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ORIGINAL ARTICLE

Assessing Chromosomal Abnormalities Caused by Brain CT-Scan Exposure of Children by Molecular and Cytogenetic Methods (QF PCR)

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ABSTRACT
Background and objectives: With advances in cytogenetic science and new molecular methods genetic assessment can be made in pediatric patients undergoing brain CT scan (the most widely used CT scan).The aim of this study was to determine quantitative and qualitative chromosomal changes in the blood of pediatric patients exposed to low dose irradiation for as well as lifetime attributable risk [LAR]. Methods: 6 children under 10 years of age referred to hospital for a brain CT scan for various medical reasons. Were selected for cytogenetic analysis all on blood samples taken before and after CT scan. The new QF PCR method was applied for quantitative evaluations on STR regions of five major chromosomes 13, 18, 21, X, and Y. The G and solid banding methods were used to acess structural and numerical alteration on all chromosome . Finally a comparison was made for results obtained from both QF_PCR and cytogenetic analyses. Results: Results showed no significant structural and numerical abnormalities in karyotype profiles on lymphocyte sample taken before and after CT scan radiation exposure and the sequencing analysis shown that of 6 children 3 had abnormality on somatic chromosome 13,18,21 with only abnormal markers. Discussion and conclusion: karyotype file show nosignificant structural and numerical alteration .none the less QF-PCR methods indicated 50% aberration on somatic chromosome. Hence13.21.18 with only one or two abnormal marker which consider cal to be also an significant . hence both molecular and cytogenetic methods can complement the genetic analysis..
Keywords; *CT, QF PCR, Karyotype, solid. G-Banding, Child*

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INTRODUCTION

The CT scanning is a medical imaging process that presents high exposure to radiation. Medical use of CT scanning is increasing on a daily base. In the mid-1990s, about 4% of medical imaging was performed with CT scans, which included 40% of total exposure dose. Today, with developing helical technology and multi-slice techniques, it contains 15% of medical imaging including 75% of the total exposure dose of medical radiation [1].

One of the most widely used imaging scans is that of brain CT imaging of children, witch applied at the mean doses of 40 mGY and 60mGY as standard protocols [2,3].

Most researches have focused on the effect of high dose radiation, such as radiotherapy and accidental radiation however the effects of low dose radiation in children have not fully explored. The majority of surveys were statistically equivalent, generalized and compared various high doses of accidental radiation exposure (radiotherapy, nuclear disasters of Hiroshima, Nagasaki and Chernobyl). Doses used in CT scanning vary from a few msv to 100 msv; and a medical dose under 100 msv is considered a low dose according to the BEIR VII report (Biologic Effects of Ionizing Radiation) [3, 5].

Children are about ten times more sensitive to radiation ill effect on their chromosomes than adults hence they are more likely to suffer from chromosomes aberration during their lifetime, this is termed as lifetime attributable risk (LAR) [6]. Today, with the help of new advances in cytogenetic science and molecular methods in studying genetic defects in paediatric patients undergoing a brain CT scan by evaluating quantitative and qualitative chromosomal changes.

The Quantitative fluorescence polymerase chain reaction (QF- PCR) is a new molecular method that replicates short tandem repeat regions (STR) on five chromosomes 13, 18, 21, X, and Y by multiplex PCR technique and then quantitatively assesses all copies of five markers for each chromosome using DNA sequencing analysis [7]. This method examines various peaks produce by STR on chromosomes using 4to 5 markers with high sensitivity and accuracy, but it does not have the ability to check the chromosome structure, therefore chromosome banding methods such as G-banding and solid karyotype were used to study structural aberration on chromosome this study [8].

The low dose radiation risk, as one of the main concepts in radiation protection; feasibility and optimization of medical imaging can support discussions on the issue. Therefore, by examining and comparing results of molecular and cytogenetic methods in each patient, a complete genomic profile of quantitative and qualitative chromosome anomalies in relation to CT scan radiation dose was prepared to assess the risks of DNA damage and associated LAR so that further measures can be taken to protect children from radiation and to check their health status after exposure.

MATERIALS AND METHODS

In this study, six volunteer children with normal health aged 0 to 10 years of age (three boys and three girls) were studied who had been referred for CT scans solely because of trauma and had no previous exposure to radiation. Four blood samples were taken from each patient in which two were taken , 10 minutes before the brain CT scan and one to 24 hour(s) after the scan.

BLOOD SAMPLING

At least 3 ml of all blood samples (before and after CT radiation) were collected in EDTA and heparinized tubes. The tube was shaken gently for 2 minutes to mix thoroughly labelled with the patient's name of age and then transferred quickly to the genetics laboratory in accordance with lab standard [50].

DOSIMETRY

Terms of radiation for brain CT were chosen based on Siemens and Philips standards and ct scan dose index (CTDI) was determined for each patient by Monte Carlo software [9,10].

PROCEDURE OF TESTS:

In summary, the following steps were taken for these two tests:

(A) Heparinized sample ►► culture ►►lymphocytic karyotype ►► analysis

(B) Non-heparinized sample ►► DNA extraction ►► QFPCR ►► sequencing analysis

A. Cytogenetic: Cultivation and harvest procedures of lymphocytes followed by the sliding preparation were performed. the prepared slides were dried at 37°C for 24 hours at 45-60°C for three hours. Slides were divided into two groups of treated and 0.05% untreated with trypsin for 10-60 seconds at 37°C. slides were washed in cold PBS liquid and placed in 10% giemsa for 5-7 minutes and finally the slides were washed. After this stage, metaphases were visible on the buses of both slid and G banding .all slides G and solid banding metaphase were than karyotype microscopically to determine structural and numerical anomalies on all chromosome induced by irradiation.

B. Molecullar method : The DNA extraction was performed on blood cell, QFPCR was done as follows:

1. Aniopak kit containing 5 chromosomes 13, 18, 21, X, and Y and two master mik S1 and S2, divided equally, were used.
2. For each microtube, 10 microliters PCR from the collection of master mix S1, S2 was used.
3. Two microliters of distilled water were added to the microtube.
4. Five microliters of DNA was added to each microtube.
5. Tubes were spun for 10 seconds to mix the contend
6. Finally Tubes were placed in PCR machine to amplity STRs on chromosome

In the final step, sequencing was done on all PCR product. At this point, QF PCR products from each sample, marked by color fluorescence, were sequenced by 4and 5 markers. chromosomes 13, 18, X, and Y were marked with 4 markers and chromosome 21 with 5 markers. For final evaluation, at least 3 markers for each chromosome was considered positive for normal or abnormal and 1 or two out of the 4 markers were considered insignificant

RESULTS

Assessment of the results of karyotype and QFPCR tests were as follows:

KARYOTYPE PROFILE

Karyotype analysis, were clone on both G and solid banding metaphases totale of 72 slides before and 72 slides after exposure to radiation were analyzed. These slides were been produced from blood lymphocytes of children less than 10 years of age. In each sample, at least 20 metaphases (solid and G banding) were assessed for aneuploidy, polyploidy and structural abnormalities on chromosomes. In case of quantitative or qualitative abnormality in the karyotype metaphases, 30 more metaphases were studied.

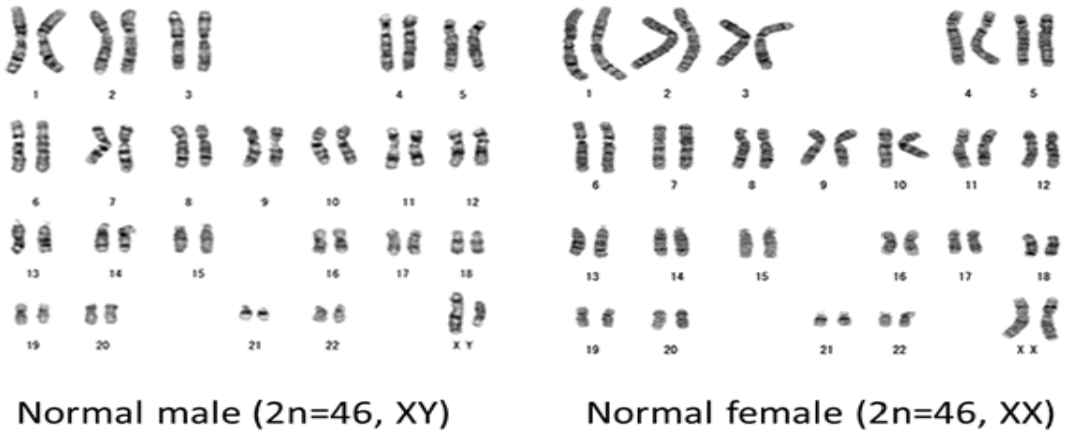

Fig 1- Karyotype profiles in metaphase with G stain technique

RESULTS OF KARYOTYPE TEST

Karyotype profile showed no aberration (qualitative or quantitative) on lymphocytic chromosomes, in both before and after CT scan exposure.

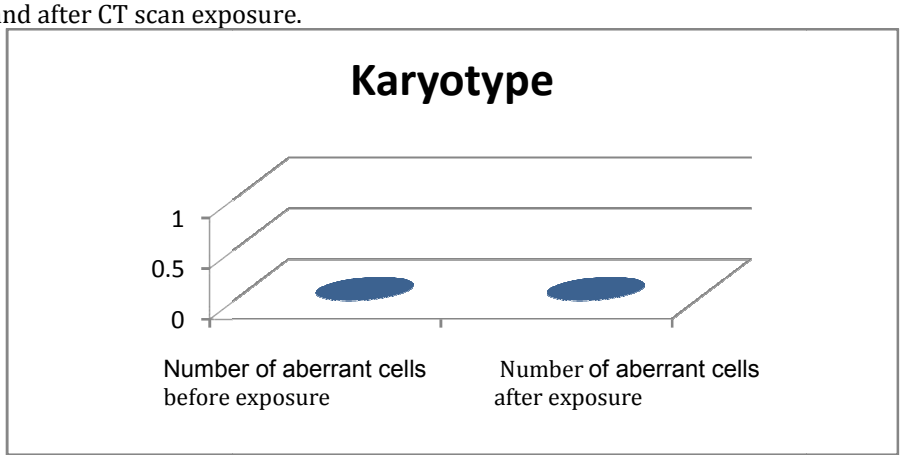

Fig 2- Comparison of cytogenetic analysis of chromosomes before and after irradiation

QFPCR

final sequencing, results of STRs on five major chromosomes 21,13, 18, X, and Y were analyzed using with 4-5 markers. In interpretations of tests, at least 3 markers for each chromosome were needed to be normal or abnormal, but if 1 or two markers were detect the result was considered as 'insignificant'. all markers were represented by peaks product by STRs chromosomes.

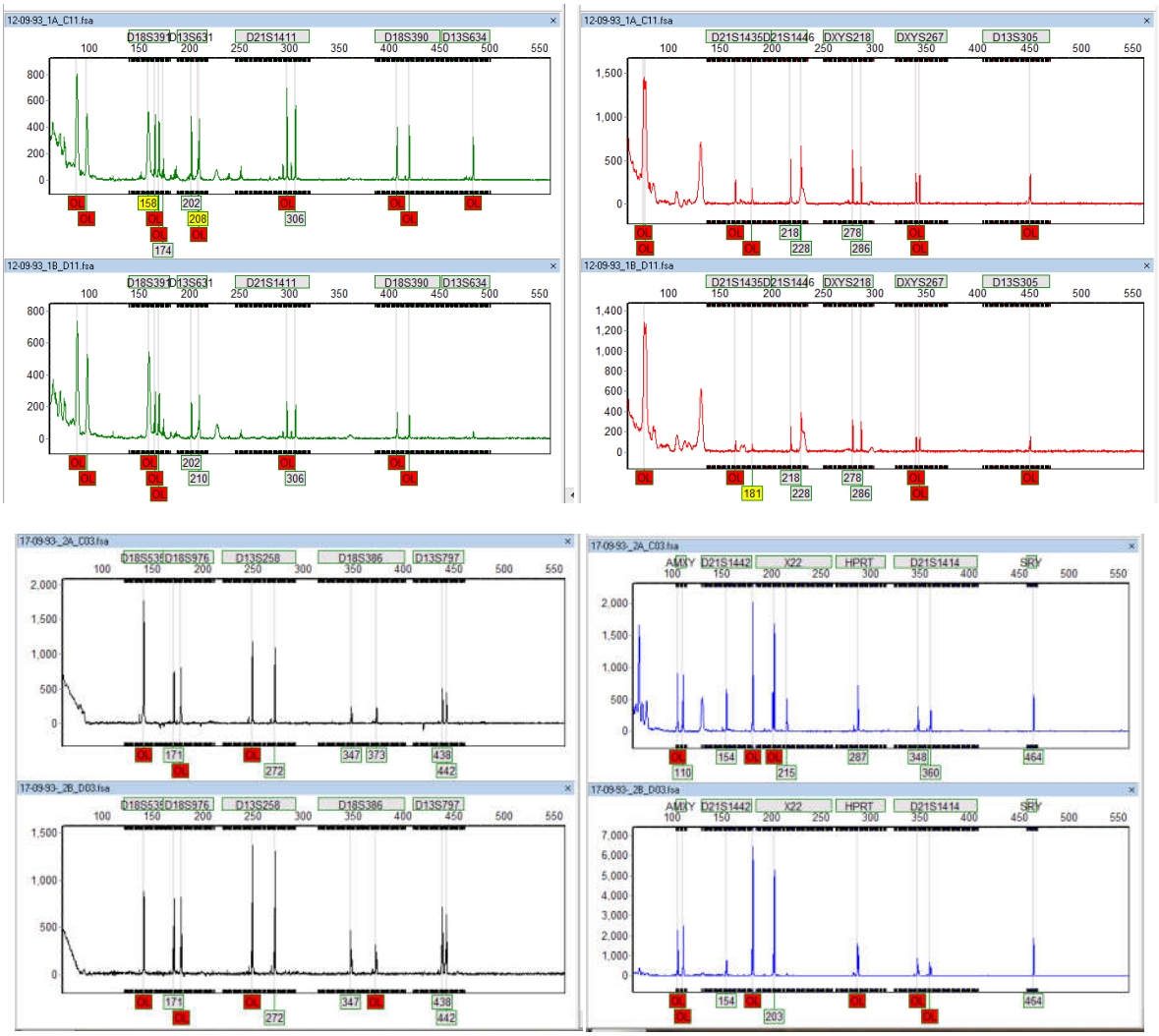

Fig 3- the sequences analysis of 4 stir regions on chromosome 21, 18 ,13, X and Y

RESULTS OF QF PCR TEST

Regarding the number of samples, non-parametric tests were used and peak based quantitative analysis were performed before and after exposure. The data of the two groups was dependent, the Wilcoxon test was used for comparing two dependent groups in non-parametric conditions.

According to P-value of the Wilcoxon test, which were larger than 0.05, no significant difference was observed in the quantity of all five chromosomes21,13, 18, X, and Y before and after irradiation.

Fig 4- Average of QFPCR's marker in paediatric samples

Average of QFPCR's marker																				
Chromosome 21				Chromosome 18				Chromosome 13				Chromosome Y				Chromosome X				
4	3	2	1	4	3	2	1	4	3	2	1	4	3	2	1	4	3	2	1	Number of marker
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Before exposure
0	0	0	0.16	0	0	0	0.16	0	0	0	0.33	0	0	0	0	0	0	0	0	After exposure
0.317				0.317				0.317				1				1				p

DISCUSSION

Ionizing radiation, whether natural or artificial, exists in the environment and affects people. Thus, the extent of our knowledge of the possible DNA damage, especially from man-made methods can improve our knowledge in management, optimization and feasibility of radiation. From the early 20th century unintended side effects have been revealed from the ever-increasing use of the recently discovered x-ray. Examinations and investigations started from the beginning and were developed with technical and laboratory progress. Progress and innovation in genetic analysis the past decade has developed modern research in this field. In this decade, different laboratory tests including cytogenic and molecular technique have been employed by physicists and radiobiologists that present more accurate analysis of the effects of ionizing radiation.

In 2003, R. M'kacher and colleagues analyzed chromosomes in lymphocytes of 10 patients after CT scans. They used common cytogenetic techniques (fluorescence in situ hybridization and premature chromosome condensation (PCC)) and concluded that no chromosomal aberrations were observed despite changes in frequency of chromosomal fragments. They suggested more careful behavior with younger patients and considering justifying; Hence in the current study, we evaluated younger patients and children with both karyotype and QF PCR tests.

Ernest KJ Pauwels, Michel Bourguignon and colleagues declared in their paper published in 2010, that estimations for cancer risk assessment in patients undergoing CT radiation were suspicious and questionable. It was claimed that radiation under 50 msv, common in CT scans, could not be generalized to higher doses [44]. As a result of this research and in accordance with accuracy of testing, we calculated that patients undergoing CT scan had a radiation of about 10-20 msv directly with no qualitative and quantitative aberration on their hromosome and this could not be generalized.

In 2012, a review article published by Michel Bourguignon and Ernest KJ Pauwels presents statistical data in solid tumors in children who underwent CT tests. They considered low probability of cancer but importance with respect to the linear effect of non-threshold(LNT) and considered justifiability in children undergoing CT evaluation [46].

In this assessment, direct radiation received by patients, their lymphocytes were collected in two banding methods and QFPCR tests before and after irradiation. The examination and analysis of QFPCR test resulted in observing various STR markers indicating the of five chromosome 21,13, 18, X, and Y. Tri-peak STRs were found mostly on chromosome13 followed by 18 and 21 but since quantitative anomalies were represented by only one or two markers it was not significant quantitative aberration was found on sex chromosomes .

Assessment and analysis of profiles by solid and G banding, showed no aberration in structure and number of chromosomes (qualitative and quantitative) before and after CT scans exposure. Therefore Both cytogenetic and molecular analysis of lymphocytic methaphase can complement each other and provide complete profile for genomic evaluation.

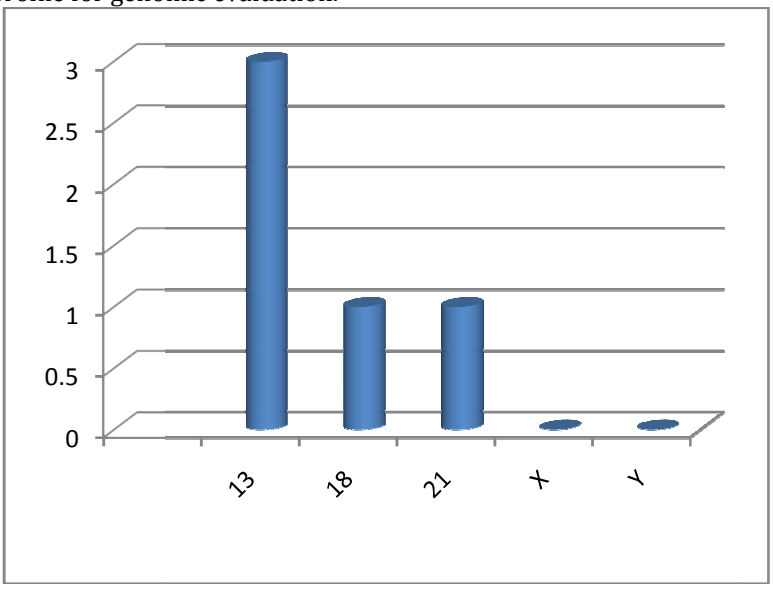

Fig 5- frequency of the sequences markers on five chromosome

CONCLUSION

The results of the current study showed that despite some changes in frequency of chromosomal fragments in QF PCR test, no significant changes were observed in structure and number of chromosomal exposure to CT scan radiation.

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