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Karyotyping: A important diagnostic tool to differentiate infertile females at Prakriti level

Dr. Lakshmi and KN Singh

Abstract
Background: According to ancient system of medicine *vataj prakriti* females are more prone for infertility. Many studies have been carried out to determine the association between *dehaj prakriti* and specific diseases, whereas to understand the direct relation between vataj prakriti and infertility still need more studies.
Objective: To investigate the association between infertility and prakriti by using modern diagnostic tool i.e. Karyotyping.
Materials and Methods: This study was conducted as randomized controlled study on 50 infertile females by evaluating their prakriti and Karyotyping. Study was conducted at Sir Sunderlal Hospital, IMS, BHU and Cytogenetic Lab, Department of Anatomy, IMS, BHU. Prakriti was assessed by Performa (questionnaire based) and Karyotyping was done by G- banding Technique. Correlation between Prakriti and Karyogram and other factors such as age, chief complaints, menstrual history, endocrine profile, were assessed.
Results: The overall vataj prakriti females was 72% out of these 01% are Turner's and 18% are Turner Mosaic. The study identified a statistically significant relation between Vataj Prakriti and infertility.
Conclusion: Results of the present study suggested that the vataj prakriti females was more prone for infertility than pittaj and kaphaj prakriti and all abnormal karyogram is also associated with vataj prakriti.

Keywords: Infertility, Prakriti, Karyotyping

Introduction
Karyotyping is a laboratory procedure that allows your doctor to examine your set of chromosomes. "Karyotype" also refers to the actual collection of chromosomes being examined. Examining chromosomes through karyotyping allows your doctor to determine whether there are any abnormalities or structural problems within the chromosomes. Chromosomes are in almost every cell of your body. They contain the genetic material inherited from your parents. They're composed of DNA and determine the way every human develops. When a cell divides, it needs to pass on a complete set of genetic instructions to each new cell it forms. When a cell isn't in the process of division, the chromosomes are arranged in a spread out, unorganized way. During division, the chromosomes in these new cells line up in pairs. A karyotype test examines these dividing cells. The pairs of chromosomes are arranged by their size and appearance. This helps your doctor easily determine if any chromosomes are missing or damaged. A normal test result will show 46 chromosomes. Two of these 46 chromosomes are sex chromosomes, which determine the sex of the person being tested, and 44 of them are autosomes. The autosomes are unrelated to determining the sex of the person being tested. Females have two X chromosomes, while males have one X chromosome and one Y chromosome. Abnormalities that appear in a test sample could be the result of any number of genetic syndromes or conditions. Sometimes, an abnormality will occur in the lab sample that's not reflected in your body. The karyotype test may be repeated to confirm that there's an abnormality [1]. *Prakriti*, refers to genetically determined physical and mental constitution of an individual. Every person has his/her own unique constitution which determines the biological functions, response to environmental factors, drugs and also susceptibility to diseases making it one of the earliest known concepts of preventive and personalized medicine. The knowledge of *prakriti* and the ability to subgroup individuals based on their predominant *prakriti,* in Ayurveda system of health care, thus, is one of its important and unique specialties and essential tools. This not only helps to understand the mental and physical nature of a person in health but also to know the susceptibility to diseases which assists in promotion of health, prevention and cure of diseases [2].

It may also be mentioned that Ayurveda system primarily aims at treating the cause of the disease (and not just the symptoms) by identifying the imbalance of the *Tridoshas* (*Vimana Sthana* 8, *Charaka Samhita,* 2003). As Prakriti represented by three bio humours that are present in every single cell of human body and alteration in these biohumours leads to disease, what if these biohumours disturbed at time of birth of a baby, it will leads to genetic defect on the basis of disturbance of biohumor

Materials and Methods

Karyotyping

To detect any chromosomal aberration in cases of infertility karyotyping of Patients blood sample was done. Karyotyping procedure was done at the Cytogenetics Lab of Department of Anatomy, I.M.S., B.H.U., Varanasi. Following procedure was used.

Media Preparation

Following chemicals were used to prepare complete media for Lymphocytes (for 100 ml).

Table 1: Media preparation

Chemical/Reagent	Amount
RPMI 1640 (gibco)	70ml
Fetal Bovine Serum (gibco)	30 ml
Streptomycin/Penicillin antibiotic (gibco)	800µl
Sodium bi carbonate (Himedia)	0.1gm
Total	100ml

Objectives

1. To demonstrate a micro technique for reliable chromosomal analysis of leucocytes obtained from peripheral blood.
2. To prepare a karyotype from the chromosomes of female patients.
3. To use the karyotyping techniques for diagnosing a chromosomal disorder.
4. Introduction to Karyotyping

Materials and Methods

(1) Materials required

- Peripheral human blood
- Heparin sodium injection 25000units/vial
- Karyotype medium RPMI-1640
- Foetal Bovine Serum
- Penicillin –Streptomycin 10000 U/ml penicillin and 10000 mg/ml streptomycin
- Phytohemagglutinin-M (PHA-M)
- Sodium bi carbonate
- Colcemid solution 10mg/ml
- Hypotonic solution (3X KCl + 1XTri sodium Citrate)
- Fixative solution (3X Absolute Methanol +1X Glacial Acetic Acid)
- Giemsa stain
- Phosphate buffer saline
- Trypsine
- Slides and microscope

Methods

(1) Peripheral blood media preparation

Blood culture media; Add 70 ml RPMI 1640 with 30ml foetal bovine serum, 800µl penicillin – streptomycin, 0.1 gm Sodium bi-carbonate in flask and filter it.

(2) Blood preparation

i. Wash 5ml syringe with Heparin solution and collect blood aseptically to the syringe (0.5ml for each tube).
ii. Remove the blood to sterile vacuette tubes or to sterile, Heparin prewashed tube.
iii. Store at 4 ^{O}C until required (up to 7 hours).
iv. Before use, invert the tube with blood several times.

(3) Karyotyping procedure

i. Inoculate 300µl of heparinized whole blood into tube with 5ml of karyotyping medium.
ii. Incubate the tubes in incubator with 5% CO_2 at 37 ^{O}C for total of 70 hours.

(4) Harvesting

i. After total of 70 hours from seeding add 30µl of Colcemide Solution to each culture tubes.
ii. Incubate the tubes at 37 ^{O}C for an additional 1 hour.
iii. Spin at 1000 RPM for 10 minutes.
iv. Remove the supernatant and re-suspend the cells in 5ml of hypotonic solution pre wormed to 37 ^{O}C.
v. Incubate at 37 ^{O}C for 20 minutes.
vi. Spin at 1000 RPM for 10 minutes.
vii. Remove the supernatant and add drop-by- drop (with vortexing) 5ml fresh ice cold fixative
viii. Spin at 1000 RPM for 10 minutes.
ix. Remove the supernatant, agitate the cellular sediment and add drop-by drop (with continuous vortexing), 5ml of fresh, ice-cold fixative.
x. Leave at 4 ^{O}C for 20 minutes.
xi. Repeat steps 9 and 10, until the supernatant is clear.
xii. Spin at 1000 RPM for 10 minutes.
xiii. Re-suspend the cell pellet with a 1.5ml of fresh fixative.
xiv. Drop 4-5 drops, from a high of approximately 50 cm onto a clean chilled slide and blow carefully on the drops for spreading them on the slide.
xv. Heat the slides to 55 ^{O}C for overnight.

(5) Staining Procedure

Put the slides in a staining rack (e.g. coupling staining jar) and treat as follows:

i. Four Second in Trypsin solution.
ii. Wash in 100ml Buffer solution pH 6.8.
iii. Wash several times with running water.
iv. Put slides in Giemsa stain for 20 minutes.
v. Wash slides several times with running water.
vi. Air dries the chromosome's slides.
vii. Check for chromosome spreads in a phase contrast lab microscope.

(6) Karyotype analysis

Obtain a set of chromosomes. Match the chromosomes with their homologous mate. One chromosome of each pair is numbered, as you match your chromosomes number the homologous pairs. You need to be very systematic. The number one chromosome is the largest. Its corresponding mate should be of the same size, with the same banding pattern, and have the same centromere location. (The Applied Imaging Cytogenetic Workstation)

Fig 1: Showing method of peripheral blood withdrawal from patient.

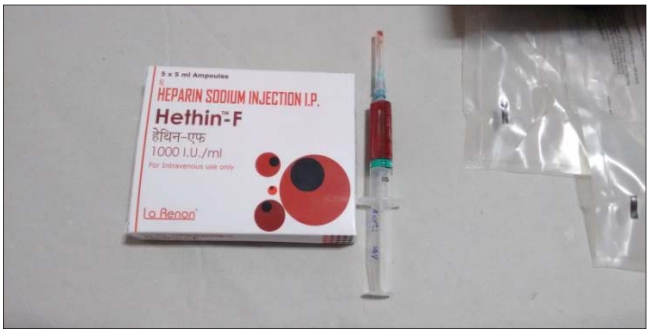

Fig 2: Showing withdrawal blood sample in heparinised vial.

Fig 3: Showing chemicals used for making culture media.

Fig 4: Blood culture media; Add 70 ml, RPMI 1640 with 30ml fetal bovine serum, 800µl penicillin – streptomycin, 0.1 gm Sodium bi - carbonate in flask and filter it.

Fig 5: Showing Laminar Air Flow.

Fig 6: Showing Incubator used in Karyotyping.

Fig 7: Showing Centrifugation Machine.

Fig 8: Showing equipments/chemicals required for harvesting of blood media.

Fig 9: Showing slide preparation from harvested cells (WBC pallet).

Fig 10: Showing prepared slides kept for aging at 37˚C temperature.

Fig 11: Showing Final Prepared Slides in slide box.

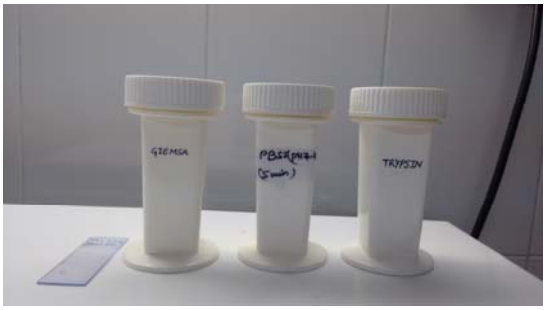

Fig 12: Showing chemicals used in staining process of prepared slides.

Fig 13: Showing the Applied Imaging Cytogenetic Workstation used in analysis of chromosomal slides.

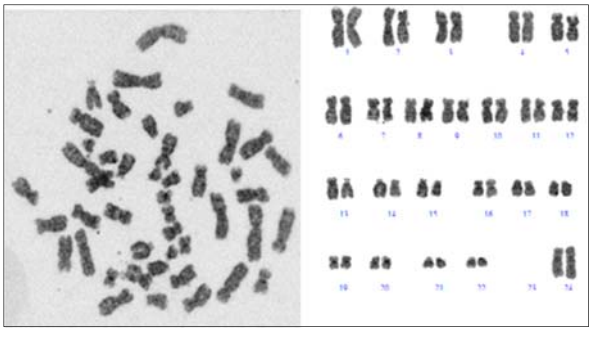

Fig 14: Showing Report-*46*, *XX*; Normal Female Karyotype.

Result

According to Karyotype, women were classified into three categories, the first one included women with normal karyotype and they represented the 80% of the infertile women sample, whereas the second category included women with XO this group accounted for only 02%infertile women sample. Women with turner mosaic constituted the third group and they accounted for 18% of the entire sample. These categories are outlined in table -1.

Table 1: Showing the distribution of cases according to Karyotype

Karyotype	Number of cases	Percentage
Normal(XX)	40	80.0
Turner(XO)	01	02.0
Turner mosaic	09	18.0
Total	50	100.0

According to prakriti, women were classified into three categories, the first one included women with vataj prakriti and they represented the 72% of the infertile women sample, whereas the second category included women with pittaj prakriti this group accounted for 16% infertile women sample. Women with kaphaj prakriti constituted the third group and they accounted for 12% of the entire sample. These categories are outlined in table -2.

Table 2: Showing the distribution of cases according to *Prakriti* of patient

Prakéti	**Number of cases**	**Percentage**
VÁtaja	36	72.0
Pittaja	8	16.0
Kaphaja	6	12.0
Total	50	100.0

On the comparison of prakriti & Karyogram, out of 50 patients (52%) *VÁtaja*, (16%) *Pittaja*, (12%) *Kaphaja* represent normal karyotype (80.0%), 20 *VÁtaja*, represent turner & turner mosaic (20.0%). table -3.

Table 3: Showing the comparison of cases as per *Prakriti* and karyotype report

Karyotype	***Prakéti***			**Total**
	Vátaja	***Pittaja***	***Kaphaja***	
Normal	26 (52.0%)	8 (16.0%)	6 (12.0%)	40 (80.0%)
Turner & Turner mosaic	10 (20.0%)	0 (0.0%)	0 (0.0%)	10 (20.0%)
Total	36 (72.0%)	8 (16.0%)	6 (12.0%)	50 (100.0%)

$\chi 2=4.861$, $p=0.08$

Discussion

Prakriti is formed at time of birth of a baby by three bio humours (vata, pitta, kapha), these biohumours are present in every single cell of baby, manifestation in any of these humours leads to defect in baby, chromosome distribution in a cell also occur at very initial level and vata is responsible for division of cell and its content according to ayurveda.so, if at time of birth there is disturbance in vata dosha it will lead to defect in division of cell and its content and hence lead to defect on the basis of its severity it can produce defect in single cell and in no. of cells. we can make this ancient system of health care easily available and acceptable to 21st century generation, we must transformed it into a burgeoining cash cow on worldwide soil for that we need minds who can think outside the box. By connecting these methods to present time requirement and need and thoughts we can introduce this ancient holistic medical system worldwide, as people need simple ways to achieve health and prevent disease for that people need healthy diet and healthy life style, people also need mental piece and relaxation, all these demands are fulfil by the Ayurveda but to make this pathy easily understandable to all in present time we need to innovate its content and procedures, as so in this paper we asses prakriti by questionnaire Performa and after assessing prakriti we also asses karyogram and we try to co-relate both.

Conclusion

Ayurveda has universal value and potential to provide innovative, holistic and affordable health care, but this good will energy need to be properly channelled. If we can aware people to follow preventive aspect of ayurveda all in present time we need to innovate its content and procedures, this is our one step in bringing ayurveda and modern pathy together.

Limitations

This study was done on limited no. of patients due to time limitation for my research work to make it more clear this study requires comparison of large no. of patients *prakriti* with Karyogram.

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