ANALYSIS OF THE USAGE OF PLATELET - RICH PLASMA IN THE DEPARTMENT OF COSMETOLOGY AT A TERTIARY CARE HOSPITAL

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LIST OF ABBREVIATIONS

PRP	Platelet Rich Plasma
RBC	Red Blood Cell
WBC	White blood Cell
AGA	Androgenic alopecia
MPHL	Male Pattern Hair Loss
FPHL	Female Pattern Hair Loss
PDGF	Platelet Derived Growth Factor
TGF	Transforming Growth Factor
EGF	Epidermal Growth Factor
FGF	Fibroblast Growth Factor
CPDA	Citrate Phosphate Dextrose Adenine
HIV	Human Immuno Deficiency Virus
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus

INTRODUCTION

INTRODUCTION

In 1930s, the Transfusion Medicine field began as a simpler discipline that focused on collecting, processing, storing and distributing blood and blood products to patients[1].The field has evolved with advancements like aphaeresis technology, peripheral stem cell collection, plasma fractionation and recently in to the area of Regenerative Medicine[1].In the past platelets were considered to be exclusively as haemostatic cells. Later research by scientists concluded that the platelets had proliferative effect[3].

Witte et al. (1978) found and coined the term platelet derived growth factor (PDGF) and Kaplan found PDGF were present in alpha granules by subcellular fractionation method[3]. Slowly, transforming growth factor beta (TGFβ), insulin-like growth factor (IGF)-1, basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) were identified in platelet alpha granules[3]. Platelet suspensions in plasma began to be used for therapeutic intravenous transfusion. But the idea to use platelet concentrates for non-haemostatic therapy only arose in the late 1990s, after the discovery of these growth factors[3]. Coincidentally ‘‘Regenerative Medicine’’, a new field was born during the late 1990s[3].

Regenerative Medicine includes four main categories like gene therapy, tissue engineering, cell-based therapies and platelet rich plasma (PRP)

therapies, with different success in clinical translation[4]. Tissue engineering refers to producing tissues exvivo and transplanting them as functional tissues[4].

In Regenerative Medicine, the raw materials necessary for a ‘‘scarless repair’’, or regeneration, is supplied to the affected site[3]. Here the concept is to augment and optimize the natural healing response, rather than ‘‘insertion’’ of an engineered product[3]. They provide at least 1 out of 3 components considered essential for tissue regeneration—namely cells, growth factors and scaffold[3].

Currently, Regenerative Medicine represents a shift toward more affordable, approachable, and often bed-side strategies to tissue restoration[3]. Field of Tissue engineering and regenerative medicine are intimately related and are now often referred to as ‘‘Tissue Engineering and Regenerative Medicine’’, or ‘‘TERM’’[3]. Platelet-rich plasma (PRP) comes under the field of Regenerative Medicine as it provides 2 components – growth factors and scaffold[3].

PRP, an autologous fraction of peripheral blood with platelet concentration above baseline, is the simplest regenerative medicine intervention[4]. Normal blood has 93% red blood cells, 6% platelets, and 1% white blood cells.[20] The idea behind PRP treatment is to reverse this red blood cell: platelet ratio so that red blood cells which are less useful in the healing process are reduced and platelets are concentrated to about 94% which on activation provides a powerful concoction of growth factors needed for tissue

regeneration and healing.[20]As it is autologous, there is no risk of an immune response and hence thought to have a high margin of therapeutic safety[3].

PRP is an attractive option as it is autologous, easily available and affordable, can be rapidly and freshly prepared when needed at the point of care and requires minimal specialized equipment[3]. Platelet-rich plasma is theoretically not mutagenic, as the growth factors released do not enter the cell or its nucleus but acts by binding to the membrane receptors on the target cells[18]. Since PRP prepared from each and every patient at different time intervals have a different platelet concentration level, WBC count and the growth factor level, till now there is no standardised protocol for PRP preparation. [12]

Numerous variables like preparation method, activation status, activation method, platelet concentration, leucocyte concentration, the concerned individual, physical form of PRP, method of application, time of application influence the desired clinical outcome[3,12]. These properties make it difficult to analyse the effectiveness of PRP in the studies across literature. Although it seems apparently that PRP could never be a standardised product, it could be tailored according to the specific requirements of a patient, tissue, anatomic site or lesion type[3].

Although PRP preparation, dose, timing of application has not been standardised, its use has preceded the clinical research because of easy use,

safety, cost effectiveness and successful treatment of various clinical conditions[4]. Moreover, such therapy causes no significant complications.PRP preparation is widely used in dentistry, cosmetology, dermatology, plastic surgery, orthopaedic surgery, otorhinolaryngology and certain other fields of medicine for its supportive role in regenerative tissue process[4].

Since PRP preparation has demonstrated positive effect in certain cases of androgenic alopecia and augmented wound healing in chronic ulcers, this study was carried out mainly on these two conditions. There are studies substantiating similar outcome in literature.[25,26,35] Androgenic alopecia is hereditary thinning of the hair induced by androgens in genetically susceptible men and women[5]. Leg ulcers are the most common among chronic non healing ulcers. Ulcer is defined as a chronic ulcer when the loss of skin and subcutaneous tissue takes more than 6 weeks to heal.[6] These conditions are quite a challenge to treat and cause considerable psychological stress to the patients. Many treatment modalities have been used in the past but an autologous product like PRP which could be affordable with minimal risk can revolutionize the cosmetic and wound healing procedures currently available.

In a developing country like India, novel modalities like PRP if found effective can change the people's perspective of cosmetic treatment available only to the elite. It could play a major role in improving the self confidence and quality of patient's life. This study analyses the effect of autologous PRP in the treatment of androgenic alopecia and chronic non-healing ulcers.

AIM AND OBJECTIVES

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AIM

To evaluate the effectiveness of autologous Platelet Rich Plasma therapy in androgenic alopecia and chronic ulcer patients in the Department of Cosmetology, Stanley Medical College, Chennai.

OBJECTIVE

To find out the influence of factors like age, gender and socioeconomic status of the patient on the treatment outcome of autologous Platelet Rich Plasma therapy.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

From time immemorial man has tried to understand the functions of blood and its role in maintaining life. Slowly they began to experiment animal to animal transfusion and then transfusing animal's blood into human's body[2]. Many of them were failure. James Blundell, in 1818, performed the first human to human blood transfusion and also suggested that the transfusing blood must come from the same species[2]. In 1901 Karl Landsteiner discovered the ABO blood group system[2]. Then the discovery of anticoagulants, improvements in blood storage and transfusion devices, PVC blood bags revolutionized the blood transfusion field[2].

Whole blood transfusion was a life saving procedure in the management of massive blood loss during an accident or child birth, during and after major surgeries. Now blood is separated in to it's various components and are used according to the specific requirements of a patient's disease. The shelf life of each component varied, so packed red blood cells are stored at 2-6^{0}C for 35-42 days(35 days with CPDA-1 and 42 days with CPDA and SAGM), fresh frozen plasma is stored at -18^{0}C or lower for 12 months, platelets are stored at 22-24^{0}C with continuous gentle agitation for 5 days, cryoprecipitate at -18^{0}C or lower for 12 months[7].

With advancements like aphaeresis technology, stem cell separation, cellular therapy and regenerative medicine, the transfusion medical field has

grown leaps and bounds. It is still evolving and the era has come where certain specific cells are used to regenerate tissues.

PLATELETS

Platelet discovery dates back to 1880 & 1882, when Bizzozero identified platelets anatomically & its role in hemostasis.[8]He also identified bone marrow megakaryocytes but never recognised them as precursors of platelets.[8]Wright in 1906 identified megakaryocytes to be the precursors of platelets.[8]Platelets are 2.0 to 4.0 μm in diameter, 0.5 μm in thickness, 6 to 10 femtoliters in volume, with a life span of 7-10 days.[45]About 100 billion platelets are produced per day by the human body of which only a small fraction is utilised under normal physiological conditions and the remaining are cleared mainly via Kuppfer cells and hepatocytes. [3,8]The proplatelet extensions of megakaryocyte form the platelets. Through the endothelial pores of the bone marrow sinusoids these extensions become interwoven and are fragmented by shear forces[3]. This leads to the formation of heterogenous population of nascent small, discoid, anucleate platelets.[3]

Platelet structure includes an extensive cytoskeleton, mitochondria, lysosomes, ribosomes, smooth endoplasmic reticulum, granules[3]. The granules include alpha, dense and lysosomes[3]. There are about 50-80 Alpha granules per platelet[10], most of them are synthesized or endocytosed by the parent megaryocyte[3]. It contains about 300 different proteins but the distribution of

these proteins is not uniform, suggesting that distinct subpopulations of alpha granules exist with different release kinetics[3]. Platelets contain few dense granules which contain serotonin, ADP, ATP, GDP, GTP, histamine, calcium, magnesium, and polyphosphate[3]. Platelet lysosomes are thought to have a role in the clot lysis.[3]

The complex network of invaginations in the platelet membrane that that results in the shape change when platelet gets activated are called as open canalicular system (OCS).[3] When platelets get activated, its cytoskeleton gets reorganised and it's granules move towards the cell center and fuses with the OCS via a vSNARE and tSNARE mechanism, resulting in the release of their contents into the extracellular environment. Dense tubular system (DTS), in the platelet sequesters intracellular calcium in the resting cell and is analogous to the sarcoplasmic reticulum of muscle cells[3].

The platelet cytoskeleton consists of spectrin membrane skeleton, a circumferential microtubular coil, and an abundant network of actin filaments[3]. Platelets have a high degree of contractility when the actin network interacts with non-muscle myosin IIA[3]. This contractile force contributes to the dense packing of platelets in a primary hemostatic plug[3]. The same force plays a role in clot retraction during secondary hemostasis[3].

Platelets can be activated by physical, chemical stimuli, or a combination of both[3]. The main in vivo platelet agonists are subendothelial

collagen, von Willebrand's factor (vWF), thrombin, ADP, or a combination of these[3]. Under experimental conditions, collagen, thrombin, and ADP (as well as their synthetic substitutes and calcium ionophores) are the main agonists used in platelet research[3]. The collagen receptors on the platelets include integrin α2β1, the GPIb-V-IX complex, and GPVI. When vWF binds to GPIb, collagen binds to GPV in the same complex, slows the platelet and allows further collagen binding by α2β1 and GPVI[3]. So platelets get arrested and activation ensues. The thrombin receptors on the platelets are PAR (protease-activated receptor)-1 and PAR-4[3].

Final pathway through any of these receptors is the phospholipase C signaling cascade, which ultimately results in the release of calcium from the dense tubular system[3]. The increase in cytoplasmic Ca^{+2} activates gelsolin to severe actin filaments, and reassembles into a new cortical ring[3]. During this process granules are centralized and subsequently released[3]. As the cytoskeleton reorganizes, the intracellular protein, talin, binds to the cytoplasmic tail of the main platelet integrin, αIIbβ3[3]. The integrin becomes active and helps in the binding of platelet to fibrinogen, in a phenomenon referred to as ''inside-out signaling''[3]. Then these integrins cluster together on the platelet surface and transduce an ''outside-in'' signal back to the interior of the cell: focal adhesion plaques are formed around the intracytoplasmic tails of the β3, linking the external fibrin strand to the internal actin cytoskeleton of the platelet. The prothrombinase complex assembled on the platelet membrane,

forms thrombin and the platelet is activated via the PAR receptors. Now fibrin monomers assemble into fibers[3].

The reorganization of the actin cytoskeleton upon platelet activation includes 4 steps- 1) rounding into a sphere, 2) extension of pseudopodia, 3) adherence to a surface, and 4) spreading[3]. These help in the sealing of a vessel hole, primary platelet thrombus formation, and fibrin clot formation for definitive hemostasis. Then platelets contract against the fibrin network and stabilize the clot further. So the wound margin gets diminished.[3]

When platelets get activated phosphatidylserine and specific receptors for the coagulation factors IX, VIII, X, V and II are exposed on the platelet surface creating a procoagulant surface[3]. The clotting cascade reactions which ensue, results in fibrin formation[3]. The thrombus formation has three main phases, platelet-collagen binding (‘‘initiation’’), recruitment and activation of other platelets (‘‘extension’’), and finally, densely packed, platelet-rich fibrin clot (‘‘stabilization’’)[3].

In the center of clot platelets are maximally activated and towards the periphery of the clot activation gradient reduces[3]. In the center of the clot, there is direct platelet–platelet communication via contact-dependent signalling. Studies show that platelets are dynamic, living cells within the clot, in contrast to the concept of platelet activation as a rapid, disintegrating, ‘‘kamikaze’’-like

process[3]. Platelets in the center of clot may synthesize protein for 18 hours and those on the periphery may disaggregate and re-enter the circulation[3].

PLATELET RICH PLASMA

Platelet-rich plasma (PRP) is defined as a portion of the plasma fraction of autologous blood having a platelet concentration above baseline.[13]

Platelets use in the wound treatment has begun in the mid 1980's itself. In 1998, an oral surgeon incorporated autologous PRP in oral and maxillofacial surgery graft and published about its enhanced tissue healing effects.[51] Soon PRP became popular in the dental field. Its use spread to orthopedics, dermatology, plastic surgeries and the healing of ulcers due to diabetes, vasculitis etc[9]. Recently PRP is increasingly used in the field of cosmetology for facial rejuvenation,[41] treating acne scars[49] and hair growth in androgenic alopecia.[34] Many studies on PRP have shown that PRP could be a novel modality where rapid healing & regeneration is required.[25,26,29]

Normal platelet count is between 150,000 and 350,000 cells per microliter of blood in a normal person.[10] Many studies have suggested that if Platelet count is less than $1{,}000 \times 10^6$/ml in PRP, it will not be helpful for enhancing wound healing. Studies suggest, in PRP the platelet concentration should be four to seven times[10] the normal concentration of platelets and much higher concentrations did not show any additional benefits. The ideal concentration required for clinical benefits still remains to be defined. Across

the literature PRP is prepared by different equipments, methods and these may influence platelet degranulation characteristics which will have effect on clinical outcomes, making interpretation of the results challenging.

The PRP includes various growth factors like platelet- derived growth factors (PDGF), transforming growth factor (TGF)-β, insulin-like growth factor (IGF), vascular endothelial growth factors (VEGFs), epidermal growth factor (EGFs) and fibroblast growth factor (FGF)-2[10].PDGF and its three isomers PDGF-αβ, PDGF-αα and PDGF-ββ[10] functions include angiogenesis and macrophage activation, fibroblasts proliferation, chemotaxis for fibroblasts and collagen synthesis.[11]TGFβ has three different isoforms: β1, β2 and β3.[3] TGF-β1 and PDGF stimulate mesenchymal cell proliferation. TGF-β1 stimulates extracellular matrix production, fibroblasts proliferation, type I collagen and fibronectin synthesis and bone matrix deposition[11].VEGF and FGF-2 along with other factors play role in neo-vascularization[11]. PRP also contains Leukocytes in a varied concentration according to the preparation method. Leucocytes synthesize cytokines which have catabolic or inflammatory properties.[4]These catabolic cytokines play a role in extracellular matrix breakdown and activates leucocytes[4].

Neutrophils contain cytokines such as collagenases, gelatinases, lysozymes, elastases, serprocidins, and myeloperoxidase[4]. Studies show MMP-9 from Neutrophils can degrade collagen and extracellular matrix molecules and could be used as a predicator of poor healing[4].

T-lymphocytes contain interleukins, such as IL- 2, 4, 5, 6, 13, 17, 21, and 22; interferon-γ (IFN- γ), and tumor necrosis factor–α (TNF-α). B-lymphocytes contain cytokines, such as IL-6, IL-8, TNF-α and IL-1β[4]. Monocytes are of 2 phenotypes, one is proinflammatory and the other is proinflammatory and proangiogenic.[4]Proinflammatory type contain cytokines such as cathepsin B, L, and S; MMP-2, 3, 9, 13; and TNF-α.

Studies show platelets alone do not influence the clinical effects of PRP, but influenced by the WBCs present also[4]. Cytokines and growth factors from platelets are mostly anabolic and that from WBCs are catabolic[4]. The ratio of platelets to WBCs in PRP will in turn affect the amount of anabolic and catabolic signaling molecules in PRP and influence the clinical outcome[4]. This ratio for varied clinical applications is yet to be determined. More in vivo studies are needed to determine the platelet and WBC level in PRP that could result in the desired clinical outcome in different clinical conditions.

CLINICAL APPLICATIONS

The clinical applications of PRP has been on the rise, is used in the treatment of chronic skin and soft tissue ulcerations, periodontal and oral surgery, maxilla facial surgery, orthopaedic and trauma surgery, cosmetic and plastic surgery, spinal surgery, heart bypass surgery and burns.[4]

Contraindications for PRP usage may include platelet dysfunction syndrome, septicemia, anemia (Hb < 10 g/dL), critical thrombocytopenia, hemodynamically unstable patients and pregnancy.[21]

Indications to the use of PRP usage in dermatology may include Androgenetic alopecia, Alopecia areata, Skin rejuvenation, Acne scars and contour defects, Wound ulcers, Connective tissue disease associated ulcers, striaedistensae, Lipodermatoslerosus, Lichen sclerosus.[18]

WORKING DEFINITION

Marx et al proposed a working definition of PRP stating a platelet count of about 10 lakhs/microliter in PRP.[10,12,16]

NOMENCLATURE

Across the literature, PRP has been referred to by various names like platelet-enriched plasma, platelet-rich concentrate, autologous platelet gel and platelet releasate, plasma rich in platelets, plasma very rich in platelets, and even plasma very very rich in platelets.[16] A consensus regarding the correct term for various platelet derived products should be reached to avoid confusion while analysing the product's clinical efficacy and to standardize each product in the near future. In 2007, the term and definition of PRP was introduced in Pubmed as a medical subject heading(MeSH) to be used for indexing articles.[4]

CLASSIFICATION

Ehrenfest *et al*. (2009) had classified the various PRP products which was validated by a multi-disciplinary consensus conference published in 2012.[12]

1. Pure Platelet-Rich Plasma (P-PRP) - this has only platelets with no leucocytes.[12]

2. Leucocyte and PRP (L-PRP) products – this has both platelets & leucocytes forming a low-density fibrin network after activation. Most of the commercial systems available today are for preparation of this type.[12]

3. Pure platelet-rich fibrin (P-PRF) – this does not have leucocytes and forms a high-density fibrin network.[12]

4. Leucocyte and platelet-rich fibrin (L-PRF) – these are the second-generation PRP products. [12]

PRINCIPLE OF PRP PREPARATION

The basic principle behind PRP preparation is the differential centrifugation.[12]It is the process by which different cellular constituents of blood sediment based on specific gravity by adjusting the acceleration force applied.

PRP can be prepared by the PRP method or by the buffy coat method.[12]

In the PRP method, first/soft spin separates blood into plasma (upper part) having platelets and WBC, an intermediate thin buffy coat rich in WBCs, and a bottom layer having RBCs. To prepare pure PRP (P-PRP), upper layer and superficial buffy coat are collected. For leucocyte rich PRP (L-PRP), the entire layer of buffy coat and few RBCs are collected. The second/hard spin step forms pellets (erythrocyte-platelet) at the bottom of the tube. The upper portion - PPP (platelet-poor plasma) is removed. Pellets are homogenized in lower 1/3rd to create the PRP (Platelet-Rich Plasma).[12]

In the buffy coat method, first hard spin is done and three layers are formed. The supernatant plasma is removed. Buffy coat layer alone is collected and a soft spin is performed to separate the WBC's. When a small volume of 10 ml is used for PRP preparation difficulty lies in separating the buffy coat.[12]

METHODS OF PRP PREPARATION

PRP can be prepared by manual method as well as by using automated devices. Commercially available automated devices include Biomet GPS II and III, Arthrex ACP, Regen PRP and Harvest smart Prep[12]. Each system varies widely in their method of producing platelet rich plasma depending on method of centrifugation. Hence the resultant products also vary in their WBC and platelet concentration.[12]

Across the literature numerous methods of PRP preparation has been mentioned.[12] Till date no particular method has been standardised. Different centrifugation protocols are used by different authors. There is no consensus regarding the centrifugation and duration. Authors suggest 'trial and error approach' to find the appropriate centrifugation parameters and duration to standardise our individual PRP preparation protocols.[12]This is of paramount importance when a table top centrifuge is used for PRP preparation. PRP preparation by a table top centrifuge is economical and easy to adapt in clinical settings.[12]

FACTORS INFLUENCING PLATELET YIELD IN PRP

Many factors influence the platelet count during its preparation from whole blood. Starting from collection of blood every step of PRP preparation influences the platelet count in PRP.[12] Waters and Roberts, in their study found that proper vein selection and collection of blood from the appropriate vein is essential. Improper or difficult phlebotomy reduces the platelet count. Most protocols across literature suggests use of large bore needles (>22) so that platelets don't get activated unintentionally during the draw the blood.[12]

i) CENTRIFUGATION

Different acceleration forces like RCF/rpm & duration of centrifugation greatly influences platelet yield in PRP. Calculation of RCF depends on the radius of the centrifuge rotor used.[12] Rotors vary among each type of centrifuge machines, so produces different acceleration forces making it difficult to standardise a single protocol for the preparation of PRP.[12]

$g = (1.118 \times 10^{-5}) R S^2$

Where ‘g’ is the RCF, R is the radius of the rotor in centimetres and S is the ‘revolutions per minute’.[12]

ii) TEMPERATURE

Macey *et al*. suggests preparing PRP at lower temperatures may retard platelet activation.[12]

iii) ANTICOAGULANTS

The anticoagulant of choice for preparing PRP is ACD.[12] EDTA is not preferred as it might damage the platelet membrane. Citrate phosphate dextrose-adenine can also be used.[12] But studies across literature show ACD is the preferred anticoagulant of choice.

Also during PRP preparation, it must be separated soon after centrifugation as the concentrated platelets will slowly diffuse into the PPP resulting in false low platelet count in the PRP.[16]

The literature on PRP is considerable, but the results vary. It is very difficult to sort and interpret the available data, due to a large number of preparation techniques, terminologies, forms of these materials, and the endless list of potential applications.[12]

METHOD OF USE OF PRP

There is no consensus on whether to activate platelets or not before clinical application and with which agonist.[12]Some scientists suggest PRP can be used with or without activation.[38]Across the literature many platelet activating agents like thrombin, calcium chloride or calcium gluconate have been used.[12] Some authors even suggest that there is no need for platelet activation, collagen in the skin acts as a natural activator of PRP. But this is applicable only when PRP is for soft tissue applications. Some authors even suggest that platelets without activation gives better results.[12]

PRP as a solution is given as injection or just applied topically with gauze. PRP also can be formulated as a gel and used topically or incorporated in to bone grafts.

Activation of PRP should be done only at the time of use. When activating agents are added, the clotting is initiated which activates platelets. The activated platelets start secreting their growth factors immediately and 70% is secreted within 10 minutes and close to 100% within the first hour[6]. Studies suggest that they synthesize and secrete growth factors for about 8 days until they die. Hence activating PRP only when they are ready to use is of prime importance.

PRP dose is yet another important matter of debate that has to be considered in the evaluation of its biological effects.

MECHANISM OF ACTION

On adding activating agents to prepared PRP, Platelets start secreting growth factors within 10 minutes of activation and about 95% of pre-synthesized growth factors are secreted within 1 hour.[6,21]

The growth factors present in α granules include platelet-derived growth factor (PDGF-αα, PDGF-ββ, and PDGF-αβ), transforming growth factor-β (TGF-β1 and TGF- β2), vascular endothelial growth factor (VEGF), and epithelial growth factor (EGF)[24]. Fibrinogen, fibronectin, and vitronectin which act as cell adhesion molecules are also present in PRP, not in a concentrated manner but in the same concentration as a normal blood clot. Other growth factors like insulin-like growth factor (IGF-I, IGF-II), fibroblast growth factor (FGF), endothelial cell growth factor (ECGF), platelet-derived angiogenesis factor (PDAF), osteocalcin (Oc), osteonectin (On) and thrombospondin 1(TSP 1) are also present.[10]Dense granules contain factors like serotonin, histamine, dopamine, calcium, and adenosine.[10]These factors are involved in inflammation, the first stage of wound healing. Serotonin and histamine play a role in increasing the capillary permeability, allowing inflammatory cells to migrate from the capillary lumen into the wound site and activate macrophages.[10]

PRP contains both anti – inflammatory and pro-inflammatory cytokines.[24]PRP releases pro-inflammatory cytokines, such as IL-1α, IL-1β, TNFα, IL-6, IL-8, IL-17 and IL-18, their concentrations are much lower than those of the anti-inflammatory counterparts.[24]Not only the platelets in PRP but the suspending fluid Plasma also contributes much to the beneficial effect of

PRP.[24] On activation fibrinogen and other clotting factors in the plasma form a provisional fibrin scaffold where cells adhere, migrate and proliferate.[24] Platelets aggregate along these fibrin fibers forming a scaffold and releases growth factors. [24]

The released growth factors attract undifferentiated cells and increase the cell division. PRP may suppress cytokine release and limit inflammation. Also they interact with macrophages to improve tissue healing and regeneration, promote new capillary growth and accelerate epithelialisation in chronic wounds. There are reports suggesting that platelets secrete anti microbial peptides which might have an antibiotic effect.[38] PRP also contains some erythrocytes and leucocytes on preparation and it can have influence the effect of its treatment.[39]

PRP has been recently used in the skin rejuvenation also. A study on the reduction of pain in split skin graft donor sites by miller et al [42] showed PRP can reduce pain at those sites. PRP has been used in the oral and Maxillofacial surgery and ulcers due to surgery.[43]Choukroun et al. in France, first described Platelet-rich fibrin (PRF), considered to be second-generation PRP mainly for use in oral and maxillary surgery.[10] Recently PRP was used in the treatment of atrophic acne scars also with a positive outcome.[49]

Recently L-PRF has been studied which is prepared by centrifuging the whole blood immediately without any anticoagulant it contains platelets and leucocytes.[47]

ADVANTAGES OF PRP USAGE

1. As PRP is an autologous product, infectious disease transmission risk is low/rare.[21]

2. PRP can be easily prepared for use in an out-patient setup.[21]

3. Only 8-10 ml of blood is collected, so no harvesting expense for the patient.[21]

4. Easy to handle and can be prepared gel-like also. So easy to apply with substitute materials.[21]

5. Has a short preparation time.[21]

6. Easy to prepare at the point of care as needed.[21]

DISADVANTAGES OF PRP USAGE

As PRP is safe according to most of published researches, it's clinical use has preceded the scientific research. The possibility of cancer on using PRP has been raised by some authors. But Literature suggests, no growth factor can provoke a cancer. All growth factors act on cell membrane receptors, not the cell nucleus[17]which in turn acts on the signal proteins present in cytoplasm. These leads to a normal gene expression, rather an abnormal gene expression.[14] Still further research on PRP safety is certainly needed.

As in all bioactive agents, there should be an optimal dose range of PRP. The common idea that PRP with a high concentration of platelets would be more beneficial is incorrect, as a highly concentrated PRP could actually have an inhibitory effect on wound healing. Studies have shown that, the PRP effect may change according to the application dose. Also the biological stimulation of cell growth varies with PRP dose. On application PRP enhances mesenchymal stem cell migration and proliferation. PRP overdose can result in high cellular proliferation, but its differentiation into appropriate cell lines becomes limited.

Baseline platelet value varies widely from person to person. Therefore study reports with an x-fold increase, makes it difficult to suggest a fixed concentration of platelets in PRP to use for a particular beneficial effect..The platelet count in PRP varies according to the preparation method.[46,50]

ALOPECIA

Androgenetic alopecia (AGA) is a common chronic hair loss disorder affecting both sexes.[25]As age increases occurrence of alopecia also increases but the onset may occur at puberty itself.[25] As hair is considered to be an important feature of self-image, hair loss affects self-esteem and may lead even to depression especially in women.

Androgenetic alopecia is hereditary thinning of the hair induced by androgens in genetically susceptible men and women. This condition is also

known as male pattern hair loss[27] or common baldness in men and as female pattern hair loss in women.[28] Thinning usually begins between the age of 12 and 40 years in both sexes. In men at least 50% have alopecia by the age of 50 and in women 50% have alopecia by 60 years. It is more common in men.[28]

It has a polygenetic inheritance and is multifactorial.[26] Hair is a complex outgrowth from the hair follicles in the epidermis.[27] hair growth follows a cyclical pattern starting with the anagen phase (7-94 weeks).[27] Then catagen phase when proliferation stops and apoptosis begins.[27] Lastly telogen phase when no significant apoptosis or proliferation is seen and the hair falls.[27]

Androgens are important in regulating hair growth at puberty, as they increase the size of follicles in beard, chest and limbs and decrease the size of follicle in bitemporal regions which reshapes the hair line in men and women.[5] The scalp dihydrotestosterone binds to the androgen receptor and activates the genes responsible for gradual transformation of large terminal follicle to miniaturized follicle. [5]

Treatment options are minimal for alopecia. FDA-approved drug therapies are finasteride and minoxidil. Oral finasteride, a type II 5α-reductase inhibitor lower serum, prostrate, and scalp dihydrotestosterone. [27].

Minoxidil appears to prolong anagen phase and to promote survival of dermal papilla cells and increase in hair follicle size. Finasteride also promotes

hair growth of anagen hairs leading to gradual increase in hair diameter and hair elongation and appears to activate anagen hair growth.[26]

But there are side effects like loss of libido,increase in body hairs.[27]

Hair transplants can also be done but the cost and the chances of graft failure are the disadvantages.[27]

Studies are being done to understand cellular pathways and molecular mechanisms involved in the pathophysiology of alopecia, so that appropriate treatment methods could be developed that not only stimulates hair growth, but induces formation of new hair follicles.[51]EGF & FGF activates the proliferation & causes transdifferentiation of hair stem cells & produce new follicular units. bFGF& beta-catenin, promotes the anagen phase of papilla cells & thereby plays a key role in elongating hair shaft. The antiapoptotic regulators, Bcl-2 protein and Akt signaling, that are activated by these GFs, prolongs the survival of dermal papilla cells during the hair cycle.[51]Thus, the mitogenic and antiapoptotic effects, of PRP prolong survival of dermal papillae. PRP modulates angiogenesis and enhance blood flow around hair follicles, thus facilitating hair growth.[51]

The use of PRP either alone or as an adjunct to surgical procedures in the patients of androgenic alopecia thus holds promising results.

MECHANISM OF ACTION OF PRP IN ANDROGENIC ALOPECIA[18]

Upregulation of transcriptional activity of β-catenin→Differentiation of stem cells into hair follicle cells.

Increased bcl-2 levels→Anti-apoptotic→Prolongs survival of dermal papilla cells.

Activation of Akt and ERK signalling pathways→Prolongs survival of dermal papilla cells.

Expression of FGF-7 in dermal papilla cells→Prolongs anagen phase of hair cycle.

Increased VEGF and PDGF→Proangiogenic→Increases peri-follicular vascular plexus.

ULCERS

Chronic wounds are breaks in the skin that do not heal or require a long time more than 6 weeks to heal[29] and frequently recur. Chronic wounds include pressure ulcers[30], venous leg ulcers[30,31], neuropathic ulcers[32], trophic ulcers[29] and foot ulcers in people with diabetes,[30]vasculitic[29] and traumatic ulcers.[29]PRP treatment has been studied in all these above type of ulcers.[29,30,31,32] It can also be used in the treatment of ulcers due to surgery, burns, autoimmune conditions.[37]

Wound healing is an interactive process that involves coagulation,, inflammation, formation of granulation tissue and tissue remodelling.[33]The normal process of wound healing includes three phases: inflammation, tissue formation and tissue remodelling. When the normal healing process is disrupted, a wound can become chronic in nature. Risk factors that commonly contribute to poor wound healing are: 1) local causes, such as wound infection, tissue hypoxia, repeated trauma, and presence of debris or necrotic tissue; 2)systemic diseases, such as diabetes mellitus, immunodeficiency, or malnutrition; and 3) certain medications, such as corticosteroids.

Medical management of chronic wounds should, whenever possible, involve treatment of the primary cause. This may be glycaemic control in diabetic patients, or vascular surgery for people with chronic venous disease or ischaemic vascular disease. Other measures include the removal of necrotic or infected tissue,[14]compression therapy, maintenance of a moist wound environment, management of wound infection,[14] proper wound debridement[14] and diet. Despite treatment, many chronic wounds fail to heal, persist for months or years and/or recur after healing.

Topical growth factor products are typically used as adjuvant treatments along with the treatment of diabetic foot ulceration, including debridement, offloading, frequent dressing changes, and compression for wounds with an origin of vascular insufficiency.

PRP increases the growth factors in the granulation tissue of refractory dermal ulcers when applied topically as gel or as injection. PRP, contains intra- and extra-platelet components other than GFs which might contribute to the regeneration of tissue. Fibrinogen, for example, creates the fibrin network necessary for cellular implantation and later multiplication. Autologous PRP has the advantage of low or null risk of infection or immune reactions.

According to Weibrich *et al*,[40] age and gender does not significantly influence the platelet or growth factor concentration in PRP. But some studies report that the hematocrit and total platelet count influence the platelet concentration of the PRP.

Kang JS et al[48] study showed the effect of CD34+ cell containing PRP preparations in the androgenic alopecia. They concluded that CD34+ cell containing PRP had a positive outcome in the treatment of alopecia.[48]

Schiavone*et al*[34] in their study used PRP in the treatment of male and female patients of androgenic alopecia. They used two injection approaches with three months interval. Patient reaching a clinically important difference was assessed by 2 evaluators was 40.6% and 54.7%. The median age was 28 in the male group and 32 in the female group.

Gkiniet al[25] study included 20 patients in their study. The mean age of patients was 34 years. Patients with grade 2-3 showed better results with PRP

treatment. Hair loss decreased after the second session of treatment. The mean satisfaction questionnaire score was 7.1.

Vasconcelos RCF et al[26] studied a total of 18 patients were studied. Uneven results were observed between the genders in the present study, with a clearer and more satisfactory response in the female group (mean = 42.9%) as compared to the men's (mean = 25.6%). In their study all patients showed some improvement with an average improvement of 33%.

Khatu S et al[35]- a pilot study A significant reduction in hair loss was observed between first and fourth injection as noticed by patients. Global pictures also revealed a moderate improvement in hair volume and coverage.

Sarvajnamurthy S et al[6] studied the use of autologous platelet rich plasma in chronic venous ulcers in 12 patients. The total number of ulcers was 17. The mean age of the patients was 33.5 years. The mean of the time duration of healing of ulcers was 5.1 weeks. The mean of the improvement percentage of area and volume of ulcers was 94.7% and 95.6% respectively.

Frykberg *et al*[30]studied the use of PRP in the healing of chronic ulcers and showed that 63 out 65 ulcers showed improvement. The mean percentage of improvement in the area and the volume of venous ulcers were 56.1% and 43.1% respectively. The mean of time duration for healing of ulcers was 2.3 weeks.

Suryanarayan S *et al*[29] in their study used PRP in the treatment of chronic non healing ulcers at weekly intervals for a maximum of six sitting. The mean age of the patients was 42.5 years. The type of ulcers included 19 venous ulcers, 7 traumatic ulcers, 2 ulcers secondary to pyoderma gangrenosum, 2 diabetic ulcers, 2 trophic ulcers and 1 vasculitic ulcer. The mean of healing time of ulcers was 5.6 weeks. The mean of percentage improvement in area and volume of ulcers was 91.7% and 95% respectively. 25(76%) of the ulcers showed complete healing.

Xuan Tran TD *et al*[36]studied the use of autologous PRP in the healing of diabetic foot ulcers. Their protocol was to give PRP 2 times with 14 day interval. They studied 6 patients with diabetic ulcer and found that all ulcers completely closed after about 7 weeks.

MATERIALS AND METHODS

MATERIALS AND METHODS

STUDY DESIGN

Ours is a prospective study conducted at the department of cosmetology, Stanley Medical College, Chennai, Tamilnadu. The department of cosmetology, at Stanley Medical College is the first of its kind among the government hospitals in the whole of India. The Institutional Ethical Committee of Stanley Medical College and the University Ethical Committee of The Tamil Nadu Dr. MGR Medical University approved this study.

STUDY POPULATION

Patients attending the cosmetology department for the treatment of androgenic alopecia and chronic ulcers were included in this study.

INCLUSION CRITERIA

Patients aged above 18 who are advised PRP for androgenic alopecia and chronic non healing ulcers.

EXCLUSION CRITERIA

1. Patients with Anaemia (Hg < 10 g/dL), Critical thrombocytopenia, were excluded.

2. Patients who are not willing to participate in the study were excluded.

3. Patients aged less than 18 were excluded.

4. Pregnant and lactating mothers were excluded.

5. Patients with keloidal tendency, active infection at the treatment site were excluded.

6. Patients who were on minoxidil, finasteride for the past 6 months were excluded.

7. Chronic Ulcer patients who have uncontrolled diabetes, infection wereexcluded.

Sample size

Purposive sampling was done in this study

42 androgenic alopecia patients and 4 chronic ulcer patients were included in this study.

Study period

The study period was from July 2015 to June 2016

Statistical analysis

Data analysis was done using SPSS software

Demographic details were given in descriptive statistics

Quantitative data was given in summary statistics

$P<0.05$ was considered significant

METHODOLOGY FOR ANDROGENIC ALOPECIA PATIENTS

Androgenic alopecia (AGA) patients both males and females, irrespective of their initial grades were included in this study. Regarding diagnosis, there is no gold standard for AGA.[26] In addition to a physical examination focusing on the pattern and degree of alopecia involvement, full medical history was taken in order to rule out other causes. The pattern of hair loss and the degree of alopecia were thoroughly examined and graded according to Norwood Hamilton classification[26] for males and Sinclair classification [27] for females.

Traditionally, across the literature the grading of androgenic alopecia in female patients (also called FPHL-Female Pattern Hair Loss) has been done using the Ludwig scale, which has only three grades (Ludwig 1977).[26,28] More recently, Sinclair scale was developed simplifying the Ludwig and Savin scale[28] making the grading even simpler. Hence in our study Sinclair scale was used to classify FPHL.

Global photographs were taken before starting and after completion of PRP treatment for analysing its outcome. Patients were tested for HIV, HBV

and HCV. For alopecia patients PRP injections were advised monthly once for a total of 6 sittings.

NORWOOD-HAMILTON CLASSIFICATION[26]

Grade 1 - Very minor or no recession of the hair line

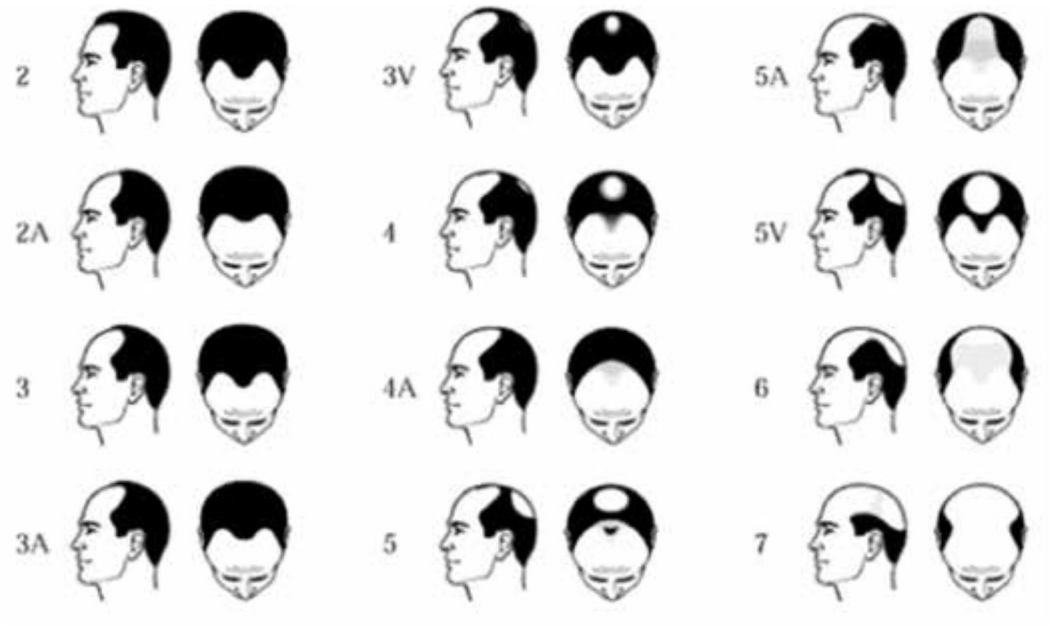


Figure 1:Norwood Hamilton classification

SINCLAIR SCALE[27]

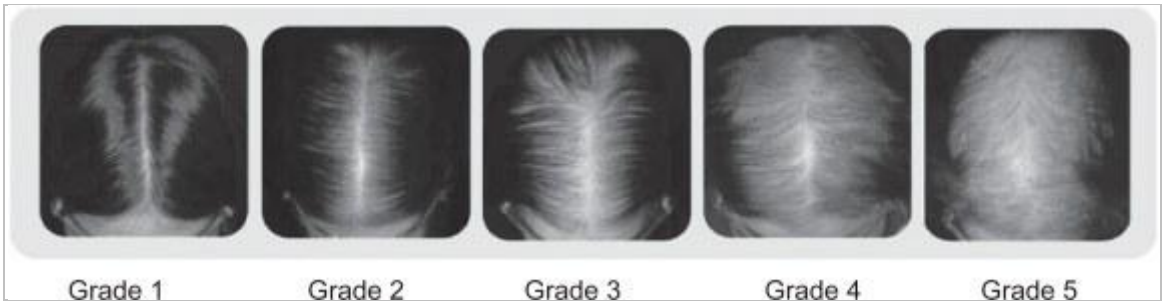


Figure 2: Sinclair Scale

METHODOLOGY FOR CHRONIC ULCER PATIENTS

Chronic Ulcers were diagnosed based on their medical history and their nonhealing period. For chronic ulcer patients PRP was advised once every 2 weeks with proper dressing. Demographic details were collected from these patients. Global photographs were taken before starting and after completion of PRP treatment for analysing its outcome. Measurement of Length, breadth and depth of ulcer was recorded before and after the treatment. The area and volume of ulcers were measured using the formulas:

Area of ulcer = length x breadth x 0.7854

Volume of ulcer = length x breadth x depth x 0.7854

PRP treatment outcome was measured by percentage improvement in area and volume by the formula:

Initial value – final value/ initial value

METHODOLOGY OF PRP PREPARATION

1. At every sitting, 8-10 ml of autologous whole blood was collected from the patients & mixed with acid citrate dextrose (ACD) in the ratio 1:9(anticoagulant :blood=1:9).[29]

2. 1ml whole blood was kept aside for assessing its Hemoglobin, RBC count, WBC and platelet count using an automatic analyser.

3. To date there is no universally accepted protocol. Each laboratory must standardise its own protocol by trial and error method.[12] In our study first centrifugation is done at 2000 rpm for 10 minutes. The plasma is collected and centrifuged again at 3000 rpm for 10 minutes to obtain platelet rich plasma.

4. Platelet-poor plasma (PPP) removed was used to resuspend the platelets.

5. 1ml of prepared PRP was analysed for platelet count, RBC and WBC count using an automatic analyser.

6. Calcium chloride (10%)[21] was used as an activator (1:9).

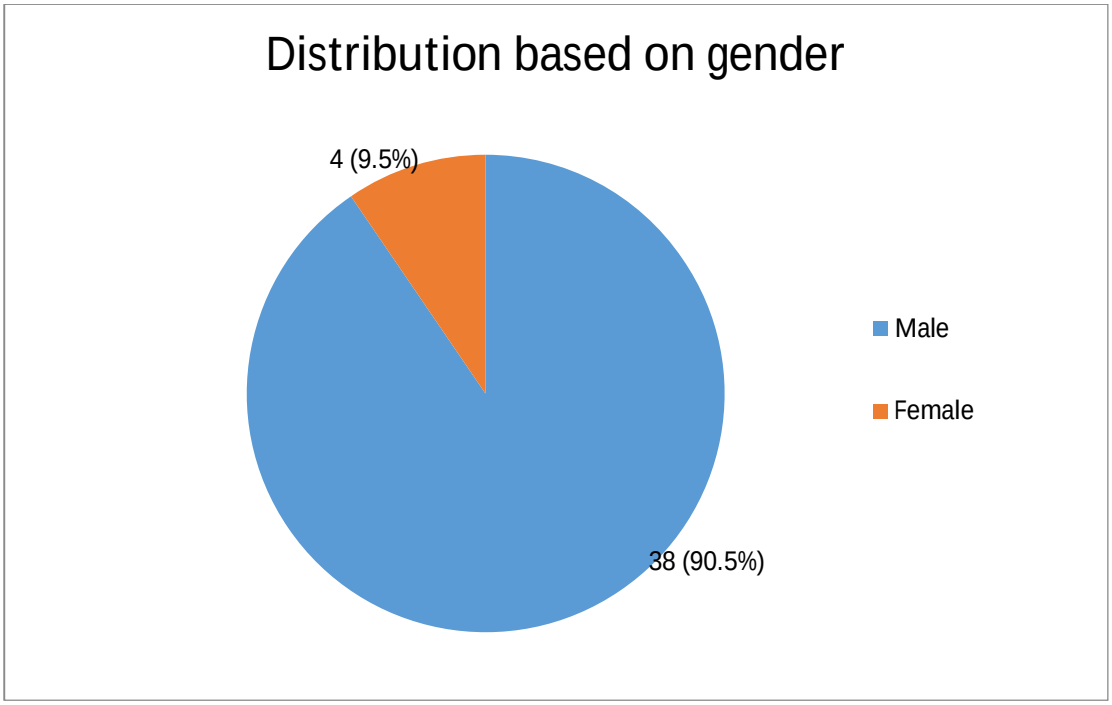
7. After activating the PRP, it was injected 0.1ml/cm^2 intradermally[25] in the affected scalp areas for “alopecia” patients using insulin syringe.

8. In “chronic ulcer” patients; initially proper wound debridement was done. Then, activated PRP was injected perilesionally and the remaining autologous PRP was sprayed over the ulcer followed by proper dressing. No topical antibiotics were applied. No oral antibiotics were prescribed for the ulcer patients.

RESULTS

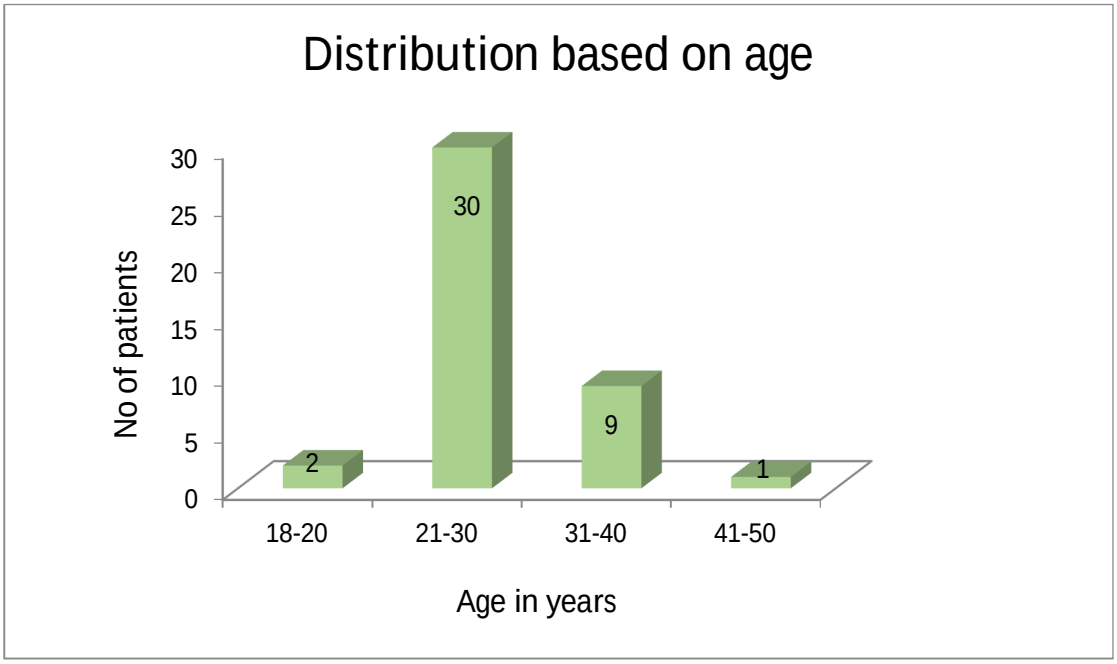
RESULTS

Figure 3: Distribution of androgenic alopecia patients based on gender



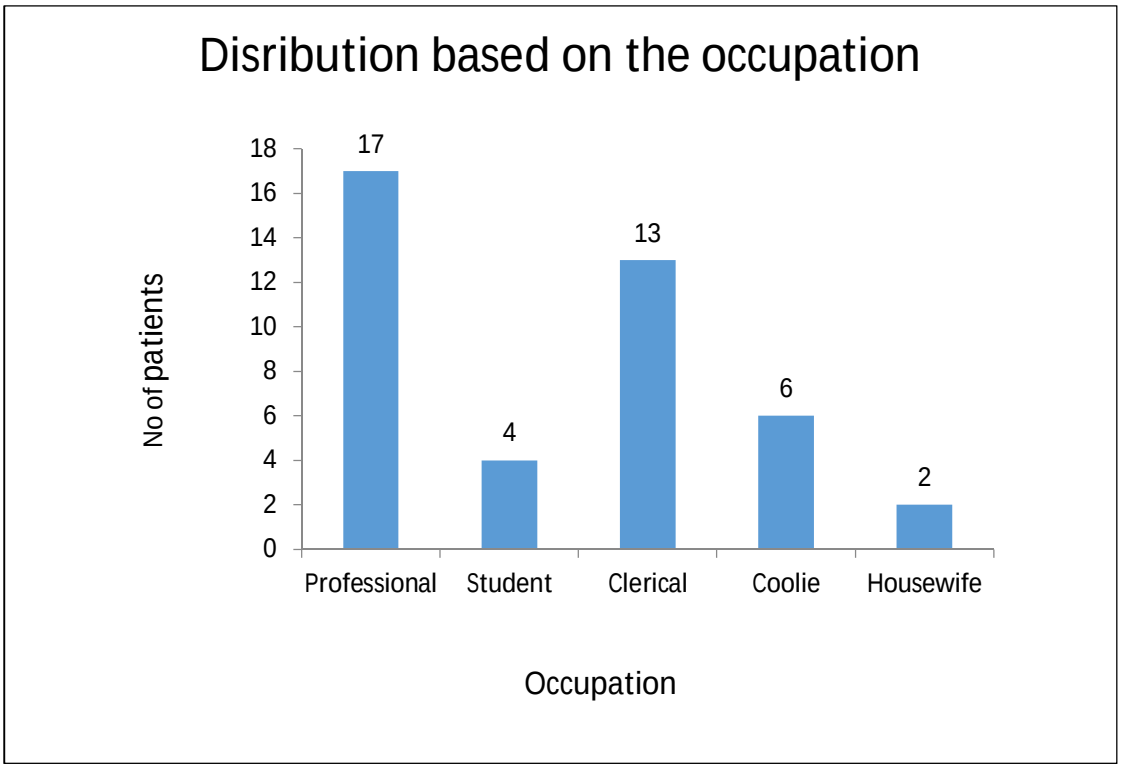
Demographic analysis of the 42 androgenic alopecia patients showed 90.5 % (38) male and 9.5 % (4) female.

Figure 4: Distribution of androgenic alopecia patients based on age



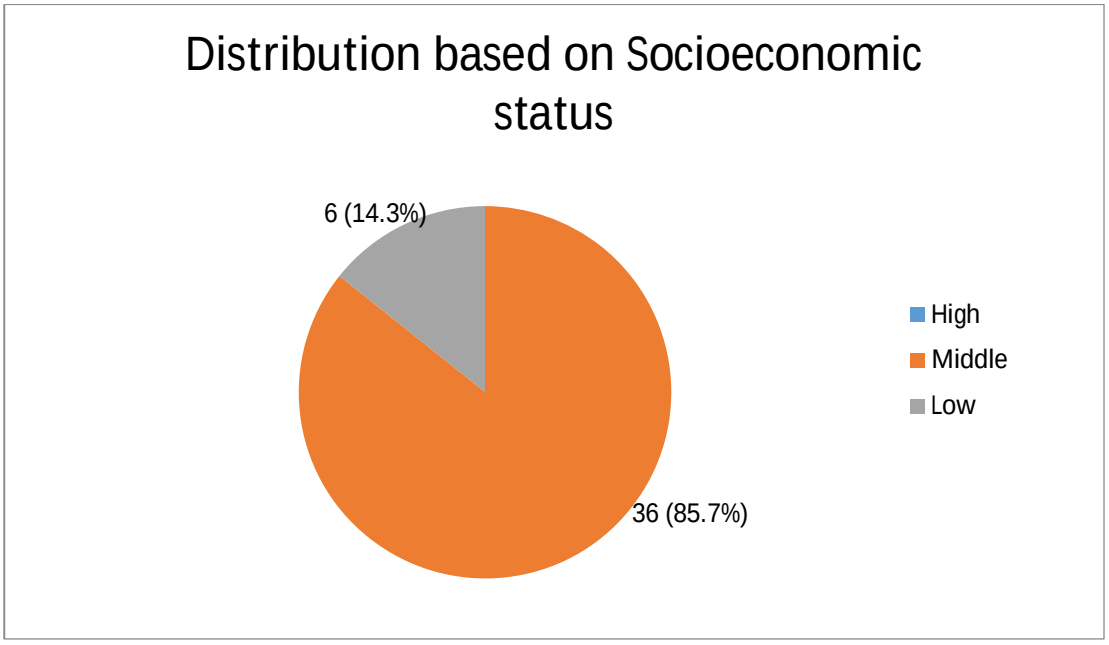
Age distribution among the androgenic alopecia patients were 4.8% (2) in 18-20 years, 71.4% (30) in 21-30 years, 21.4% (9) in 31–40 years and 2.4% (1) in 41-50 years (Figure 4).

Figure 5: Distribution of androgenic alopecia patients based on the occupation



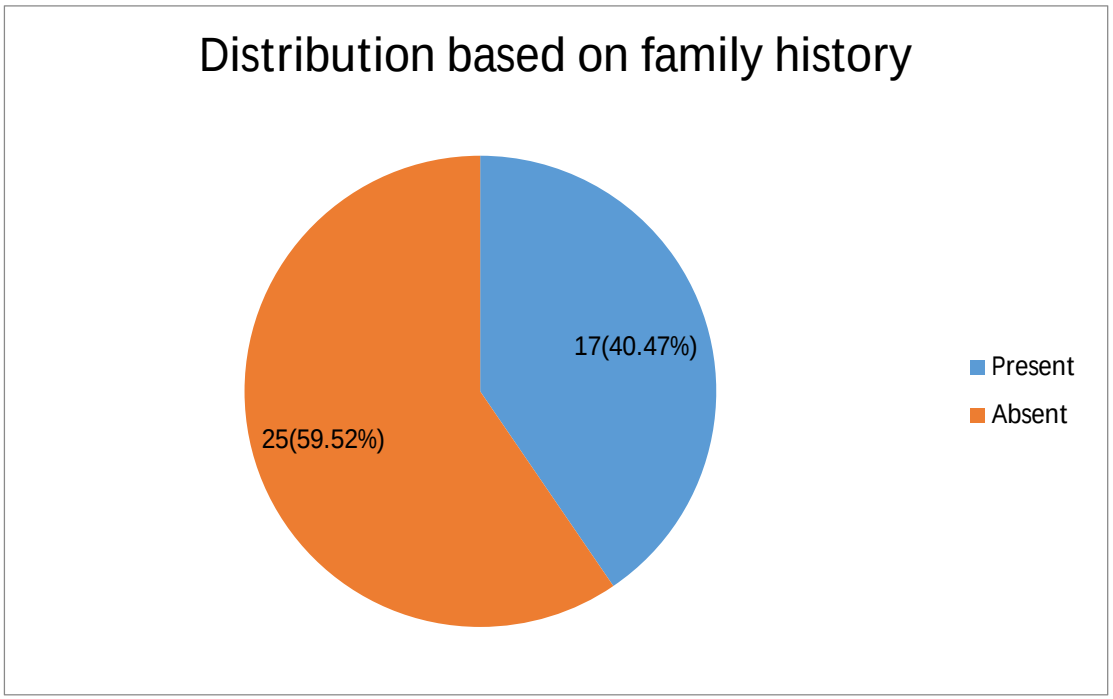
Distribution of androgenic alopecia patients on the basis of occupation were 40.5% (17) professionals, 9.5% (4) students, 31.0% (13) clerical, 14.3% (6) coolie and 4.8% (2) housewife (figure 5).

Figure 6: Distribution of androgenic alopecia patients based on the socioeconomic status



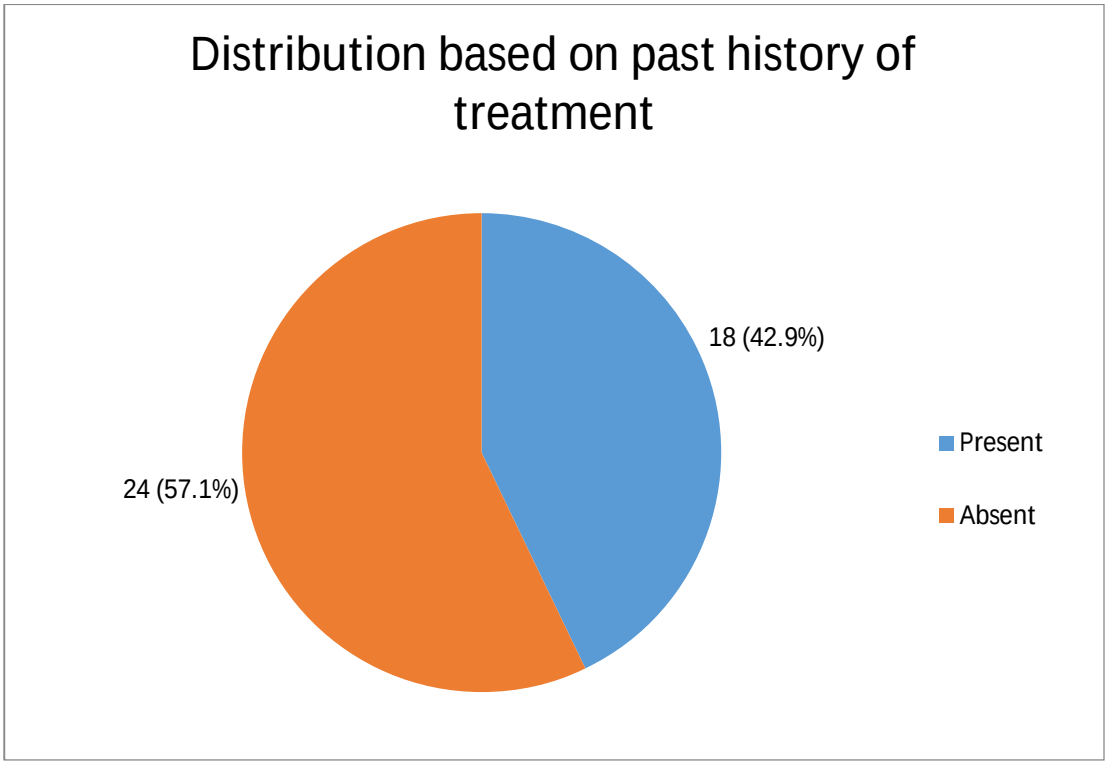
Distribution of androgenic alopecia patients based on the socioeconomic status showed 85.7% (36) and 14.3% (6) belonged to middle and low socioeconomic status respectively (figure 6).

Figure 7: Distribution of androgenic alopecia patients based on the family history of alopecia



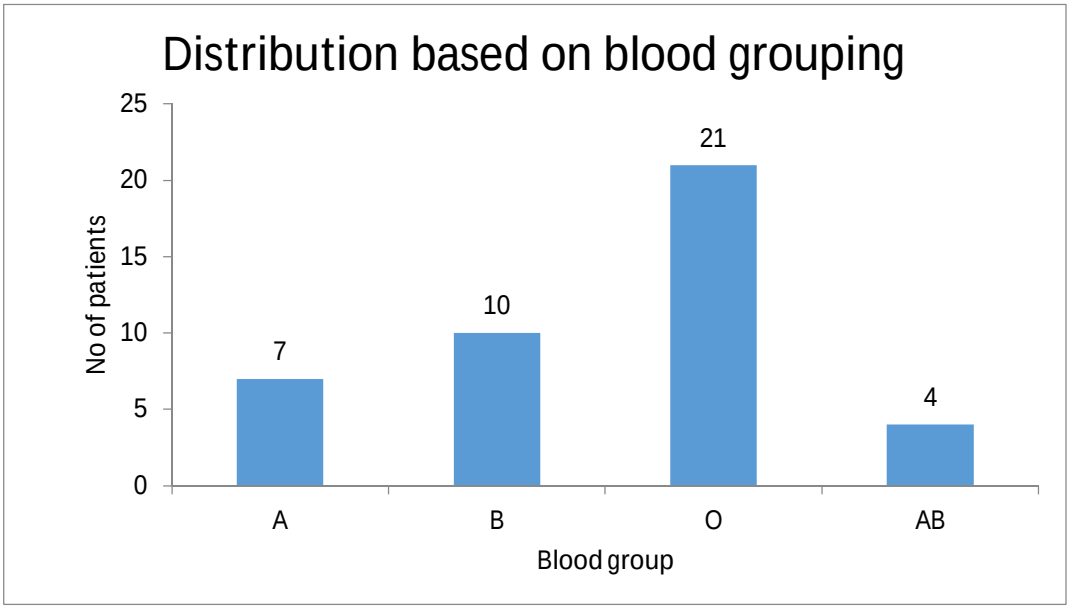
Distribution of androgenic alopecia patients based on the presence or absence of family history of alopecia were 40.47 %(17) and 59.52 %(25) respectively.

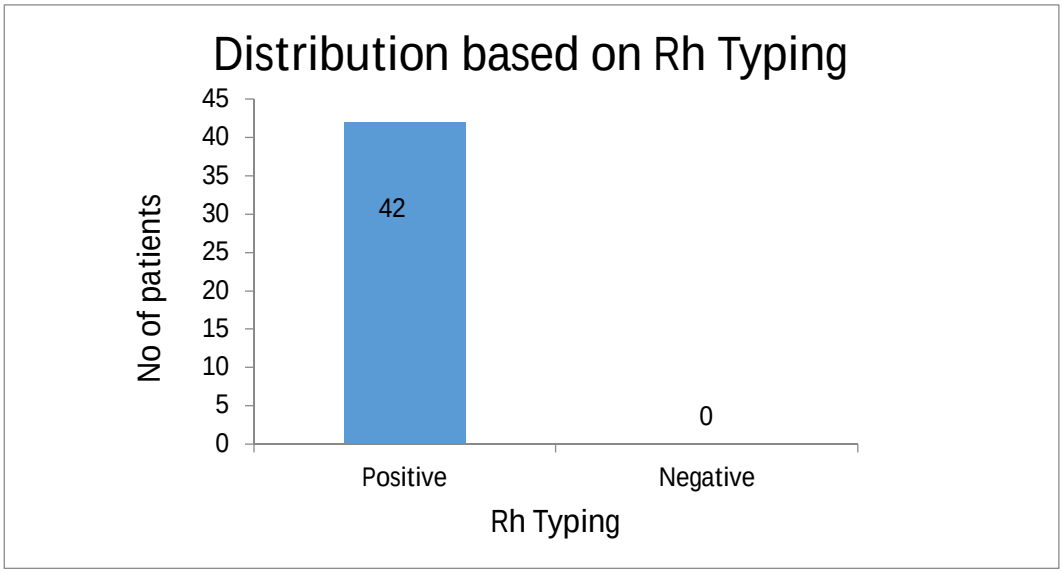
Figure 8: Distribution of androgenic alopecia patients based on past history of treatment for alopecia



Distribution of androgenic alopecia patients based on the presence or absence of past history of treatment for alopecia were 42.9%(18) and 57.1%(24) respectively (figure 8).

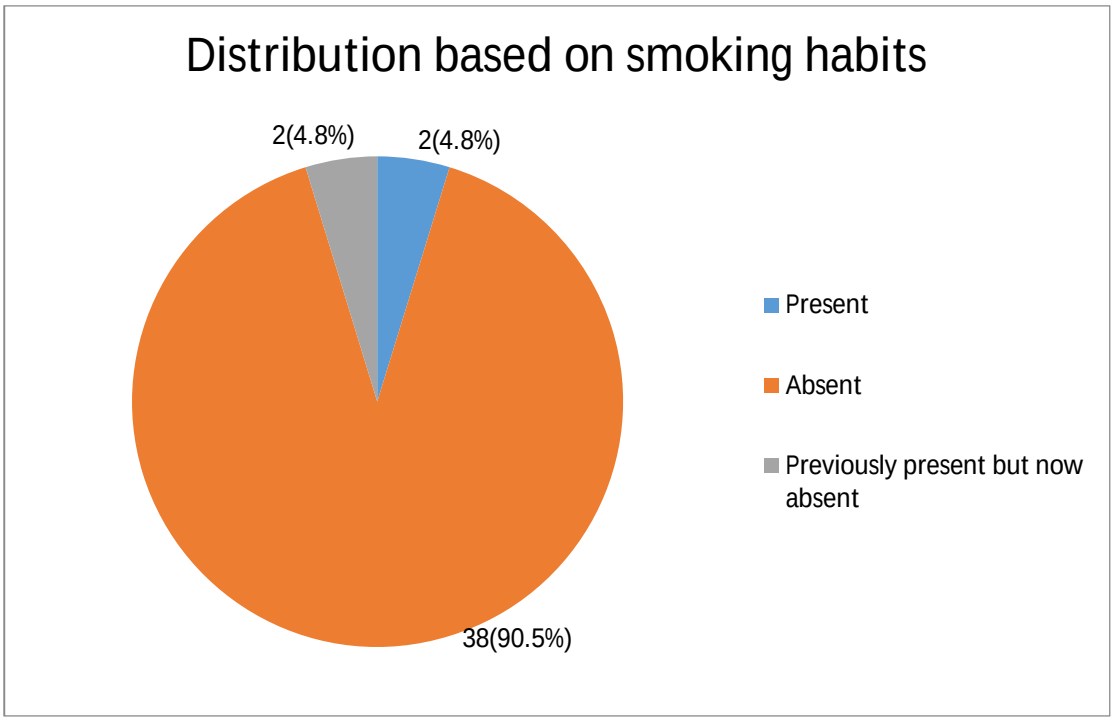
Figure 9 & 10: Distribution of androgenic alopecia patients based on blood grouping and Rh typing





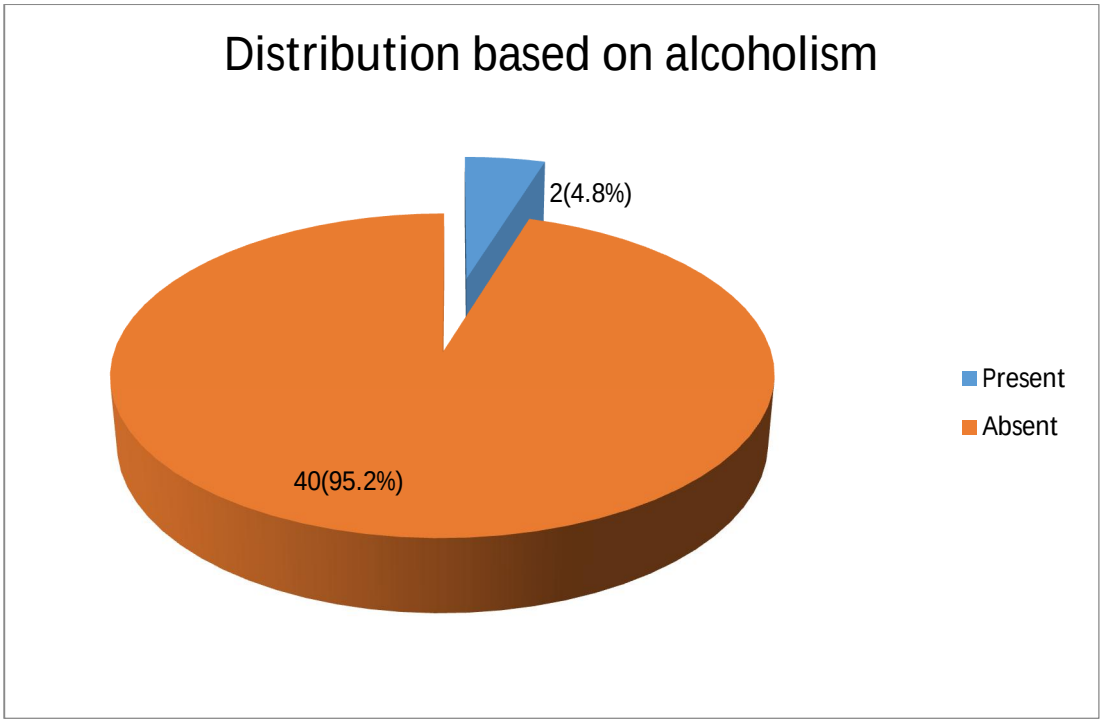
ABO Blood group Distribution among the androgenic alopecia patients showed 16.7%(7) to be 'A' group, 23.8%(10) to be 'B' group, 50%(21) to be 'O' group and 9.5%(4) to be 'AB' group. Rh typing distribution showed 100% Rh(D) positive.

Figure 11: Distribution of androgenic alopecia patients based on smoking habits



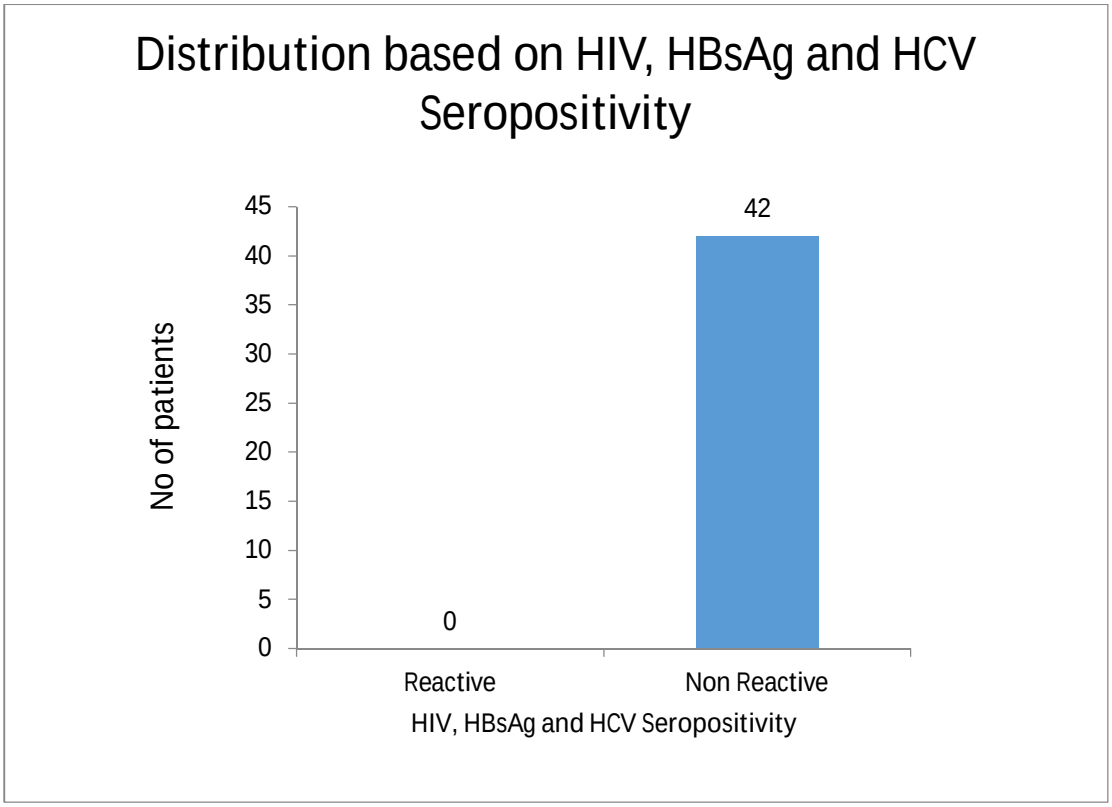
Distribution of androgenic patients based on smoking habits showed that 4.8%(2) were smokers, 90.5%(38) to be non smokers and 4.8%(2) had smoking habit but now abstinence.

Figure 12: Distribution of androgenic alopecia patients based on alcoholism



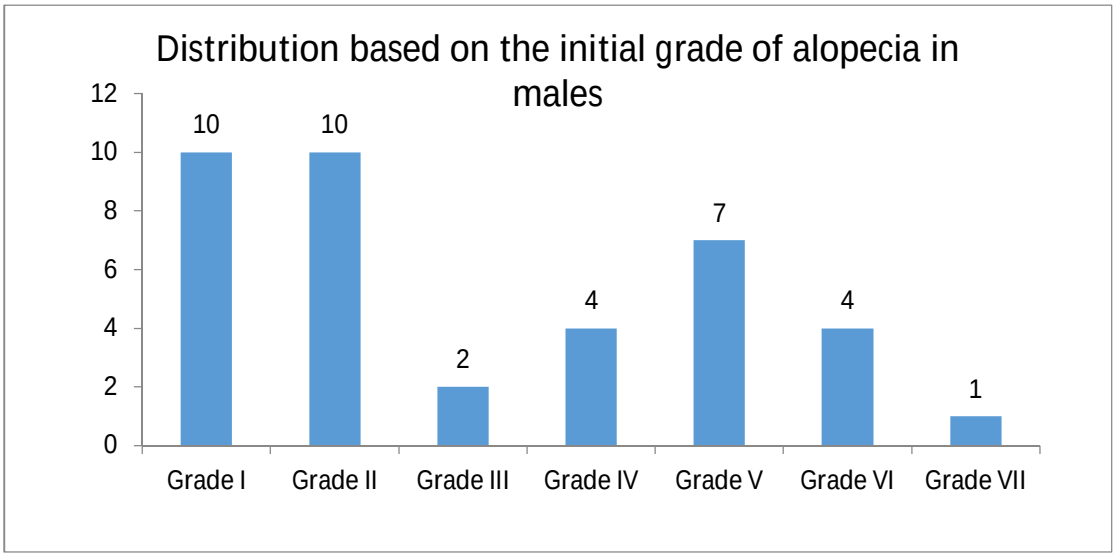
Distribution of androgenic alopecia patients based on alcoholism showed that 4.8 %(2) had the habit of taking alcohol frequently and 95.2 % (40) were non alcoholics.

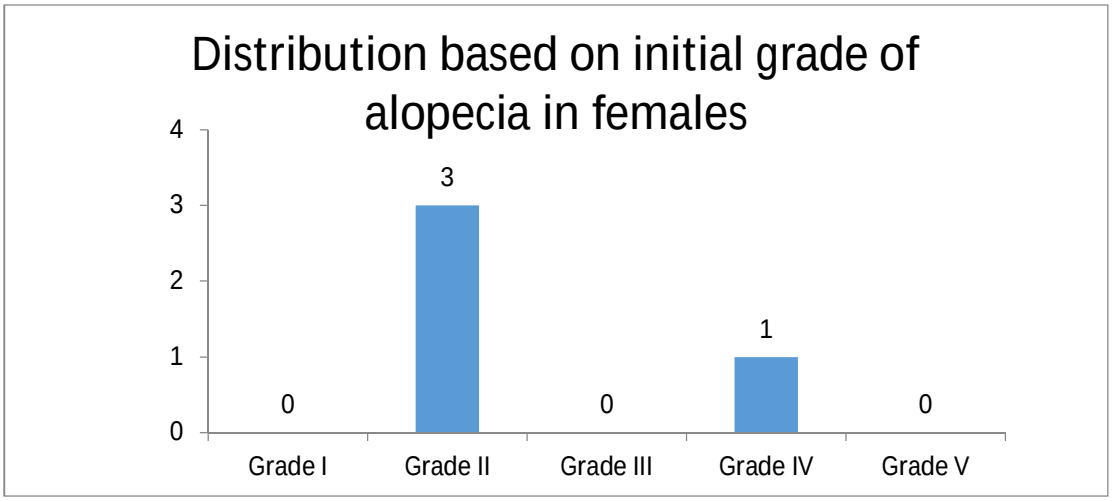
Figure 13:Distribution of androgenic alopecia patients based on seropositivity of HIV, HBsAg and HCV



Distribution of androgenic alopecia patients based on seropositivity for HIV, HBsAg and HCV showed all them were non reactive.

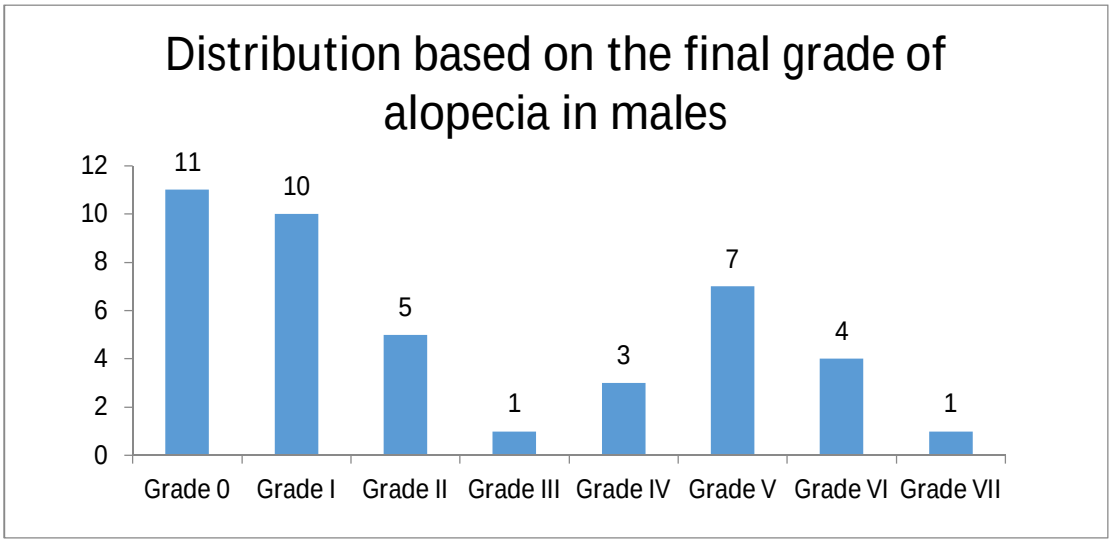
Figure 14 & 15: Distribution of androgenic alopecia patients based on the initial grade of alopecia in males by Norwood Hamilton Classification and females by Sinclair Scale

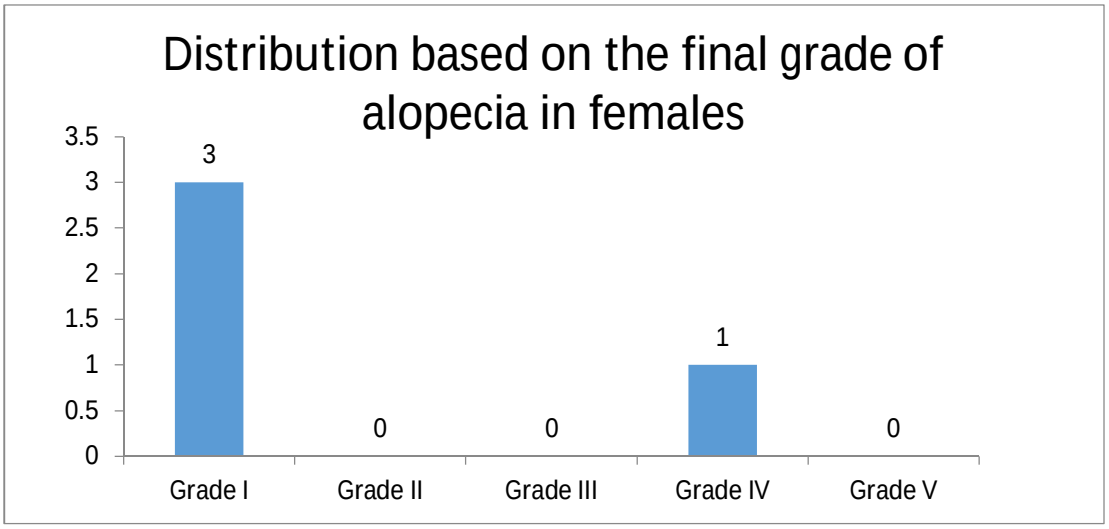




Distribution of male androgenic alopecia patients based on the initial grade of alopecia by Norwood Hamilton Classification showed 26.31%(10) – Grade 1, 26.31%(10) –Grade 2, 5.26%(2)- Grade 3, 10.53%(4) – Grade 4, 18.42%(7) – Grade 5, 10.53%(4) – Grade 6 and 2.63%(1) – Grade 7. Distribution of female androgenic alopecia patients based on the initial grade of alopecia by Sinclair Scale showed 75%(3) – Grade 2 and 25%(1) – Grade 4 (figure 14 & 15).

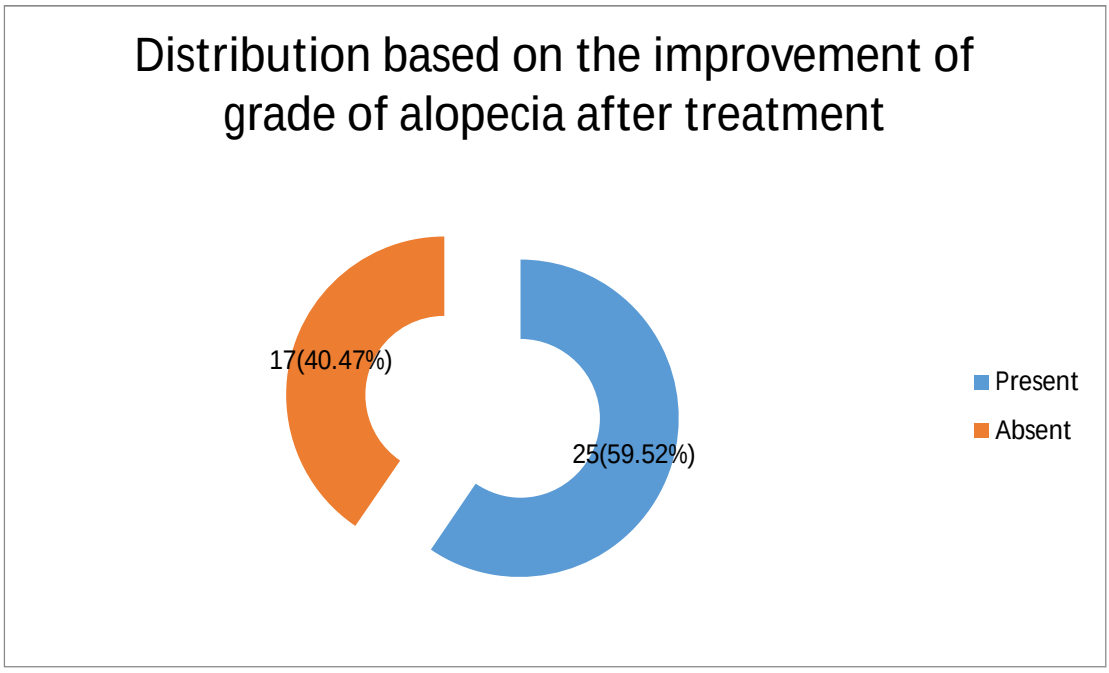
Figure 16 & 17: Distribution of androgenic alopecia patients based on the final grade of alopecia in males by Norwood Hamilton Classification and females by Sinclair Scale





Distribution of male androgenic alopecia patients based on the final grade (outcome of PRP treatment) of alopecia by Norwood Hamilton Classification showed 28.95%(11) – persons showing improvement from grade 1, 18.42%(7) –Grade 1, 13.16%(5) –Grade 2, 2.63%(1)- Grade 3, 7.89%(3) – Grade 4, 18.42%(7) – Grade 5, 10.53%(4) – Grade 6 and 2.63%(1) – Grade 7. Distribution of female androgenic alopecia patients based on the final grade of alopecia by Sinclair Scale showed 75%(3) – Grade 1 and 25%(1) – Grade 4 (figure 16 & 17).

Figure 17: Distribution of androgenic alopecia patients based on the presence or absence of improvement in their grade of alopecia after treatment



Distribution of androgenic alopecia patients based on the presence or absence of improvement in their grade of alopecia after treatment - 59.52%(25) showed improvement and 40.47%(17) showed no improvement.

Figure 18: Distribution of androgenic alopecia patients based on gender and final outcome

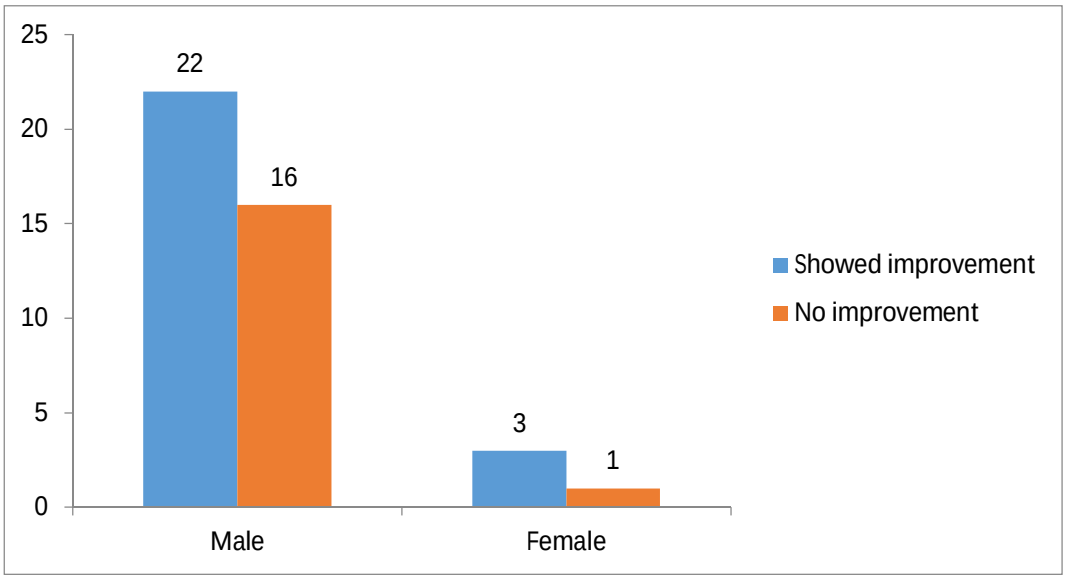


Table: 1

Gender	No. of patients who showed improvement	No. of patients who did not show improvement
Male	22	16
Female	3	1

In our study among the 38 male patients, 22(57.89%) showed improvement and 16(42.11%) showed no improvement. Among the 4 female patients who, 3(75%) showed improvement and 1(25%) showed no improvement. The p=0.50 which is >0.05 hence it is not a significant finding.

Figure 19: Distribution of androgenic alopecia patients based on age and final outcome

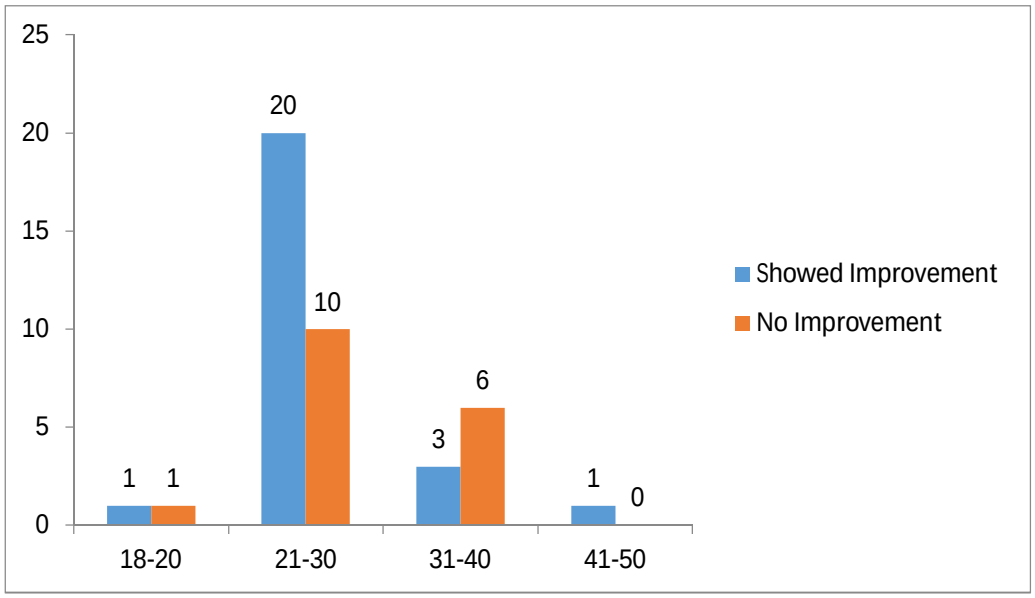


Table:2

Age	No. of patients who showed improvement	No. of patients who did not show improvement
18-20	1	1
21-30	20	10
31-40	3	6
41-50	1	0

In our study among the 2 patients belonging to the age group of 18-20 years, 1(50%) showed improvement and 1(50%) showed no improvement. Among the 30 patients belonging to the age group of 21-30 years, 20(66.67%) showed improvement and 10(33.33%) showed no improvement. Among the 9 patients belonging to the age group of 31-40 years, 3(33.33%) showed improvement and 6(66.67%) showed no improvement. Only one patient belonged to the age group of 41-50 years and he didn't show any improvement. The p=0.27 which is >0.05 hence it is not a significant finding.

Figure 20: Distribution of androgenic alopecia patients based on occupation and final outcome

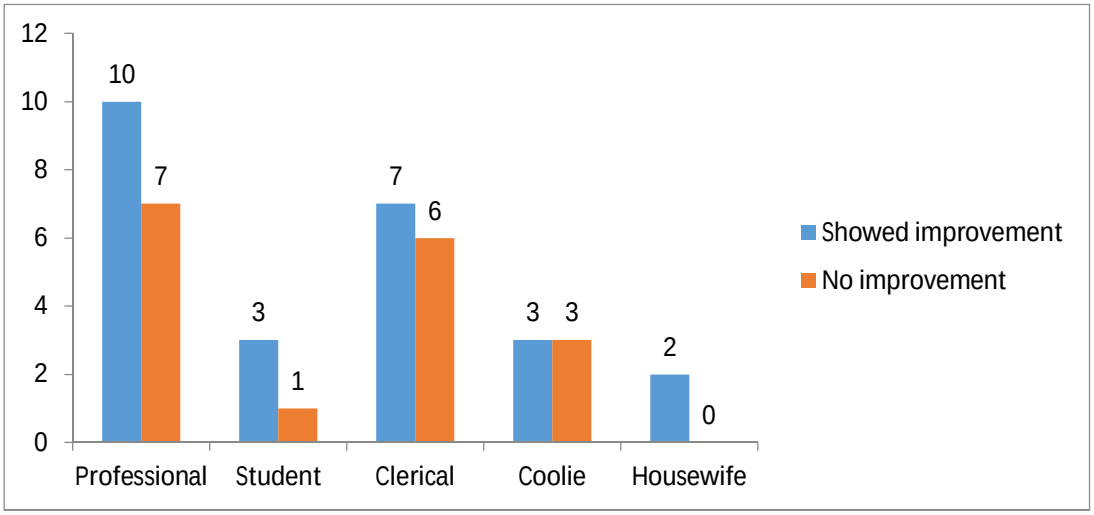


Table: 3

Occupation	No. of patients who showed improvement	No. of patients who did not show improvement
Professional	10	7
Student	3	1
Clerical	7	6
Coolie	3	3
Housewife	2	0

In our study among the 17 professionals, 10 (58.82%) showed improvement and 7(41.18%) showed no improvement. Among the 4 students, 3(75%) showed improvement and 1(25%) showed no improvement. Among the 13 clerks, 7(53.84%) showed improvement and 6(46.15%) showed no improvement. Among the 6 coolies, 3(50%) showed improvement and 3(50%) showed no improvement. Among the 2 housewives, 2(100%) showed improvement. Occupation does not seem to affect the final outcome. The p=0.71 which is >0.05 hence it is not a significant finding.

Figure 21: Distribution of androgenic alopecia patients based on socioeconomic status and final outcome

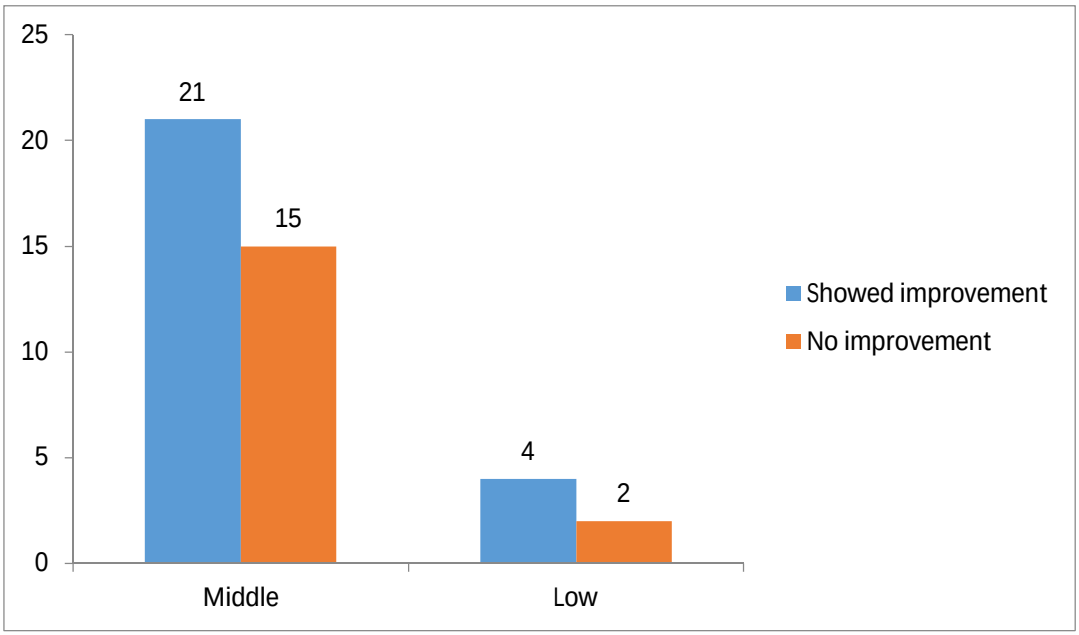


Table: 4

Socioeconomic status	No. of patients who show improvement	No. of patients who did not show improvement
Middle	21	15
Low	4	2

In our study 36 patients belonged to middle socio economic status and 6 belonged to lower socio economic status. Among the middle socio economic status patients 21 (58.33%) showed improvement and 15(41.66%) did not show improvement. Among the low socio economic status 4 (66.66%) showed improvement and 2(33.33%) did not show improvement. Socio economic status does not seem to affect the final outcome. The p=0.70 which is >0.05 hence it is not a significant finding.

Figure 22: Distribution of androgenic alopecia patients based on family history and final outcome

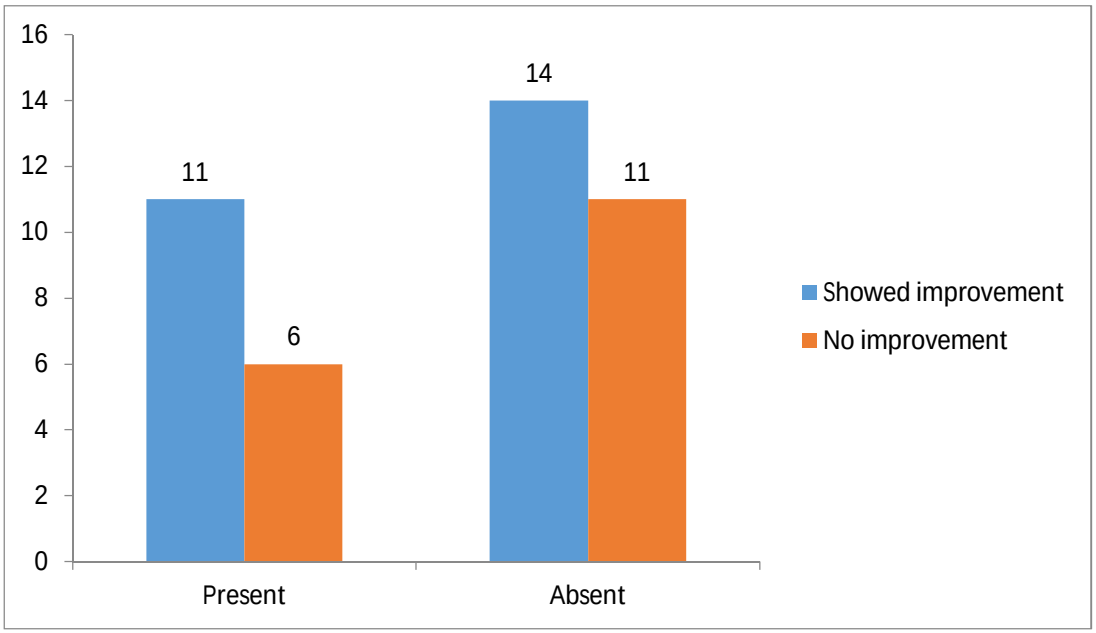


Table: 5

Family history	No. of patients who show improvement	No. of patients who did not show improvement
Present	**11**	**6**
Absent	**14**	**11**

In our study among the 17 patients who had family history of alopecia, 11(64.70%) showed improvement and 6(35.29%) showed no improvement. Among the 25 patients who had no family history of alopecia, 14(56%) showed improvement and 11(44%) showed no improvement. Family history of alopecia does not seem to affect the final outcome. The p=0.50 which is >0.05 hence it is not a significant finding.

Figure 23: Distribution of androgenic alopecia patients based on past history of treatment for alopecia and final outcome

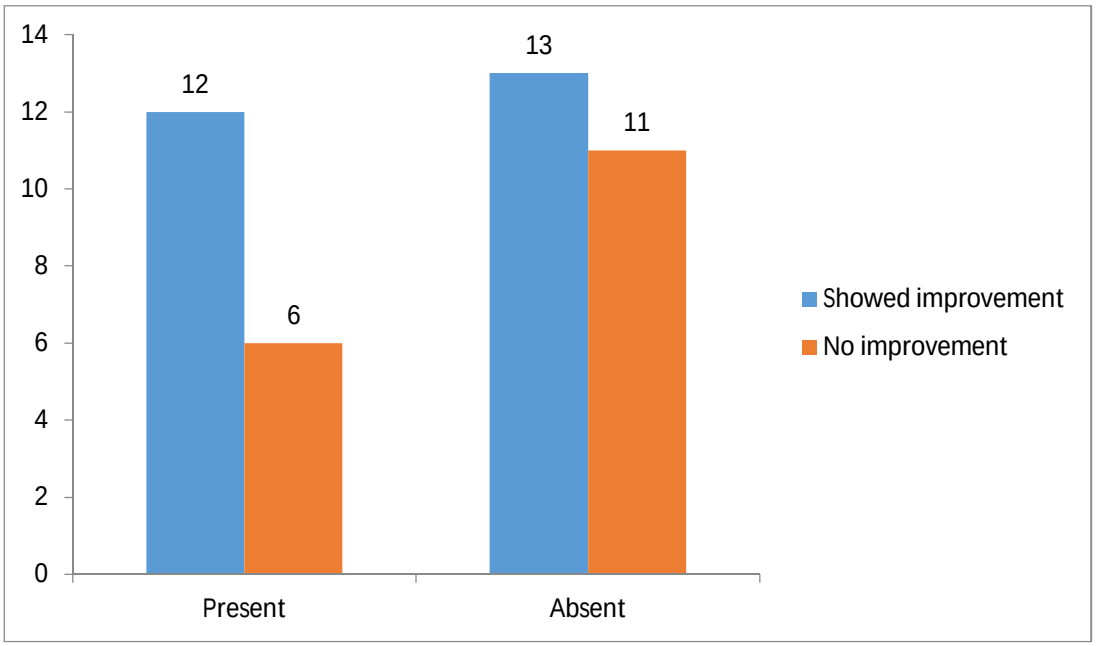


Table:6

Past history of alopecia treatment	No. of patients who show improvement	No. of patients who did not show improvement
Present	12	6
Absent	13	11

In our study among the 18 patients who had past history of treatment for alopecia, 12 (66.67%) showed improvement and 6(33.33%) showed no improvement. Among the 24 patients who had no past history of treatment for alopecia, 13(54.16%) showed improvement and 11(45.83%) showed no improvement. Past history of treatment for alopecia does not seem to affect the final outcome. The p=0.41 which is >0.05 hence it is not a significant finding.

Figure 24: Distribution of androgenic alopecia patients based on ABO blood grouping & Rh typing and final outcome

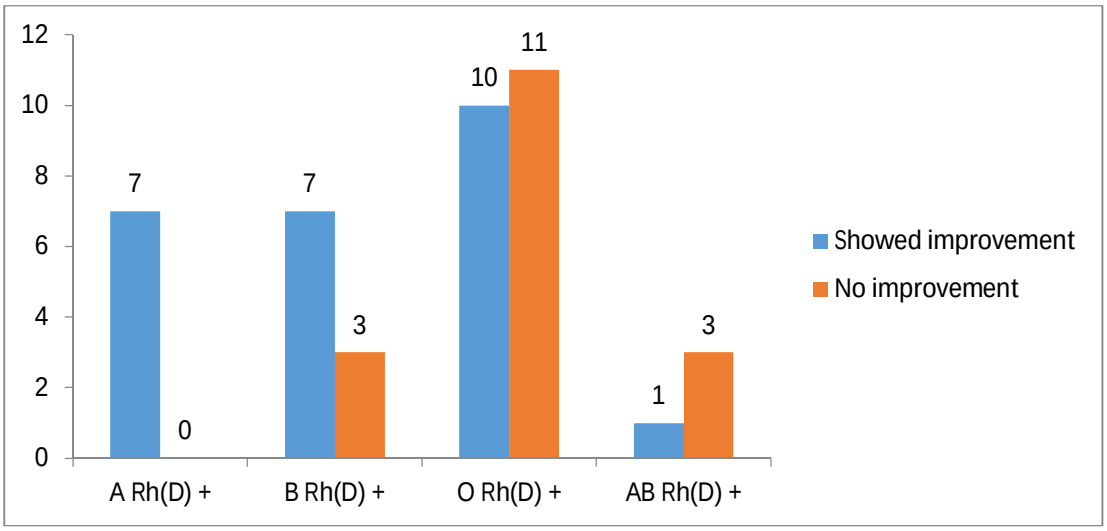


Table: 7

Blood Grouping and Rh typing	No. of patients who show improvement	No. of patients who did not show improvement
A Rh(D) +	7	0
B Rh(D) +	7	3
O Rh(D) +	10	11
AB Rh(D) +	1	3

In our study all 7(100%) A Rh(D) positive patients showed improvement. Among 10 B Rh(D) positive patients, 7(70%) showed improvement and 30% showed no improvement. Among the 21 O Rh(D) positive patients, 10(47.62%) showed improvement and 11(52.38%) showed no improvement. Among the 4 AB Rh(D) positive patients, 1(25%) showed improvement and 3(75%) showed no improvement. Blood grouping and Rh typing does not seem to affect the final outcome.

Figure 25: Distribution of androgenic alopecia patients based on smoking habits and final outcome

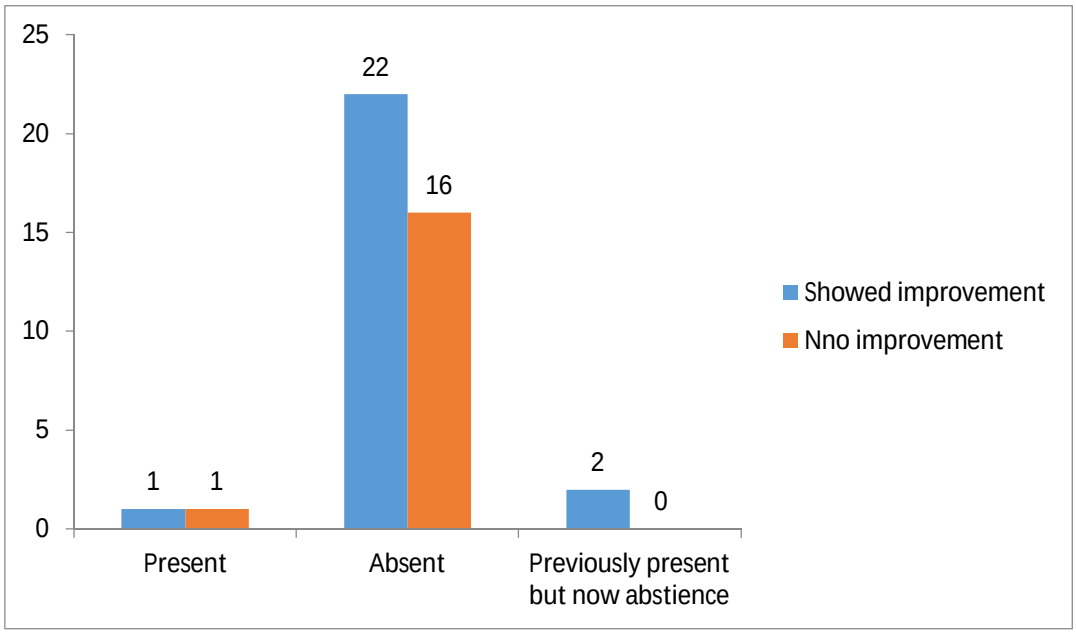


Table: 8

Smoking habit	No. of patients who show improvement	No. of patients who did not show improvement
Present	1	1
Absent	22	16
Previously present but now abstinence	2	0

In our study among the 2 patients having smoking habit, 1(50%) showed improvement and 1(50%) showed no improvement. Among the 38 patients who do not have smoking habit, 22(57.89%) showed improvement and 16(42.11%) showed no improvement. Among the 2 patients who previously had smoking habit but abstinence from it for past 2 years, 2(100%) showed improvement. Smoking habit does not seem to affect the final outcome. The p=0.48 which is >0.05 hence it is not a significant finding.

Figure 26: Distribution of androgenic alopecia patients based on alcoholism and final outcome

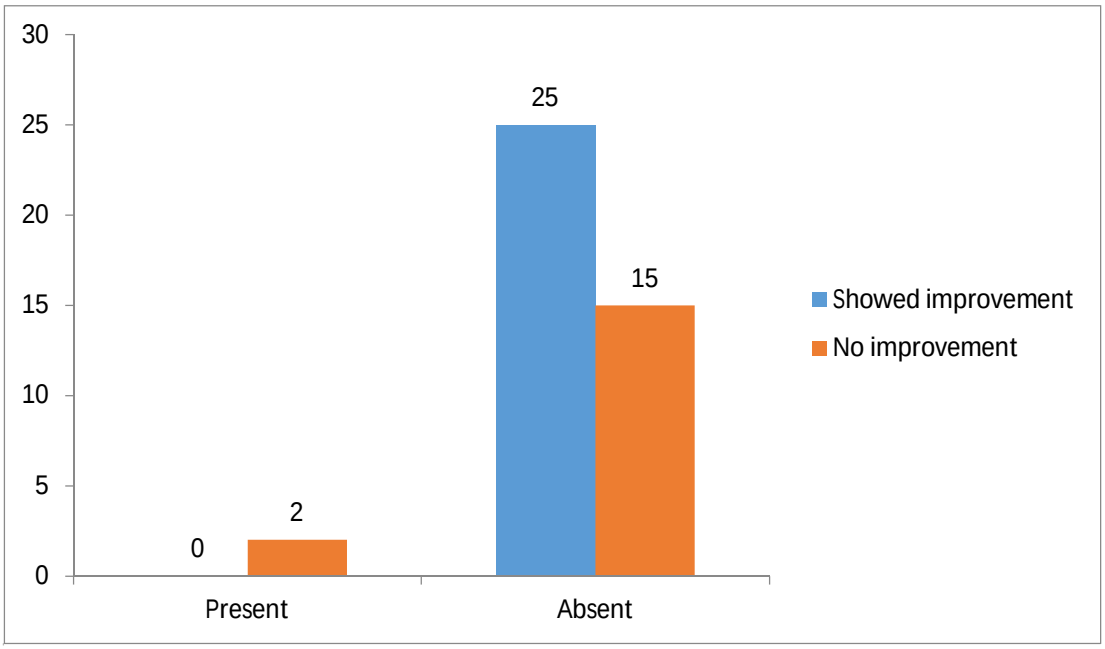


Table: 9

Alcoholism	No. of patients who show improvement	No. of patients who did not show improvement
Present	0	2
Absent	25	15

In our study among the 2 patients who had past history of alcoholism, 2 (100%) showed no improvement. Among the 40 patients who had no history of alcoholism, 25(62.5%) showed improvement and 15(37.5%) showed no improvement. Alcoholism does not seem to affect the final outcome. The p=0.079 which is >0.05 hence it is not a significant finding.

Figure 27: Distribution of androgenic alopecia patients based on seropositivity of HIV, HBsAg, HCV and final outcome

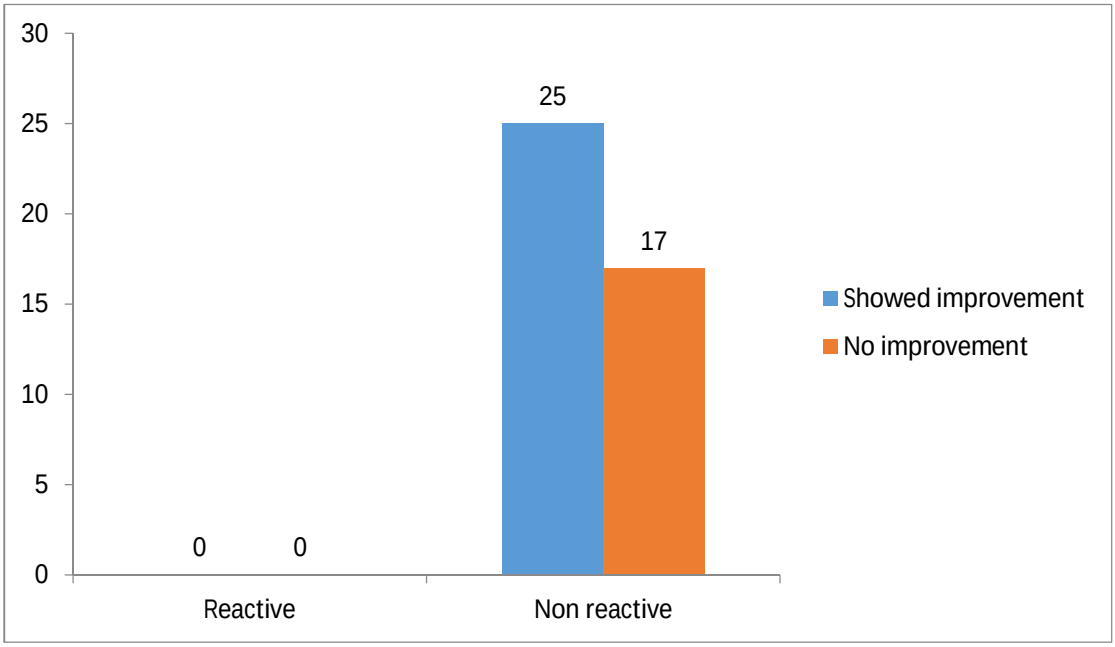


Table: 10

Seropositivity of HIV, HBsAg and HCV	No. of patients who show improvement	No. of patients who did not show improvement
Reactive	0	0
Non reactive	25	17

In our study all patients were HIV, HBsAg and HCV non reactive. Hence comparison of final outcome between reactive and non reactive patients could not be done.

Figure 28: Distribution of androgenic alopecia patients based on Initial grades of alopecia in males by Norwood Hamilton classification and final outcome

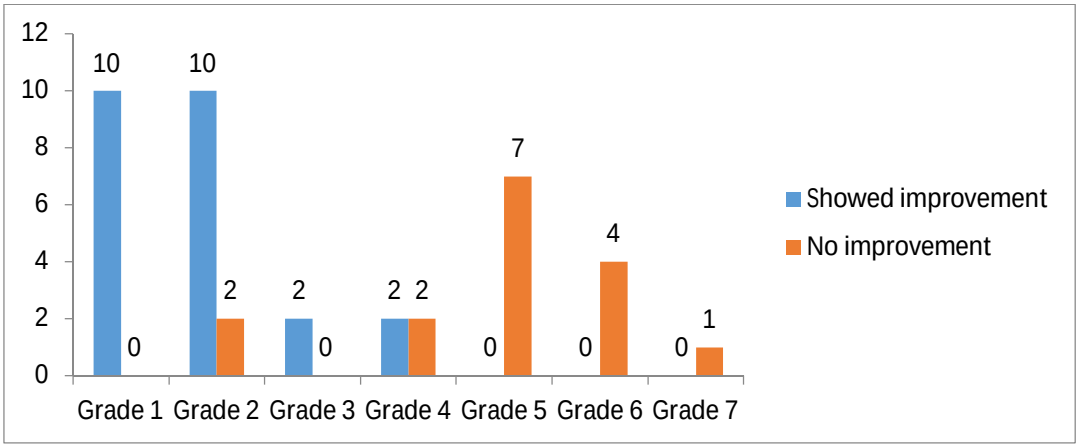


Table: 11

Initial grade of androgenic patients	No. of patients who show improvement	No. of patients who did not show improvement
Grade 1	10	0
Grade 2	8	2
Grade 3	2	0
Grade 4	2	2
Grade 5	0	7
Grade 6	0	4
Grade 7	0	1

In our study, males were classified according to Norwood Hamilton classification and our study had all grades of alopecia. Among the 10 Grade I patients, 10(100%) showed improvement. Among the 10 Grade II patients, 8(80%) showed improvement and 2(20%) showed no improvement. Among the 2 Grade III patients, 2 (100%) showed improvement. Among the 4 Grade IV patients, 2(50%) showed improvement and 2(50%) showed no improvement. Among the 12 Grade V, VI and VII patients, 100% showed no improvement. In our study lesser grades of androgenic alopecia (I, II, III) seems to have better outcome by PRP treatment.

Figure 29: Distribution of androgenic alopecia patients based on Initial grades of alopecia in females by Sinclair Scale and final outcome

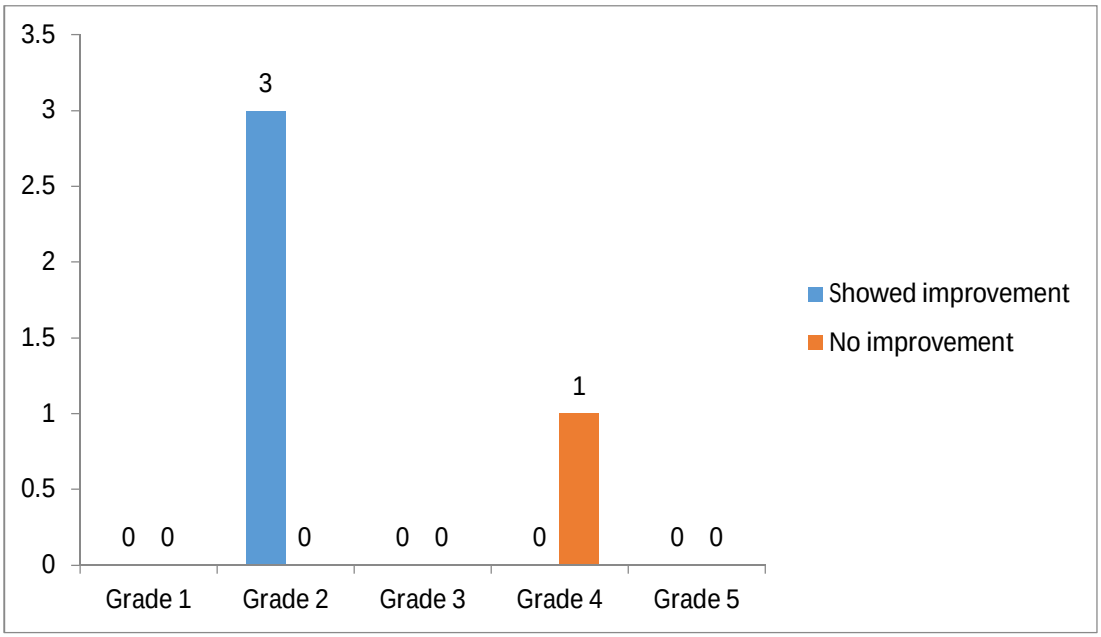


Table: 12

Initial grade of androgenic patients	No. of patients who show improvement	No. of patients who did not show improvement
Grade 1	0	0
Grade 2	3	0
Grade 3	0	0
Grade 4	0	1
Grade 5	0	0

Among the 4 females, 3(75%) belonged to Grade II and showed improvement with PRP treatment. 1(25%) belonging to Grade IV showed no improvement.

Mean values of the androgenic patients

The mean of the age of the androgenic alopecia patients was 22.79 ± 5.38 (18-41) which includes males 27.92 and females 26.5.

Table 13. Mean values of Platelet count in the whole blood from first to sixth sitting.

SITTING	Platelet count Mean ± Standard deviation $\times 10^3/\mu L$
1st	142.07±40.83
2nd	148.21±33.10
3rd	151.71±28.94
4th	153.52±31.12
5th	150.40±29.74
6th	155.74±34.26

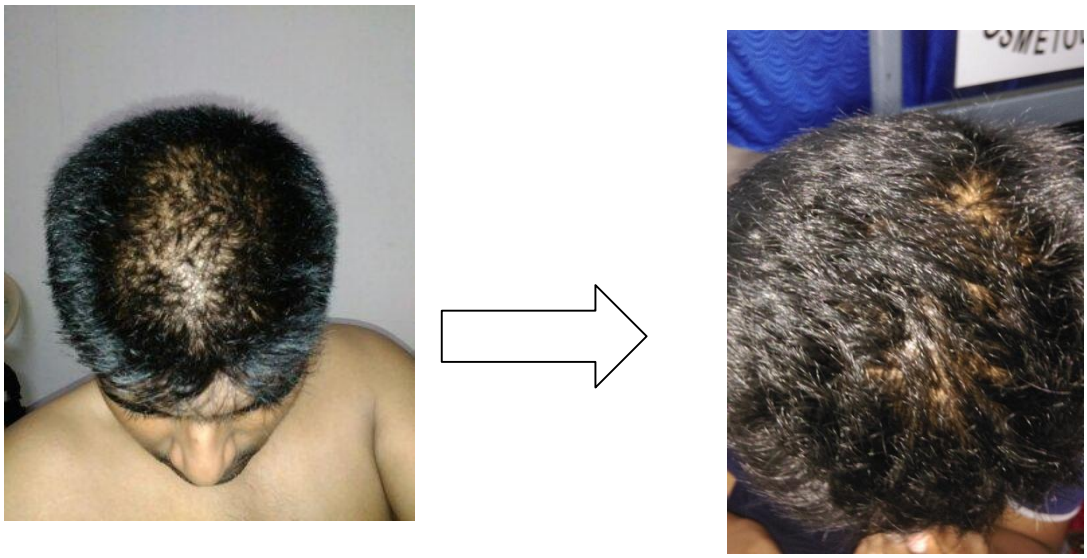
The mean baseline platelet count was 150.275 × 10^3/µL.

Table 14 Mean values of Platelet count in the platelet rich plasma from first to sixth sitting.

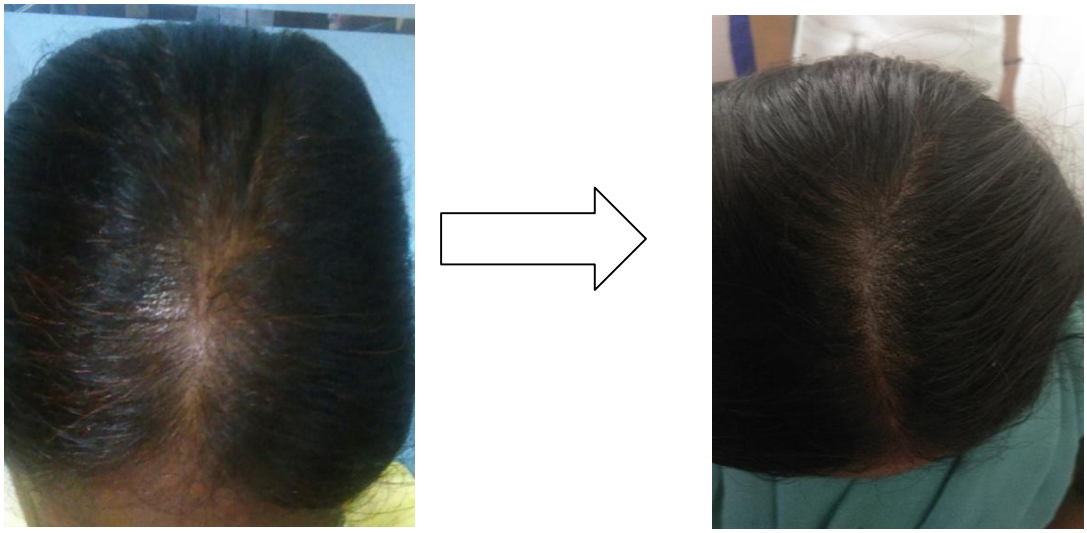
SITTING	Platelet count Mean ± Standard deviation $\times 10^3/\mu L$
1st	1381.81±356.12
2nd	1357.17±348.00
3rd	1184.74±338.08
4th	1239.24±282.80
5th	1283.90±336.36
6th	1397.48±338.92

The mean PRP platelet count was 1307.39 × 10^3/µL.

The mean of Self-Assessment Questionnaire score was 11.07.



Grade III **Grade II**



Grade II **Grade I**

Figure 30 Global photograph of a male and female androgenic alopecia patient before and after PRP treatment.

CHRONIC ULCER

Table: 15

Patient	Gender	Age	Past H/Otreatment	Site of ulcer	Duration of ulcer	Type of ulcer
Patient 1	Male	36	Present	Medial side of right foot	20 years	Trophic
Patient 2	Female	40	Present	Dorsum of right foot	3 months	Traumatic
Patient 3	Male	52	Present	Above left medial malleolus	1 year	Traumatic
Patient 4	Female	55	Present	Dorsum of right foot	6 months	Venous

Table: 16 Duration of healing time of patients

Patient	Duration of healing time
Patient 1	12 weeks
Patient 2	4 weeks
Patient 3	12 weeks
Patient 4	12 weeks

Table: 17 Comparison of Dimension of ulcer before and after PRP treatment

Patient	Length(cm)		Breadth(cm)		Depth(cm)		Area of ulcer(cm^2) L xB x 0.7854			Volume of ulcer(cm^3) L xB xD x0.7854		
	*	**	*	**	*	**	*	**	Percentage improvement in area	*	**	Percentage improvement in volume
Patient 1	7	5	6	3	0.5	0.2	33	11.8	64%	16.5	2.4	85.7%
Patient 2	8	1	5	1	0.2	0.0	31.4	0.8	97.5%	6.3	0	100%
Patient 3	3	2	2	1	0.6	0.2	4.7	1.6	66.7%	2.8	0.3	88.9%
Patient 4	6	3	5	2	0.3	0.1	23.6	4.7	80%	7.1	0.5	93.3%

*before PRP treatment

**after PRP treatment

Mean values of chronic non-healing ulcer patients:

The mean of the age of the chronic ulcer patients was 45.75 ± 5.38 (18-41) which includes males 27.92 and females 26.5.

Table 18 Mean values of Platelet count in the whole blood from first to sixth sitting.

Sitting	**Mean baseline Platelet count $\times 10^3/\mu L$**
1^{st}	195.75
2^{nd}	175
3^{rd}	192.5
4^{th}	201
5^{th}	192.75
6^{th}	166.75

The mean baseline platelet count was 186.79 × 10^3/µL.

Table 19 Mean values of Platelet count in the platelet rich plasma from first to sixth sitting

Sitting	Mean PRP Platelet count $\times 10^3/\mu L$
1st	1354.75
2nd	1525.75
3rd	1540.25
4th	1334.25
5th	1351
6th	1138

The mean platelet count in PRP was 1374 × 10^3/µL.

The mean improvement was calculated by (initial measurement – assessment day measurement) / initial measurement.

The mean percentage improvement in area was 77.05%. The mean percentage improvement in volume was 91.97%.

The mean duration of healing time of ulcers was 10 weeks.

DISCUSSION

DISCUSSION

Our study was undertaken to analyse the effectiveness of Platelet Rich Plasma (PRP) in the treatment of androgenic alopecia and chronic non-healing ulcers. Both conditions affect the quality of life and are difficult to treat. Autologous PRP treatment is simple and does not cause significant side effects.

ANDROGENIC ALOPECIA

In our study the median age for males was 28 and females was 27. Gkiniet al[25] study included 20 patients in their study. The mean age of patients was 34 years. In Schiavone et al[34] study total number of patients included were 64, 42 male and 22 female. The median age for males was 28 and females was 32. In our study among the 38 male patients, 22(57.89%) showed improvement and 16(42.11%) showed no improvement. Among the 4 female patients who, 3(75%) showed improvement and 1(25%) showed no improvement. In our study among the 2 patients belonging to the age group of 18-20 years,1(50%) showed improvement and 1(50%) showed no improvement. Among the 30 patients belonging to the age group of 21-30 years, 20(66.67%) showed improvement and 10(33.33%) showed no improvement. Among the 9 patients belonging to the age group of 31-40 years, 3(33.33%) showed improvement and 6(66.67%) showed no improvement. Only one patient belonged to the age group of 41-50 years and he didn't show any improvement. In our study and Schiavoneet al[34] study, age and gender does not seem to influence the outcome.

In Vasconcelos RCF et al[26] study, 18 patients showed a clearer and more satisfactory response in the female group (mean = 42.9%) as compared to the male group (mean = 25.6%).

In our study among the 17 professionals, 10 (58.82%) showed improvement and 7(41.18%) showed no improvement. Among the 4 students, 3(75%) showed improvement and 1(25%) showed no improvement. Among the 13 clerical by occupation, 7(53.84%) showed improvement and 6(46.15%) showed no improvement. Among the 6 coolies, 3(50%) showed improvement and 3(50%) showed no improvement. Among the 2 housewives, 2(100%) showed improvement. Occupation does not seem to affect the final outcome.

In our study 36 patients belonged to middle socio economic status and 6 belonged to lower socio economic status. Among the middle socio economic status patients 21 (58.33%) showed improvement and 15(41.66%) did not show improvement. Among the low socio economic status 4 (66.66%) showed improvement and 2(33.33%) did not show improvement. Socio economic status does not seem to affect the final outcome.

In our study among the 17 patients who had family history of alopecia, 11(64.70%) showed improvement and 6(35.29%) showed no improvement. Among the 25 patients who had no family history of alopecia, 14(56%) showed improvement and 11(44%) showed no improvement. Even though percentage wise improvement in final outcome was seen in patients with family history of alopecia but p value is not stastically significant.

In our study among the 18 patients who had past history of treatment for alopecia, 12 (66.67%) showed improvement and 6(33.33%) showed no improvement. Among the 24 patients who had no past history of treatment for alopecia, 13(54.16%) showed improvement and 11(45.83%) showed no improvement. Past history of treatment for alopecia does not seem to affect the final outcome.

In our study all 7(100%) A Rh(D) positive patients showed improvement. Among 10 B Rh(D) positive patients, 7(70%) showed improvement and 30% showed no improvement. Among the 21 O Rh(D) positive patients, 10(47.62%) showed improvement and 11(52.38%) showed no improvement. Among the 4 AB Rh(D) positive patients, 1(25%) showed improvement and 3(75%) showed no improvement. Blood grouping and Rh typing does not seem to affect the final outcome.

In our study among the 2 patients having smoking habit, 1(50%) showed improvement and 1(50%) showed no improvement. Among the 38 patients who do not have smoking habit, 22(57.89%) showed improvement and 16(42.11%) showed no improvement. Among the 2 patients who previously had smoking habit but abstinence from it for past 2 years, 2(100%) showed improvement. Smoking habit does not seem to affect the final outcome.

In our study among the 2 patients who had past history of alcoholism, 2 (100%) showed no improvement. Among the 40 patients who had no history of alcoholism, 25(62.5%) showed improvement and 15(37.5%) showed no improvement. Alcoholism does not seem to affect the final outcome.

In our study all patients were HIV, HBsAg and HCV non reactive. Hence comparison of final outcome between reactive and non reactive patients could not be done.

In our study, males were classified according to Norwood Hamilton classification[26] and our study had all grades of alopecia. Among the 10 Grade I patients, 10(100%) showed improvement. Among the 10 Grade II patients, 8(80%) showed improvement and 2(20%) showed no improvement. Among the 2 Grade III patients, 2 (100%) showed improvement. Among the 4 Grade IV patients, 2(50%) showed improvement and 2(50%) showed no improvement. Among the 12 Grade V, VI and VII patients, 100% showed no improvement. Among the 4 females, 3(75%) belonged to Grade II and showed improvement with PRP treatment. 1(25%) belonging to Grade IV showed no improvement. In our study lesser grades of androgenic alopecia (I, II, III) seems to have better outcome by PRP treatment. Similarly KhatuS et al[35] study showed improvement in lesser grade of alopecia.

Throughout the literature different studies have used different time intervals between initial and subsequent of PRP treatment sessions. In our study PRP injections were given monthly once for 6 months and at the end of 6months treatment outcome was studied. Gkini et al[25] study the treatment protocol was to give three sessions with an interval of 21 days and a booster session at 6 months. In Schiavoneet al[34] study 2 injection plan was followed with 3 months interval. During the first session they used PRP prepared by

single spin technique using the commercial device GPS III platelet separation system. To this they added plasmatic protein concentrate (platelet poor plasma filtered through mini hemoconcentrator). After inducing a cutaneous inflammatory response through gentle pressure of a 1.0 mm scalp roller, the mixture was injected. During the second session after 3 months PRP was prepared by double spin centrifugation method and injected the same way after mixing it with plasma protein concentrate. In Khatu S et al[35] study PRP was prepared by double spin technique and injected every 2 weeks for 4 sessions and evaluated after 12 weeks. In Vasconcelos RCF et al[26] 3 treatment sessions with 21days interval was followed. In our study the PRP was prepared by double spin centrifugation method.

In our study, the mean baseline platelet count was 150 × 10^3/µL. In Gkiniet al[25] study the mean baseline platelet count was 190 × 10^3/µL. In our study baseline platelet count does not seem to influence the outcome. Similarly Schiavone et al[34] study, they found that baseline platelet count did not affect the clinical outcome of PRP treatment. In our study, the mean PRP platelet count was 1307 × 10^3/µL. In Gkini et al[25] study, the mean PRP platelet count was 1102 × 10^3/µL. In many of the studies for PRP to be effective in clinical treatments, it should have approximately 10 lakh platelets or more per µL of PRP. In our study we were able to achieve this working definition of PRP.

In our study based on self assessment questionnaire and global photographs 59.52% of patients showed improvement. In Schiavone et al[34] study, patient reaching a clinically improvement difference was assessed by two evaluators, which was 40.6% and 54.7% respectively, based on global photographs. In our study, the mean self-assessment satisfaction score was 11.07. Gkini et al[25] study also reported increase in hair density significantly and patient mean satisfaction rate was 7.1 on a scale of 1-10. Khatu S et al[35] showed improvement in hair volume and coverage by global pictures.

COMPARISON OF OUR STUDY WITH OTHER STUDIES – ANALYSIS OF PRP USAGE IN ANDROGENIC ALOPECIA

	Our study	Gkini et al[25]	Schiavone et al[34]	Vasconcelos RCF et al[26]	KhatuS et al[35]
Place of study	India,Chennai	Greece	Italy	Brazil	India
Year of study	June 2015 – June 2016	October 2012 – September 2013	2012 – 2013	2015	August 2013-November 2013
No of patients	42	20	64	16	11
Method of PRP preparation	Double spin	Single spin	Single and double spin	-	Double spin
Method of PRP application	Injection in the scalp	Injection in the scalp	Injection in the scalp	Injection in the scalp	Injection in the scalp
Treatment session	Monthly once for 6 months	3 sessions with an interval of 21 days and a booster session at 6 months	1st injection – single spin 2nd injection after 3 months – double spin	3 injections at the interval of 21 days	4 sessions at 2 weeks interval
Alopecia grading	Male – Norwood Hamilton scale Female – Sinclair scale	Male – Norwood Hamilton scale Female – Ludwig scale	Male – Norwood Hamilton scale Female – Ludwig scale	Male – Norwood Hamilton scale Female – Ludwig scale	Norwood Hamilton scale
Outcome	Macroscopic photograph showed overall improvement Hair loss reduced at 3 months The mean patient self assessment questionnaire score was 11.07.	Macroscopic photograph showed overall improvement Hair loss reduced at 3 months Patient satisfaction rate on linear analog scale of 1 – 10 was 7.1	Macroscopic photograph - Jaeschke rating of clinical change by 2 evaluators were 40.6% and 54.7% respectively	Macroscopic photograph and dermoscopy. In Dermoscopy - thickening of hairs, improved local circulation and increased number of follicles. Female - average improvement was 42.85% (patients) and 35.71% (external observer). Male - average improvement was 25.55% (patients) and 18.88% (external observer).	Macroscopic photograph showed moderate improvement in hair growth. Trichoscopic hair count showed increase in hair follicular density by 15.1%
Adverse effects	No remarkable adverse effects	No remarkable adverse effects	No remarkable adverse effects	No remarkable adverse effects	No remarkable adverse effects

CHRONIC ULCERS

In our study, the mean age of chronic ulcer patients was 46 years. Among 4 patients, two of the patients were males and another two were females. Among these 4 patients with chronic non-healing ulcers, 1 had trophic ulcer, 2 had traumatic ulcer and 1 had venous ulcer. All of them belong to lower socioeconomic status.

In our study, the mean duration of healing time was 10 weeks (PRP injected at once in two weeks' time interval). In our study, the mean percentage improvement in area was 77.05% and mean percentage of improvement in volume was 91.97%. In Suryanarayan S et al[29] study on 24 chronic ulcer patients, the mean duration of healing time was 5.6 weeks (PRP injected at weekly interval). The mean percentage improvement in area was 91.7% and mean percentage improvement in volume was 95%. 100% resolution in the area was seen in 76% of the ulcers and 100% reduction in volume was seen 73% of ulcers. In Sarvajnamurthy S et al[6] study on 12 patients, the mean age of patients was 33.5 years. The mean duration of healing time of ulcers was 5.1 weeks (PRP injected at weekly interval). The mean percentage improvement in area was 94.7% and mean percentage improvement in volume was 95.6%. In Frykberg et al[30] study on 49 patients, The mean duration of healing time of ulcers was 2.6 weeks. The mean percentage improvement in area was 39.5% and mean percentage improvement in volume was 51%.

In our study, the mean baseline platelet count was $186.79 \times 10^3/\mu L$. The mean platelet count in PRP was $1374 \times 10^3/\mu L$.

COMPARISON OF OUR STUDY WITH OTHER STUDIES – ANALYSIS OF PRP USAGE IN CHRONIC NON-HEALING ULCER PATIENTS

	Our study	SarvajnamurthyS et al[6]	Frykberg et al[30]	SuryanarayanS et al[29]
Place of study	India, Chennai	Karnataka, India	USA	Karnataka, India
Year of study	June 2015 – June 2016	April – June 2013	2010	2015
No of patients	4	12	49	24
Method of PRP preparation	Double spin	Double spin	PRP prepared by commercial system	Double spin
Type of ulcers studied	1 venous 2 traumatic 1 trophic	17 venous ulcer	65 – venous, pressure, diabetic foot ulcers	33 – venous, traumatic, trophic, vasculitic, diabetic foot ulcer and ulcer due to pyodermagangrenosum
Mean Duration time of healing	10 weeks	5.1 weeks	2.8 weeks	5.6 weeks
Mean Improvement percentage	Area – 77.05% Volume – 91.97%	Area – 94.7% Volume – 95.6%	Area – 39.5% Volume – 51%	Area – 91.7% Volume – 95%
Outcome of PRP treatment	Improved	Improved	97% of wounds improved	Improved

SUMMARY

SUMMARY

In our study

I. on analysis of the usage of PRP in the treatment of androgenic alopecia:

- Out of total number of 42 patients, thirty eight (90.5%) were males and four (9.5%) were females.

- Two (4.8%) patients were in 18-20 years age group, thirty (71.4%) patients were in 21-30 years of age group, nine (21.4%) patients were in 31–40 years of age group and one (2.4%) patients was in 41-50 years of age group.

- 10 (58.82%) out of 17 professionals, 3 (75%) out of 4 students, 7 (53.84%) out of 13 clerical occupation, 3 (50%) out of 6 coolies and 2 (100%) housewives showed improvement.

- 21 (58.33%) out of 36 patients belonged to middle socio economic status and 4 (66.66%) out of 6 belonged to lower socio economic status showed improvement.

- 11(64.70%) out of 17 patients who had family history of alopecia and 14(56%) out of 25 patients who had no family history of alopecia showed improvement.

- 12 (66.67%) out of 18 patients who had past history of treatment for alopecia and 13(54.16%) out of 24 patients who had no past history of treatment for alopecia showed improvement.

- As per Norwood Hamilton Classification for male androgenic alopecia.

i) Before treatment, 10 patients had Grade I alopecia, another 10 patients had Grade II alopecia, 2 had Grade III, 4 had Grade IV, 7 had Grade V, 4 had Grade VI and 1 had Grade VII alopecia.

ii) After treatment, all 10 patients (100%) with Grade I, 8 out of 10 (80%) patients with Grade II, 2 out of 2 patients (100%) with Grade III, 2 out of 4 patients (50%) with Grade IV alopecia showed improvement.

- As per Sinclair Scale for female androgenic alopecia,

i) Before treatment 3 patients had Grade II and 1 had Grade IV alopecia.

ii) After treatment, 3 out of 3 female patients (100%) with Grade II showed improvement, a single patient with Grade IV alopecia showed no improvement.

- The mean baseline platelet count of the patients was $150 \times 10^3/\mu L$.

- The mean platelet count of the autologous PRP prepared and used for treatment was $1307\times 10^3/\mu L$.

- In total out of 42 patients with androgenic alopecia, 25 (59.52%) of them showed improvement.

- On the basis of scores given for the response to self assessment questionnaire, the mean satisfaction rate was 11.07.

II. On analysis of the usage of PRP in the treatment of chronic non-healing ulcer

- Out of total number of 4 patients, 2 (50%) were males and 2 (50%) were females.

- The mean of the age of the patients with chronic ulcer was 46± 5

- The mean baseline platelet count was 186.79 × 10^3/µL.

- The mean platelet count of the autologous PRP prepared and used for treatment was 1374× 10^3/µL.

- Dimensions measured before and after treatment showed significant reduction in the ‘area’ and ‘volume’ of the chronic ulcer with mean improvement percentage of 77. 05% and 91.97% respectively.

- The chronic ulcers showed mean of healing time of 10 weeks.

CONCLUSION

CONCLUSION

The emerging science of transfusion medicine is a growing field, as it applies to regenerative therapy and the platelet rich plasma treatment. PRP in particular is part of a new biotechnology. In a developing country like India, autologous PRP treatment could be an affordable alternative therapy for androgenic alopecia and chronic non-healing ulcers as it provides necessary growth factors and cytokines required for tissue healing and regeneration.

Strict vigilance at maintaining sterility and regular cross-checking of the platelet values are a must to obtain consistent results. It is important to motivate both patients and clinicians to attempt these more advanced treatment modalities.

It is the responsibility of the clinician to gain a thorough understanding of this biotechnology, to use it correctly and wisely for the benefit and well being of patients, who trust our judgment.

In country like India, where diabetes mellitus is prevalent such alternative therapy could provide additional treatment options to augment healing of one of the commonest complications - a non-healing leg ulcers.

However, further studies on larger number of patients with long term follow-up are essential to exactly assess the treatment outcome.

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ANNEXURE

PROFORMA

Name/ID : Address:
Age: Education:
Sex: Annual income:
Diagnosis (onset of age, duration):
Past h/o treatment for the same (details)
Associated medical conditions:
On any medications (details)
Treatment advised: PRP – predicted no. of sittings:
With any medication/procedure:
INVESTIGATIONS:

Blood group: HIV/HBsAg/anti HCV:

			Whole blood				Platelet – rich plasma				
AD.D	SITG	DOP	HB Gm%	TC	DC	PLT.C	VOL ml	PH	RBC.C	WBC.C	PLT.C
	1st										
	2nd										
	3rd										
	4th										

ACNE SCAR (Quantitative global scarring assessment scale)

Sitting	Type of scar	No. of scars	Score calculation	Score (done pre procedure)	Improvement in %
1st					
2nd					
3rd					
4th					

ULCER ASSESSMENT

Sitting	Ulcer site	No. of ulcers	L	W	D	w. area (LXW)cm^2	w.vol (LXBWXD)cm^3	Improvement in Area	vol
1st									
2nd									
3rd									
4th									

ANDROGENIC ALOPECIA - grade

(Patient self assessment questionnaire):		score
1) My bald area is getting smaller -	Strongly agree	1
	Agree	2
	No opinion	3
	Disagree	4
	Strongly disagree	5
2) Appearance of my hair after PRP -	Better	1
	Little better	2
	Same	3
	Little worse	4
	Worse	5
3) New hair growth -	Greatly increased	1
	Moderately increased	2
	Slightly increased	3
	No change	4
	Slightly decreased	5
	Moderately decreased	6
	Greatly decreased	7
4) Slowing down of hair loss -	Effective	1
	Somewhat effective	2
	Not very effective	3
	Not effective at all	4
5) Satisfaction with appearance of hair -	Very satisfied	1
a. Frontal area	Satisfied	2
b. Vertex	Neutral	3
	Dissatisfied	4
	Very dissatisfied	5

PATIENT INFORMATION SHEET

ANALYSIS OF THE USAGE OF PLATELET-RICH PLASMA IN THE COSMETOLOGY DEPARTMENT OF A TERTIARY CARE HOSPITAL

PROCEDURE:

PRP is used in various medical fields such as dentistry, orthopaedics, plastic surgeries and ulcer management. In recent times, PRP is used increasingly in cosmetology like for treating acne scars and hair growth in alopecia. This study is done to analyse the usage of PRP in treating alopecia, acne scars and chronic non healing ulcers.

BENEFITS AND RISKS

There is no risk for patients enrolled in this study as their treatment protocols are not interfered with.

CONFIDENTIALITY

Your privacy will be protected in so for as permitted by law. Only your researcher and ethics committee members will have access to the data collected during the study.

PARTICIPATION

Your participation in this study is voluntary and you are free to decide now or later whether to continue or discontinue from the study.

CONSENT

I confirm that I read and understood the information about the above research study dated ----------And I received the chance to questions.

My participation in this study is voluntary and I know that I am free to withdraw from the study at any time, without affecting of my legal rights.

I agree to this access. I know that my identification will not be revealed in any detail,that is released to third person or published.

I agree not to restrict or interfere with any data or results that are obtained from this study. I agree to participate in this research study for the above listed purpose.

Patient's name:

Signature: Date:

Patient IP number:

Signature of the person who obtains consent /date:

பங்கேற்பாளர்க்கான தகவல் படிவம்.

Platelet–Rich Plasma-எவ்வாறு அழகியல் துறையில் உபயோகமாகிறது என்று இந்த ஆராய்ச்சி ஆய்கிறது.

குறிக்கோள்:

Platelet –Rich Plasma வழுக்கையை குணப்படுத்துவதிலும், பருக்களினால் ஏற்படும் தழும்புகளை சரி செய்யவும், பல நாட்களாக ஆறாத புண்களை ஆற்றுவதற்கும் எவ்வாறு பயன்படுகிறது என்று இந்த ஆராய்ச்சி ஆய்கிறது.

செய்முறை:

- சிகிச்சை பெறுபவரின் விவரங்கள் மற்றும் இரத்த பரிசோதனைகளின் விவரங்களும் பெறப்பட்டு

ஆய்வுக்கு உட்படுத்தப்படும்.

பலன்களும் பாதிப்புகளும்:

நோயாளிகளின் சிகிச்சை முறையில் ஆராய்ச்சியாளர் தலையிடுவதில்லை. ஆகையால் நோயாளிகளுக்கு எவ்வித பாதிப்பும் இல்லை.

இரகசியப் பாதுகாப்பு:

சட்ட வரைமுறையின்படி தங்களின் சொந்த விஷயங்கள் பாதுகாக்கப்படும். தங்களின் ஆராய்ச்சியாளர் மட்டும் இந்த ஆராய்ச்சியின் போது கிடைக்கும் புள்ளி விவரங்களை பயன்படுத்த இயலும்.

பங்களிப்பு:

இந்த ஆராய்ச்சியில் தங்களின் பங்களிப்பு தன்னார்வமானது. இந்த ஆராய்ச்சியில் தங்களின் பங்களிப்பினைத் தொடர்வதற்கும், விடுபடுவதற்கும் எந்த நேரமும் தங்களுக்கு உரிமையுண்டு.

ஒப்புதல்:

மேற்கண்ட ஆராய்ச்சியினைப்பற்றி பற்றிய தகவல்களைப் படித்து புரிந்து கொண்டேன் என்பதையும் வினாக்களைக் கேட்பதற்கான வாய்ப்பும் எனக்கு அளிக்கப்பட்டது என்பதை நான் உறுதி செய்கிறேன்.

இந்த ஆராய்ச்சியில் எனது பங்கு சொந்த விருப்பத்தின் பேரில் மட்டுமே சட்ட ரீதியான எனது உரிமைகள்பாதிக்கபடாமலும் எந்த நேரத்திலும் இந்த ஆராய்ச்சியிலிருந்து என்னை விடவித்துக் கொள்ள முடியும் என்பதை நான் அறிவேன்.

எனது தனிப்பட்ட விஷயங்கள் மூன்றாவது பேருக்கோ அல்லது எந்த பிரசாரத்திற்கும் வெளியிடப்படமாட்டாது என்பதை நான் அறிவேன்.

இந்த ஆராய்ச்சியின் முடிவுகள் மற்றும் புள்ளி விவரங்கள் போன்றவற்றை தடை செய்யவும் மாட்டேன் குறுக்கிடவும் மாட்டேன் என்பதை ஒத்துக்கொள்கிறேன்.

மேற்கூறிய நோக்கத்திற்காக இந்த ஆராய்ச்சியில் பங்கெடுத்துக் கொள்ள நான் ஒத்துக்கொள்கிறேன்.

சிகிச்சை பெறுபவரின் கையொப்பம்: உறுதி மொழி பெறுபவரின் கையொப்பம்:

தேதி:

THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY

No.69, ANNA SALAI, GUINDY, CHENNAI - 600 032.

Website : www.tnmgrmu.ac.in Ph : 22353574, 22353576 - 79, 22301760 - 63, 22353094
E-mail : mail@tnmgrmu.ac.in Fax : 91-44-22353698

INSTITUTIONAL ETHICS COMMITTEE

Address of Ethics Committee: The Tamilnadu Dr MGR Medical University Chennai, India	
Presenter: Dr. D. Deepa MBBS	
Analysis of the usage of platelet rich plasma in the Department of Cosmetology at a Tertiary Care Hospital. (ECMGR0309044)	
Documents filed	
Protocol	✓
Informed consent documents	
Any other documents	PROFORMA .

THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY

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INSTITUTIONAL ETHICS COMMITTEE 25.06.15

NAME OF MEMBER	DESIGNATION	SIGNATURE
Prof. D. SHANTHARAM **M.D., D. Diab** VICE CHANCELLOR, THE T.N. DR.MGR MEDICAL UNIVERSITY	**Chairman**	
MR. ANAND DAVID UNIVERSITY STANDING COUNSEL THE T.N. DR.MGR MEDICAL UNIVERSITY	**Member**	
Dr. GEETHALAKSHMI, MD PhD DIRECTOR OF MEDICAL EDUCATION, CHENNAI.	**Member**	—
Dr. PERIANDAVAR MD INSTITUTE OF DIABETOLOGY GOVERNMENT GENERAL HOSPITAL, CHENNAI	**Member**	
DR.SABARATNAVEL, MD DEPARTMENT OF MEDICINE, MADRAS MEDICAL COLLEGE & GOVERNMENT HOSPITAL.	**Member**	
DR. SARAVANAN MDS. DEPT. OF ORAL SURGERY GOVERNMENT DENTAL COLLEGE, CHENNAI	**Member**	—
DR. M. LOGAMANIAN, M.D.,Ph.D. NATIONAL INSTITUTE OF SIDDHA, CHENNAI.	**Member**	25/6/15
Dr. R. P. ILANGHO, M.D DEPT. OF RESPIRATORY MEDICINE, APOLLO HOSPITAL, CHENNAI.	**Member**	
Dr. S. MINI JACOB, M.D DEM, THE T.N. Dr. MGR MEDICAL UNIVERSITY	**Member Secretary**	

DR. N. RAJASEKARAN (ECMGR0309044) DDME — Member representing DME — 25/6/15

THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY

No.69, ANNA SALAI, GUINDY, CHENNAI - 600 032.

Website : www.tnmgrmu.ac.in Ph : 22353574, 22353576 - 79, 22301760 - 63, 22353094
E-mail : mail@tnmgrmu.ac.in Fax : 91-44-22353698

DECISION

Opinion of the institutional Ethics Committee-PLEASE CHECK ONE

✓ Approved

_____ Modification required prior to approval (please specify on the space below)

_____ Disapproved

Date of review: 25.06.15

Signed : _____ (please print name) DR.D. SHANTHARAM M.D., D.Diab
(please delete as appropriate, Chairperson, Secretary)

Modification needed

The research proponent is hereby informed that the Institutional Ethics Committee will require the following:

1) All adverse drug reaction (ADRs) that are both serious and unexpected to be reported promptly to the IEC within 7 working days.

2) The progress report to be submitted to the IEC at least annually.

3) Upon completion of the study, a final study status report to submitted to the IEC.

(ECMGR0309044)

INSTITUTIONAL ETHICAL COMMITTEE,
STANLEY MEDICAL COLLEGE, CHENNAI-1

Title of the Work : Analysis of the usage of platelet rich plasma in the department of Cosmetology at a tertiary care Hospital

Principal Investigator : Dr. Deepa.D

Designation : PG M D (IH & BT)

Department : Department of Blood Transfusion
The TN Dr MGR Medical University,
Chennai-32

The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 09.07.2015 at the Council Hall, Stanley Medical College, Chennai-1 at 2PM

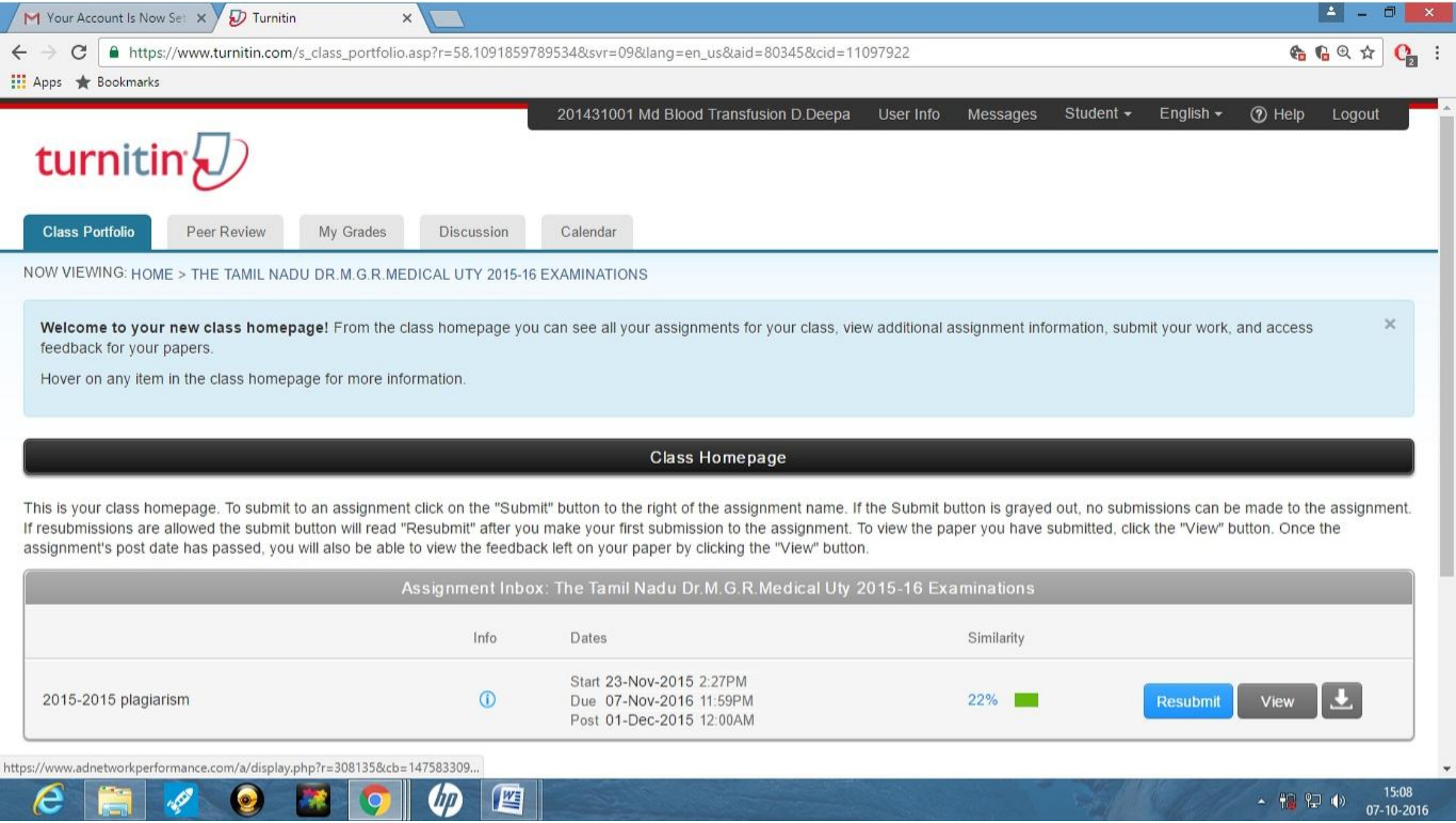
The members of the Committee, the secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The Principal investigator and their team are directed to adhere to the guidelines given below:

1. You should inform the IEC in case of changes in study procedure, site investigator investigation or guide or any other changes.
2. You should not deviate from the area of the work for which you applied for ethical clearance.
3. You should inform the IEC immediately, in case of any adverse events or serious adverse reaction.
4. You should abide to the rules and regulation of the institution(s).
5. You should complete the work within the specified period and if any extension of time is required, you should apply for permission again and do the work.
6. You should submit the summary of the work to the ethical committee on completion of the work.

MEMBER SECRETARY,
IEC, SMC, CHENNAI

MEMBER SECRETARY
ETHICAL COMMITTEE,
STANLEY MEDICAL COLLEGE
CHENNAI-600 001.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Assignment Inbox: The Tamil Nadu Dr.M.G.R.Medical Uty 2015-16 Examinations
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1	25	1	1	2	1	3	1	2	2	2	2	2	197	199	188	190	190	192	996	783	1038	998	1088	1080	5	5	15	2
2	41	1	1	2	1	2	1	2	2	2	2	2	168	180	184	192	200	198	1083	1648	1068	844	848	1430	2	1	8	1
3	23	1	3	2	1	2	1	2	2	2	2	2	131	155	145	160	165	178	844	1844	884	1086	846	1674	5	5	14	2
4	23	1	3	2	2	1	1	2	2	2	2	2	226	210	203	215	216	221	1871	888	1383	844	1649	1248	2	1	8	1
5	25	1	3	2	2	3	1	2	2	2	2	2	190	174	175	180	180	186	998	708	1088	1648	988	1767	2	2	13	2
6	30	1	4	3	1	2	1	2	2	2	3	2	134	184	165	168	150	160	1768	1705	806	1330	1679	1767	1	0	12	1
7	25	1	1	2	1	3	1	2	2	2	2	2	131	130	167	172	164	140	1668	1660	988	708	1208	1885	5	5	16	2
8	37	1	3	2	1	1	1	2	2	2	3	2	219	180	204	212	199	216	1783	1348	788	1380	1811	788	2	1	10	1
9	28	1	1	2	1	2	1	2	2	2	2	2	102	132	142	117	125	130	996	1648	1038	1330	1638	998	2	0	7	1
10	25	1	2	2	1	3	1	2	2	2	2	2	115	133	152	110	121	128	1568	1065	1867	1088	1096	1698	1	0	9	1
11	23	1	1	2	1	3	1	2	2	2	2	2	65	104	113	118	138	118	1096	1495	788	868	1076	1886	2	1	11	1
12	27	1	3	2	1	3	1	2	2	2	2	2	100	114	97	110	145	139	1098	1810	824	1211	888	884	1	0	7	1
13	30	1	4	2	2	2	1	2	2	2	2	2	165	201	186	156	210	176	966	964	1007	1087	1468	1108	6	6	18	2
14	18	1	2	2	2	1	1	2	2	2	2	2	156	148	155	130	142	128	1448	989	1760	1768	1488	780	1	0	5	1
15	29	1	3	2	2	3	1	2	2	2	2	2	190	210	176	185	195	198	1780	1422	996	1008	889	1248	2	2	11	2
16	23	1	3	2	1	2	1	2	2	2	2	2	115	136	132	144	108	128	1688	708	1096	1098	1483	1083	1	0	9	1
17	25	2	5	2	1	4	1	2	2	2	2	2	106	132	167	155	132	142	884	1185	1062	1638	1698	1148	2	1	8	1
18	21	1	2	2	2	3	1	2	2	2	2	2	80	104	110	98	124	130	1480	1096	1768	1686	1786	1648	4	2	9	1
19	22	1	3	2	2	3	1	2	2	2	2	2	166	158	156	182	128	210	1688	1088	1780	1490	1560	1660	1	0	8	1
20	27	1	3	2	2	1	1	2	2	2	2	2	110	90	137	176	157	145	1183	1680	1064	1388	1380	1588	2	1	11	1
21	32	1	3	2	1	4	1	2	2	2	2	2	95	117	135	132	129	110	1488	1786	1085	1289	1208	1380	7	7	17	2
22	35	1	4	3	1	3	1	2	2	2	2	2	78	70	118	106	126	135	1680	1686	1760	1068	809	1768	5	5	13	2
23	19	1	2	2	2	3	1	2	2	2	2	2	135	112	130	133	125	126	1380	1788	1048	998	1248	1190	5	5	14	2
24	32	1	1	2	2	3	1	2	2	2	1	1	134	145	146	135	116	148	1198	1260	708	1240	1168	908	6	6	13	2
25	40	1	4	3	2	4	1	2	2	2	2	1	115	148	138	127	98	119	1488	1790	646	1168	922	1786	6	6	14	2
26	29	1	1	2	1	3	1	2	2	2	2	2	106	134	125	112	116	120	1760	1190	928	814	726	964	3	2	10	1
27	28	1	4	3	2	3	1	2	2	2	2	2	114	126	134	105	125	118	786	1009	1760	986	1160	1380	2	1	11	1
28	25	1	1	2	2	3	1	2	2	2	2	2	155	160	135	150	160	142	1680	1386	1560	1085	1480	988	5	5	10	2
29	37	1	4	3	2	2	1	2	2	2	2	2	135	160	142	158	157	146	1686	1008	1798	1160	1468	1065	1	0	11	1
30	26	1	1	2	1	3	1	2	2	2	2	2	160	132	158	173	149	166	1788	1108	979	1580	1660	1789	4	4	12	2
31	27	2	5	3	2	3	1	2	2	2	2	2	120	143	156	132	136	140	678	998	1106	1350	1580	1760	2	1	8	1
32	26	1	1	2	2	1	1	2	2	2	1	2	112	108	155	138	127	132	1688	1206	1078	1878	889	1108	1	0	9	1
33	29	1	1	2	2	2	1	2	2	2	2	2	172	155	136	166	198	187	1480	1780	1105	1180	1086	1686	4	3	11	1
34	32	2	1	2	2	3	1	2	2	2	2	2	134	148	146	189	138	145	1580	1760	1205	1308	1766	1086	2	1	10	1
35	25	1	3	2	2	2	1	2	2	2	2	2	223	165	148	206	133	215	1588	886	1086	1168	1028	1668	5	5	13	2
36	30	1	1	2	2	1	1	2	2	2	2	2	172	155	138	167	158	190	856	1588	1568	1360	1660	1760	3	2	8	1
37	25	1	3	2	1	3	1	2	2	2	2	2	134	156	132	168	155	140	1660	1460	1096	1786	1208	1668	1	0	9	1
38	27	1	1	2	2	2	1	2	2	2	2	2	168	167	200	143	176	155	1066	1206	1096	1308	896	1586	1	0	9	1
39	24	1	1	2	1	1	1	2	2	2	2	2	172	155	138	165	160	89	1686	1468	1180	1568	689	1180	2	1	10	1
40	22	2	1	2	2	4	1	2	2	2	2	2	212	218	238	187	182	228	780	1756	1660	1068	1460	1560	4	4	16	2
41	35	1	3	2	2	3	1	2	2	2	2	2	96	117	106	128	127	155	1768	1580	1108	1108	1566	1360	6	6	16	2
42	35	1	1	2	2	3	1	2	2	2	2	2	159	160	164	158	137	172	1386	1568	1106	1080	1680	1686	4	4	12	2

S.no	Age	Sex	Occup	SE Status	WB 1 PLT	WB 2 PLT	WB 3 PLT	WB 4 PLT	WB 5 PLT	WB 6 PLT	PRP1PLT	PRP2PLT	PRP3PLT	PRP4PLT	PRP5PLT	PRP6PLT	ini leng	ini bdh	ini dep	ini area	ini vol	final leng	final bdh	final dep	final area	final vol	imp	imp area%	imp vol%	tal time dur wks	
1	36	1	4	2	227	188	220	198	228	187	1438	1383	1638	1383	1488	1338	7	6	0.5	33	16.5	5	3	0.2	11.8	2.4	1	64	85.7	12	
2	40	2	5	2	226	220	186	240	248	196	988	1667	1638	1248	1336	1088	8	5	0.2	31.4	6.3	1	1	0	0.8	0	1	97.5	100	4	
3	52	1	4	2	164	184	196	186	135	136	1630	1386	1639	1323	1169	708	3	2	0.6	4.7	2.8	2	1	0.2	1.6	0.3	1	66.7	88.9	12	
4	55	2	5	2	166	108	168	180	160	148	1363	1667	1246	1383	1411	1418	6	5	0.3	23.6	7.1	3	2	0.1	4.7	0.5	1	80	93.3	12	

Age group in years	Code
18-20	1
21-30	2
31-40	3
41-50	4

Sex	Code
Male	1
Female	2

Socioeconomic Status (SE Status)	Code
High	1
Middle	2
Low	3

Occupation (Occup)	Code
Professional	1
Student	2
Clerical	3
Coolie	4
Housewife	5

Family history of alopecia (Fam H/O)	Code
Present	1
Absent	2

Past history of treatment for alopecia (Past T.H)	Code
Present	1
Absent	2

Blood group (bldgp)	Code
A	1
B	2
O	3
AB	4

Rh typing (Rh Typ)	Code
Positive	1
Negative	2

HIV, HBsAg, Anti-HCV	Code
Reactive	1
Non Reactive	2

HIV, HBsAg, Anti-HCV	Code
Reactive	1
Non Reactive	2

History of Alcohol	Code
Present	1
Absent	2

Initial grade of alopecia (ini grade)	Code
Grade 1	1
Grade 2	2
Grade 3	3
Grade 4	4
Grade 5	5
Grade 6	6
Grade 7	7

Final grade of alopecia (final grade)	Code
Improvement from Grade 1	0
Grade 1	1
Grade 2	2
Grade 3	3
Grade 4	4
Grade 5	5
Grade 6	6
Grade 7	7

Improvement in Grade (imp in gr)	Code
Present	1
Absent	2

Ini leng	Initial Length
Ini bdh	Initial breadth
Ini dep	Initial depth
ini area	Initial area
ini vol	Initial volume
final leng	Final length
final bdh	Final breadth
final dep	Final depth
final area	Final area
final vol	Final volume
imp	Improvement
imp area%	Percentage improvement in area
imp vol%	Percentage improvement in volume
heal time dur weeks	Duration of healing time in weeks