Overview on the first human cytogenetic research in Sudan

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Abstract

Introduction: The present study is the first human cytogenetic project in Sudan which was titled: Cytogenetic and FISH analyses in Sudanese patients with dysmorphic features, ambiguous genitalia, and infertility. The aim of the present study was not only to characterize the genetic alterations in patients with dysmorphic features, ambiguous genitalia and/or infertility among Sudanese population, but also to attract the medical community attention to the importance of human cytogenetics in clinical genetics practice, and also to facilitate the introduction and clinical application of such valuable service in Sudan.

Materials and Methods: In this study chromosomal G–banding and fluorescence *in situ* hybridisation (FISH) analysis were performed on 44 Sudanese patients, 29 females, 14 males, and one patient with unassigned sex. Patients age ranging between 17 days-39 years (mean 18 years), Of the 44 patients, 20 had ambiguous genitalia, 8 dysmorphic features, 11 have puberty and/or fertility complains, and 5 were healthy individual (parents of 3 patients with dysmorphic features).

Results: Cytogenetic analysis of 20 patients complaining of ambiguous genitalia (13 females and 6 males, and one case with unassigned sex) showed that 8 has karyotypes different from their assigned sex and the other cases showed karyotypes consistent with Edward syndrome (47,XX,+18) (case 7), and a case with 45,Xdel(X)(p11) (case 11) respectively, when using FISH the 45,Xdel(X)(p11) case showed translocation of the SRY (sex-determining region Y), gene to the active X chromosome. For the 8 patients of dysmorphic features; five showed karyotypes consistent with Down syndrome, of which one showed Robertsonian translocation, with both FISH and ordinary G-banding, and the other three showed normal karyotypes. All the parents showed normal karyotypes. Among the infertility cases all showed normal karyotypes, except for two which showed karyotypes consistent with Turner syndrome and one which showed a male karyotype although the case was raised as a female; ultrasound showed a mass in the position of prostate.

Discussion: The study, the ever first one in Sudan, assured the importance, the possibility, and the need for cytogenetic and FISH analysis in diagnosis, management and genetic counseling of genetic diseases caused by constitutional chromosomal changes among Sudanese patients.

Introduction

Clinical cytogenetics is defined as the study of abnormalities in chromosome structure, number and the inheritance of such aberrations. Over the past few decades, cytogenetic analyses have become an important role in the diagnosis, clinical management, and prognosis of several human diseases especially those associated with numerical and/or structural chromosomal changes, leukaemia, and lymphomas, soft and bone tissue tumors. The advances in cytogenetic technology, the progress on the clinical cytogenetics field and our understanding of the link between chromosomal alterations and diseases have improved dramatically over the last 50 years. As a mature science, cytogenetics now works in the settings of human genomics, diseases and cancer genetics, tumour pathology, chromosome evolution and the relationship of nuclear structure to function[1].

Fluorescent *In situ* Hybridization (FISH) is a new hybrid of molecular cytogenetic technique which is based on the principle that target nucleic acid sequences that can be hybridized with appropriately labelled DNA or RNA probes[2]. In 1986, Pinkel and co-workers introduced FISH as novel technique to explore alterations in specific nucleic acid sequences in individual cells or chromosomes[3]. The advantages of FISH over ordinary banding technique include the ability to assess sample cells for their integrity at specific nucleic acid sequences even in non-dividing cells (interphase FISH), thus FISH technique can be applied to standard cytogenetic preparations,

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formalin-fixed tissue, blood and bone marrow smears, and directly fixed cells[4].

FISH is more sensitive than ordinary banding technique which can only detect changes of chromosomes having size of 4Mb, while FISH can detect aberration within at least 1-2 Mb. The increased resolution and specificity provided by FISH enables also the study of minute chromosomal deletions, usually too small to be detected using conventional banding techniques. Although FISH is an extremely useful and powerful technique, some prior knowledge of the nature of the rearrangement to be investigated is usually needed to aid in the selection of FISH probes. FISH probes are either whole chromosome painting probes, or locus specific probes or cosmid, or centromeric specific repetitive DNA probes or telomeric specific repetitive DNA probes.

Though it is an extremely important clinical and research tool, human cytogenetic techniques have never been introduced in Sudan before this study. Hence the aim of the present study, "Cytogenetic and Fish analyses in Sudanese patients with dysmorphic features, ambiguous genitalia, and infertility", was not only to characterize the genetic alterations in patients with dysmorphic features, ambiguous genitalia and/or infertility in Sudanese population, but also to facilitate initiation of treatment and/or habilitation for patients with chromosomal abnormalities in Sudan

Material and Methods

Collection of samples, culturing and harvesting were in the Institute of Endemic Disease, University of Khartoum, Sudan. Staining, cytogenetic, and FISH analysis were done in the Department of Clinical Genetics, Lund University Hospital, Sweden.

The total number of patients was 44; 29 were females, 14 males, and one patient with unidentifiable sex. Patients age ranged between 17 days and 39 years (mean 18 years). Of the 44 patients, 20 were clinically diagnosed as intersex (cases 1-20) (Table 1); 8 with dysmorphic features (Cases 21-28) (Table 2) of which 5 had features consistent with Down syndrome, one with DiGeorge syndrome, and 2 with Edward syndrome; 11 patients complained of infertility, delayed puberty or habitual abortion (cases 29-39) (Table 3); also, 5 parents (cases 40-44) of 3 Down's syndrome cases

Cytogenetic analysis

Peripheral blood samples from all cases were processed for cytogenetic analysis according to standard protocols. In brief, 10 drops of patient peripheral blood were added to 10 ml of culture medium and incubated in 5% CO2 at 37 ˚C for 72 hours. The culture medium used was RPMI 1640 supplemented with L-glutamine, penicillin (100 IU/ml), streptomycin (200 µg/ml), 25% foetal bovine serum, and 3.4 ml of phytohemagglutinin (PHA). All cultures were harvested within 72 hours. To achieve metaphase arrest, Colcemid 0.1 ml was added 30 min before harvesting. After sedimentation and removal of the supernatant, cells were re-suspended in hypotonic solution 0.05 M KCl and fixed three times in methanol: acetic acid (3:1) before dropped onto wet clean slides. The slides were incubated at 60°C for 15 h and kept in 2x SSC in water bath for 4 h. G-banding was obtained with Wright's stain, and at least 10 metaphase cells were analysed for each case. The clonality criteria and the karyotypic descriptions followed the ISCN[5] recommendations

Fluorescent *in situ* hybridization (FISH)

Fluorescent *in situ* hybridization (FISH) were done according to standard protocol[6] as the slides were treated with 0.1% Tween 20 detergent/2xSSC at 60 C˚ for 1 hour, and then washed for 5 minute in phosphate buffered saline(PBS). After that the slides were treated with 1 mg/ml pepsin/0.01 M HCL for 10-20 minutes, washed for 5 minutes in PBS and immersed in1% formalin/PBS for 10 minutes, then rewashed for 5 minutes in PBS, dehydrated by ethanol-series and air dry.

FISH probes cases 11 and 37 respectively, and subtelomeric probe for chromosome 21 were used after labelling with FITC and digoxigenin. The probes were denatured together with the slides on a hot plate at 75Cº for 5 minutes and hybridized at 37Cº overnight. After washing and detection, the slides were counterstained with 0.1 ml 4,6-diamino-2-phenylindole (DAPI) in an antifade solution. The analysis was performed using an olympus epifluorescence microscope equipped with a CCD camera (Photometrics).

FISH with whole chromosome painting, an Xcentromeric probe, and the SRY gene specific probe was applied to fixated metaphases cells according to standard procedures.

Results

Ambiguous genitalia: Cytogenetic analysis of patients with ambiguous genitalia (in total 20 patients) showed male or female karyotype in 18

Table 1. Cytogenetic data of patients with ambiguous genitalia

Case	Age	Sex	Complain	Karyotypes	FISH results
1	1 Year	Unidentified	Intersex	46,XY	
2	22 Year	Grown up as F	Intersex	46,XY	
3	3 Year	Grown up as F	Intersex	46,XX	
4	9 Month	Grown up as F	Intersex	46,XY	
5	3 Year	Grown up as F	Intersex	46,XX	
6	20 Year	Grown up as M	Intersex	46,XX	
7	17 Days	Grown up as F	Intersex	47,XX+18	
8	11 Year	Grown up as F	Intersex	46,XX	
9	3 year	Grown up as M	Intersex	46,XY	
10	1 year	Grown up as M	Intersex	46,XX	
11	7 month	Grown up as M	Intersex	46,Xdel(X)(p11)	Negative for Y materials
12	15 year	Grown up as F	Intersex	46,XY	
13	15 year	Grown up as F	Intersex	46,XX	
14	12 year	Grown up as M	Intersex	46,XX	
*15	12 year	Grown up as F	Intersex	46,XY	
16	1 year	Grown up as M	Intersex	46,XY	
+17	3 year	Grown up as F	Intersex	46,XX	
+18	1 year	Grown up as F	Intersex	46,XX	
+19	5 year	Grown up as F	Intersex	46,XX	
*20	12 year	Grown up as F	Intersex	46,XY	

Table 2 Cytogenetic results of the patients with dysmorphic features

Case NO	Age	Sex	Clinical presentation	Karyotypes
21	25 Year	M	Azoospermia	46,XY[11]
22	22 Year	F	Primary amenorrhea	45,X[6]/46,XX[80]
23	28 Year	F	Primary amenorrhea	45,X[10]/47,X,idic(X)(p11)x2[4]
"24	23 Year	F	Secondary amenorrhea	46,XX[11]
"25	24 Year	F	Primary amenorrhea	46,XX[11]
26	21 Year	F	Primary amenorrhea	46,XY[27]
27	21 Year	F	Primary amenorrhea	46,XX[11]
28	37 Year	F	Secondary amenorrhea	46,XX[11]

"Both cases have sisters with primary amenorrhea banding, which was later corroborated by FISH with whole chromosome 21 painting

Table 3 Clinical and cytogenetic data on 11 patients with infertility and delayed puberty

Case	Age	Sex	presentation	Karyotypes	FISH
*29	34 Year	F	Habitual abortion	46,XX[11]	
*30	39 Year	M	Infertility	46,XX[11]	
31	30 Year	M	Azoospermia	46,XY[11]	
32	10 month	F	DS	46,XX,der(21;21)(q10;q10)[22]	46,XX,der(21;21)(q10;q10)
33	6 year	F	DS	47,XX+21[10]	
34	2 month	F	DS	47,XY+21[11]	
35	1 year	M	DS	47,XY+21[11]	47,XY+21[11]
36	7 month	F	DS	47,XX+21[11]	
37	6 month	M	DiGS	46, XY[11]	46, XY[11]
38	2 year	M	EDS	46, XY[11]	
39	63 days	F	EDS	46,XX[11]	

DS = Downs' syndrome; DiGS = DiGeorge syndrome; EDS = Edward syndrome**,** *Husband and wife

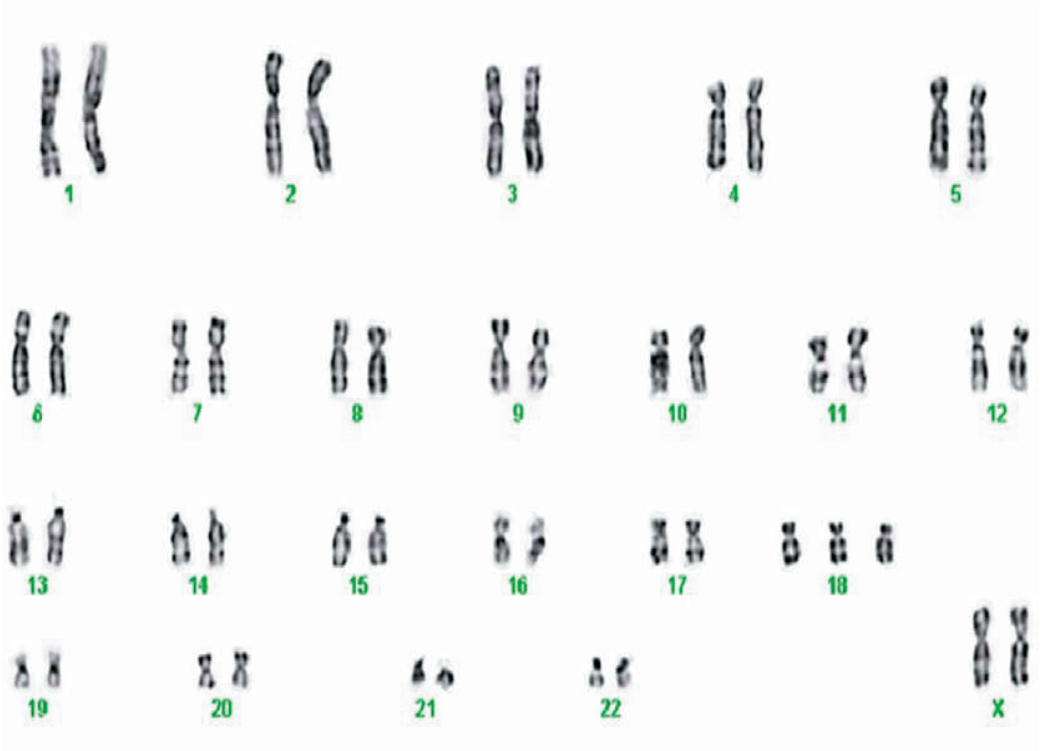

Figure 1. The karyogram of case #7 (Trisomy 18)

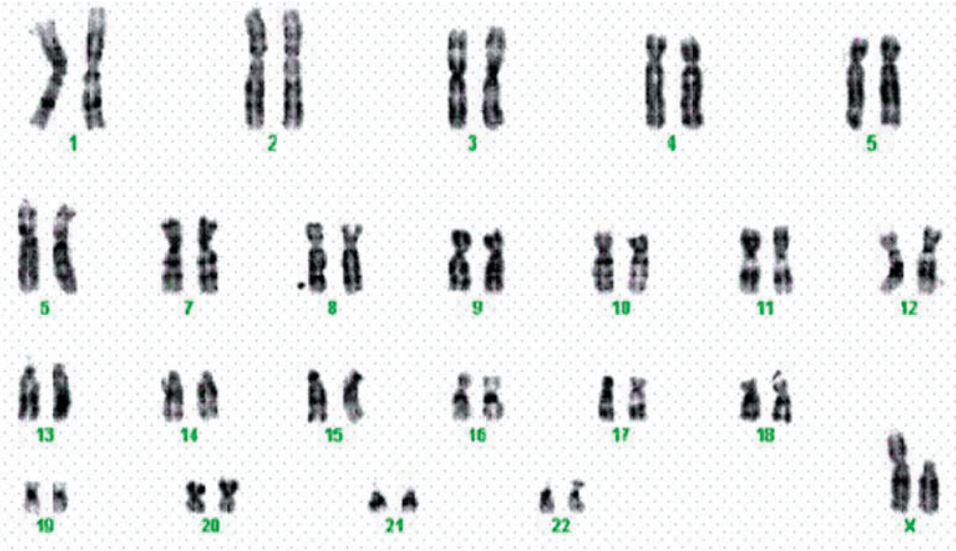

Figure 2. The karyogram of case #11[46,X,del(X)(P11)

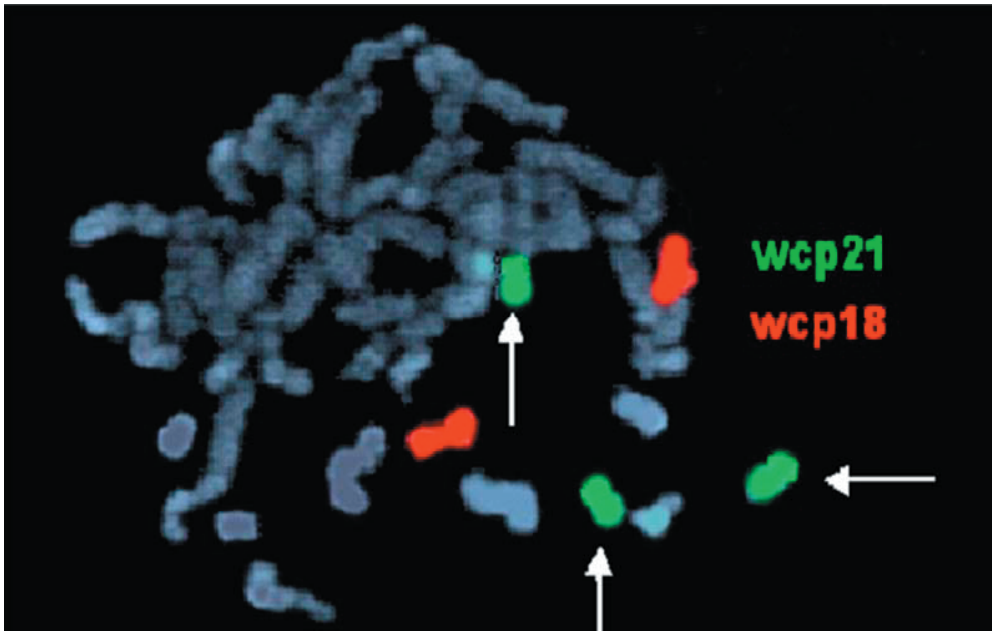

Figure 3. FISH analysis of case #35 using whole chromosome painting (wcp) painting probes specific for chromosome #21.

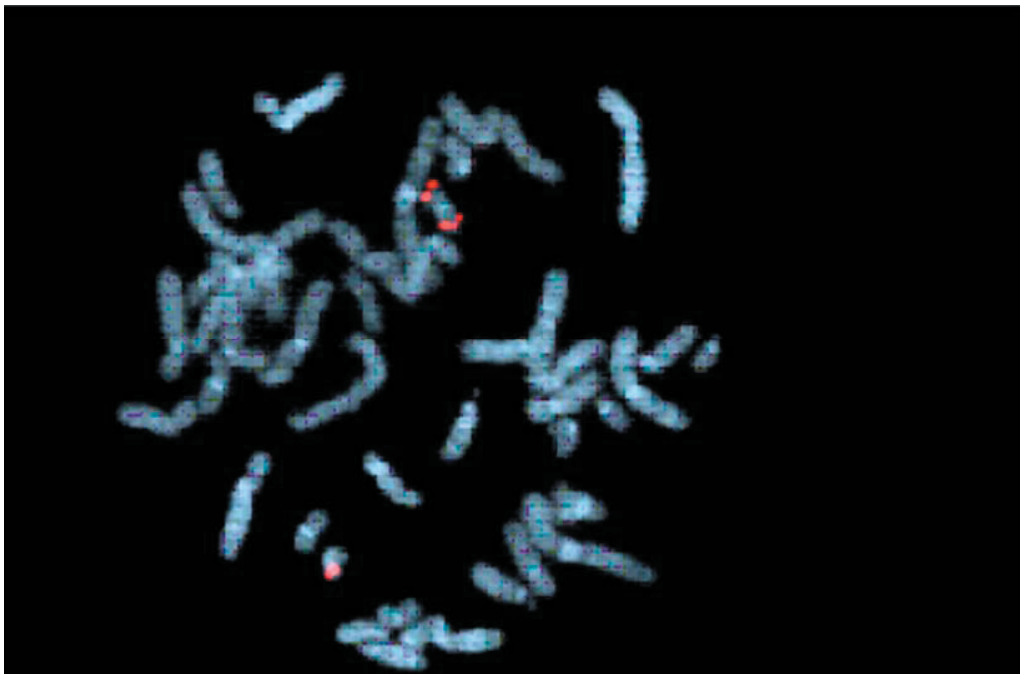

Figure4. Karyogram of case #32 with Robertsonian translocation

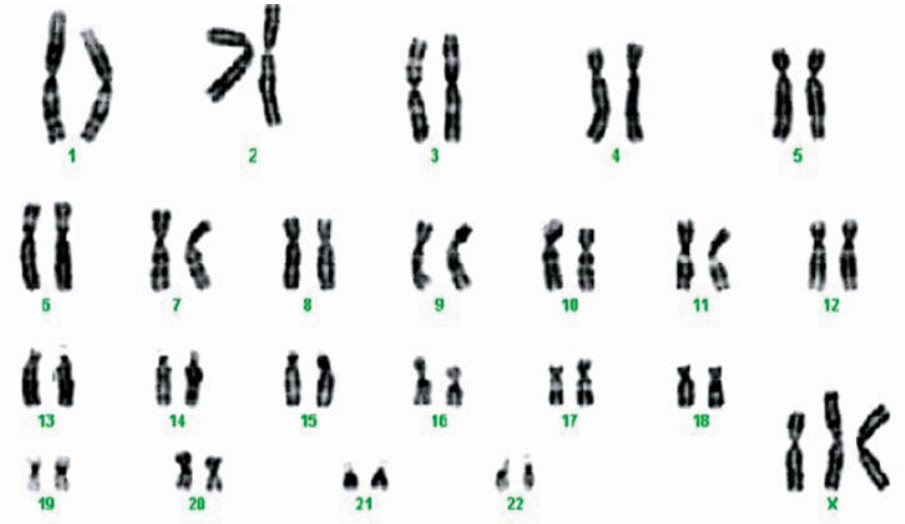

Figure 5. The karyotype of case #23

Patients (Table 1). In the remaining two cases the karyotypes were consistent with Edward syndrome (47,XX,+18) (Figure 1), and the other one was with 45,X,del(X)(p11) (Figure 2), FISH for this case showed *SRY* gene translocation to the abnormal X.

Dysmorphic features: Three of 8 patients with dysmorphic features showed normal karyotypes (Table 2). The remaining 5 revealed abnormal karyotypes consistent with Down syndrome of which 4 had gain of a whole copy of chromosome 21. One patient (Case 32) showed Robertsonian translocations 21;21 translocation by G-(Figure 3). FISH was also applied to one of the other five Down syndrome cases to corroborate the trisomy (Figure 4), because the G-banding preparation quality was suboptimal.

Fertility and pubertal complains: Eight patients with fertility and pubertal complains showed normal male/female karyotypes, 2 patients showed karyotype consistent with Turner syndrome; 46,XX/45,X0 (case 22) and 45,X0/47,X,idic(X)(p11)x2 (case 23) (Figure 5), and one case showed male karyotypes which was not consistent with the assigned sex, when doing further analysis the ultrasound showed a mass in the position of the prostate, clinical examination of this case was difficult because of the female genital infibulations that was performed on the patient during childhood.

Discussion

Ambiguous genitalia

Diagnosis and management of patients with ambiguous genitalia remain difficult and controversial, especially in Muslim societies such as Sudan, due to the sensitivity of gender assignment, which is an issue that possess and plays a valuable social, cultural, and even economical role. The classical view of early gender assignment has been challenged by the results of clinical and basic researches.

Both have shown evidence that the development of gender identity begins in the uterus[7]. Today most of the clinical studies point towards a delay intervention policy. The majority recommend puberty as the optimal timing for gender assignment because the child at that stage can and should be involved in the decision[8]

An interesting finding in the present study was familial ambiguous genitalia seen in 2 families (3 and 2 siblings) cases (15, 17, 18, 19 and 20). Before this study, De Vaal[9] and Simpson *et al*[10]., have simultaneously described 3 intersex brothers . Familial male pseudohermaphroditism due to 5 alpha-reductase has also been reported in a Swedish family with three male siblings[11]. In similar reports tru e hermaphroditism in an XY individual caused by familial point mutation of the *SRY* gene resulting in gonadal dysgenesis has been described[12] .However, fewerthan 10% of true hermaphrodites carry an XY karyotype, and so far only two patients have been documented to car r ya mutation in the *SRY* gene. Although, further molecular analysis was beyond the theme of this study, it is alluring and perhaps scientifically rewarding doing such analysis in the two families.

The real debate revolves around the issues of early gender reassignment since in Sudan it has been mostly decided by midwifes involved after the delivery process. However, the reassignment is challenged by the ultimate question of gender preference of the patient in adulthood, and thus thi s process should be postponed until adulthood as gender assignment does not only based on clinical examinations but also on a team-approach from various specialties such as neonatology, pathology, genetics, endocrinology, pediatric surgery, urology, radiology, psychology and medical ethics. In Sudan another challenge resides in the possibility of providing ideal management for those patients in the absence of any national multidisciplinary management protocols or guidelines, although ambiguous genitalia problem is not negligible and an inappropriate approach to this problem raises an essential question about the outcome of the physiological and psychological complications. It is now beyond doubts that the multidisciplinary approach in management of patients with ambiguous genitalia is superior to all other approaches and hence a specialists group on Sudanese intersex should be initiated

Dysmorphic features

Among the cases with dysmorphic features, 5 of the 8 cases showed abnormal karyotypes consistent with Down syndrome. The classical trisomy 21 was see n i n 4 patients, and one case (case 32) showed a Robertsonian translocation(Figure 3), usually seen only in 4% of all Down syndrome. Robertsonian translocations imply translocation of chromosome 21 to a D group chromosome or another G group chromosome

and in rare instances results from the formation of an isochromosome

Although an isochromosome may originate *de novo* through a recombination of two chromosomes at the region of the centromere, the translocation can be inherited from a healthy carrier, either the mother or the father, with balanced translocation involving chromosome[13] 21 . In such cases chromosomal analysis of the parents plays a crucial role in genetic counselling and risk estimation for the second child. In our case cytogenetic analysis of the parents showed normal karyotypes, indicating *de novo* mechanism. Thus the possibility of having another child with Down syndrome will be within the ratio of other normal couples who has a Down syndrome child with trisomy[14] 21,. Cytogenetic and FISH analysis of the patient with clinical features of DiGeorge syndrome revealed normal karyotype. Although over 90% of patients with DiGeorge syndrome have a microdeletion at 22q11.2 and that the usual deletion is quite large (2-3 Mb) in more than 75% of the cases, FISH usually detects around 85% of the cases. For the remaining cases, the mutation can only be detected using molecular techniques[15]

Pubertal delay and infertility

Disorders of reproduction represent a significant social, medical, and economic burden for individuals and society. Approximately 1 in 10 couples in USA are infertile, and each partner is equally likely to be affected. In Sudan the problem is not clear due to the lack of systematic records. Defining the genetic basis of disease has significant benefits for the patients, as appropriate and educated counselling can be provided and treatment tailored to the individual. Cytogenetic analysis has proven important tool in the detection of chromosomal anomalies *(e.g.* XO, XXY) involved in fertility disorders, thusthe karyotyping of fetal tissue should be done subsequent to a pregnancy loss to identify such chromosomal abnormalities. Unfortunately such investigation requires high level of logistic coordination between clinicians of various specialties and the cytogenetic laboratory.

In the present study, 11 patients (3 males and 8 females) have been referred for chromosomal analysis based on fertility disorders. Of the 3 males, two had azoospermia, and the third was a husband of a woman with habitual abortion and his karyotpyes showed unbalanced translocation. Although, one of the patients has the clinical feature of Klinefelter syndrome, the cytogenetic analysis showed normal male karyotypes in all 3 patients. Thus azoospermia can be due to other physiological or anatomical abnormality of these males genital system.

The cytogenetic analysis of the 8 females with pubertal and fertility disorders showed normal female karyotypes in 5 patients whereas 3 patients showed abnormal karyotypes. Of course menstrual abnormality could also be caused by wide variety of physiological or anatomical irregularities, including mutations in genes involved in the regulation of the menstrual cycle. This might explain the primary amenorrhea in cases 25 and 27 and also can explain the cause of irregularity of menstrual cycle in case 24. For the 5th case (case 28) with female karyotype and secondary amenorrhea her ultrasound showed atrophic ovaries, which could be the cause of the disorder, on the other hand cases 24,25, and 28 had a family history of the same condition. Case 22 who was also complaining form primary amenorrhea showed 45,X[6]/46,XX[80] karyotype, consistent with mosaic Turner syndrome. This explains the mild clinical features of the syndrome in this case as she has few XO cells and most of her cells showed a normal female chromosome complement. The other case was case 23 which showed 45,X[10]/47,X,idic(X)(p11)x2[4] karyotype (Figure 5), which was also consistent with Turnersyndrome, although the second cell line was with one normal X chromosome and two isodicentric X chromosomes, resulting in gain of Xq and loss of Xp. Although females carrying multiple X chromosomes do not complain from primary amenorrhea, the missed short arm of the X chromosome could explain the cause of the amenorrhea. The last case which was also complaining from primary amenorrhea showed 46,XY karyotype. In this patient, there was difficulty in the identification of genital ambiguity clinically because she was circumcised. This made the clinical diagnosis of her condition almost impossible. Her ultrasound showed a prostate, and this matched with her genotype. This case was very crucial because of the aggressive type of female genital mutilation (FGM) which was performed on her when she was a child that

limited the possibility of clinical assignment. The male chromosome complement suggests that the patient suffered from a type of male pseudohermaphroditism. This case suggest a very new and dangerous outcome from FGM which can threaten and limits the possibility of gender assignment of intersex conditions which forces more psychological trauma among them.

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