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Cytogenetic study of types anemia patients

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***Abstract*---**Background and Objectives: studying chromosomal changes for anemia patients of children age (3month-12years) in the city of kut and diagnosing those abnormalities resulting from having anemia Methods: The chromosomes of anemia Patients to were analyzed and studied using cytogenetic analysis to detect chromosomal aberrations caused by having anemia after collecting blood samples from children patients in Al-Karama Teaching Hospital as well as from the to the patients clinics in Kut.Results: Chromosomal analysis of all subjects showed chromosomal aberrations, and most of the aberrations were structural and numerical changes that structural in the (1 out28)of the chromosomal abnormality was for the gap chromosome type, chromosomal-aberration dicentric (2 out 28) ,chromosomal-aberration deletion (1-28) addition to the short chromosomal and clumped metaphase of patient .As well as changes chromosomal aberrtion were numerical in the chromosomal –aberration absent (1-28)it was due to leukemia that was anemia causative .And classification of anemias based on Size of red blood cells Microcytic, Normocytic , Macrocytic The most commn prevalent and preventable form of microcytic anemia is iron deficiency anemia Conclusion: Chromosome abnormlity (abnormal number ,structural)it occure (abnormlity structural)in our study present short chromosomes and clumped metaphase,deletion ,chromsomes gap ,chromosome dicentric ,and when an individual loses achromosome from one pair than to chromosme (abnormal nuber) chromosome absent .in the anemia patients.

***Keywords*---**Anemia, chromosomal aberrations,and thier cytogenetic anemia.

Introduction

Anemias are the blood disorders characterized by lacking in the number of circulating red blood cells, the amount of hemoglobin, or the volume of packed

red cells in blood. Chromosomal aberrations have often been reported from the bone marrow as well as cultured lymphocytes of the anemic patients. It is a very heterogeneous group of diseases that may be either acquired (mostly due to iron deficiency) or inherited. (Upma, et al.2010).An estimated 20 percent of American children will have anemia at some point in their childhood.1 Anemia is defined as a hemoglobin (Hgb) concentration or red blood cell (RBC) mass less than the 5th percentile for age. Hgb levels vary by age, and many laboratories use adult norms as references; therefore, the patient's Hgb level must be compared with age-based norms to diagnose anemia2

Awareness about anaemia and its consequences for the health and development of women and children has increased in the past few decades. In 2012, the 65th World Health Assembly approved an action plan and global targets for maternal, infant, and child nutrition, with a commitment to halve anaemia prevalence in women of reproductive age by 2025, from 2011 levels. As such, attention to nutritional interventions, such as the Scaling Up Nutrition initiative, has increased. Furthermore, emphasis has been placed on the reduction of risk factors that adversely affect women and children, for example in the UN Secretary-General's Every Woman Every Child initiative and the accompanying Global Strategy for Women's and Children's Health. To plan for these programmes and prioritise interventions, information is needed about haemoglobin and anaemia in women and children, and how they have changed over time .(WHO,2011)

Globally, it is estimated that 273 million (approximately 42.6%) of children under five years are anaemic, whilst 60.2% of children under five years in the African region are anaemic (WOH.2015) .Used sample collection blood of the patients children , carried out in the laboratories of university 0f wasit our study.To date, there are few studies that can determined type of anemia common and provide an overview of the prevalence of chromosomal abnormalities and using cytogenetic analysis, to determine the type of chromosomal abnormalities either structural or numerical, in chelidren in Iraq, . Therefore Sought study to determine changes chromosomal.

The objectives of this study : Studying chromosomal changes by examining the chromosomal by body means 0f G-band and micronuclei.

Method

Methods Participants

This study was conducted for a period of one year from October 2021 to October 2022in the Wasit University College of Education for Pure Science Biology Department, Complete clinical data and blood samples were taken from the anemia injured children age (3month -12year) at Al-Karama Teaching Hospital and private clinics. The study included 70 blood samples, 50 patients,29 female and 21males, and the control group was 20 blood samples .We collected data regarding sex (male,female),the age, family history and type of treatment . Cytogenetic analysis of peripheral blood samples was performed using traditional cytogenetic methods according (short time culture), and the use of culture media

(LymphoPrime Medium) and colchicine solution, reagents, and stains (KCL, PBS, fixative solution, trypsin solution, and others), many laboratory equipment and tools. Cellular proliferation and chromosomal analyses were performed according to (Geleick, et al. 1990 ; Yassen, 1990) with some modifications.

Blood samples for cytogenetic analysis

Peripheral venous blood samples were collected from all study participants between October (2021-2022). Blood samples (5 ml) were collected in heparinized tubes using a 5 ml syringe under aseptic conditions. The samples were cultured and evaluated for karyotyping.

Karyotyping

Chromosome culture was carried out by adding the blood sample to the artificial chromosome media (LymphoPrime Medium) which were ready to use media. After 70 h of incubation at 37 °C, 100 µl of colcemid (Merck, Darmstadt, Germany) was added. After a passage of 60 min, cells were harvested (1,500 rpm for 7 min). Then, a 0.075 M KCl solution was added, mixed and incubated at 37 °C for 15 min. After centrifugation (1,500 rpm for 7 min), the hypotonic supernatant was removed. Then, 10 ml of cold fresh fixative solution (3:1 methanol: glacial acetic acid) was added dropwise to the cell pellet for the first 2 ml to the cell pellet. Centrifugation was then performed and the supernatant was removed. The last two steps were repeated until a clear pellet was obtained. Finally, the obtained cells were dropped onto clean slides and stained with Giemsa (Salih et al., 2018).
Chromosomal status was analyzed using the (microscope -micros) Austria system of karyotype. Metaphases and chromosome preparations from peripheral blood cultures were examined according to standard cytogenetic protocols. Cytogenetic analysis was performed by G-banding. (28) metaphases were analyzed in all participants. Chromosomal abnormalities were reported according to the International System of Human Cytogenetic Nomenclature (McGowan-Jordan, Hastings & Moore, 2020

Results

During the period of study (20) control (normal) and (50) pateints children anaemia was total (70) sample. Their ages range from (3months - 12 years). There were 21 male and 29 female 0f group pateints children, and (10) male,(10) female of group control. found in the study no significant difference($p<0.05$) as shown in Table (3-1)

Table (3-1)
classification of patients according to age and gender

Age group	Gender				Total	
	Male		Female			
	%	N	%	N	%	N
3months-1 year	5	23.8	10.3	3	16	8
2 year-5year	28.5	6	27.5	8	14	28
6 year-10 year	4	19	34.4	10	28	14
11 year-12 year	28.5	6	27.5	8	14	28
Total	100	21	100	29	100	50
Control (normal) (3-12 year)	50	10	50	10	20	
Total	31		39		70	
Chi-Square Tests	P-value				0.344	

Of the 50 cases in the present study , The hemoglobin levels ranged from(HB < 11 g/dl), the age group ranged between (3month-12years)
Found number of chromosome changes in number and morphology in patients with anamia.

Found in the study :

*Chromosome deletion: chrmosmal aberration of male with anemia case No.(32) show under microscope(1000X) during metaphase as Figure (3-2) A and present karyotype in the chromosomal 46,XY,del (17)(p) as shown figure: (3-2B).
Chromosme break and gapa: Chromosomal aberration of female with anemia case No. (2) under microcope (1000 X) show in the metaphase, Present that chromosomal aberration karyotype 46 in the XX.gap(9).(10) as shown figuar : (3-3)A,B

* Chromosome dicentric : Chromosomal aberration of male with anemia case No. (15)and femalein case No.(19) under microcope (1000 X) show of the metaphase present karyotype in the chromosomal 46,dicentric (9) as showe Figuar:(3-6)A,B

* Chromosome absence : during Study found Chromosomal aberration of male with anemia case No. (43) under microcope (1000 X) showe absence chromosomal 22, Of metaphase and present in the(XY 22) change of chromosome number in Karyotype 45 in XY, -22, as showe in the figuar : (3-3) A ,B.
found after diagnosis of anemia type(39)cases iron deficiency ,(3)cases thalassemia minor,(3)cases infection anemia, (2)cases plastic anemia ,(1)case

megloplastic ,(1)case bone morro failur,(1)case myeloid leukemia sampleS total (50) cases of anemia patients.

So this study aim determina changes chromosomal by cytogenetic analysis because of the danger chromosomal aberration on survival.
Micronuclei (MN) are extra-nuclear bodies that contain damaged chromosome fragments and/or whole chromosomes that were not incorporated into the nucleus after cell division.

In this study found micronuclei and polymorphic nuclei in case number(43) ,micronucle in case No.(15) ,micronuclei in case No.(2) ,and micronuclei and multinuclear in case No.(32).As shown figuar (3-6)A,B,C,D.

Family history factore plays a part in risk in the anemia (genetic diseases) in children .
During study family history found (9 out 50) samples of anemia as shown in the table (3-2) has a history of aninherited anemia, and presence was observed (thalassaemia minor)cases three(15, 21 ,33) due to the synthesis of abnormal haemoglobin.

In this study we found after diagnosis of types anemia (39)cases iron deficiency ,(3)cases thalassemia minor,(3)cases infection anemia, (2)cases plastic anemia ,(1)case megloplastic ,(1)case bone morro failur,(1)case myeloid leukemia samples total of chelidren patients (50) cases of anemia patients. group of diseases that may be either acquired or inherited.

Table (3-2)
chromosomal abnormality in patients with anemia

No. of Patient	Age year	Gender	Chromosomal aberration	Micronuclei	Family history
1	9	F	46, XX	Nil	None
2	4	F	46, XX, gap (9), (10)	Presence	Father
8	6	M	46, XY	Nil	Mother
10	3	M	46, XY	Nil	None
11	9	M	46, XY	Nil	None
12	12	M	46, XY	Nil	None
13	12	M	46, XY	Nil	None
15	5	M	46, XY, dice (9)	Presence	Mother
17	11	F	46, XX	Presence	Mother
19	4	F	46, XX, dice (9)	Presence	Mother
21	12	M	46, XY,	Presence	Mother
23	12	F	46, XX	Nil	None
25	9	M	46, XY	Nil	None
27	11	M	46, XY	Nil	Mother
28	5	M	46, XY	Nil	None
30	9	F	46, XX	Nil	None
31	4	F	46, XX	Presence	None

32	9	M	46, XY, del (17), (p)	Presence	Father
34	8	F	46, XX	Nil	None
39	11	M	46, XY	Nil	None
43	12	F	45, XX, -22	Presence	None
44	7	F	46, XX	Nil	None
45	6	F	46, XX	Nil	Mother
46	11	M	46, XY	Nil	None
47	3	F	46, XX	Presence	None
48	3	F	46, XX	Presence	None
49	5	F	46, XX	Nil	None
50	11	F	46, XX	Nil	None

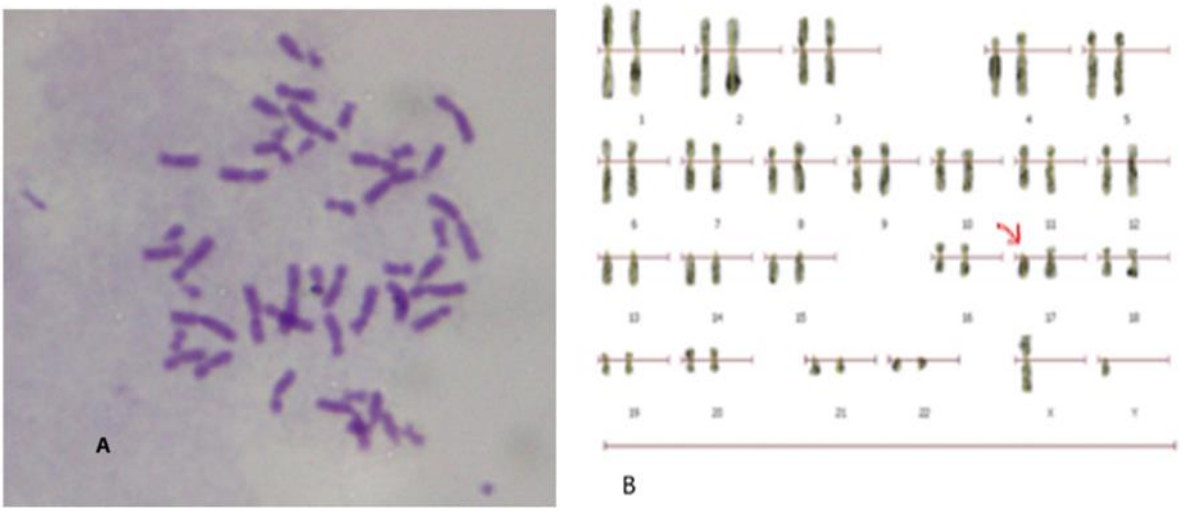

Fig. (3-2) Chromosomal aberration of male with anemia case No. (32) (1000 X) A: Metaphase B: Karyotype 46, XY, del (17) (p)

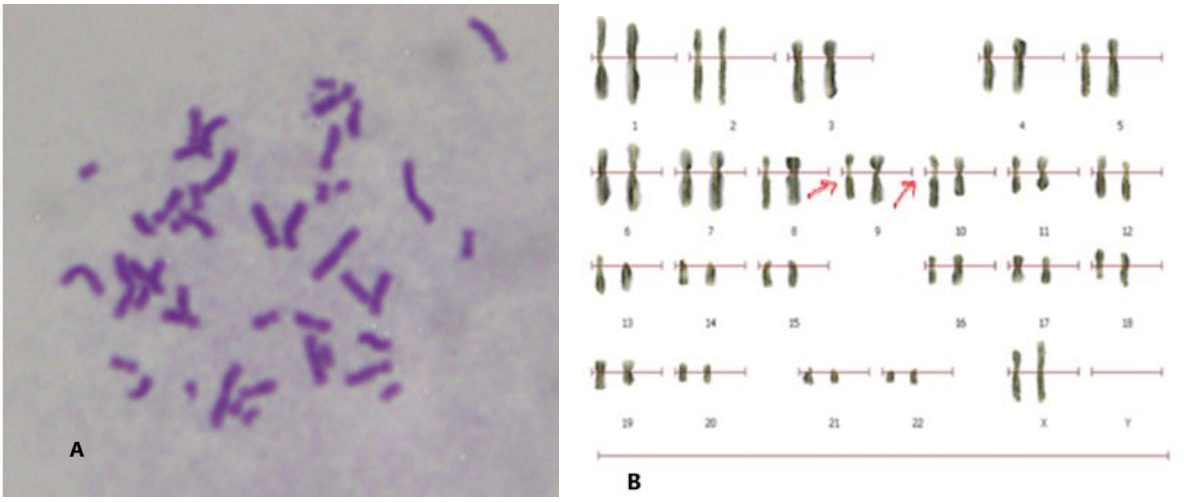

Fig. (3-3) Chromosomal aberration of female with anemia case No. (2) (1000 X) A: Metaphase B: Karyotype 46, XX, gap (9), (10)

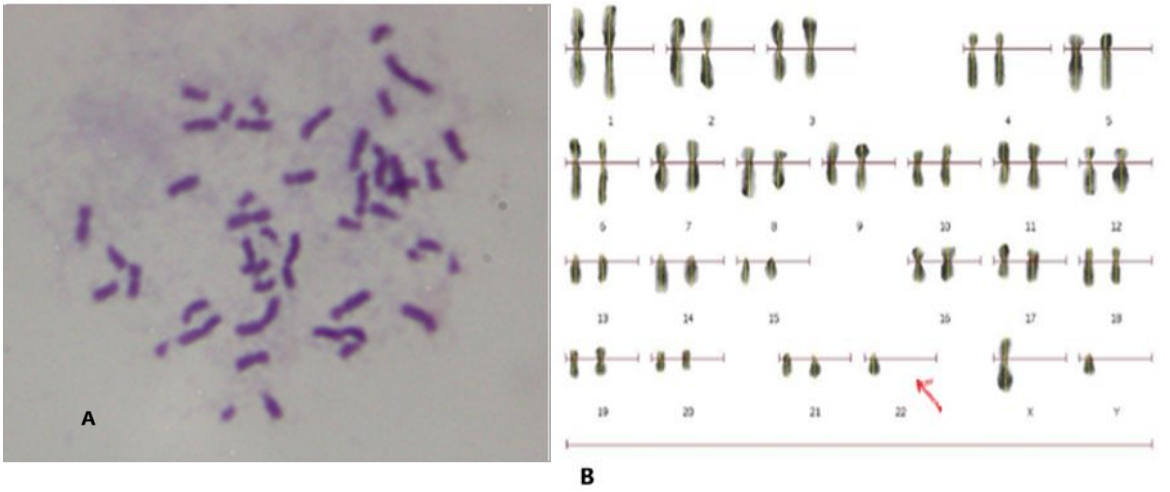

Fig. (3- 4): Chromosomal aberration of male with anemia case No. (43) (1000 X) Metaphase:A, B: Karyotype 45, XY, -22

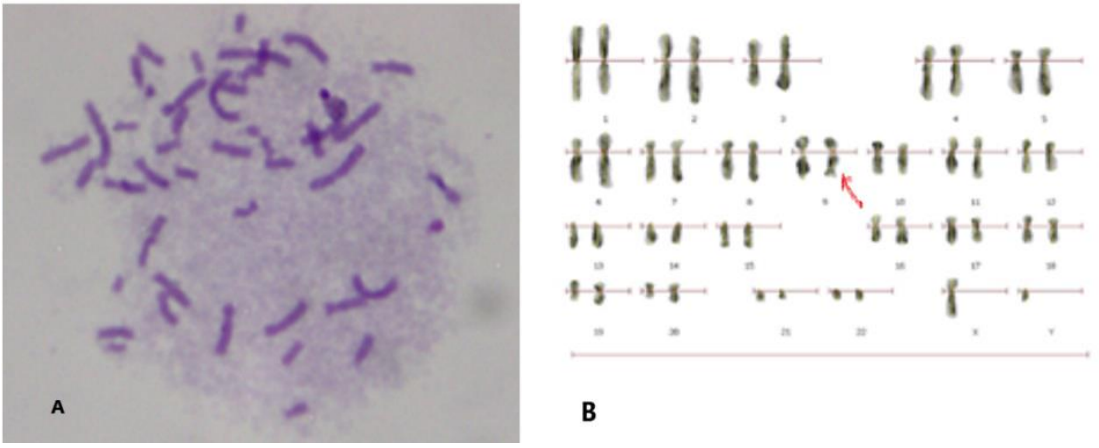

Fig. (3-5) Chromosomal aberration of male with anemia case No. (15) (1000 X) A: Metaphase B: Karyotype 46, XY, dice (9)

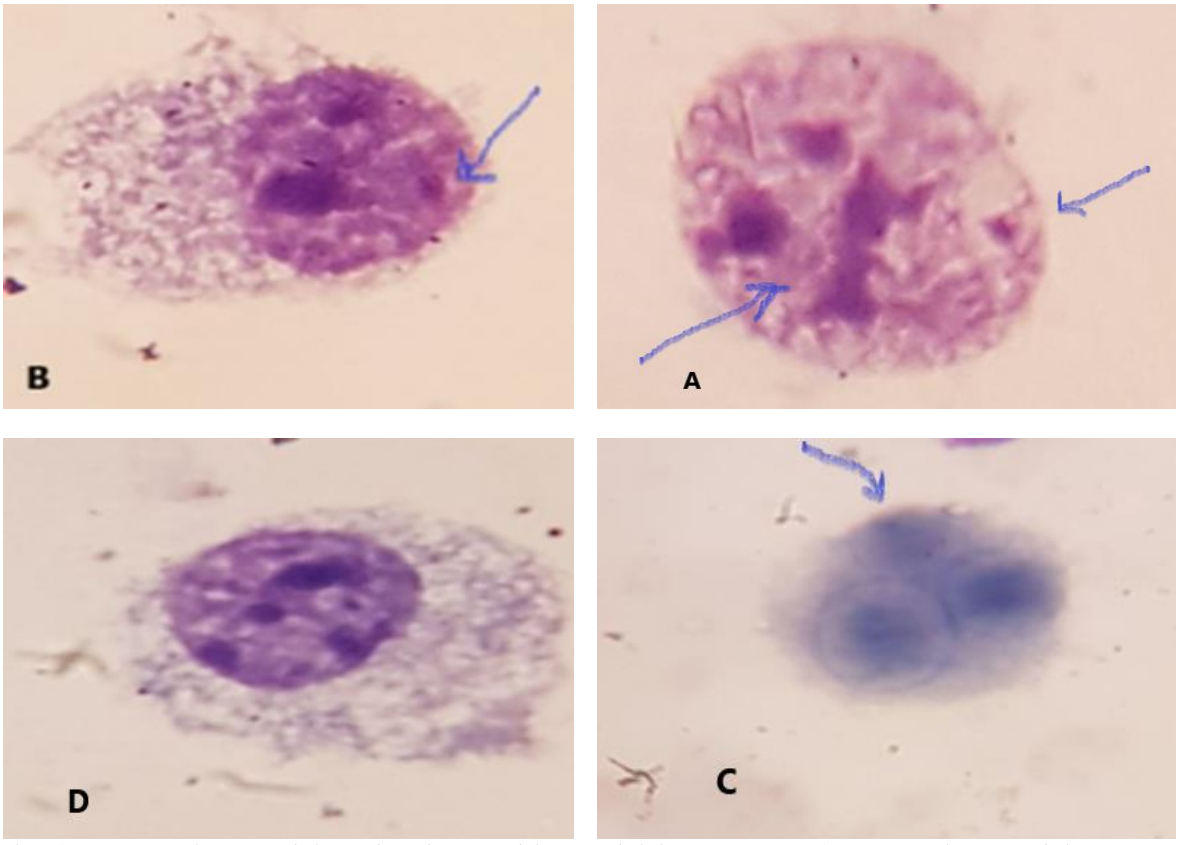

Fig. (3-6) A-micronuclei and polymorphic nuclei in case No. (43). B-micronuclei in case No. (15) C- Micronuclei in case No.(2) D- Micronuclei and multinuclear in case No. (32

Discussion

During study found few studies about chromosmeal changes of anemia disease in Iraq ,Althought of anemia risk in chromosomeal aberration (morphaloge and number) .Studies indicte ,Fanconi anaemia (FA) is an inherited disease with congenital and developmental abnormalities, bone marrow failure, and extreme risk of leukemic transformation.(Stefar M.et al 2012). Fanconi Anemia (FA) is an inherited bone marrow failure syndrome characterized by congenital abnormalities, progressive marrow failure and predisposition to myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), and solid tumors .(Parinda A.et al,2010). In this study no significant difference ($P<0.005$). The relationship between the age and types of anaemia are shown in table(3-1).. Similar study in Karbala 2021 indicted findings revealed that there was no relation between the type of anaemia and age or sex(albaroodi K,2021). And study in Bagdad indicte ,The statistical analysis shouwed no significant relation between age and prevalence of anemia and no significant relation was noticed between gender with anemia (Zeki;Warid ,2019).Study in Tanzania indicate that younger children(under 2 years) were more likely to be anemia compared to their older peers (Kejo D.et al, 2018).Anemia is major source of morbidity and mortality worldwide (Vijay G.,2015). Globally it is estimated that 273 million (approximately 42.6%)of children under five years are anemia whilst 60% of children are anemia in the African region are anemia (WHO,2015).

This study similarity in 1965 Blood American Socity of Hematology, indicate Numerical and morphologic chromosomal aberrations were demonstrated in three cases of pernicious anemia in relapse. The morphological abnormalities including chromatid breaks, gaps , "giant" chromosomes,translocation,dicentric chromosmes,acentric fragments,ring chromosmes, were reduced in remission following vitamin B_{12} therapy. The numerical changes consisted of aneuploidy (45 and 44 chromosomes) with the most common finding encountered (6 to 100 per cent of the cells) being monosomy involving the G 21 chromosome.And in that study they found numerical changes consisted of aneuploidy(45 and 44 chromosomes) . The numerical anomalies persisted in remission.(KOSMAS. A,et al 1965). And similar studies in Kashmir2010 indicate . There have been reports of aneuploidy and loss of Y chromosome in case of sideroblastic anemias; trisomies of 6 and 8 and loss of chromosome 7 in aplastic anemia.

Association of reversible cytogenetic abnormalities with megaloblastic anemia have also been observed.Where he found it several changes chromosomal were Chromosme breaks and gaps ,Centromere stretching, Centromere spreding , Chromosome elongation (Upma et al,2010). And study in India indicate The spontaneous chromosome breakage was detected in 21 (63. 64%) patients FA anemia.(Seema Korgaonkar, 2013). 2022 American College of Pysicians indicte Cultured peripheral lymphocytes showed a high prevalence of chromosomal breaks characteristic of Fanconi anemia.(Richard J,et al 2022).And studies indicte Aplastic anemia The most common chromosome abnormalities are trisomies of 6 and 8 and loss of chromosome 7. Trisomy 6 is more common at diagnosis while loss of chromosome 7 is more common after therapy. Am. J. Hematol.And Although the number of cases studied has been small, most authors contend that

clonal cytogenetic abnormalities in aplastic anemia carry increased risk for subsequent development of myelodysplastic syndrome and leukemia . However, larger studies are required to confirm and assess the magnitude of the risk in these patients with abnormal karyotypes (Yi-Kong,et al, 2001).In 1960, Peter Nowell and David Hungerford discovered the first chromosomal abnormality associated with cancer using cytogenetics , (Ingrid Lobo, 2008).Gene deletions or inactivations are responsible for initiating cancer progression (Mitelman, 2005).So this study aim determina changes chromosomal by cytogenetic analysis because of the danger chromosomal aberration on survival.
The micronucleus technique was proposed as a reliable method for measuring chromosomal damages after treatment with cytochalasin-B (Cyt-B) was postulated as a procedure of choice.where Cyt-B, an inhibitor of the spindle assembly, was used to prevent cytoplasmic division after nuclear division had occurred.Chromosome segregation errors during mitosis lead to the formation of structures called micronuclei. (PMID.2013) .Micronuclei(MN) known as Howell-Jolly bodies were first identified at the end of nineteenth century in red cell precursors by William Howell,an American and Justin Jolly aFrenchman (Sears and Udden,2011).

Studies indicte in the Unniversity ,AB,Canada 2013 found The influence of the micronutrient deficiency on MN formation and the initiation of hematological diseases was also shown. Therefore, understanding the link between the micronutrient status and MN frequency may be useful for the future development of novel therapeutic approaches (Lal and Ames, 2011).And in the same study of Canda indicate Today, numerous studies include micronucleus scoring for measuring DNA lesions and genotoxicity of almost any possible chemical and radioactive compound (Lau et al., 2009; Watters et al., 2009; Cveticanin et al., 2010; Lal and Ames, 2011). Considered anemia from hereditary blood diseases . Family history factore plays a part in risk in the anemia (genetic diseases) in children .Study from Najaf indicted, There are a large number of genetic mutations that lead to thalassemia and that common genetic disease in the provine ofNajafin Iraq(Furqan et al 2019).

In the Kurdstan Iraq ,given the high prevalence of hemoglobinopathies in the region, and the high rates of consanguineous marriages, a preventive program was initiated in 2008, and results of its first 5 years were promising, though there are still many outstanding challenges that require addressing.(Sarah Al Allawi ,et al 2021).Study in Indian indict The genetic analysis through pedigree revealed a significantly (P,0. 000927) high frequency (36. 4%) of consanguinity compared to controls (3. 33%).(Seema korgaonkor, et al .2013)

Some people are born with genetic abnormalities that can cause certain types of anemia, including sickle cell anemia, thalassemia, and Fanconi anemia.(Jennifer Acosta Scott 2010)and found during study one cases of the patients Bone Marrow failure that also goes back to family history .Study in USA indicte, the inherited bone marrow failure syndromes (IBMFSs) are a group of genetic disorders associated with inadequate bone marrow (BM)(Tarek Elghetany ,2021).And found in the study three cases of patients Aplastic anemia this is diseases was accure by inherited or acguired. Study indicteAplastic anemia classified in constitutional

or acquired syndromes based on pathophysiology.(Valentina Giudice;Carmine Selleri 2022).

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