Physical Properties of Surrogate's Blood

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Abstract: ***The present paper discusses methods to determine Coefficient of Viscosity, Volume flow rate, Surface Tension, Turbidity, Size and shape of Surrogate's Red Blood Cells with the help of different techniques like "Capillary tube method" & "Laser diffraction technique" designed and developed in our Biophysics Laboratory and comparison with the normal pregnant women's blood. Significant variations are observed in the trails. This variation may be attributed to the number of factors including past reproductive history, maternal age, reason of fertility and life style factors of surrogate's and normal pregnant women. This investigation identifies grounds of several adverse effects/conditions being associated with Surrogacy and compare with Normal pregnancy. Further discussed the more interesting results along with future directions.***

Keywords : Human Blood, Viscosity, Volume flow rate, Surface Tension, Size and shape of Red blood cell, Turbidity, Surrogacy, Capillary tube method, and Laser Diffraction Technique.

I. INTRODUCTION

The term Surrogacy arrangement or Surrogacy agreement means the carrying of pregnancy for intentional parents who are not blessed with privilege by natural means. There are two key types of Surrogacy, gestational Surrogacy also known as host or full surrogacy [1] which was first achieved in mid 1980s[2] and traditional Surrogacy also known as partial, genetic, or straight Surrogacy. Many developments in Medicine, Social Customs, and legal proceedings worldwide surfaced the way for modern commercial Surrogacy [3]. Different Religions take different approaches to Surrogacy, often related to their stances on assisted reproductive technology in general. Many Ethical issues that have been raised with regards to Surrogacy [4]. Surrogacy in India is relatively low cost and previously laws were flexible. But as of 2014 a Surrogacy ban was imposed on homosexual couples, and single parents. Commercial Surrogacy is now illegal in India after a bill No. 257-C of 2016 passed in Loksabha on 19th Dec 2018; however in India the first Surrogacy case was found/reported on 23rd June 1994.

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II. MATERIALS AND METHODS

The Blood is heterogeneous bio fluid as a result it is very complex in nature. Studying the size and shape of Red Blood Cells (RBC) is clinical importance not only to different type of cells but also in differentiating Surrogates from Normal pregnant women blood cells. There can be change in the shape of the cells as it is reported in the case of Sickle cell anemia [5]. Size and Shape of Red Blood Cells(RBC) are usually determined by microscope eriometer technique and many other methods. But all these methods are complex and tedious one, cannot be extended to a huge number of cells and samples. In view of this a simple and rapid method is developed in Biophysics Laboratory, Nizam College (Autonomous) Osmania University, Hyderabad for the determining the average size and shape of Red Blood Cells (RBC) using PC based Laser Diffraction Technique [6, 7]. This new technique is free from the practical difficulties of the past techniques like optical and eriometer methods with accurate results and in agreement with Laser Diffraction method. The changes in many morphological parameters provide clues to many disease conditions & possible side effects and also advise additional diagnostic information.

A. Laser Diffraction Technique

Fresh samples of Surrogate's and normal healthy pregnant blood of 2-8 ml was collected from different IVF/Surrogacy/Fertility and Conception/ Test Tube Baby Research centers. EDTA was added to the collected blood samples to avoid coagulation and samples are stored in a non-conducting flask/test tubes for the measurement of different physical parameters. On well cleared slides, uniform smears of these samples were made. Sample collection and slide making process duration is less than 10-60 minutes. The PC based Laser Diffraction Technique is based on Babinet principle that gives Fraunhafer diffraction pattern[8, 9]. This experimental arrangement consists of specimen slide, Laser, screen and PC based embedded system for the determination of size and shape of Surrogates and healthy normal pregnant women's erythrocytes. For sample preparation freshly collected blood drop from the subject is smeared uniformly on glass microscope slide in the form of thin film and then it is kept in between the laser and the screen with the smeared surface facing the screen [10]. A 2 mw power He-Ne Laser source when passes through the blood sample gives the well-defined Laser Diffraction rings pattern on the screen and it will be captured by web-cam arranged with PC based embedded system as shown in Figure.1

"The radius r of the first order diffraction minima was measured for different samples for a known sample to screen distance D by using this PC based embedded system".

The angle of diffraction is given by

Tan θ = r/D

Where r = radius of the first circle

D = **distance** between sample slide and Screen

"The mean diameter (d) of the Red Blood Cells was calculated using the image processing toolbox of MATLAB taking account the wavelength of the Laser light. By making use of Rayleigh's criterion, the diameter (d) of the cell can be written as **d = 1.22 λ/Tan θ = 1.22 λ D/r** where λ = 6328 Å "

Figure1. Experimental set up of PC Based Laser Diffraction Technique

B. Capillary Tube Method

For the experiment, the Viscometer is a simple glass capillary tube of length of 30 cm with inner radius 0.05cm was marked with two points A & B and is clamped to be held vertical in a mechanical arrangement as shown in Figure.2 below.

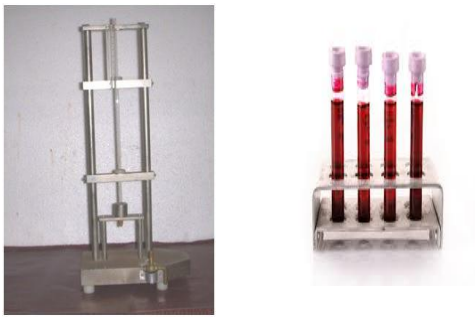

Figure2. Viscometer and plastic blood test tubes for experimentation

To measure the time flow of Surrogate's and normal pregnant women's blood a digital watch of LC 0.1 second was used.

The distance between points A and B on glass tube is 10 cm. The column of Surrogate's and normal pregnant women's blood sample about 1 to 8 cm in length was sucked in to the capillary tube. The flow of blood column was controlled by closing and opening the upper end of the tube by right hand thumb. The time was switched on the moment the meniscus of the blood column just touches mark A. The time was switched off at moment the meniscus just reaches the mark B. The timer records the time (t) of the blood sample which travelled a fix distance of 10 cm. The velocity was calculated as 10/t cm/Sec. for different lengths (L) of the surrogate's and normal pregnant column the time of travel (t) was recorded and velocity (V) was calculated. A plot was drawn between L^{-1}on X-axis and V on Y- axis which approves the theory. The intercept and slope of the straight line were measured. Viscosity and Surface tension of the blood sample were calculated from the intercept and slope of the straight line knowing radius of the capillary tube (R) and angle of contact (θ) of the sample with the capillary wall. The contact angle is approximated to 30º for blood samples.

Coefficient of viscosity (η) is calculating the using the relation **(η) = R^2ρ g / 8 V_0**

Volume flow rate (Q) of sample is computed from the relation

Q = V_0 (Π R^2)

Surface tension is (T) is calculated using the relation

T = 4 η tan α / R Cos θ

Where R- Radius of capillary tube,

ρ – Density of Surrogates and Normal pregnant women's blood,

g- Acceleration due to gravity,

V_0- Maximum velocity,

α - Slope of the straight line drawn between Velocity (V) on Y-axis and 1/L on X- axis,

θ – Angle of contact for blood sample.

C. Turbidity

The phenomenon of absorption and scattering of light by particles present in normal pregnant woman & surrogate's blood serum is called turbidity of biofluid. This turbidity is caused due to suspended ingredients in the blood sample. In this present investigation turbidity is determined by comparing the intensity of scatter light by water solution sample with reference surrogate's blood sample using Digital Turbidity Nephelometer.

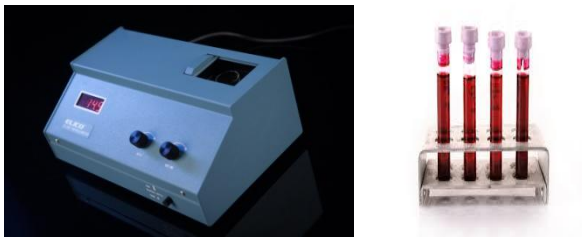

Figure3. Nephelometer and Blood Samples

The aforementioned method is employed in the total of fifty blood serum samples of surrogates and normal pregnant women. Significant variation was observed in turbidity measurements. The large variation in turbidity figures were observed, This also true in a number of inflammatory lesions, losses of blood proteins, nutritional defects, toxemias of pregnancy etc. The intensity of the turbidity variation in surrogates and normal pregnant women (21 to 53%) depends from the qualitative and quantitative relations of the serum proteins,

albumin being the most important; however the qualitative changes of albumin also make themselves felt. The results were compared with data obtained from further laboratory examinations and clinical diagnosis and the following biochemical and clinical correlations of the albumin turbidity reaction were found.

III. RESULTS

The results of the present investigation were tabulated in Table.1, 2 and 3.

Table.1 Size (d) of Normal healthy pregnant Women and Surrogate's erythrocytes taking Average and STD for twenty samples each.

S.No	Type	Size and Shape of RBC in Diameter (µm)
1	Normal healthy pregnant Women	7.23 ± 0.54 (µm) (Spherical Shape)
2	Surrogacy Women	6.78 ± 2.15 (µm) (Slightly differ from regular Spherical Shape)

Average ± STD

The table gives the average data on Coefficient of Viscosity, Surface Tension and Volume Flow rate of 25 surrogates and normal pregnant women's blood sample along with standard deviation.

Table:2 Data on Viscosity, Volume flow rate and Surface tension of Surrogate's and normal pregnant women samples

Sample Name	Viscosity (Poise)	Surface tension (dyne/cm)	Volume flow rate (X 10^{-3} cm^3/sec
Surrogate's Blood	0.051 ± 0.023	2.54 ± 1.25	0.90 ± 0.066
Normal Pregnant Women's Blood	0.037 ± 0.008	1.84 ± 0.011	0.65 ± 0.025

Average ± STD

Table.3 Turbidity of Normal healthy pregnant woman and Surrogate's blood serum samples taking Average and STD for twenty five samples each.

S.No	Type	Turbidity in NTU Nephelometer Turbidity Units
1	Normal healthy pregnant Women	189 ± 23.55
2	Surrogacy Women	283 ± 41.23

Average ± STD

IV. DISCUSSION

Viscosity is ascribed to the intrinsic properties of the bio fluids itself however surface tension is exclusively a molecular phenomenon and volume flow rate is a main parameter in bio-fluid dynamics. The study suggests that protein concentrations greatly affect the viscosity of Surrogate's blood, however its value changes from person to person (surrogates). The health risks of surrogates are uncertain but the long term health effects are not known. In the case of surrogates the Viscosity is drastically increased due to variations taken place on the RBC cell membranes, because of increased metabolic activity due to surrogacy and this leads to the changes in the cell size and morphology. High level of RBC is characterised as Polycythemia situation and very high RBC mass causes reduction in blood flow speed and upsurge the risk of intravascular clotting, coronary vascular resistance, reduced coronary blood flow and also predisposition causing thrombosis.

Hematocrit values were also significantly high in surrogates than those of normal pregnant and consistent with previous findings. The drastic increased in the Viscosity of erythrocytes of Surrogate's may be attributed to clotting of blood cells and it leads to damage in nervous system especially the spinal cord and brain. The surrogate's viscosity method is simple and easy method and can be used as an adjunctive measure for the diagnosis of various diseases with involvement of central nervous system and spinal cord. Surface tension significantly varies compare to other bio fluids. Coefficient of viscosity can be potential parameter to detect contamination in bio fluids like blood. Volume flow rate of surrogates appears to be significant in characterizing different bio-fluids.

The PC based Laser Diffraction method is very rapid and simple technique. The size of the diffraction pattern is inversely proportional to the particle size and the width of the diffraction pattern is a function of the variations in the size of the particles.

Likewise, the sharpness of the minima depends upon the consistency in cellular size and shape.

This PC based embedded method is not only simple but also sophisticated, can be readily handled and easily demonstrated to a large gathering at a time.

Especially to moderately sophisticated health science/Medical Students which would offer them to look at physically relevant phenomenon. This could also be viewed as improvement in diagnostic tool for assessing abnormal variation in the size distribution of Surrogate's RBC from the width of diffracting ring.

The mean diameter of RBC of normal healthy pregnant women obtained by this PC based Laser Diffraction method is **7.23 ± 0.54 µm** and whereas for Surrogate's women RBC cells size is **6.78 ± 2.15 µm** The shape of RBC of Normal healthy pregnant women is spherical one, where as in the case of Surrogacy Women it slightly differ from regular spherical shape. It was noticed that the average mean value of standard deviation is very high in the case of Surrogacy Women. In the case of Surrogate's the size of RBC obtained is significantly decreased or increased due to changes taken place on the RBC cell membrane, because of high metabolic activity and various steps involved in Surrogacy/ (IVF) process and this leads to the changes in the cell morphology and size.

The drastic size decreased or increased in erythrocytes of Surrogate's may be attributed to a number of factors including past reproductive history, maternal age, reason of infertility and life style of Surrogate, it is also important to comprehend that genetic Surrogacy is inherently more problematic and more likely to fail than gestational Surrogacy.

The turbidity is made by serum albumin and comes about when the serum is diluted. The reaction can be rightly used as a screening test of pathological anomalies of serum proteins, especially of albumin. The turbidity has been found to be formed by the surrogate's serum albumin under described conditions, subject to molecular changes with following changes of disparity. This occurrence has been used to create the albumin turbidity reaction of surrogate's blood serum applicable in clinical diagnostics. The fact itself which of the globulin fractions were increased was of no significance. The strength of the reaction was not influence in cases of higher bilirubin levels in the surrogate serum.

V. CONCLUSIONS

From the present investigation of physical properties of surrogate's blood like viscosity, surface tension, volume flow rate, size and shape of Red blood cells, and turbidity the following interesting conclusions were drawn.

From the present investigation it is clear that Viscosity of Surrogate's blood **(0.051 ± 0.023)** poise higher than the normal pregnant. **(0.037 ± 0.008)** poise.

The average size and shape of Surrogate's (**6.78 ± 2.15 µm)** and normal pregnant women **(7.23 ± 0.54 µm)** Red Blood Cells, was calculated by using the PC based Laser Diffraction Technique. Significant variation was observed. It is also found that the shape of RBC of Surrogate's is slightly differing from regular spherical shape.

Turbidity can be potential parameter to detect adulteration in biofluids like blood. The turbidity of Surrogate's blood serum is high (**283 ± 41.23) NTU** when compared to normal pregnant (**189 ± 23.55) NTU** women.

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