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Use of Autologous Plasma as a Hair Follicle Holding Solution with Clinical and Histological Study

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Abstract **-**

Background *- One of the crucial aspects of hair transplant procedure is graft holding solution. We need a media which have same osmolality as of grafts cells, prevent acidosis, provides energy and prevent release of free radicals*

Aims and Objective *- To evaluate plasma as a graft holding solution and its efficacy in terms of hair growth and graft survival.*

Material and Method *- Equal number of Hair follicles were divided in two groups. Test group grafts were kept in freshly prepared autologous plasma, while the control group grafts were kept in ringer lactate. Under same conditions both group grafts were implanted male patients on right and left frontotemporal region in same density. Results were analysed on patients global photographs, tricoscan for hair density and thickness. The grafts were also send for histology by MTT stain to see viable cells in follicles.*

Results *- on all parameters the results in plasma as graft holding solution were much better as compare to ringer lactate.*

Conclusion *-As per our study the freshly prepared autologous plasma is a better graft holding solution as compare to ringer lactate in terms of cells viability, clinical results of early hair growth, decrease in anagen effluvium, and better quality of hair.*

Conflict of Interest *-The authors have no conflict of interest and study self-funded.*

Keywords **- *PRP, Plasma, graft holding solution, tricoscan, MTT stain, anagen effluvium*.**

Introduction

Success of hair transplant depends on many factors. Main factors are trauma to grafts at multiple stages of manipulation and ischemic injury during outside body period. All these factors are unfavourable and will lead to ischemic damage and finally apoptosis. The apoptosis will affect graft survival and or quality of hair growth. To get best results we need to focus on all factors and improve on them.

One of the crucial aspects of hair transplant procedure is graft holding solution. We need a media which have same osmolality as of grafts cells, prevent acidosis, provides energy and prevent release of free radicals. Mainly there are two types of holding solutions –one is extra cellular- Examples are normal saline, lactated ringer and plasma like fluids. Second type is intra cellular- example is hypothermosol. Extra cellular fluid does not require chilling rather chilling causes sodium pump failure leading to cell swelling. While intra cellular fluids needs chilling.[6]

An ideal graft holding solution- It should prevent Acidosis. Osmolality- maintains Ionic balance- to prevent cell swelling. Energy-for regeneration of high energy compounds.[4] Prevention or treatment for free radicals (Free radical causes apoptosis leading to anagen effluvium. Ultimately affect hair mass per hair follicle).[5] Scavengers to fight against organism.

At our centre we are using autologous plasma as a graft holding solution. After noticing good clinical results we did a study based on histological, clinical and tricoscan analysis. There are numerous papers and presentation advocating use of PRP to promote hair growth with logic that platelets have growth factors which affect stem cells of hair follicles.

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Aims and Objective -

To evaluate plasma as a graft holding solution and its efficacy in terms of hair growth and graft survival.

Method

Autologus plasma is used to keep grafts at a temparature aroud 12+2 degree celcious.

Another graft holding solution used was lactated ringer as a control.It is also at temp 12+2 degree celcious.

Room temprature was around 18 degree Celcious.

Bilateral fronto temporal area was selected as recipient site. Left side grafts were kept in lactated ringer and right side graft kept in plasma. Both sides were implanted with same number of grafts with nearly same quality and similar implantation time. Both side two surgeons of same experience and efficiency using loupe did implantation.

Parameters were taken into consideration for study.

1. Histological study in MTT stains to see viability of cells in grafts at 12hr and 72 hrs.
2. Post-operative patient were followed periodically and Photographs taken to see anagen effluvium.
3. Tricoscan study for hair density at three months for anagen effluvium.
4. Hair thickness at 6 months for quality of hair growth.

First step is to prepare plasma - The 23 cc of blood was collected from same patient in a syringe having 2 cc ACD solution as anti-coagulant. The blood was transferred to an innovated high quality glass container, specialy designed by author. (fig.01)The blood was centrifuged in a temperature control centrifuge machine for separation of RBC and plasma having platelets in it.(fig.02)After preparing plasma, it was kept in an sterile stainless steel bowl which was kept on a cool gel to keep plasma temprature around 14 degree Celcius.

Fig.01, Blood **Fig. 02, After First spin**

Fig.03, Plasma in syring to be used as graft holding solution

Grafts harvested from a strip were divided randomely in 2 groups.same number of grafts were chosen in both group. Group (A) grafts were kept in Ringer lactate and Group (B) grafts were kept in Plasma.(Fig.04)

Patients of male pattern baldness aging 25 to 40 yrs were selected for study. Bilateral fronto temporal area were site for implantation of grafts. Grafts kept in plasma were implanted on Right FT area while grafts kept in RL were implanted on left side. Same number of grafts were implanted at density of 40 Grafts per square cm. by two surgeon of almost same efficiency, in same time using magnification loup.

All other factors were same as a routine protocol.

Study conducted as follows -

1. The graft sample A and B were sent for MTT stain at 12 hrs and 72 hrs of graft holding time.
2. Pateints were followed as a routine at 1,2,3,4,6 months post op.
3. At three months photographs were taken for comparision on left and right fronto temporal area using flash and without flash in same situation.
4. Hair count and density taken on both side using tricoscan.
5. At 6 months follow up, photographs and trico scan for hair thickness was done again.During tricoscan only terminal hair were studied.

Fig. 04, Grafts kept in Plasma

OBSERVATIONS

A. Observation of MTT Stain

1. MTT- STAINING AT 12 HRS - Grafts kept in plasma were well stained at 12 hrs (fig.-06)while stored in RL showed poor staining (fig.-05),indication good cell viability in plamsa group while relatively less viability in RL group

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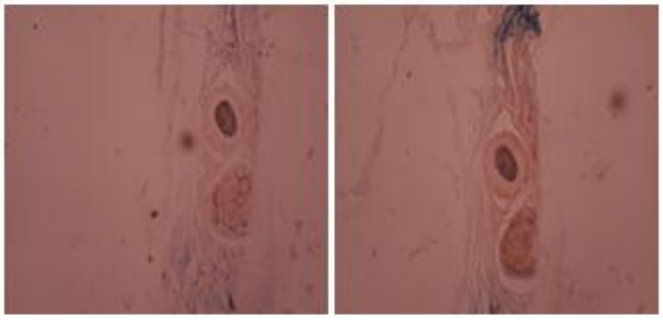
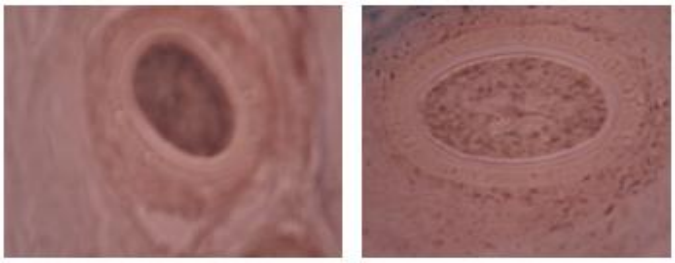
Fig.5, 12 hrs, Ringer **Fig.6, 12Hr Plasma**



2 MTT- STAINING AT 72 HRS - Grafts stored in Plasma Fig.08 showed good staining while very poor stain of graftes of RL group Fig.07. Means grafts cells were still viable at the end of 72hrs when kept in plasma while there was very poor viability of graft cells in RL group.

Fig. 07, 72 hrs Ringer **Fig.08, 72 hrs Plasma**



B. Tricoscan study -

Tricscan study for hair density done at 3 months, Table1, shows average hair growth on plasma side was 71.5% as conpare to ringer lactate side where it was only 13.75%. Hair thickness at 6 months, table2. Shows on plasma die 62.33 micromm on plasma side while on ringer side it was 40.6 micromm.

Table-1 Hair Density - At 3months

	Hair Count		Hair Density per square cm		Average % of hair growth	
G.H.S.	RL	Plasma	RL	Plasma	RL	Plasma
P	2	9	6.6	29.7	13.75 %	71.5%
P	1	10	3.3	33		
P	2	7	6.6	23.1		
AV	1.7	8.7	5.5	28.6		

Fig .09, hair counts by tricoscan at 50 days

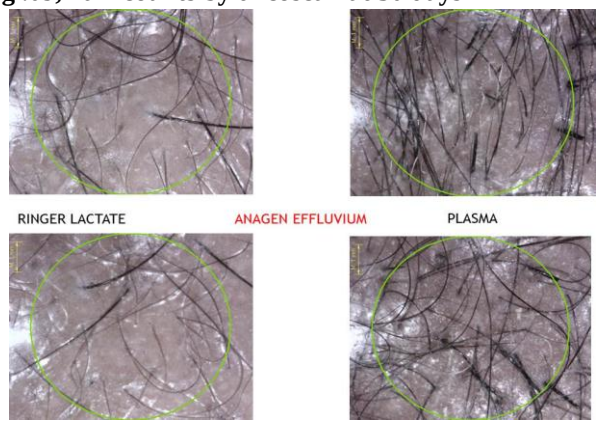
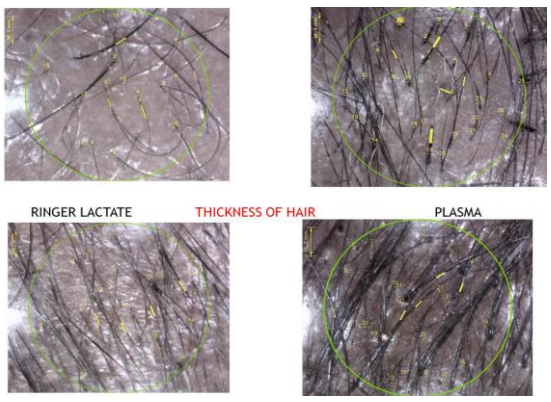


Table -2 Hair thickness at 6 month on right side (plasma) and left side (rl)

PAT IEN(P)					AV THICK NESS RL					AV THICK NESS PLASMA
P-1	56	36	30	42	41	95	86	50	48	69.75
P-2	60	38	38	28	41	63	60	60	52	58.75
P-3	52	38	40	30	40	60	58	48	68	58.5
AVERAG					40.6					62.33

Fig. 10, hair thickness after 6 months



C Clinical Evaluation –

After hair transplant patients photographs were taken on both left and right side fronto temporal region for hair growth and anagen effluvium. Photographs on both Right and Left fronto temporal area were taken at 50 days, and 4 months. At 50 days - On Right side (plasma) implanted hair were present ,while on left side means ringer lactate side most of implanted hair were lost means they entered in anagen effluvium.(fig-11,12)

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Fig. 11, Post-operative after 50 days of HT case I after 50 days of hair transplant showing significant Anag

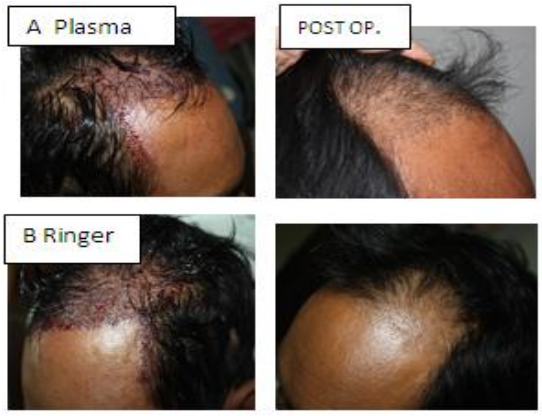

Fig. 12 A,B,C,D, Case II after 50 days of hair transplant showing Anagen effluvium on left side

Fig. 13 A,B after 4 months of hair transplant

RINGER SIDE **PLASMA SIDE**

Fig. 14, All grafts stored in plasma , growth at 60 days after hair

Fig. 15, Results after four months.

All grafts (1876 grafts, FUT) stored in plasma-shows good growth at 4 months

BEFORE **AFTER**

Discussion

The most important benefit of holding solution would be an increase in hair yield from the transplanted grafts.The optimum holding solution would reduce the trauma to tissue from reperfusion injury and free radical formation as well as from ion and osmolarity abnormality created by ischemic phase.

INTRACELLULAR v/s EXTRACELLULAR - REQUIRMENT OF BOTH GRAFT HOLDING SOLUTIONS ARE OPPOSITE - Intracellular graft holding solution6 needs chilling which is not a user friendly, as well as graft may suffer from reperfusion injury3 and they are expensive. While extra cellular solutions are widely used, economical and does not need chilling. Intra cellular fluids like hypothermasol added with ATP has significant benificial effect when graft holding time is more then 10 hrs and this is a very rare condition to face. Autologus Plasma is an extra cellular fluid which is isotonic, with nutrients and platelet drived growth factors. It does not cost much. This can be prepared by surgeon himself or he can ask his pathologist. Plasma may not be an ideal graft holding solution but it is better then any other extra cellular fluid. Dessication of grafts immmerged in plasma is delayed, grafts looks shiny and more hydrated even at the end of 4hrs holding time. Fibrinogen is converted into Fibrin and fibrin2 gets attached to grafts which prevents dehydration of grafts. This is an imortant point especially for novice surgeon or a technitian as they may not pay much attention on dessication of grafts during implantation.

It was a great surprice to us when we got histological results of MTT staining.

The MTT assay is a colorimetric assay for assessing cell metabolic activity. NAD (P) H-dependent cellular oxidoreductase enzymes may, under defined conditions, reflect the number of viable cells present. These enzymes are capable of reducing the tetrazolium dye MTT 3-(4,5-dimethylthiazol-2-yl) - 2,5-diphenyltetrazolium bromide to its insoluble formazan[09]. Samples of follicles were sent for

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histological study (MTT assay) for detection of live cells. Results of staining showed that at 12 hrs the plasma grafts were better stained then RL grafts. Results at 72 hrs staining were surprising. Plasma grafts showed good staining while RL grafts showed very poor staining indicating that cells were viable in plasma grafts even at the end of 72 hrs.

Clinical obervation results showed at three months. The hair count by Tricoscan, on RL side was Average 1.7 (DENSITY 5.5G/SQCM) while on plasma side Average hair count was 8.7 (DENSITY 28.6G/SQCM).So on plasma side 71.5 % hair grew while on RL side only 13.75 hair grew. This proves that anangen effluvium on plasma side was only 28.5% while on RL side the effluvium was 86.25%.So effluvium was controlled by 57.75% which is significant. In the first seven days after hair transplantation there occurs an inflammatory process involving neutrophils, eosinphils, macrophages, platelets, fibroblasts and growth factors[2]. both erythma and oedema occur in the scalp, after this period apoptosis occur and micrograft enters into an involution phase resulting in hair shedding. This all happens because of ischemia. Next growth cycle begins after the third month and continues upto seven month.

Prevention of anagen effluvuim would be the result of the prevention of apoptosis of the more metabolically active progeny of the stem cells. This observation may help us in development of an ideal holding solution by some other bio enhancemnt of plasma.

Hair thickness results were engouraging at six months. The hair thickness taken by trico scan showed on RL side with Average was 40.66 micromm while on plasma side it was 62.33 micro mm.so the difference of thickness on both side is 21.67 micro mm, which is good differene. The hair thickness on plasma graft side was better then RL side. This may be because of effect of multiple factors of plasma. Hair diameter depends on number of viable cells in the matrix. These delicate mesodermal stem cells are very sensitive to ischemia. Ischimia leads to accumulation of free radicals and anarobic metabolic pathways resulting into apoptosis of cells there by affecting hair thickness.

Conclusion

This was small study group to analyse. Although at our centre in all cases grafts are kept in plasma and undoubtly the results in terms of anagen effluvium, patient satisfaction, hair growth hair thickness are much better then our previous one when we were using lacted ringer.

The plasma is an easily available graft holding solution with growth factors, isotonic in nature having nutrients in it with fibrin as well. Fibrin coating around grafts make it sticky and prevents dehydration. The growth factors and nutrients also control the Anagen effluvium. The thickness of hair and yield of graft is also better in plasma. So there are certainly advantages of using plasma over other extra cellular graft holding solutions. At the same time it is not an ideal graft holding solution where chilling cannot be done and availability of energy source is not clear.But this can be developed as an ideal graft holding solution by some bio enhancement.

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