A study of some cytogenetic variables in a sample of patients with polydactyly and acromegaly in Anbar Governorate

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Abstract

Local gigantism (acromegaly) and polydactyly are one of the genetic problems that some members of society suffer from, due to the various psychological and health damages that result from it. The growth hormone, insulin-like growth factor-1 axis are among the most important reasons that overlap in the emergence and development of this case. Also, the genetic causes related to mutations and chromosomal Abnormalities have an effective role in the formation and development of the case. The current study indicates that there is a positive history of the case represented by (5) individuals out of (22) cases, in addition to a significant increase ($P \leqslant 0.05$) in the concentration of insulin-like growth factor-1 in the serum of the study sample Compared with its concentration in the serum of the control sample, and there was no significant difference between the concentration of growth hormone in the serum of the study sample compared with its concentration in the serum of the control sample. The study also showed a significant increase in the Mitotic index among the study sample compared to the control sample, and the presence of numerical chromosomal Abnormalities (9%) and structural (31%). The causes of these Abnormalities may be due to a variety of external and internal influences.

Keywords: *Acromegaly, Polydactyly, GH, IGF-1, Chromosome.*

INTRODUCTION

Normal physical growth requires balanced cellular processes among themselves, and these processes are regulated by several factors, such as internal factors that include hormones, growth rates, genetic factors, psychological (social) factors, and external factors, including nutrition and the natural environment (1). One of these acting hormones is growth hormone, a peptide hormone secreted by somatic cells in the anterior lobe of the pituitary gland. It is also expressed in extra pituitary tissues. It has direct, delayed, or local effects at these sites, promoting cell division and growth. It has a central function to regulate growth and metabolism after birth. It presents multidirectional effects on different human tissues(2 (Through the complex hormonal exchange, which includes the hypothalamus, pituitary gland, and peripheral tissues, the secretion of growth hormone is regulated, and thus the regulation of physical growth(3).The bones and soft tissues of the hands and feet can be affected by various conditions, including genetic, metabolic or systemic disorders. (4)Acromegaly is a rare syndrome of disturbed growth and physical proportion in adults that occurs everywhere in the world. This disease results from elevated levels of growth hormone (GH) secretion or overproduction of the mediator insulin-like growth factor 1 (IGF-1) in more than 90%. In some cases, the origin of excess growth hormone is a benign pituitary

adenoma and in very rare cases (less than 1% of all cases) tumors in the body that release a component known as a growth hormone-releasing hormone (GHRH) that causes the pituitary gland to overproduce growth hormone (5).The total prevalence of the disease ranges between 2.8 and 13.7 cases per 100,000 persons, and the annual incidence rates range between 0.2 and 1.1 cases/100,000 persons. The peak of diagnosis is during the fifth decade of life (6).Regardless of etiology, elevated levels of both GH and IGF-1 lead to clinical abnormalities, swelling of soft tissues, arthritis, hypertension, heart failure, diabetes, and sleep apnea) 7).But there are other conditions that can mimic the clinical features seen in acromegaly without an abnormality in the GH/IGF-1 axis termed pseudo acromegaly (4). It is believed that there are many factors that play a role in the occurrence of acromegaly, including genetic factors and environmental factors. Among the known genetic disorders associated with pituitary tumors that secrete growth hormone, McCune-Albright syndrome (MAS) , Carney complex (CNC) types of endocrine tumors multiplayer 1 and 4 (MEN 1, MEN 4) (8).

GH)) Growth Hormone

Human growth hormone is a pituitary hormone that is essential for normal postnatal growth and has many organisms across physiological systems and is also expressed in extra pituitary tissues Pituitary hormones have local effects at these sites)2 (It is a polypeptide protein with two disulfide bonds (S-S-disulfide bonds) on a single protein base and through the Somatotroph cells, growth hormone is produced, stored and then secreted in the anterior lobe of the pituitary gland(9). It mostly rotates as a protein with a molecular weight of 22 kDa, as this hormone consists of 191 amino acids (10). Growth hormone (GH) belongs to the same family of hormones as prolactin (PRL), prolactin 2 (PRL2), and somatolactin (SL), which have It exerts a wide range of influences including development, regulation, metabolism, and growth stimulation (11) as it exerts a variety of physiological actions that include prominent roles in growth and metabolism with a significant contribution by stimulating the synthesis of IGF-1, which is a transcriptional target. Key to growth hormone signaling in the liver and other tissues (12) Growth hormone is expressed by the growth hormone gene, GH1, which belongs to a family of five genes that are homogeneous and linked with each other. It is the basic gene responsible for the secretion of human growth hormone to regulate all postpartum. The structure of this hormone includes four helices necessary for functional interaction with its receptors (56). Because of its stimulating effects on body growth, growth hormone is known as somatotropin, as it is one of the most abundant hormones in the pituitary gland, as 30-40% of cells in the anterior pituitary are somatotropes, and this name is also given to the growth hormone produced by recombinant DNA technology. Techniques (13)Its release and release are regulated during puberty and decrease with age at a rate of 14% every 10 years over the age of 40. As for its importance, it stimulates growth and cell proliferation in humans and other animals (14). Growth hormone has two mechanisms of action: direct action and indirect action (15). The direct effects of human growth hormone on the body through its action on binding to target cells to stimulate the response, while the indirect effects occur primarily through the action of insulin-like growth factor-1 or somatomedin, as it is induced to secrete in peripheral tissues, especially the liver mainly in response for elevated growth hormone binding to surface receptors (16).

Insulin like Growth Factor (IGF-1)

Insulin-like growth factor (IGF) is a complex system consisting of two insulin-like growth factors (IGF-1 and IGF-2) and their receptors (IGF-1R and IGF-2R) as well as binding

proteins (IGFBPs) whose components work together to influence growth where they occur. IGF actions predominantly through activation of IGF-Rs bound to the plasma membrane by circulating ligands (IGFs) released from IGFBPs that stabilize their serum levels (17)Insulin-like growth factors (IGF-I and IGF-II) and their receptors are widely expressed in neural tissues from the beginning of embryonic life and they cross blood-brain barriers by active transport, thus their regulation as endocrine factors differs from that of other tissues (18). IGF1 and IGF2 have been named as insulin-like growth factors because of their structural homology to insulin (~50% sequence identical) and similar metabolic actions and are functionally related to insulin but have much higher growth-promoting activity (19) (20); also It has an insulin-like effect in the body as it was found that the post-receptor signaling mechanisms involving tyrosine kinase and insulin receptor substrate-1 (IRS-1) are also similar to insulin-like growth factor and insulin (21) Both insulin-like growth factors (IGF-1 and IGF-2) are peptides necessary for normal growth and development before and after birth. They are mainly produced in the liver, but are also secreted by most tissues, where they can act as autocrine or paracrine and act as endocrine hormones (22) Quantitatively, IGF2 is the dominant circulating IGF, present in adults at up to three times that of IGF1, a mature peptide hormone of which is 67 amino acids and has a molecular weight of 7.5 kDa, whereas IGF2 stimulates placental growth and promotes fetal development, which is lower. Growth hormone dependent on IGF1 but recent studies indicate that in adults IGF-II is also important for muscle, brain and other tissues by transmitting signals through the(23,24) IGF-IR receptor While (IGF1), which is a small peptide consisting of 70 amino acids with a molecular weight of 7649 Daltons, acts as a mediator of the growth-promoting effects of pituitary growth hormone (GH) during postnatal life and has a GH-independent growth-stimulating effect, as this hormone has indirect effects in the growth of a number of Body cells and tissues (25; 23) It is mainly expressed by the IGF-1 gene located on chromosome 12q23.2)) It is a gene composed of 7 spliced exons encoding three protein isoforms (pro-IGF-1A and pro-IGF-1A). -IGF-1B and pro-IGF-1C) and all transcription isoforms lead to the same 70-amino-acid mature IGF-I peptide that uses the same receptor. (17)

Methods

Sample collection:

The study samples were collected from (22) blood samples from individuals with gigantism and (5) samples from healthy people who were used as a comparison sample (control group) from the reviewers in a special laboratory for pathological analyzes - Ramadi for the period from 4/8/2022 to 4/10 / 2022 by drawing (5) ml of each person’s blood divided into special tubes containing heparin to conduct genetic tests with a slight shake to make sure it is mixed with heparin, then the tubes were transferred to the laboratory to conduct tests, and the pathological cases were diagnosed by doctors specializing in hormonal diseases In the Women's and Children's Hospital - Ramadi / Growth Hormone and Laboratory Tests Unit , In addition to relying on the clinical diagnosis represented by physical symptoms and family history of the disease, information was collected according to an information form and according to the rules of scientific research ethics and after obtaining the consent of all the individuals included in the study.

Estimation of hGH concentration in serum

The hormone was estimated in the blood serum using the ELISA technique, according to the method attached to the measurement kit prepared by Monobind Inc.

Estimation of insulin-like growth factor (IGF-1) concentration in blood serum

The hormone was estimated in the blood serum using the ELISA technique, according to the method attached with the measurement kit provided by INVITROGEN.

Isolation and identification of human chromosomes from peripheral blood chromosomal aberration

The chemical solutions used in the analyzes of cytogenetics were prepared, the solutions were prepared, and the work methods of cytogenetics were carried out as followed in the Iraqi Center for Cancer Research and Medical Genetics, and the blood lymphocytes were cultivated and harvested for each of the 22 individuals in the sample who had gigantism and 5 for a group. Control based on the method (57) and the method modified by the Iraqi Center for Cancer Research and Medical Genetics (58).A comprehensive examination was carried out for each slide and at the rate of 30 metaphases for each individual, as each chromosome was examined by an optical microscope (Altay-chine) modified by the researcher by adding a digital camera on a holder attached to the eyepiece, and pictures were taken on the oily objective lens (100X). Directly, the results of the examination for each equatorial phase were compared with a global database using an electronic system (3.0 smart type), as the captured images were entered in the form of (TIFF Image) into the program and after making the required adjustments to the image according to the requirements of the program

Results and Discussion

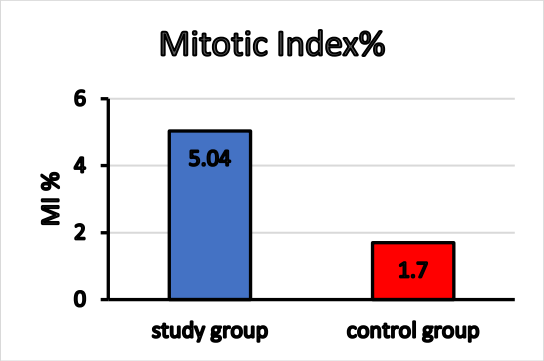
Mitotic index

The current study showed Figure (4-1) Table (1-4) that there is a high significant effect of the pathological condition, as the statistical results indicated that there was a significant increase ($P \leqslant 0.05$) in the cell division coefficient of the study sample compared to the control sample, where the ratio of the coefficient of Their cell division was (5.04 ± 1.01)%, while the percentage of the division coefficient in the control sample was (1.7 ± 0.49)%. The reason for this may be the stimulation of the immune system due to the disturbance of the GH/IGF-1 axis, which causes an increase in the rate of cell differentiation and division. (53).

Table (4-1) Number of patients and percentage of infertile patients by age group

IGF-1 ng/ml	GH ng/ml	Mitotic Index%	History		Number			sample	
			Positive	Negative	Total	♀	♂		
573.1±106.7	0.36±0.13	5.04±1.01	5	17	22	2	20	Study	1
246.8±19.9	0.32±0.16	1.7±0.49	0	5	5	2	3	control	2

Figure (4-1) the percentage of the mitotic index of lymphocytes for the study and control samples.



Malarkey and his group indicated that the imbalance in growth hormone levels led to a disorder in the differentiation of immune cells, specifically T cells, and the reason for this hyperdifferentiation may be a defect in interleukins, as it stimulates the production of IFNγ with a small positive effect on the production of IL-10(61), as another study indicates that the axis of GH/IGF-1 has an important role in accelerating the differentiation and division of cells and reducing programmed death, in addition to the important role that the axis plays in the development and division of body cells in special organs such as ovaries and testes (27,45) GH promotes maturation and activation of dendritic cells as antigen-exposed cells to participate in the organism's immune response (26), where Weigent and colleagues have published data suggesting that GH produced in lymphocytes plays a role in lymphocyte growth and survival and cytokine production (29) Lymphocyte isoforms are present in different compartments. Under normal culture conditions, Lymphocyte GH is present mainly in the cytoplasm as an isoform (about 100 kDa) and in the nucleus as an isoform (about 48 kDa) and it is likely that it represents a function Important in mediating intracellular growth hormone activity, the complex regulatory circuit within leukocytes is important for the production and function of leukocyte-derived growth hormone and IGF-I within the immune system. So This circuit can satisfy local tissue needs for these hormones independently of the pituitary gland or liver without disturbing the homeostasis of the system of other organs. Thus, cells of the immune system recognize the increase in oncogenic growth hormone as an oxidative stress event and signal the release and translocation of a low-molecular-weight isoform. for GH in the nucleus (48 or 65 kDa). And in the nucleus, GH would be free to influence transcription in response to a stress event, defending the cell against oxidative damage, and that lymphatic growth hormone stimulates IFNγ production with a small positive effect on IL-10 production. TH-1-mediated cellular immunity (30)It is possible that GHR and/or GH-binding protein (GHBP) serve specific roles in the intracytoplasmic actions of growth hormone produced by lymphocytes in cells of the immune system.

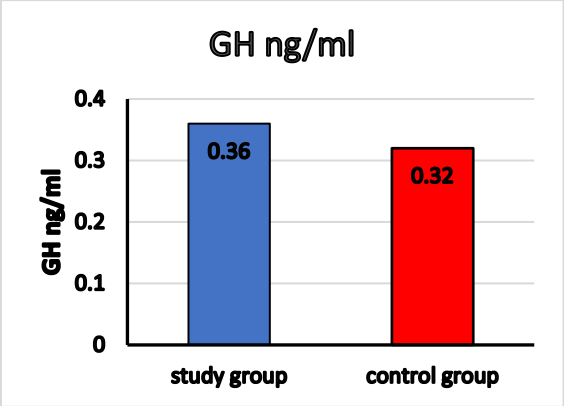
Serological tests:

The study, Appendix (4), indicates that among (22) patients (10) patients with gigantism and (12) patients with polydactyly had hormonal disorders distributed as follows.

Growth Hormone concentration

The results of the GH concentration test, Figure (2-4), Table (4-1), indicated that there were no significant differences ($P \leqslant 0.05$) between the study and control samples. Its concentration in the study sample was (0.13±0.36) ng/ml, while its concentration in the control sample was (0.16±0.32) ng/ml.

Figure (4-2) the concentration of growth hormone for the study and control samples.



The reason for this result may be due primarily to the fact that the study did not follow the scientific method used in collecting samples for measuring the concentration of growth hormone, as it is assumed that blood draws are made without and after stimulation on several withdrawals (4), but the refusal of study samples to undergo stimulation or to give a blood sample to more than Once, it may be the main reason for the lack of significant differences between the two study samples, while the second reason that supports this result is that hypertrophy or multiplicity in all members of the study sample occurred early in the age of the sample when the concentration of the hormone was on the rise that led to this condition and it is known The concentration of growth hormone decreases with age. The decrease in concentration may be attributed to the natural consumption of the hormone with age. Classic growth hormone deficiency can be the result of a mutation in the GH releasing hormone receptor (GHRH-R) gene (31) or a defect in one of the genes that plays a role in The emergence of growth hormone-producing cells in the anterior pituitary (POU1F1, PROP1, HESX1, LHX3, LHX4, etc.)) or a mutation or deletion in the GH1 gene(59).Variants in different growth hormone resulting from heterozygous missense mutations in the GH1 gene have been described. Takahashi et al (60) recently described the first homozygous missense mutation in the GH1 gene (GH-C53S((31). This mutation results in the absence of a Cys-53 to Cys-165 disulfide bridge, resulting in decreased GHR binding and signaling. As a result, some new genotypes were associated with decreased gene expression, while other haplotypes were associated with increased expression(31). One could speculate that the haplotype, associated with reduced expression of GH, results in decreased spontaneous growth hormone secretion and thus lower IGF-I levels, while stimulating growth hormone secretion may be normal. Recently, mutations in the membrane and intracellular Identification of the receptor domain, resulting in a growth hormone insensitivity syndrome with normal or high levels of a growth hormone-binding protein(32). Kofoed et al published the first report of a specific molecular defect in GH signal transduction (33).. The authors describe a patient homozygous for a missense mutation in the highly conserved SH2 domain of the STAT5b gene, which is essential for the GH signaling cascade and IGF-I transcription. At the moment of writing this review, many patients are homozygous Mutations in the STAT5b gene have been described: a frameshift mutation (38) (35)a nonsense mutation (36), another frameshift mutation (37), and a splice site mutation (34). All patients appear to show hyperprolactinemia. Some of them have a serious immunodeficiency, while others do not show such clinical symptoms. Recently, a mutation was heterozygous in the IkB gene(39،40) IkB is part of the NFk B signaling pathway that plays a key role in immune responses. Besides severe immunodeficiency, the patient also had signs of partial insensitivity to growth hormone, suggesting that the NFk B pathway could play a role in GH signal transduction.

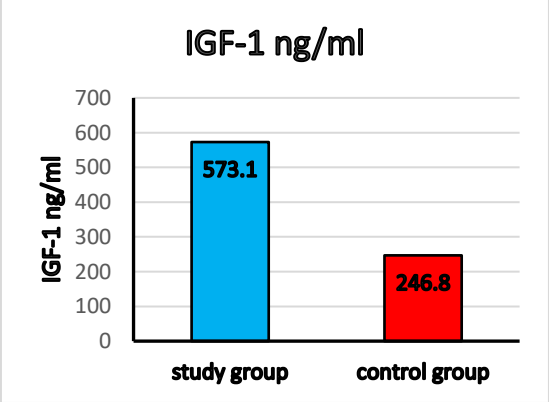
Pseudo acromegaly are conditions with clinical manifestations of excess growth hormone that have no abnormality in the GH/IGF-1 axis (41).

Insulin-like growth factor I

The results of the current study, Figure (4-3), Table (4-1), showed that there was a high significant difference in the concentration of insulin-like growth factor-1 between the two study samples, as its concentration in the study sample was (106.7 ± 573.1) ng/ml compared to its concentration in the subjects. In the control sample (19.9 ± 246.8) ng/ml, the basic nature of insulin-like growth factor-1, in contrast to growth hormone, continues to be secreted over the life of the individual and has a longer half-life and shows a more stable concentration in the blood. Moreover, it can Determining its levels at any time of the day, so the concentration of IGF-1 is the first recommended test for people with acromegaly (54),

The reasons for this increase may be attributed to the continuous excessive secretion of growth hormone (consumer), which causes a continuous increase in insulin-like growth factor (IGF-1), especially since it has a half-life of up to 20 hours per day (55).

Figure (4-3) concentration of insulin-like growth factor I for the study and control samples.



Continuous exposure to elevated hormone levels may lead to a decrease in the number of receptors by enhancing internalization as well as degradation of the receptors occupied by the hormone (42).When a hormone stimulates its target cell, simultaneously through negative feedback, it may reset the target cell's response to subsequent doses of the hormone, resulting in functional hormone resistance. Desensitization, the ability of the stimulus to re-establish the response of target cells(43). In this regard, IGF-I and IGF-IR may show similarities to insulin and the insulin receptor: thus in chronic active acromegaly elevated levels of IGF-I may reset the IGF-IR response and lead to a degree of IGF-IR resistance. Indeed, it has been found that chronic and prolonged stimulation with IGF-I may lead to functional resistance to IGF-IR through negative feedbac (42). In determining resistance to IR-like growth factor in the pituitary/ hypothalamus, negative feedback of IGF-I on the pituitary and hypothalamus would be ineffective, leading to higher levels of growth hormone and thus a further rise of IGF-I levels, Cellular responses resulting from elevated growth hormone levels overwhelm intracellular mechanisms that attenuate GH signaling, because of excessive growth hormone secretion, circulating (immune) IGF-I concentrations rise in active acromegaly(7)،. Diagnosis of acromegaly requires elevated total circulating IGF-I concentrations And high concentrations of IGF-I may be linked to some types of cancer. However, opposite effects of elevated IGFBP-3 have been reported. Hypothetically, potential risk assessment can be determined using tertiles of IGF-I SDS and IGFBP-3 SDS. Patients who have levels in the potential risk area of IGF-I SDS with concomitant low IGFBP-3 SDS may need to reduce their GH dose in order to avoid any potential risk of adverse events (44).

Chromosomal study

Table (4-2) shows the numerical and structural chromosomal variations of the study and control samples. The chromosomal profile of patients with gigantism and hypertrophy was studied and compared with the control sample by using the electronic system (Smart type 3.0) with the global data stored in the World Genetic

Information Bank, where the results showed The presence of chromosomal disorders in (9) cases, with a rate of (40.1%), while the study did not record the presence of numerical or structural variations in the control sample. The results of the study, Table (4-2), pictures (4-1) and (4-3), showed the presence of numerical chromosomal disorders in (2) patients with a rate of (9.02%), while the study did not record the presence of numerical variations in the control sample, including numerical disorders (1) Molecular polyploidy of an entire set of chromosomes (46,XY/92,XY) and (1) Molecular case of loss of chromosome No. (18) 45,XY,del(18) / 46,XY)

Structural chromosomal Abnormalities:

The study, Table (2-4), indicates that there are structural chromosomal changes in (7) patients, with a rate of (31.08%), represented by (2) cases of inversion, as shown in pictures (4-2) and (4-5) (5) cases of chromosomal deletion. They are shown in the pictures (4-4, 6, 7, 8, and 9). No structural chromosomal changes were recorded in the control sample

Table (4-2) Structural and numerical chromosomal Abnormalities in the peripheral blood lymphocytes of the study and control sample.

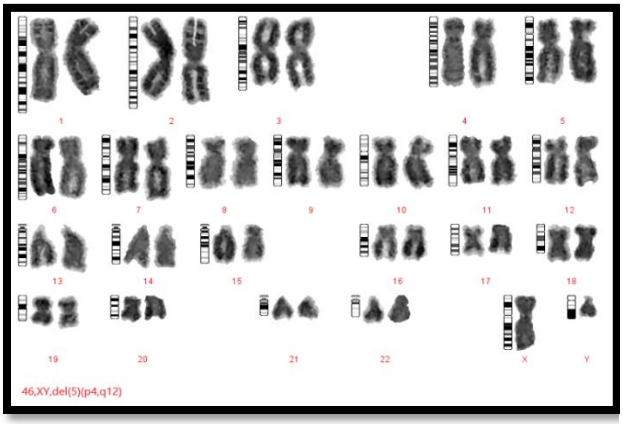
Numerical Abnormalities%			Structural Abnormalities%					Number	sample	
Total	Lose	Add	Total	Tran.	Ring	Inver.	Del.			
2	1	1	7	0	0	2	5	22	**Study**	1
0	0	0	0	0	0	0	0	5	**control**	2

Case No. (7): (sample No. (13) - 46,XY,del(5)(p4)(q12): The results of the current study, picture (4-7) and figure (4-9) indicated that deletion cases occurred in chromosome 5. And in both the short and long arms.

Studies (52,62) indicate that the diversity of the causes of local gigantism or polydactyly may be due, depending on the case, to damages or mutations resulting from a defect in meiosis, causing damage to the X chromosome, specifically (Xq26). 3), while familial isolated pituitary adenoma (FIPA) resulting from damage to the AIP gene located on chromosome (11q13.3), on the other hand, damage to autosomal chromosomes is involved in mutations in suppressor genes. For tumors, such as the PRKAR1A gene, which is located on the chromosome (17q22-24) and its allele located on the chromosome (2p16), While other mutations cause multiple endocrine tumors, such as damage to the Multiple endocrine neoplasia 1 (MEN1) gene located on chromosome (11q13) and the Multiple endocrine neoplasia 4 (MEN4) gene located on chromosome (12p13).

Photo (4-7) equatorial phase of sample No. (13) using G-packing technology, with 100X magnification.

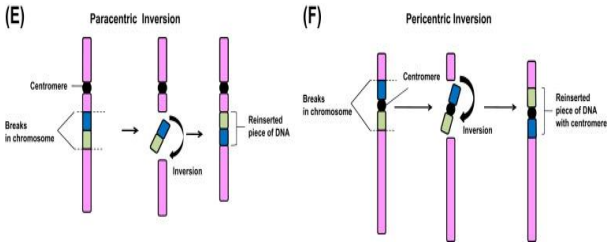
Figure (4-9) the chromosomal structure of sample No. (13) using the Smarttype3.0 automatic analysis program



and early segregation (in which homologous chromosomes or sister chromatids separate early) and the difference in the percentage of aneuploidy that arises from the formation of female and male gametes plays an important role in the differences that make meiosis in the oocyte more prone to error, including the crossing of the oocytes and the formation of structures during Recombination, decreased control of the prophase check point and decreased efficiency of the spindle assembly checkpoint. Decreased requirements for chromosome alignment at the spindle equator to initiate anaphase have a role in heterochromia (27, 45). Obesity, smoking, radiation exposure, and the use of contraceptives as factors that may lead to an increase in mitotic errors(28).

Some of the reasons for the occurrence of chromosomal changes may be due to the exposure of one chromosome to fracture, then it is reversed and rearranged within itself, or what is known as inversion and inversions of two types: paracentric and pericentric. Paracentric inversions do not involve the centromere and both breaks occur on the same arm of the chromosome (Fig. 4-13). Eccentric reflexes include the centromere and there is a break point in each arm (p and q arm) (46).

Figure (4-13) Inversion of chromosomes) Chowdhury et al.,2020))



Or it may be due to overexpression of the pituitary tumor transforming gene 1 (PTTG1) in pituitary adenomas leading to chromosomal instability and aneuploidy and that aneuploidy is frequently observed in invasive adenomas (46). (47) In addition, whole exon sequencing revealed somatic copy number changes at arm level in 42 pituitary adenoma samples at genome-wide sites in 29% of samples. Chromosomal alterations have also been shown to be more frequent in hormone-producing adenomas, particularly somatic adenomas and null-cell adenomas(48). Damage to the chromosome region that contains the GHR gene encoding the growth hormone receptor protein (5p13.1-p12) or the growth hormone binding protein (GHBP) by cleavage or proteolysis of an extracellular binding domain may affect the hormone receptor protein or growth hormone mature (NCBI) and that partial or structural aneuploidies directly affect sub-sections of chromosomes. These abnormalities may arise from wrong corrections of chromosome damageDamage occurs during DNA synthesis when replication forks stop and collapse for reasons such as DNA damage, absence of DNA synthesis components, or stress associated with DNA secondary structure(50,49). In gametes, there are strand breaks programmed to enable meiosis recombination which can go awry and lead to chromosomal breakage.These cases result from abnormalities that change the normal arrangement of chromosomal segments

(the fourth level of chromosomal epitaxy) in vulnerable regions, and then genes are placed in different regions of the chromosome and can be regulated differently, Chromosome breakage with subsequent recombination in a different configuration and can be balanced or unbalanced with balanced rearrangements such that the chromosome is complete without loss or gain of genetic material. And therefor, such rearrangements are generally harmless except in rare cases when a breakpoint damages a functionally important gene. However, a balanced rearrangement is often at risk of having children with an unbalanced chromosomal complement. In the case of an unbalanced rearrangement, there is a loss or gain of chromosomal material (and the reason for the chromosome rearrangement may be due to exposure to radiation and external influences that play a role in rearranging the chromosomes (51).

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