

## Detection of Steroid Sulfatase Gene Deletion (STS) in Egyptian Males with X-linked Ichthyosis

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### ABSTRACT

**Introduction:** Ichthyosis is a disorder of keratinization characterized by diffuse uniform and persistent scales resulting from abnormal epidermal differentiation or metabolism. Ichthyosiform dermatoses are classified into four major types, ichthyosis vulgaris, X-Linked ichthyosis, congenital recessive ichthyosis and lastly epidermolytic hypekeratosis which was previously called bullous ichthyosiform erythroderma. The identification of steroid sulfatase as the cause of X-Linked ichthyosis points to the importance of this enzyme in skin desquamation. Fluorescent in situ hybridization (FISH) analysis is a good diagnostic technique to detect a common deletion of the STS gene. Most patients with X-Linked ichthyosis have large deletions of the STS locus.

**Aim of the work:** In this study, we aimed to detect the X-Linked type of ichthyosis, diagnosed by detection of STS gene deletions among Egyptian males.

**Patients and Methods:** We performed this study on Egyptian males complaining of X-linked ichthyosis who were subjected to clinical examination, pedigree analysis of the family, cytogenetic studies using G-banding technique and fluorescent in situ hybridization (FISH) using locus specific probe for steroid sulfatase (STS) gene which is located at chromosome Xp22.3. Our results showed that 11.11% of patients had nocturnal enuresis and 33.33% showed STS gene deletion by FISH study.

**Conclusion:** The current study underlines the difficulty of diagnosis of X-Linked ichthyosis on the clinical features or pedigree analysis of the family in Egypt and the importance of cytogenetic and molecular cytogenetic studies for diagnosis. Fluorescent in situ hybridization (FISH) technique is a good, reliable, and rapid diagnostic tool to detect STS gene deletion. Since FISH will not detect partial deletion or point mutations, we recommended further molecular studies to reach the proper diagnosis of X-linked ichthyosis.

#### Key Words:

Ichthyosis- X-Linked, gene deletion, fluorescence in situ hybridization.

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## INTRODUCTION

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Ichthyosis is a disorder of keratinization characterized by diffuse uniform and persistent scales resulting from abnormal epidermal differentiation or metabolism. Ichthyosiform dermatoses are classified into four major types, ichthyosis vulgaris, X-Linked ichthyosis, congenital recessive ichthyosis and lastly epidermolytic hyperkeratosis which was previously called bullous ichthyosiform erythroderma.<sup>1</sup>

In addition to the genetic difference between X-linked ichthyosis and autosomal dominant ichthyosis vulgaris, clinical and histologic differences exist. In ichthyosis vulgaris the onset is not since birth and the flexures are characteristically spared. In the X-Linked form onset is at birth or during the first three months of age, and scalp, ears, neck and one or more flexures are involved, with more striking scaling on the abdomen than on the back. Palms and soles are usually normal. Histologically the epidermis is atrophic in ichthyosis vulgaris and hypertrophic in the X-Linked variety.<sup>2</sup>

Congenital recessive ichthyosiform erythroderma or non bullous ichthyosiform erythroderma is a severe autosomal recessive inflammatory ichthyosis characterized by generalized erythroderma which is apparent and persistent since birth, palmoplantar hyperkeratosis, ectropion and hypohidrosis. Lamellar ichthyosis is considered a non-erythrodermic type of recessive ichthyosis with typically large scales, palmoplantar keratoderma, ectropion and scarring alopecia with characteristically absent erythroderma. Collodion baby is the

clinical manifestation of lamellar recessive ichthyosis which occurs at birth, characterized by generalized glistening, taut, yellowish film stretched over the skin. Bullous ichthyosiform erythroderma is now known as epidermolytic hyperkeratosis resulting from mutation in keratin gene, and now classified with epidermolysis bullosa, is characterized by generalized erythroderma at birth, flaccid blisters, peeling, erosions and palmoplantar hyperkeratosis.<sup>3</sup>

Approximately 1 in every 6000 males is affected with X-linked ichthyosis.<sup>4</sup> An increased incidence of testicular maldescent, abnormalities of sperm count or motility and testicular cancer have been reported in patients with X-Linked recessive ichthyosis.<sup>5</sup>

Ballabio et al.<sup>6</sup> found apparent close linkage of steroid sulphatase (STS) of X-Linked ichthyosis and Kallmann syndrome which is characterized by hypogonadotropic hypogonadism, anosmia and a variety of neurological defects. Kallmann syndrome may be caused by a large deletion of the short arm of the X-chromosome proximal to and including the STS gene.

Solomon and Schoen<sup>7</sup> reported a patient with XO Turner syndrome and ichthyosis which by the pedigree and by its clinical features was X-Linked.

Clinical manifestations of microdeletion of Xp22.3 include ichthyosis, chondrodysplasia punctata, hypogonadotropic hypogonadism, anosmia, ocular albinism, short stature and mental retardation.<sup>8</sup>

Zettersten et al.<sup>9</sup> investigated the significance of the fact that patients with X-Linked ichthyosis display a 10-fold increase in cholesterol sulfate in squamous keratinizing epithelia, as well as a 50% reduction in cholesterol. They suggested that cholesterol sulfate accumulation rather than cholesterol deficiency is responsible for the barrier abnormality of stratum corneum which is responsible for abnormal cornification and scale formation in steroid sulfatase deficiency.

The identification of steroid sulfatase as the cause of X-Linked ichthyosis points to the importance of this enzyme in skin desquamation.<sup>10</sup>

The gene locus for steroid sulfatase (STS) has been identified at the distal end of the short arm of the X-chromosome (Xp22.3) i.e. the third subband of the second band of the second region of the short arm of X-chromosome.<sup>11</sup>

Most patients with X-Linked ichthyosis have large deletions of the STS locus.<sup>11</sup> However partial deletion, point mutation and translocation had been reported. Valdes-Flores et al.<sup>12</sup> reported patients with X-Linked ichthyosis and partial STS deletion. Mohandas et al.<sup>13</sup> demonstrated that in XX males in whom the testis determining factor gene (TDF) is transferred from a Y chromatid to an X chromatid, the break point is distal to the STS locus, which is retained on the TDF-bearing X-chromosome.

In more than 85% of patients with STS deficiency, a large deletion involving the entire gene and its flanking sequences is responsible. However a point mutation at nucleotide 1317, resulting in substitution of tryptophan (TGG) by

arginine (AGG) was reported.<sup>14</sup> They demonstrated also substitution of serine 341 by leucine.

Alperin and Shapiro<sup>15</sup> found a 19-bp insertion starting at nucleotide 1477 of the STS gene resulted in frame shift causing premature termination.

Fluorescent in situ hybridization (FISH) analysis is a good diagnostic technique to detect a common deletion of the STS gene. FISH is the application of fluorescently labeled DNA molecules to metaphase chromosomes and interphase nuclei for the detection of chromosomes and their alteration. It is a rapid, reliable and direct approach for identifying patients with microdeletion or microduplication.<sup>16</sup>

## AIM OF THE WORK

Is to detect the X-Linked type of ichthyosis, diagnosed by detection of STS gene deletions located at Xp22.3 among Egyptian males.

## PATIENTS AND METHODS

This study was carried out on nine Egyptian males complaining of ichthyosis manifested by diffuse and persistent scales. These patients were recruited from Dermatology and Andrology outpatient clinic, National Research Centre (NRC).

The patients were diagnosed clinically as X-Linked ichthyosis and selected from other types of ichthyosiform dermatoses according to the following criteria:

- Onset is since birth or during three months of age.

- Flexures of elbow and knee are affected.
- Palms and soles are normal.
- No erythroderma.
- No ectropion.

All patients under the study were subjected to the following:

- Thorough history taking including history of onset.
- Pedigree analysis of the family.
- General clinical examination including sense of smell, ectropion and secondary sexual characters.
- Genital examination for testicular maldescent.
- Dermatological examination including shape, site of scales, palms and soles.

#### Cytogenetic and molecular cytogenetic studies:

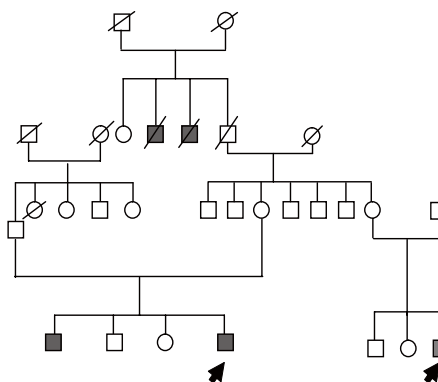
Cytogenetic analysis was done for every case using G-banding technique.<sup>17,18,19,20</sup> karyotyping nomenclature was according to the international system for human cytogenetic nomenclatures (ISCN) (2005).<sup>21</sup>

Fluorescent in situ hybridization (FISH) was performed using locus specific probe for Steroid Sulfatase Region (STS) spectrum Orange/CEP X (DXZ1) spectrum Green control probe (Vysis). The experiment was carried out according to Pinkle<sup>22</sup> and the manufacture's instructions.

The absence of a red hybridization signal at Xp22.3 indicates a deletion of STS gene.

## RESULTS

The present study was carried out on nine Egyptian males complaining of ichthyosis. These patients have fulfilled the selection criteria in order to exclude ichthyosiform dermatoses other than X-linked type of ichthyosis clinically. The age of the studied patients ranged from 1.5 to 20 years. All patients were males. Pedigree of the family of cases 1 & 2 showed the X-linked pattern of inheritance (Figure 1).



**Fig. 1:** Pedigree of the X-Linked ichthyosis family. Two uncles of the mothers and aunt of cases 1 & 2 are affected.

Family history was positive for ichthyosis in 4 patients (4/9) (44.44%). Only one patient (1/9) (11.11%) had nocturnal enuresis and STS gene deletion. Three patient 3/9 (33.31%) showed STS gene deletion by FISH study.

Chromosomal analysis using G-banding for all patients revealed a 46, XY karyotype and confirmed a non mosaic karyotype without any chromosomal rearrangement in all metaphases analyzed. (Figure 2).



**Fig. 2:** Metaphase spread and karyotype showing normal 46, XY chromosomal complement.

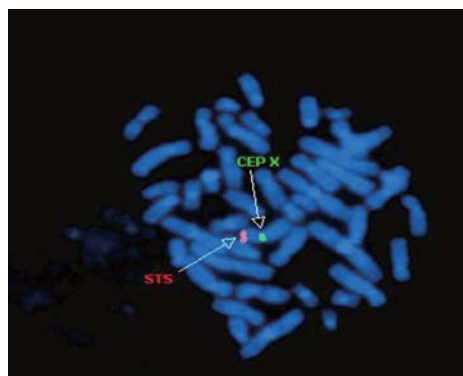
FISH analysis using (Locus specific) LSI STS probe showed one green signal for the control region (X chromosome centromere) and a red signal for the STS gene on the X-chromosome. The presence of the STS gene was confirmed in six cases numbers 3, 4, 6, 7, 8 & 9 (Figure 3). The probe also revealed a green signal for the control region of X chromosome and no red signal for the STS gene on chromosome X which indicate a deletion of the STS gene in three cases numbers 1,2 & 5 (Figure 4).

Table (1) shows the individual data of each case.

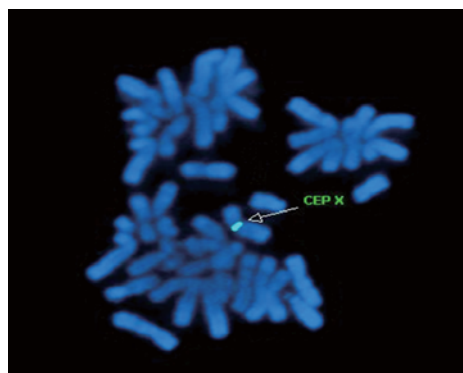
**Table 1 :** The data of the studied cases.

Case No.	Age (yrs)	Family hist.	General exam	Undescended testis	FISH STS deletion
1	17	+	Nocturnal Enuresis	No	+Ve
2	20	+	NAD	No	+Ve
3	6	+	NAD	No	-Ve
4	4	+	NAD	No	-Ve
5	14	-	NAD	No	+Ve
6	9	-	NAD	No	-Ve
7	1.5	-	NAD	No	-Ve
8	2	-	NAD	No	-Ve
9	6	-	NAD	No	-Ve

*NAD (No Abnormality Detected)*



**Fig. 3:** LSI STS probe hybridized to metaphase cell. Presence of the red signal on the X chromosome indicates no deletion of the STS gene. (Cases, 3,4,6,7,8 & 9).



**Fig. 4:** LSI STS probe hybridized to metaphase cell. Absence of the red signal on the X chromosome indicates a deletion of the STS gene. (Cases 1,2 & 5).

None of the studied cases showed anosmia, corneal opacity or undescended testis.

## DISCUSSION

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X-Linked ichthyosis (XLI) is an inherited metabolic disorder resulting from steroid sulfatase (STS) deficiency characterized by adhesive and regular scales of skin.<sup>23</sup>

Approximately 1 in every 6000 males is affected with X-linked ichthyosis.<sup>4</sup>

X-linked recessive is a mode of inheritance in which a mutation in a gene on the X-chromosome causes the phenotype to be expressed only in males who are necessarily hemizygous for the gene mutation because they have only one X-chromosome and in females who are homozygous for the gene mutation i.e. they have a copy of the gene mutation on each of their two X chromosomes. Most of cases of X-linked ichthyosis are males. Few females were described with XLI who were homozygote daughters of a father with the disorder and a mother who was a carrier.<sup>24</sup>

Solomon and Schoen<sup>7</sup> reported a female patient with XO Turner syndrome and X-linked ichthyosis.

The occurrence of a recessive X-linked disorder in a girl was explained on the hypothesis of uni-parental disomy.<sup>25</sup>

All patients in our study with X-linked ichthyosis were males. This is consistent with X-linked inheritance pattern.<sup>10</sup>

Males with interstitial or terminal deletions involving Xp22.3

show contiguous gene syndrome characterized by a variable association of apparently unrelated clinical manifestations including ichthyosis, chondrodysplasia punctata, hypogonadotropic hypogonadism, anosmia, ocular albinism, short stature and mental retardation.<sup>8</sup>

None of the patients in our study had anosmia, corneal opacity, undescended testis, ocular albinism, short stature or mental retardation. Case 1 in our study aged 17 years had nocturnal enuresis with normal intelligence This observation needs more investigations about the relation between STS gene deletion and nocturnal enuresis. Nocturnal enuresis may be a clinical feature of STS gene deletion. The involvement of other genes that might operate as a part of polygenic system is another explanation for nocturnal enuresis.

Diagnosis of recessive X-Linked ichthyosis and to be differentiated from autosomal dominant ichthyosis vulgaris was done in 1965 on the basis of pedigree analysis and clinical features.<sup>26</sup>

Our data figure (1) showed that the pedigree of cases 1 & 2 is compatible with X-Linked ichthyosis as two uncles of the mother and aunt are affected. On the other hand case 5 showed STS gene deletion using FISH but gave a negative history of any other affected family member. This underlines the difficulty to diagnose XLI on the clinical ground or on the basis of pedigree of the family.

Histopathological diagnosis of X-linked ichthyosis is controversial.

The epidermis is atrophic in ichthyosis vulgaris and hypertrophic in the X-linked variety.<sup>2</sup> On the other hand, Frost<sup>27</sup> stated that both ichthyosis vulgaris and recessive X-linked ichthyosis have normal epidermal cell proliferation.

Although diagnosis of steroid sulfatase deficiency can be made readily by fibroblast culture, this procedure is expensive and slow.<sup>28</sup>

Most of the patients affected by X-Linked recessive ichthyosis [MIM 308100] have a microdeletion of the STS gene.<sup>29</sup> Fluorescent insitu hybridization (FISH) analysis is a good reliable, rapid diagnostic tool for diagnosis of deletion of the STS gene.<sup>16</sup>

The STS gene contains 10 exons spread over 146 kilobase pairs of the short arm of X-chromosome.<sup>15</sup> Most XL-ichthyosis patients have large deletions of the STS gene and flanking sequences.<sup>30</sup>

In our study, we studied the presence of deletion of STS gene in a suspected nine Egyptian X-linked ichthyosis male patients. The selection of the cases was based on history onset, clinical examination of flexures, palms and soles and presence or absence of erythroderma and ectropion to exclude other types of ichthyosiform dermatoses. Three patients out of nine (33.33%) showed STS gene deletion by FISH study. The low percentage of STS gene deletion among Egyptian patients in our work compared to other studies might be due to small number of patients or due to racial or ethnic differences.

Another explanation is probably due to environmental disruptors that may affect the STS gene other than deletion.

The other genetic defects of STS gene apart from large deletion comprises partial deletion<sup>12</sup>, point mutations<sup>14,15</sup> and frameshift due to insertion of 19 bp leading to premature termination<sup>15</sup> All these mutations needs more investigations at the molecular level e.g DNA sequencing, which are out of the scope of this study.

## **CONCLUSION & RECOMMENDATIONS:**

The current study underlines the difficulty of diagnosis of X-Linked ichthyosis on the clinical features or pedigree analysis of the family in Egypt and the importance of cytogenetic and molecular study for diagnosis.

Fluorescent insitu hybridization (FISH) technique is a good, reliable, and rapid diagnostic tool to detect STS gene deletion.

Since FISH will not detect partial deletion, frameshift or point mutations, other molecular detection strategies e.g DNA sequencing are needed to reach the proper diagnosis of X-Linked ichthyosis.

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