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Molecular genetic analysis role in diagnosis of primary amenorrhea patients

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Abstract: Twenty one cases of primary amenorrhea, with poor secondary sexual characters, were studied clinically and genetically to assign *SRY* gene defect incidence. Different primary amenorrhea categories were diagnosed but only one case shows abnormally positive SRY gene in a 46 XX intersex patient. This case shows defected maleness internal genitalia and variable degree of external genitalia development. However this single case represent an incidence rate of (0.047(4.7%)). Molecular analysis shows accurate and applicable investigation, and recommended to enrolled as a routine investigation in diagnosis of all cases of intersexual risk.

Key wards: Primary amenorrhea, Sry gene, Sex determination.

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Introduction:

Amenorrhea defined as the absence of period bleeding and may be primary or secondary. A girl of 14 years old with the absence of menstrual bleeding and secondary sexual characteristics (such as, breast development and pubic hair) or the absence of menstrual bleeding with normal development of secondary sexual characteristics in a girl by age 16 years known as primary amenorrhea.

The presence or absence of sexual development should direct the evaluation of primary amenorrhea patients. Delay of growth and puberty commonly causes primary amenorrhea in patients with no sexual development. Congenital amenorrhea causes expressed as normal pubertal development and a uterus but outflow tract obstruction with a transverse vaginal septum or imperforate hymen. But If the patient has abnormal uterine development, müllerian agenesis is the likely cause and a karyotype analysis should confirm that the patient is 46,XX.(1).

Turner syndrome ((TS) 45(X0)) which is considered as a major karyotypic cause of primary amenorrhea is due to a chromosomal abnormality in

which all or part of one of the X chromosomes is missing or defected. The chromosomal abnormality may be present in just some cells in which case it is known as TS with mosaicism. In these cases the symptoms are usually less and possibly none. Diagnosis is based on physical signs and genetic testing (2, 3). Adoption of molecular and karyotyping investigation in evaluation of females with menorrhea for better management and counseling has been emphasized by several investigators (4).

In the present study, the molecular analysis profile of patients referred with complaints of primary amenorrhea, short stature, poorly developed secondary sexual characters, and growth retardation to correlate the genetic background with the phenotypic features.

Sex determining genes on Y chromosome induce testicular development by unknown mechanism. It is postulated that Y-linked genes initiate a cascade effect of both X-linked and autosomal genes to promote the indifferent gonad to become testis. The sry gene (sex-determining region Y) is located on the short arm of the Y chromosome very near the pseudoautosomal border and code for highly conserved DNA binding protein. (5, 6, 7) .In the absence of the sry gene, the gonads develop as ovaries.

Thus Y chromosome plays a crucial role in sex development, the embryo inheriting a y chromosome develops as male, whereas the embryo lacking Y-chromosomes develops as females (8).

Materials and methods:

Twenty one primary amenorrhea with secondary sexual characters defect were subjected to ; extensive questionnaire ,lab investigations and clinical examination in Baghdad Hospitals.

Each patient was examined for potential gonad anomalies, external genitalia anomalies and levels of androgen hormones; which relied on in this study, were measured using Radioimmuno assy & ELISA technique.(9).

Molecular study (PCR) suggested normal female and normal male (normal external and internal genitalia confirm the presence of sry gene) as a control group, the final decision for selection of normal male was based on fathering a child.

However, Five ml of blood was collected by venipuncture from all patients and control group .The collected blood was divided into two aliquots for molecular & cytogenetic studies. 1St aliquots was sent to medical city laboratories for karyotyping study while the second aliquot of blood was subjected to DNA extraction & molecular study to visualize the presence or absence of sry gene locus.

Sry primer (5-GAA TAT TCCGC TCT CCG GA-3, 5-GCT GGT GCT

CCA TTC TTC AG-3) was selected according to the EAA/EMQN guidelines(10).

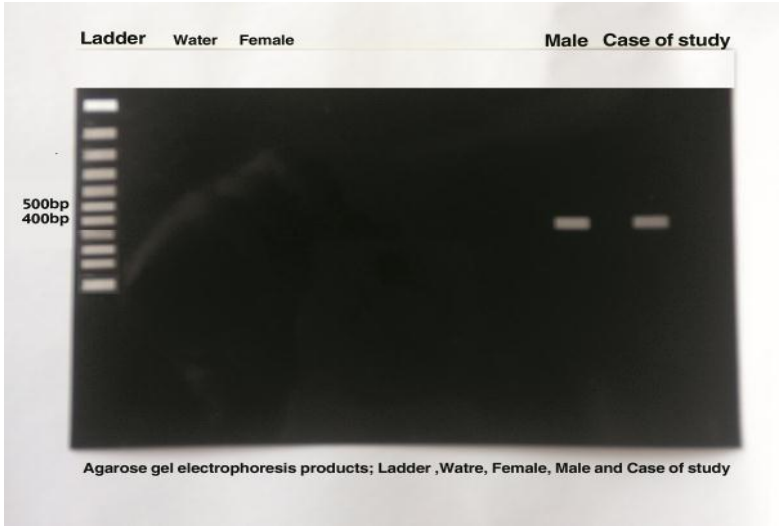
PCR program : (Table 1)

Necessary interventions were applied on the EAA/EMQN guidelines and virtually the following program was adopted. (10).

Table (1): PCR program.

No.	Steps	Temperature	Time	No. Of cycles	No.
I	Denaturation 1	95C°	3min.	1	I
First loop					
II	Denaturation 2	94C°	1min.	II	35 cycles
III	Annealing	55C°	1min.	III	
IV	Extension 1	72C°	1min.	IV	
IVI	Extension 2	72C°	5min.	1	IVI

The PCR products & the ladder marker were resolved by electrophoresis on 1-% agarose gel. The resolved bands were indicative for sry (472bp), and the molecular weight identification of deferent bands was based on their correspondence to the ladder bands.



Agarose gel electrophoresis products; Ladder ,Watre, Female, Male and Case of study

The decision of successful reaction was based on the results of external control (normal male and female).

Results & discussion:

Primary amenorrhea cases attendance to hospital was depends on the severity and type of secondary

sexual characters defect, hence the age of the 21 patients was of wide range (14 years -35 years). Patients who are young almost presenting suffering of obvious secondary sexual character defect overlapped by intersexual characters.

Virtually clinical and lab diagnosis shows, variable causes of primary amenorrhea. Hormonal imbalance perform the major cause of primary amenorrhea (13 (61.9%)). Moreover Congenital adrenal hyperplasia performed the prominent picture of most primary amenorrhea associated with ambiguous genitalia cases (5 cases 23.8 %).two cases diagnosed as turner syndrome (X0)performing (2/21(9.5%))of all cases that under study . whereas only one case was diagnosed as intersex case of XX karyotype with sry gene positive performing only 4.7% of all cases .

The XX male syndrome case (XX with positive sry gene) understudy was subjected for intensive clinical and radiological modalities. These investigations shows ambiguous external genitalia (big clitoris associated with hypospadias urethral opening), and normal feminine secondary sexual sexual characters (body hair distribution, pubic hair, voice and breast buds). But internal genitalia shows absence of feminine features with incomplete descended atrophied testes located at the inguinal regions.

Negative family history of male syndrome case denoting the de novo incomplete recombination of Y and X chromosomes (11).

Many factors explaining the small sample volume of this study, but two factors are of a great impact, Primary amenorrhea that associated with prominent variable degrees of sexual characters defects considered as stigmata in our community, and second factor represented by dropout of many cases during this study.

This study express Sry gene incidence as (0.047(4.7%)) within primary amenorrhea cases ,which reflect the significance of consideration of sry gene defect as an important cause in primary amenorrhea and suspected intersexual cases.

Positive predictive value, negative predictive value and false discovery rate of molecular analysis was 0.047, 0.46 and 0.95 respectively. The last value (false discovery rate (FDR)) express he high validity and accuracy of diagnostic value of these test.

Conclusion:

Although the small size percentage (4.7%) of detected intersex cases among other causes of primary amenorrhea in the present study, but still the consideration and adoption of genetic investigation of both karyotyping and molecular analysis of a great help in approachment for perfect diagnosis and case management which obviously expressed in FDR value (0.95)(12).

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