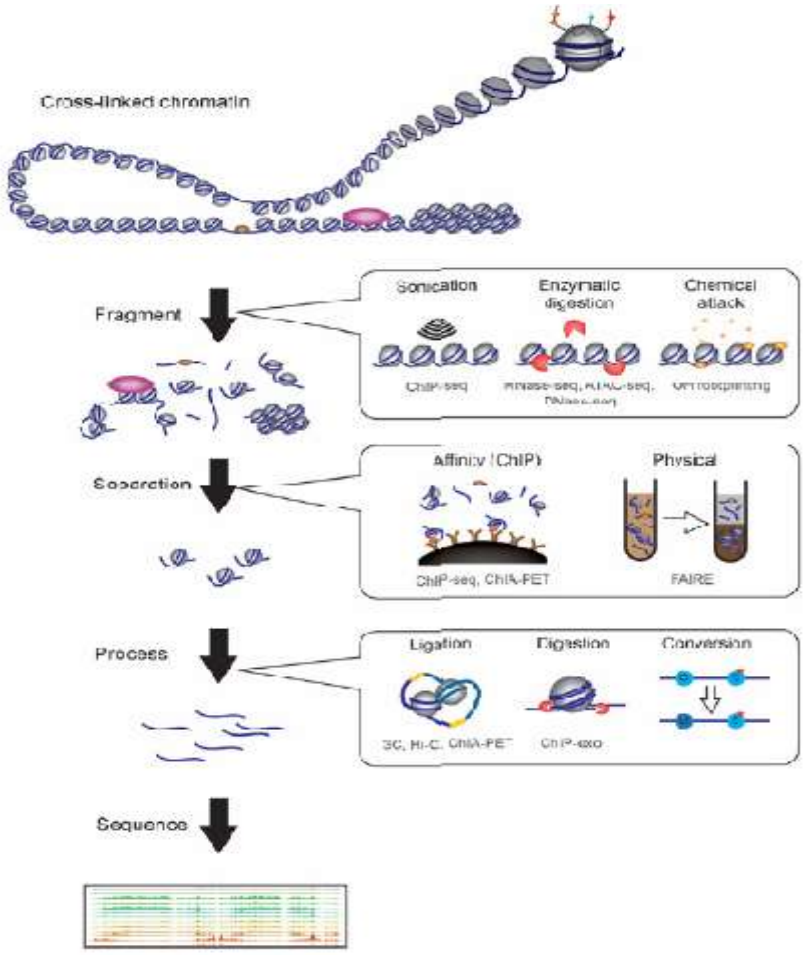
The nucleosomal proteins are not uniform in nature; rather they are varied with multiple isoforms. This occurs mostly in H2A, and H3. This is in addition to the process of chemical modification which occurs on the nucleosomal proteins by residue specific enzymes. These modifications are in the form of (methylation, phosphorylation, acetylation). These modification can have a magnificent repercussion on the process of transcription and hence gene expression ***(Tan et al., 2011)***.

Mapping of nucleosomal modifications occurs through the following steps

1- Fragmentation; through mechanical processes e.g. sonification, enzymatic reactions e.g. nucleosomes which attacks the DNA at specific sequences.

2- Separation of the nucleosome with the specific modification of interest through the process of chemical immunoprecipitation (a process through which specific antibodies are used to attach to the specific proteins or peptides of interest that are covalently bound to the DNA).
3- Sequencing to identify the specific genome under study.

(Friedman and Rando 2015)



(Friedman and Rando 2015)

The resulting map can identify the specific regions of DNA with epigenetic modification. The accuracy of this map depends on the specificity of the antibodies used in the chemical immunoprecipitation and the fragmentation protocols used ***(Egelhofer et al., 2011, and Fuchs et al., 2011)***.

There are around 60 chemical processes which can modify the tail of the Histone ***(Katalin S., 2014)***. These processes are grouped as sumoylation (Small ubiquitin like modifier), ubiquitylation ***(Viduth K et al., 2016)***, methylation and phophorylation ***(Katalin S., 2014)***.

The Histone code hypothesis states that the DNA expression regulation occurs through the process of interaction between the DNA and the chromatin. These interactions occur through the 60 modifications on the Histone tail which was mentioned before ***(Yi-An Ko et al., 2013)***.

These 60 modifications can be summarized into the most common 9 modifications which underwent a thorough examination ***(Yi-An Ko et al., 2013)***.

Chromatin Mark	Chromatin State
CTCF	Insulator/repetitive/CNV
H3K27me3	Poised promoter/repressed promoter
H3K36me3	Transcription transition and elongation
H4K20me1	Transcription transition
H3K4me1	Enhancer
H3K4me3	Active promoter
H3K4me2	Promoter/enhancer
H3K27ac	Enhancer>>promoter
H3K9ac	Enhancer>> promoter

CTCF, CCCTC-binding factor; CNV, copy number variation

For example: the modification symbolized as H3K4me3 means trimethylation of the fourth lysine in H3 histone. This particular modification encodes for an active promoter for the expression of specific genes ***(Heintzman ND et al., 2007)***.

It is worth noting that the behavior of these modification codes is different in between two sets of cells; the differentiated cells and the embryonic cells. That is while H3K27me3 is considered an enhancer in differentiated cells, in embryonic cells it is quite the contrary. This phenomenon is called ***bivalent domains***. It is presumed that during the differentiation phase, cells will decide which domain it will pursue whether it is the promoter or the repressive, depending on the path ***(Mikkelsen et al., 2007), (Bergsland et al., 2011)***.

Several enzymes which have been proposed to be involved in the chemical reactions of modifications have been isolated. These include the N-methyltransferase family which

are responsible for the methylation of Arginine. Histone acetylation is thought to be catalyzed by Histone acetyltransferase, and deacetyled by Histone deacetylase. Histone deacetylase can be targeted by inhibitors e.g. valproic acid ***(Boyer et al., 2006) (Yi-An Ko et al., 2013)***.

The ENCODE project represents a database collection of epigenetic characterizations which govern the process of gene regulation. It has been retrieved through cultured human cell lines ***(Consortium et al., 2012)***.

2. **DNA methylation**

DNA methylation is often referred to as 5mc (methylated cytosine in position number 5) ***(Yi-An Ko et al., 2013)***.

DNA methylation was first described in the genomic material of tuberculous bacillus in 1925 ***(Johnson TB et al., 1925)*** and the calf thymus 1948 ***(Hotchkiss RD 1948)***.

The significance of this discovery was kept unknown till 1975 two different studies highlighted the outstanding role of DNA methylation as a main mechanism for epigenetic characterization of the genomic material ***(Meng et al., 2015)***.

In prokaryotics DNA methylation occurs mainly in Adenine and Cytosine, and its main role is the protection of the genomic material from different competitive bacterial and viral genome, while in multicellular eukaryotic organisms DNA

methylation plays an important role as an epigenetic mechanism regulating gene expression. DNA methylation in eukaryotic has been implicated in various pathophysiological steps towards the development of various diseases including malignancies ***(Meng et al., 2015)***.

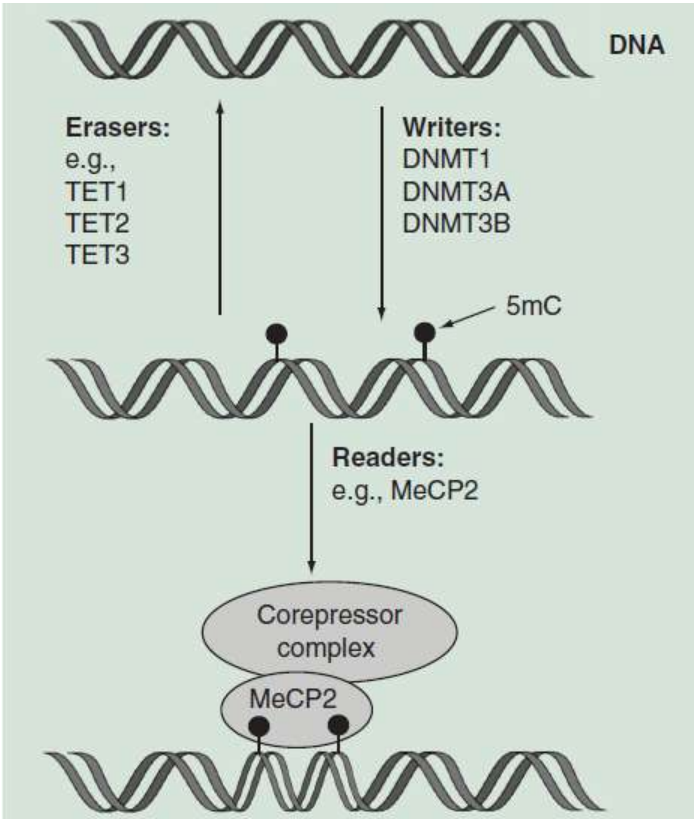
In health, DNA methylation plays an outstanding role in the process of X chromosome inactivation, genomic imprinting ***(Smith et al., 2013)***.

DNA methylation Machinery

Most of the genomic material contain low amount of cytosine/guanine content. Most of the cytosine is methylated. CGI (cytosine guanine islands) are found before gene promoters in a stretches ranges between 300-3000 base pairs. Hypermethylated CGI acts as an inhibitors to the gene promoter, hence limits its expression ***(Yi-An Ko et al., 2013)***.

There are two main mechanisms behind hypermethylation induced transcriptional silencing (in other words inhibition of gene expression). These includes; DNA methylation can directly block transcriptional activators from binding to the genetic sequences to be expressed. The second mechanism is through specific methyl proteins which can recognize methylated DNA and then recruit other co-factors to co-inhibit the transcription of the Gene ***(Yi-An Ko et al., 2013)***.

As expected from the examination and description of most of the biochemical reaction; each one should be under strict control by two different groups of rate limiting step enzymes. The promoters and the inhibitors. Regarding our topic; DNA methylation machinery, it is under regulation by two groups of enzymes and a group of proteins called the readers. The following figure summarizes the entire process.



Adapted from Hamidi et al., 2015

As from the above figure, the process of DNA methylation is under the control of three different enzymes DNMT1, DNMT3A and DNMT3B. While the first enzyme is

the main enzyme responsible for DNA methylation in mammals. DNMT1 acutally performs what is known as the hemimethylation, which means that it copies the methylation pattern from the parent cell to the daughter cell, thus ensuring the heritable pattern of epigenetic characterization. DNMT3A and DNMT3B have different functions; they act as a denovo methylators in the context of gametogenesis and embryogenesis ***(Hamidi et al., 2015)***.

As mentioned before the methylation process shouldn't be left unabated, hence the discovery of the TET enzymes became logic.

Although the DNA methylation was perceived as a stable modification, a demthylation process was observed in zygotic cells, and primordial germ cells ***(Bogdanović et al., 2013)***. The process of demthylation wasn't fully elucidated until the recent discovery of the TET family of dioxygenases ***(Hamidi et al., 2015)***. The term TET stands for *ten eleven translocation protein* ***(Yi-An Ko et al., 2013)***. Etymologically the name arose from the discovery of a specific chimeric protein as a result of chromosomal translocation between chromosome 10 and 11. The resultant protein was found to be highly expressed in selected cases of myeloid leukaemia. Its main function wasn't known until 2003 when it was discovered to be a major mediator of DNA demethylation ***(Lorsbach RB et al., 2003)***.

The process of demethylation occurs through multi-steps leading to multiple intermediary products these reactions are under control by the TET family of deoxygenases. First 5mC to 5-hydroxymethylcytosine (5hmc), then 5- formylcytosine (5fC), and 5-carboxylcytosine (5caC) ***(Tailiani et al., 2009), (Pastor et al., 2013) and (Wu et al., 2014)***.

Another passive mechanism was proposed to be responsible for demethylation; it was through the replication of the DNA leading to the dilution of the 5mC compounds ***(Hamidi et al., 2015)***.

Another peculiar role for DNMT3A and DNMT3B have been discovered, they are involved in conversion of 5hmC to cytosine in vitro ***(Wu et al., 2014)***.

The previously mentioned demethylation intermediaries e.g. 5hmC, and 5fC have been recently discovered to have an epigenetic characterization, however due to their extremely nadir level their role remains yet to be extrapolated ***(Hamidi et al., 2015)***.

There are three different TET genes encoding three TET proteins. They are expressed heavily in the human brain and TET 2 in uniformly expressed in the bone marrow ***(Zhao et al., 2013)***.

The DNA hypermethylation transcription silencing effect can be induced through the direct inhibitory effect of

methylation, or by another more common route, through the action of methylated DNA binding protein ***(Yi-An Ko et al., 2013)***.

The methylated DNA binding proteins are a set of proteins which main function are the recognition of the methylated cytosine and the induction of the transcription silencing process. There are three families of proteins assigned with this task; the methyl binding domain (MBD) including the MeCP2, the zinc figure protein (znF) and the SET and RING figure associated domain (SRA) ***(Hamidi et al., 2015)***.

Role of DNA methylation in embryonic development

The role of DNA methylation in embryonic development was first pursued through the assessment of the amount of 5mC in the genome of embryo of sea urchin in 1975 ***(Bogdanović et al., 2013)***.

Early studies failed to detect any difference between the levels of DNA methylation at different developmental stages. The late development of restriction enzymes such as MspI and HpaII was pivotal in the isolation of hypermethylated DNA sequences ***(Bogdanović et al., 2013)***.

The first laboratory isolation and quantification of the amount of DNA methylation and demethylation occurring during different developmental stages in mouse, was carried out by ***Monk et al., 1987***. The latter study was able to demonstrate

the difference between methylated DNA amount between sperm and ova genome. Also it was able to demonstrate the accumulating amount of methylated DNA through the progressive stages of development.

Moreover; late studies have demonstrated difference in the methylated DNA between parental and maternal DNA, as the former exhibits significant demethylation while the latter exhibits significant methylation. It appears that TET3 was the main orchestrator off the process of demethylation in paternal DNA, followed by selective inhibition of DNMT1 and later passive dilution of methylated DNA (through simply DNA replication) ***(Bogdanović et al., 2013), (Daniel Messerschmidt et al., 2014)***.

Selective knocking down of DNMT1 and DNMT3 and MeCP2 have been associated with developmental defects and later on embryo death ***(Tittle et al., 2011)***.

It is proposed that DNA methylation has a role in transposon repression, X chromosome inactivation and imprinted gene silencing. All of which has a pivotal role during embryogenesis ***(Daniel Messerschmidt et al., 2014)***.

This field is still in its infancy and further studies are needed to further elucidate the role and mechanisms of DNA methylation throughout different developmental stages in vertebrates ***(Daniel Messerschmidt et al., 2014)***