A COMPARATIVE STUDY ON THE PROTECTIVE EFFECTS OF ASCORBIC ACID AND TUALANG HONEY ON THE SKIN FLAP OF REVERSE SURAL FASCIOCUTANEOUS FLAP IN A SMOKING RABBIT MODEL

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III Preface

Trauma involving the lower limb is in the rising trend. Similar trend is also seen in the number of cigarette smokers. Reconstruction of lower limb soft tissue defect could be a challenging task especially amongst patient whom are smokers as has been reported in the literature. We are in an era of promoting limb salvage surgery as compared to amputation which may be the only option in the past.

Free tissue transfer would be the better option for reconstruction in area with precarious blood supply. However this form of reconstruction may be far from reach in many places. Introduction of reverse sural neurocutaneous flap has open a new dimension for lower limb reconstruction. It is a relatively easy flap to raise with lesser steep learning curve. However the complication are similar to any pedicled flap. Smoking is a known factor for flap failure and it is attributed to vasoconstrictive effect of nicotine and carbon monoxide found in the mainstream cigarette smoke. Both these agents contribute to the release of inflammatory mediators and later give rise to ischaemic reperfusion injury leading to flap loss.

We studied the effect of two anti-oxidant (Ascorbic acid and “Tualang honey”) in improving the outcome of flap survival using smoking rabbit model. We hope to establish the beneficial effect of both these agents which later could be extended its use in our clinical practice among the active smokers in our patient population.

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3.0 MATERIALS AND METHODOLOGY

V ABBREVIATIONS

%	Percent
α	Alpha
BC	*Before Christ*
Cm	centimetre
cor	Co-relation
2D	Two dimension
df	Degree of freedom
EDCF	Endothelial-derived contracting factor
EDRF	Endothelial derived relaxing factor
FAMA	The Federal Agriculture Marketing Authority
g	gram
IL	Interleukin
kg	Kilogram
L/min	Litre per minute
mm^2	millimetre square
mg	milligram
n	Numbers

PVC	Polyvinylchloride
pH	A measure of the acidity or basicity of a solution. It approximates p[H], the negative logarithm (base 10) of the molar concentration of dissolved hydronium ions
p value	is the probability of obtaining the same or more extreme data assuming the null hypothesis of no effect; p-values are generally (but arbitrarily) considered significant if $p < 0.05$
PDGF	Platlet derived growth factor
SV	Smoking with ascorbic acid
SH	Smoking with “Tualang” honey
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
TNF	Tumour necrosis factor
TGF	Transforming growth factor
USM	Universiti Sains Malaysia
VEGF	Vascular endothelial growth factor
Vs	Versus

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VIII ABSTRAK

Trauma anggota badan yang melibatkan kaki sememangnya cabaran yang besar untuk pakar bedah rekonstruktif. Kerumitan dalam komposisi tisu anggota badan yang lebih rendah dan kebimbangan mengenai keberkesanan pengaliran darah telah menjadi cabaran dalam proses rekonstruktif. Pengenalan " pedicled fasciocutaneous flap" berdasarkan aliran songsang telah menyediakan alternatif untuk memudahkan proses rekonstruktif demi menggantikan tisu yang hilang disebabkan oleh trauma. Flap sural songsang semakin popular dan digunakan secara meluas dalam proses rekonstruktif melibatkan pembinaan semula tisu melibatkan kaki. Teknik ini lebih mudah jika di bandingkan dengan penggunaan teknik pemindahan tisu bebas.

Menjalankan pembedahan flap keatas perokok telah terbukti menghasilkan keputusan yang kurang memuaskan. Komponen dalam asap rokok memberi kesan negatif kepada sel endothelium salur darah. Nikotin dan karbon monoksida dalam asap rokok menghasilkan kerosakan oksidatif dan melepaskan spesies oksigen reaktif yang mengakibatkan kecederaan iskemia.

Asid askorbik dan madu Tualang adalah dua agen yang telah dikaji secara meluas untuk fungsi anti oksidatif mereka dan sifat-sifat penyembuhan luka. Kajian sebelum ini bertujuan untuk mengkaji kesan asap rokok dan asid askorbik pada endothelium salur darah berkaliber besar seperti karotid dan aorta. Kajian ini telah dijalankan untuk membandingkan kesan anti oksidatif kedua-dua agen ini dalam meningkatkan kadar kelangsungan hidup flap sural songsang menggunakan model arnab yang terdedah kepada asap rokok.

Kajian ini adalah bertujuan untuk mengolah sifat antioxida kedua- sebut di kalangan perokok demi meningkatkan peredaran mikrovaskular dalam flap sural songsang menggunakan model arnab merokok.

Semua sampel arnab terdedah kepada asap rokok menggunakan kebuk asap untuk tempoh sejumlah 5 minggu. Sampel kajian telah dibahagikan kepada tiga kumpulan. Kumpulan kawalan hanya menerima asap rokok manakala dua kumpulan lain menerima asid askorbik dalam bentuk suntikan intramuskular dan minum madu Tualang selain di dedahkan kepada asap rokok. Pembedahan Flap sural songsang dilakukan keatas semua arnab pada hari ke 30 pendedahan kepada asap rokok.

Penilaian sampel dilakukan serta merta selepas pembedahan, hari pertama selepas pembedahan, hari ketiga dan hari ketujuh. Parameter seperti suhu flap, tempoh isian semula peredaran kapilari, warna flap, bukti terkumpulnya darah beku dibawah flap atau bukti kehadiran jangkitan dirakamkan. Kadar kelangsungan hidup flap dikira pada hari ketujuh. Ketiga tiga kumpulan masih didedahkan kepada asap rokok serta diberi suntikan asid ascorbic dan madu tualang mengikut kumpulan masing-masing sepanjang tempoh eksperimen.

Kami mendapati bahawa, kedua-dua kumpulan yang didedahkan dengan asid askorbik dan madu Tualang menunjukkan peningkatan yang ketara dalam kadar kelangsungan hidup flap. Analisa statistic menunjukkan peningkatan yang ketara dari aspek suhu, peningkatan warna dan pengurangan dalam kadar pembentukkan darah beku di bawah flap. Kadar kelangsungan hidup flap melonjak daripada 62% di lengan merokok kepada 88.9% dan 81.0% dalam kumpulan asid askorbik dan madu tualang. Walau bagaimanapun analisa menggunakan ANOVA sehala menunjukkan nilai p 0.075 yang menghampiri nilai yang ketara.

Kesimpulannya adalah asid askorbik mempunyai lebih kesan positif berbanding madu Tualang dalam mengurangkan kesan kerosakan oksidatif yang dihasilkan oleh asap rokok serta menperbaiki peredaran mikrovaskular flap sural songsang.

IX ABSTRACT

Lower limb traumas impose a great challenge for reconstructive surgeons. Complexity in the tissue constituent of lower limb and the concern regarding vascularity has been the barrier in the process of reconstruction. Introduction of pedicled fasciocutaneous flap based on reverse flow has provided an alternative to free flap reconstruction. Reverse sural fasciocutaneous flap is gaining popularity and widely being used in the reconstruction of distal third of lower limb due to its ease of elevation compared to free flap.

Performing flap surgery in smokers has been proven to yield poor outcome. Components in cigarette smoke have an established negative impact on the vascular endothelium of blood vessels. Nicotine and carbon monoxide in cigarette smoke produce oxidative damage and release reactive oxygen species resulting in ischaemic injury.

Ascorbic acid and “Tualang honey” are two agents which have been extensively studied for their anti oxidative and wound healing properties. Previous studies were aimed at studying the impact of cigarette smoke and ascorbic acid on the endothelium of large calibre vessel like carotid and aorta. The present study was undertaken to compare the anti oxidative properties of both these agents in improving the outcome in flap survival using a smoking rabbit model. This study is aimed at assessing the beneficial anti oxidative property to improve microvascular circulation in a reverse sural fasciocutaneous flap in a smoking rabbit model.

All samples were exposed to cigarette smoke using a smoke chamber for a total of 5 weeks. Study sample was divided into three groups. Control group only received cigarette smoke while the other two groups each received ascorbic acid in the form of intramuscular injection and oral “Tualang honey” on top of exposure to cigarette smoke. Reverse sural fasciocutaneous flap was raised in all the rabbits at day 30 of exposure to cigarette smoke.

Samples were evaluated on immediate post operative period, post operative day 1, 3 and 7. Parameters documented throughout the study were temperature, capillary refill time, colour of flap, presence hematoma or infection, and on post operative day 7 the flap survival was calculated. Intervention with smoking, ascorbic acid and “Tualang honey” were continued throughout the period of experiment.

We found that, both group intervened with ascorbic acid and “Tualang honey” showed marked improvement in the flap survival. Statistically significant improvement were documented with regard to temperature, improvement in colour of flap and reduction in hematoma. Flap survival improved from 62% in the smoking arm to 88.9% and 81.0% in the ascorbic acid and “Tualang honey” arm. However oneway ANOVA test showed *p* value of 0.075 which was near to significant value.

In conclusion both ascorbic acid and “Tualang honey” improved the survival of reverse sural fasciocutaneous flap in smoking rabbit model. However ascorbic acid is superior compared to “Tualang honey” in improving the oxidative damages produced by cigarette smoke and microvascular circulation of the reverse sural fasciocutaneous flap.

A comparative study of the effects of ascorbic acid and Tualang Honey on the skin flap of the reverse sural fasciocutaneouss flaps in a smoking rabbit model.

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Research background

Injuries following motor vehicle accident are common in accident and emergency department practice. Lower limb trauma is a common injury sustained in these accidents. Besides that diabetic patient with diabetic foot ulcer is increasing in number in our clinical setting. Injuries and soft tissue defect to lower third of lower limb pose a difficult challenge to both orthopaedic and reconstructive plastic surgeon. They present as a complex wound and soft tissue defect over distal third of the leg, ankle and foot thus it remains as difficult problem to solve (Hassanpour *et al.*, 2008). Despite free tissue transfer being the choice of treatment, technical difficulty and lack of expertise lead to choosing a less complicated option in the reconstructive ladder to address the soft tissue defect in those areas mentioned above (Tharayil and Patil, 2012).

Masquelet *et al* in 1992 described neuroskin island flap which form the basis of reverse sural flap (Masquelet *et al.*, 1992b). Reverse sural flap have been extensively studied for its application in the reconstruction of distal leg and heel defects. However the issue of skin viability has remained a concern in this type of flap due to its dependence to the reverse blood flow following the transaction of its axial blood supply or underlying microangiopathy. It is also a poor choice of reconstruction in situation with extensive

injury taking place 5 to 13 cm proximal to the lateral malleolus as most fasciocutaneous perforators emerge along this area. However various modifications has been described to overcome those shortfalls (Almeida *et al.*, 2002; Tharayil and Patil, 2012).

"Plastic surgery is a constant battle between blood supply and beauty" (Gillies and Millard 1957). Plastic surgery practise is a constant battle between blood supply and wound healing. It is a well accepted assumption that cigarette smoking leads to necrosis of cutaneous skin flaps (Thomas Lawrence *et al.*, 1984). Smoking is becoming a trend among Asian male and female. Performing surgeries in patients with cigarette smoking habit is inevitable and impose a challenge towards optimal wound healing. Our aim in this study was to evaluate the method to improve the survivability of the skin flap in smoking patient using a rabbit model to test our hypothesis.

1.2 Evolution of flap

" Flap " originated in the 16th century from the Dutch word " *flappe* " which carries the meaning of something that hung broad and loose and fastened only by one side. Flap surgery dates as far as 600 BC described in a famous Indian surgical encyclopaedia by Sushruta Samita who described nasal reconstruction using a cheek flap. Various scriptures, historical and scientific literature have described the evolution of flap surgery over time. The surgical procedures described during the early years involved the use of pivotal flaps, which transport skin to an adjacent area while rotating the skin about its pedicle (blood supply). The French were the first to describe advancement flaps, which transfer skin from an adjacent area without rotation. Distant pedicle flaps, The French were the first to describe advancement flaps, which transfer skin from an

adjacent area without rotation. Distant pedicle flaps, which transfer tissue to a remote site, also were reported in Italian literature during the Renaissance period.

Subsequent surgical flap evolution occurred in phases. During the First and Second World Wars, pedicled flaps were used extensively. The next period occurred in the 1950's and 1960's, when surgeons reported using axial pattern flaps (flaps with named blood supplies). In the 1970's, a distinction was made between axial and random flaps (unnamed blood supply) and muscle and musculocutaneous (muscle and skin) flaps.

In the 1980's, the number of different tissue types used increased significantly with the development of fasciocutaneous (fascia and skin) flaps (which are less bulky than muscle flaps), osseous (bone) flaps, and osseocutaneous (bone and skin) flaps. Fasciocutaneous flap are still of relevance and widely used in the armamentarium of reconstruction. Various efforts have been taken to improve the outcome of this flap either by improvising the flap design, delay of flap, in-cooperation of superficial vein into the flap or microvascular anastomosis of proximal vein to superficial vein around the recipient bed to create a flow through pattern (Suzuki *et al.*, 2008; Kececi and Sir, 2012; Tsai *et al.*, 2013)

1.3 Lower limb trauma and reconstruction

Motor vehicle accident contributes to a major portion of cases seen in a day to day emergency department practice. According to the report from the Department of Statistics Malaysia, in the year 2011 the total number of accidents in Malaysia was 449040 and 18693 patients were injured and 6877 patient succumbed to death. Motorcycle rider and the pillion rider contributed the most to the figures above

amounting 18693 and 6877 patients each.(Malaysia, 2012) In another prospective study done in three major health facilities reported that motorcyclist are the most vulnerable group in a road traffic accident. Lower limb trauma contributes 58.3% of the cases seeking treatment in the accident and emergency department and from the figure above 35% involves injuries around ankle (Lateef, 2002; Malaysia, 2012).

Spectrum of lower limb trauma is variable from the mildest abrasion wound to complex tissue loss requiring soft tissue and bony reconstruction. The principles guiding lower limb reconstruction following trauma have been described 300 years ago. Amputation rates dropped from 70% during the pre modern era to 1.8% in the modern era. Such leap in the outcome is attributed to the development of greater understanding and development of modern medicine practice (Wagels *et al.*, 2013). Reconstructive options for lower limb are described based on the location of injury. They are classified as upper, middle and lower third of leg reconstruction. Various reconstructive options and techniques have been described and it's used judiciously according to the needs of reconstruction.

Reconstructive option for lower limb defect ranges from skin graft, local flap, muscle flap, fasciocutaneous flap or free tissue transfer (Baechler *et al.*, 2010; Wagels *et al.*, 2013). Among the commonly used muscle to reconstruct soft tissue defect include gastrocnemius, soleus, extensor hallucis longus, tibialis anterior, peronues brevis and extensor digitorum longus. Free tissue transfer uses either latissimus dorsi, serratus anterior or gracilis. Fasciocutaneous is also a favourite option of reconstruction which are based either on medial or posterolateral perforator of the leg. Lower third reconstruction using fasciocutaneous flap are usually distally based, reverse flow

perfused by septocutanoeus perforators either from anterior tibial, posterior tibial or peroneal perforators.

1.4 Challenges in reconstruction of distal third of leg

Traumas to the leg are divided into upper, middle and distal third for the ease of describing the management options. Trauma of the distal third leg, tendo- Achiles and heel trauma poses a great challenge to both orthopaedic and plastic surgeon. Injuries in functional important areas like the heel require meticulous reconstruction. Choice of tissue to replace the like with like is rather challenging. The uniqueness of the skin of the plantar aspect is rather indispensible. Heel pad is an important structure withstanding the axial load of the body and facilitates the biomechanics of the lower limb and significantly contribute to the gait cycle.

Extend of injury will determine the options available for reconstruction. Among the available options are local flaps, advancement flap or free tissue transfer. Defect to ankle and foot region requires more meticulous choice of tissue used in reconstruction. Replicating the functional aspect of the heel area requires tissue which can withstand sheering pressure and does not contract with time (Zgonis *et al.*, 2007; Ahmed *et al.*, 2008; Haddock *et al.*, 2010; Hamdi *et al.*, 2012).

Lower limb trauma presents as defect with multitude of soft tissue and vascular defect. Free tissue transfer is the current choice for large soft tissue defect of the distal extremities; however the availability of resources and technical expertise limits its use. Fasciocutaneous flap emerged as a preferred alternative option in coverage of skin defect of the lower leg since it was first described by Masquelet *et al* in early 1990's. It

is based on sural neurocutaneous perfusion and reverse flow from the posterior tibial and peroneal artery (Masquelet, 1991; Masquelet *et al.*, 1991; Ahmad, 2011).

1.5 Fasciocutaneous Concept and Sural flap

Fasciocutaneous flap was first described by Ponten in 1981. It was described for soft tissue reconstruction of lower leg. Inclusion of the fascia in the process of raising the flap has allowed expansion of the width to length ratio from 1:1 to 1:3. Raising this type of flap is relatively easy, safe and reproducible by younger surgeons (Ponten, 1981b; Kan, 1988). Previously deep fascia which lies between muscle and skin was regarded as an isolated layer of dense and avascular fibrous tissue. Dissection in this plane results in minimal bleeding thus this layers was not frequently studied of their blood supply and previously it was thought this layer was insufficient to perfuse a flap (Kan, 1988).

"Fascial plexus " is the confluence of subfascial, intrafascial, and suprafascial vascular plexuses within the dermal, subdermal, superficial adipofascial (above Scarpa's fascia), and deep adipofascial layers. These plexuses represent a confluence of a large array of interconnected vessels. Thus this is the reason behind the success of a fasciocutaneous flap. A fasciocutaneous flap compose of any or all of the tissue layers found between the skin and deep fascia. Cormack and Lamberty further classify all skin flaps into a tripartite system that included direct cutaneous, musculocutaneous, and fasciocutaneous flaps. Besides Cormack and Lamberty other authors like Mathes and Nahai and Nakajima have also described their classification of fasciocutaneous flap expanding on the various subtypes of fasciocutaneous flap.