Review Article

Role of cytogenetic biomarkers in management of chronic kidney disease patients: A review

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Abstract

Chronic kidney disease (CKD) is much more common than people recognize, and habitually goes undetected and undiagnosed until the disease is well advanced or when their kidney functions is down to 25% of normal function. Genetic and non-genetic factors contribute to cause CKD. Non-genetic factors include hypertension, High level of DNA damage due to the production of reactive oxygen species and nucleic acid oxidation has been reported in CKD patients. Main genetic factor which causes CKD is diabetic nephropathy. A three- to nine-fold greater risk of End Stage Renal Disease (ESRD) is observed in individuals with a family history of ESRD. This greater risk have led researchers to search for genes linked to diabetic and other forms of nephropathy for the management of CKD. Multicenter consortia are currently recruiting large numbers of multiplex diabetic families with index cases having nephropathy for linkage and association analyses using various cytogenetic techniques. In addition, large-scale screening studies are underway, with the goals of better defining the overall prevalence of chronic kidney disease, as well as educating the population about risk factors for nephropathy, including family history. Cytogenetic biomarkers play an imperative role for the linkage study using G banding and detection of genomic instability in CKD patients. Classical and molecular cytogenetic tools with cytogenetic biomarkers provide remarkable findings in CKD patients. The aim of the present review is to draw outline of classical and molecular cytogenetic findings in CKD patients and their possible role in management to reduce genomic instability in CKD patients.

Keywords: Biomarkers, CKD, Cytogenetics, DNA damage, FISH, Micronucleus frequency, Neoplasm

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Introduction

Chronic kidney disease (CKD) is a developmental pathological manifestation in which kidney functions are lost over time. Hypertension, diabetes, cardiovascular ailment, thyroidism, malnutrition, hepatitis B and C infection and life style of an individual contribute to causes CKD [1-4]. DNA damage via production of reactive oxygen species, nucleic acid oxidation, advanced glycation end products and inflammation leads to genomic instability in CKD patients. [5-7] End stage renal disease (ESRD) patients requires dialysis or renal transplantation and estimated about four to five fold increased risk of developing renal cancer in their native kidneys. [8, 9] CKD is serious public health problem and prevalence has reached epidemic proportions with 10–13% of the populations in Taiwan, [10] Iran, [11] Japan, [12] China, [13] Canada, India and the USA. [14-15]

Cytogenetic analysis of peripheral blood lymphocytes has been accepted as the suitable assay for biological monitoring of the genetic damage induced in somatic cells [16]. Due to genomic instability, increased levels of DNA damage have been reported in CKD patients; measured using different conventional and molecular cytogenetic biomarkers such as Karyotyping, G-banding, Micronucleus assay (MN), [17] COMET assay, [18] Sister chromatic exchange assay (SCE), [19] Cytokinesis-Blocked Micronucleus (CBMN) assay where as molecular cytogenetic techniques includes, Fluorescent *in-situ* hybridization (FISH) using DNA probes and protein markers, Comparative genomic hybridization (CGH), and spectral karyotyping (SKY) etc. [20, 21]

The present review provides an overview of conventional and molecular cytogenetic findings in CKD patients, reported case studies, detection of genomic instability using cytogenetic biomarkers, consequences of DNA damage and their possible management to reduce genomic instability in CKD patients.

Conventional cytogenetic studies in chronic kidney disease (CKD) patients

Karyotyping using G-banding is the primary and conventional cytogenetic technique for the detection of chromosomal abnormalities. Karyotype was first defined by Levitsky as the phenotypic appearance of the somatic chromosomes. [22] Chromosomal abnormalities in CKD patients are found to be congenital and heritable. 6q deletion has been identified by McNeal *et al* [23] in VATER association (vertebral defects, anal atresia, cardiac defects, tracheoesophageal fistula with atresia, renal defects, and radial upper limb dysplasia) patients. Sister chromatid exchange, structurally abnormal chromosomes, deletions, chromatid breaks, radial chromosomes have been reported in CKD patients using classical cytogenetics. [24, 25] Besseau-Ayasse *et al* [26] identified 22q11.2 microdeletion in 272 fetuses and reported 27 % deletion found to be heritable. Postnatal study revealed microdeletion would be a probable cause of kidney abnormalities, thymus impairment and facial dysmorphism.

Molecular cytogenetic findings in CKD patients

Classical cytogenetic technique is a gold standard diagnostic tool for the detection of chromosomal abnormalities but have some limitations. Classical cytogenetic technique fails to detect cryptic chromosomal anomalies. [27] With the advent of fluorescence *in situ* hybridization (FISH) using DNA and protein probe (Immuno-FISH), comparative genomic hybridization (CGH), CGH array, spectral karyotyping (SKY) technique, now it is possible to detect and decipher hidden numerical and structural changes in chromosomes. Molecular cytogenetic findings in CKD patients are shown in Table 3.

Fluorescence *in situ* hybridization (FISH), FISH is a cytogenetic technique developed by biomedical researchers in the early 1980. [28] FISH works on the principle of DNA probe hybridization. Probes bind to that part of chromosome which shows a maximum degree of DNA sequence complementarity. It is used to detect genetic abnormalities such as characteristic gene fusions, aneuploidy, deletion, gene mapping for the identification of oncogenes, and loss of whole chromosome. It can also help in monitoring the progression of an aberration thus assist in diagnosis of a genetic disease or suggesting prognostic outcomes. [29]

Spectral karyotyping (SKY), Spectral karyotyping is based on the principle of FISH. It helps to diagnose a variety of diseases, because of its technique to paint each of the 24 human chromosomes with different colors. [30] In SKY, the color emission of chromosomes is

determined by the combination of painting probes and fluorochromes. In this technique, new colors can be developed by extracting a pair of different fluorescent dyes. For example 31 types of colors can be generated by using five types of fluorescent dyes by implementing 2N-1 formula. (31)

Comparative Genomic Hybridization (CGH), CGH was first developed to survey DNA copy number variations across a whole genome. With CGH differentially labeled test and reference genomic DNAs are hybridized to normal metaphase chromosomes and fluorescence ratios along the length of chromosomes provide a cytogenetic representation of the relative DNA copy number variation. It is used to detect cryptic deletions and duplications. One limitation of CGH is its small resolution which is up to 10-20 MB only. (32)

Array comparative genomic hybridization (array CGH), Array CGH is an advance form of CGH technology that allows detection of micro-deletions and micro-duplications. In this genomic plasmids or cDNA clones are used for hybridization instead of metaphase chromosomes as in conventional CGH technique. In array CGH thousands of short sequences of DNA probes, arranged in a precise grid on a glass slide called a chip. Fluorescently labeled DNA from reference and patient samples are mixed together and applied to the chip. The fragments of DNA hybridize with their matching probes on the array. The chip is then scanned in a machine called a microarray. (33, 34)

Some molecular cytogenetic work has been done on CKD patients. Jimenez et al (35) reported stress-induced premature senescence (SIPS) immunocompetent cells in dialysis patients using Flow-FISH and concluded that stress-induced premature senescent cells are responsible for decrease in telomere length. 16p deletion has been reported in CKD patients using CGH technique. Afonso *et al* (36) indentified loss of 1p, 20q and 16p, gains of 5q, 6q, and 13q along with monosomy of chromosomes 19 and 22 in dialysis patients and kidney transplanted patients. Microdeletions within 16p11.2 has also been reported and suggested that this micro-deletion would be associated with renal and enteric development abnormalities. (37) Using genome-wide association studies (GWAS) Yamada *et al* (38) identified chromosome 3q28 which may be a susceptibility locus for CKD in Japanese individuals. Xia *et al* (39) identified trisomy of chromosomes 7 and 17 and loss of Y chromosome in Papillary renal cell carcinoma (PRCC) tissue using FISH technique.

Conventional cytogenetic biomarkers/techniques for the detection of genomic instability in CKD patients

High genomic stability probably due to buildup of uraemic toxins and other genotoxic endogenous substances are reported in CKD especially patients on dialysis therapy. Many studies have been conducted to explore the mechanism behind DNA damage in CKD patients. Oxidative stress via production of reactive oxygen species was found to be major cause of genomic instability in CKD patients. (40-42) Table 1 shows the cytogenetic biomarkers and their findings with reference to CKD patients. To measure the DNA damage, following different cytogenetic biomarkers were used.

Micronuclei (MN) Frequency- Micronuclei are membrane covered condensed chromatid bodies which are formed during mitosis and an indicator of chromosome breakage due to misrepaired or unrepaired DNA abrasions. (43) Micronuclei are potential *in vivo* and *in vitro* marker of exogenous and endogenous DNA damage. Apart from Micronuclei, the other nuclear abnormalities like nuclear buds and nucleoplasmic bridges are biomarkers of genotoxicity and sign of chromosomal instability that are often seen in malignancies. For the evaluation of presence and extend of chromosomal damage in human population exposed to genotoxic compounds, micronuclei frequency is extensively used in cytogenetics as a biomarker. (44)

Comet Assay**-** The comet assay or single-cell gel electrophoresis is a sensitive technique used to measures breaks in DNA strand, alkali labile sites, and relaxed form of chromatin in individual cells. (45) In this assay, electrophoresis is done on agar embedded cells. Cells with damaged DNA migrate faster toward the pole than cells with whole and intact DNA material. DNA damage is measured through length of DNA tail or computer assistance.

Sister chromatid exchange (SCE) assay**-** Sister chromatid exchange is the exchange of

genetic material between two identical sister chromatids. In SCE both DNA strands break followed by an exchange of whole DNA duplexes. SCE is the indicator of recombination repair, point mutation, gene amplification and cytotoxicity. In this assay lymphocytes are cultured with bromo-deoxy-uridine (BrdU) and further stained with Giemsa. Exchanged DNA stained light while normal DNA stain darks with giema stain in this assay and can be seen under microscope. [46]

Cytokinesis-Blocked Micronucleus (CBMN) assay, The cytokinesis-block micronucleus assay is used to measure DNA damage in human lymphocytes. This assay is same as MN frequency assay but in this assay cells are blocked in the binucleated stage using cytokinesis inhibitors. In the CBMN assay, nucleoplasmic bridges and nuclear bud are easily observed because cytokinesis is blocked with inhibitor agents. [47]

Genotoxicity and cytotoxicity in CKD patients using cytogenetic biomarkers has been reported by number of researcher. Patients on dialysis therapy are more prone to genomic instability. It is documented that patients on daily routine hemodialysis, hemodiafiltration and peritoneal dialysis have different level of DNA damage. Studies reported high MN frequency was found to be in hemodialysis and peritoneal dialysis patients [48, 49] but on the other hand Kobras *et al* [50] reported no significant change in the frequency of MN in patients who switched from hemodialysis to hemodiafiltration. High DNA damage using comet assay and high SCE frequency has been reported in chronic renal failure patients and patients on hemodiafiltration. [51-53] Not only adults but children on dialysis had cytogenetic abnormalities. MN frequency was found to be high in children on hemodialysis therapy followed by peritoneal dialysis and kidney transplant. [54]

Case studies

Case studies reported unique finding in patients. Distinctive cytogenetic findings are documented in CKD patients. There is correlation between CKD and mental retardation. Case studies showed patients suffered from kidney impairment also had mental disability. [55] Other case studies findings are summarized in table 2.

Consequences of genomic instability in CKD patients in respect to cytogenetic findings

High incidence of cardiovascular disease and cancer has been reported in patients with ESRD. [63, 64] DNA damage, which can act synergistically with oxidative stress and inflammation, might be involved in the development of long-term complications like amyloidosis, atherosclerosis, and malignancy in CKD patients. [65] A high frequency of cancer comes into view among uremic patients. Low DNA repair ability, absence of activity of Glutathione S-transferase M1 (GST M1-belongs to family of GST protein and protect cellular DNA against oxidative damage), accumulation of SIP senescent cells and supplementation of high-glucose peritoneal dialysate may promote oxidative mitochondrial DNA damage are thought to be the causes for DNA damage and malignancy in uremic patients. [66-69] High frequency of micronuclei, SCE and DNA tail has been reported in dialysis patients. [70] There is a difference in percentage of DNA damage has been noticed in dialysis patients. The different cytogenetic finding in CKD and dialysis patients reported by researchers and concluded that dialysis patients are at high risk of developing cancer due to high genomic instability. [71] Hemodialysis patients showed maximum DNA damage as compared to patients received hemodiafiltration therapy (Table-2).

MANAGEMENT OF CKD

Prevalence of CKD is increasing worldwide with the associated increase cost has profound public health and economic implications. [72] Not only the cancer is associated with CKD but cardiovascular ailments are also very prominent in patients with CKD because of the accumulation of toxins in kidney. [73] Recommendations from previous studies, such as improvement in the procedure of dialysis therapy, tailored medication regimes, inhibiting the advanced glycation end products by supplementation of antioxidants, vitamin C, oral supplementation of cysteine prodrug which reduces glutathione level in blood and vitamin E (α-tocopherol) might help in better management of CKD. [74-77] Mode of action of each regime for management of CKD is different. Vitamin E inhibits the activation of interleukin -1β and release of monocytes O_2^- which are involves in

the initiation of oxidation of lipid, platelet aggregation and adhesion of monocytes to the endothelium. These activities promote atherosclerotic plague in CKD patients. [78] Patients on hemodialysis supplemented with vitamin E reduce reactive oxygen species in plasma. This confirm with the use of 8-hydroxy 2'-deoxyguanosine test and comet assay. [79, 80] Production of ROS through upregulation of NADPH oxidase as a result of activation of Nuclear factor- κB (NF-κB) pathway is reported in CKD patients. AGEs and angiotensin II plays an important role for the activation of NF-κB pathway. By supplementing angiotensin-converting enzyme inhibitors or angiotensin II receptor antagonists, might help in reducing the effect of oxidative stress in CKD. [81] Stopper *et al* [82] conducted an experiment on tubular cells incubated with various DNA damaging advanced glycosylation end products (AGEs) and antioxidants and found antioxidant suppressed the toxic action of AGEs. Researchers also suggested that daily hemodialysis therapy can efficiently removes the glycation end products in the body and offer better control of the production of AGEs in ESRD. [83] For the better management of CKD not only medical supplements have been given to patients however hospitals and government also have a good contribution towards the betterment of CKD patients. Multicenter consortia are engaged in recruiting large numbers of multiplex diabetic families with index cases having nephropathy for linkage and association analyses using various cytogenetic techniques. In addition, large-scale screening studies are underway, with the goals of better defining the overall prevalence of chronic kidney disease, as well as educating the population about risk factors for nephropathy, including family history. [84]

Conventional versus Molecular cytogenetic techniques

Currently, it is estimated approximately 1 million classical cytogenetic and molecular cytogenetic analyses are performed for standard care of patients suffering from congenital malformations, mental diseases, cancers, reproductive problems and other diseases. [85] Human karyotype is generally studied by classical cytogenetic techniques. For G banding, one has to obtain metaphase chromosomes of mitotic cells. This leads to the unfeasibility of analyzing all the cell types, to moderate cell scoring, and to the extrapolation of cytogenetic data retrieved from a couple of tens of mitotic cells to the whole organism, suggesting that all the remaining cells possess these genomes. However, this is far from being the case inasmuch as chromosome abnormalities can occur in any cell along ontogeny. [86] Since somatic cells of eukaryotes are more likely to be in interphase, the solution of the problem concerning studying postmitotic cells and larger cell populations is interphase cytogenetics, which has become more or less applicable for specific biomedical tasks due to achievements in molecular cytogenetics (i.e. developments of fluorescence in situ hybridization -- FISH, and multicolor banding -- MCB). [87] Molecular cytogenetic techniques have been repeatedly proven effective in diagnostics and have been recognized as a valuable addition or even alternative to chromosomal banding. [88-89]

Table-1: The cytogenetic finding in CKD and dialysis patients.

Cytogenetic biomarker	Stage of disease/ treatment being taken	Findings	References
Comet assay	206 pre-dialysis CKD patients and 209 CKD patients in hemodialysis	No significant differences of DNA damage were observed between pre-hemodialysis (pre-HD) and hemodialysis (HD) patients.	Corredor *et al*[94]
Comet assay and cytokinesis-block micronucleus assay	91 CKD patients including pre-dialysis (CKD patients; n = 23) and patients undergoing peritoneal dialysis (PD; n = 33) or	Micronucleus (MN) frequency was significantly higher in the CKD group when compared with the control. A significant increase in MN frequency was also seen in PD patients	Rangel-López *et al*[95]

	haemodialysis (HD; n = 35)	versus the control group. There was no statistically significant difference for the HD group versus the control group. Comet assay data showed a significant increase of tail DNA intensity in cells of patients with CKD with respect to the control group. PD patients also have a significant increase versus the control group. Again, there was no statistically significant difference for the HD group compared with the control group.	
MN assay	Patient on hemodialysis and ESRD patients	High MN frequency was observed in hemodialysis patient followed by ESRD patients	Stopper *et al*[96]
Comet assay	Blood samples of hemodialysis patients were collected in three intervals i.e. start of dialysis (T(0)), at the end of the treatment (T(end)) and 24 hours afterwards in the interdialytic day (T(inter)).	COMET assay performed on CD34(+) cells showed a higher basal level of genomic damage in HD patients than in controls; it increased in a statistically significant manner after the hemodialysis session, while in the interdialytic period it came back to T(0) level.	Buemi *et al*[97]
Comet assay	Patient with CKD and long-term maintenance hemodialysis (MHD)	maximum damage in patients who received MHD therapy longer than 10 years than CKD patients	Stopper *et al*[98]
Comet assay	Chronic renal failure patients and dialysis patients	Dialysis patients show high DNA damage than chronic renal failure patients.	Stoyanova *et al*[99]
Comet assay and MN frequency	Patients received hemodialysis and hemofiltration therapy	Patients who switched from hemodialysis to hemodiafiltration, a significant reduction in the comet assay but not in the micronucleus frequency was observed.	Kobras *et al*[100]
Comet assay and MN assay	3 groups was included 1.standard hemodialysis (SHD),2 switch from SHD to hemodiafiltration, and 3: daily dialysis (DHD).	Initiation of SHD did not induce significant changes of genomic damage whereas the change to hemodiafiltration improved the percentage of DNA in the tail as measured by comet assay. Genomic damage evaluated by MN frequency	Schupp *et al*[101]

		was significantly lower in a patient group treated by DHD as compared with a group treated by SHD.	
SCE	HD patients on regular maintenance acetate-free bio-filtration (AFB) and samples were drawn 3 times: predialytic, postdialytic and interdialytic (24 hours after the end of the session).	In AFB patients, the per-centages of SCE was recorded 6 %. After AFB session the percentage of SCE was recorded 7.02 %. 24 hours letter a further increased was observed i.e. 9.82%. Expression of genomic damage increases gradually on AFB therapy followed by after AFB therapy.	Pernice *et al*[102]
SCE and mitotic index	Chronic renal failure patients	high frequency of SCE and low percentage of mitotic index was found in CRF patients	Lialiaris *et al*[103]
SCE and MN frequency	Patients on hemodiafiltration	SCE and MN frequency levels are significantly higher in patients on hemodiafiltration	Buemi *et al*[104]

Table 2: Findings in CKD patients case reports

Cytogenetic Techniques	Case study	Interference	References
G banding	66 year old Japanese man which was on hemodialysis and developed Acquired cystic disease (ACD)-associated renal cell carcinoma (RCC)	49, X, +X, -Y, +3, +7, +16 unusual karyotype	Kuroda *et al*[56]
FISH, CGH using auto immune regulator full gene sequencing	12-year-old Saudi boy with chronic renal failure and other symptoms	FISH results revealed telomeric deletion of chromosome 4q33 and CGH study using AIRE (auto immune regulator) full gene sequencing identified a homozygous mutation namely 845_846insC.	Al-Owain *et al*[57]
FISH	young man suffered from chronic renal failure because of urinary tract obstruction	de novo terminal deletion of chromosome 10 del(10)(q26.1).	Leonard *et al.,*[58]
Flow cytometry and karyotyping	seven year old boy having membranous glomerulonephritis, cryptic cirrhosis and mild mental retardation	diploid, triploid and tetraploid mosaicism	Topaloglu *et al*[59]

G banding and FISH	fetus with Meckel syndrome (characterized by enlarged kidneys with numerous fluid-filled cysts)	CEP290/MKS4 (MIM611134) (12q21) nonsense mutation and 12q21 microdeletion revealed that this nonsense mutation and microdeletion was inherited from maternal and parental side and associated with characteristic renal cysts along with facial dysmorphism, impaired liver and brainstem anomalies[47]	Molin *et al*[60]
G banding	35 year old male with immunoglobulin G k-type Multiple myeloma and dialysis-dependent chronic glomerulonephritis	17p deletion	Aoki *et al*[61]
G banding, FISH and CGH	21 year old Thai women having CKD stage 4 with elevated blood pressure and mental retardation.	chromosome 20p inverted duplication deletion syndrome. Conventional cytogenetic study revealed the complex structural rearrangement of chromosome 20 [der (20) dup (20) (p11.2p13) del (20) (p13.pter)]. A FISH analysis, confirmed inverted duplication of p11.2-p13 and a deletion in the subtelomere region. Array comparative genomic hybridization detected a copy loss at 20p13 co-existing with a copy gain at 20p13-20p11.22.	Trachoo *et al*[62]

Table 3: Molecular study conducted on CKD patients and their findings

Molecular Cytogenetic Techniques	Study Group	Interference	References
CGH	ESRD patients on dialysis with upper urinary tract urothelial carcinoma (UUT-UC)	gains at 5p, 7, 19q, and losses at 4q, 9p, and 15q	Wu *et al*[90]
CGH	Autosomal dominant polycystic kidney disease patients	Deletion were mostly detected on chromosomes 1, 9, 12, 16, 19, and 22 (maximum samples), DNA sequences loss on chromosomes 7, 12, and 13 (three samples) 5, 6, 10, and 14 (two cases) 1p36 (six cases) whereas gain of DNA sequences on chromosome 3 (six cases), chromosome 4 (five cases) and chromosome 2 (3 samples).	Gogusev *et al*[91]

FISH	Acquired cystic disease-associated renal tumors patients	Gains of chromosomes 1, 2, 6 and 10	Cossu-Rocca *et al*[92]
	Chronic kidney disease patient	Missense mutations on the GNAS1 gene exons 1, 4, 10, 4 and reported this type of missence mutation would be new syndrome lies between sagliker syndrome, CKD and hereditary bone dystrophies.	Yildiz *et al*[93]

Conclusion

Cytogenetic biomarkers/techniques play an important role for the detection of chromosomal abnormalities and genomic instability in CKD patients. Novel molecular cytogenetic techniques hastily provide new insights into kidney diseases, especially regarding their nosologic classification, diagnosis, mechanistic understanding, and development of new therapeutics. There is a lack of literature in the field of genetic mechanism behind the difference in level of DNA damage among patients on different dialysis therapy. For the betterment of health of CKD patient's research should be done on molecular level. In conclusion, cytogenetic finding revealed CKD patients especially patient on dialysis have high degree of DNA damage which might be path towards progression of neoplasm in CKD patients.

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