Questionnaire

Name:…….

ID:…….....

Date of Birth:…….

Grown up as:……..

Address:…….

Reason of Refer:……

Family history:

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Diagnosis:

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Cytogenetic analysis

Blood Culturing

One ml of peripheral blood was sampled from each patient under aseptic conditions and transferred to the cytogenetic laboratory within 2 hours. All samples were processed for culture by adding 8 drops of blood to 10 ml of McCoy's 5A Modified Media (Sigma®), that has been supplemented with L-glutamine, penicillin (100 IU/mL), streptomycin (200 µg/mL), 25% fetal bovine serum (Sigma®), and 3.4 mL phytohemagglutinin (10 µg/mL) (Sigma®), and incubated in 5% CO2 at 37 ˚C incubator for 72 hours.

Culture harvesting

All cultures were harvested after 72 hours by adding 100 µg/ml Colcemid (10 µl/ml) (Sigma®), 30 mints prior harvesting to arrest the cells (lymphocytes) in metaphase. After hypotonic shock in 0.075 M KCl, the cells were fixed four times in methanol: acetic acid (3:1).

Slide preparation

The cells suspension was centrifuged at 1300 rpm for 10 min before removing the supernatant and resuspended in approximately 0.5-1ml of fresh fixative (3:1 methanol: acetic acid). 1-4 drops of cell suspension were placed on a clean dry glass slide. The quality and spreading of chromosomes were assisted under a phase contrast microscope after that 2-4 slides were prepared for each patient.

Slide staining and analysis

The slide-aging was obtained by keeping the slide in the oven at 60 º C overnight. The slides were immersed in a buffer solution (2XSSC) and kept in a water bath at 60 ˚C for 2-3 h. After that the buffer solution was poured off and the slides were washed repeatedly with running tap water, air-dried for 1 hour before the staining. G-banding was obtained with Wright's stain and for each case, 5 to 25 metaphases were analyzed using CytoVision system, Applied Imaging®. The clonality criteria and the karyotypic descriptions were done according to the International System for Chromosomal Nomenclature (ISCN) (1995) recommendations.

Hormonal analysis

Measurement of Luteinizing hormone (LH)

LH was measured using Immunoradiometric assay. The reaction depends on binding of ^{125}I labeled signal-antibody to an epitope of the LH molecule different from that recognized by the unlabelled capture antibody. The two antibodies react simultaneously with the LH molecule forming a ‘’sandwich’’.

Standards and samples were incubated with a mixture of the antibodies at room temperature. At the end of a two hours incubation period, magnetic immunosorbent (MIS) was added in excess. MIS particles selectively bind the LH-signal antibody –capture antibody complex and settled out in a magnetic field. A wash step took place to reduce non- specific binding to a minimum for increased low end precision. The radioactivity of the magnetic immunosorbent pellet was measured in gamma counter and hence the LH concentration was determined using automated data processing system.

Measurement of Follicle stimulating hormone (FSH)

This hormone was measured by Immunoradiometric assay using the same method as LH, i.e. the reaction depends on binding of ^{125}I labeled signal-antibody to an epitope of the FSH molecule different from that recognized by the unlabelled capture antibody. The two antibodies react simultaneously with the FSH molecule forming a ‘’sandwich’’. F The radioactivity of the magnetic immunosorbent pellet was measured in gamma counter and hence the FSH concentration was determined using automated data processing system.

Measurement of Estrogen

This hormone was measured using radioimmunoassay (RIA). A fixed amount of ^{125}I labeled estrogen competes with the estrogen to be measured present in the sample or in the calibrator for a fixed amount of antibody sites immobilized on the wall of a polystyrene tube. After 3 hours incubation at 37ºC, an aspiration step terminates the competition reaction. The tubes were then washed with 3ml of washing solution and aspirated. Concentration of estrogen was determined using automated data processing system.