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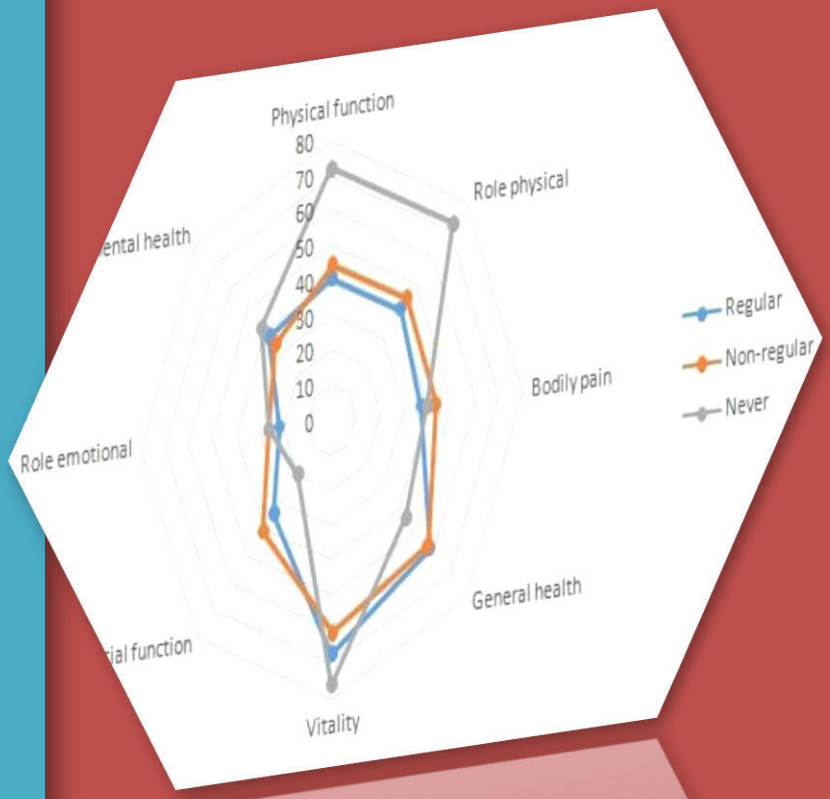

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We welcome journal submissions throughout the year but preferably by **March** and **September**. Articles submitted for publication are understood to be offered only to *JUMMEC* and which have not been sent to other journals for consideration.

Cover

Donors' QoL based on follow-up frequencies. The figure compares QoL of the donors by frequency of follow-up visits. Image courtesy of Makmor Tumin.

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The title page should contain a concise title of the article. Names of authors who have contributed to the writing of the manuscript should be written in style of initials followed by surname or preferred name, eg. Saleena VEO, Anita S or Brown J. Add at the bottom of the phrase "Address for correspondence;" followed by full name and address with postal code and email address.

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Number the references in the order of mention in text. References in the text should be indicated by a figure within parenthesis e.g. (1, 2,). Limit references to 30, if possible. Identify references in text, tables and legends.

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Foreword from the Editor

Dear JUMMEC readers,

Time really flies. In less than a week, we are closing in towards the end of 2015, while counting down to the arrival of 2016. For the past year, we have seen some significant scientific progress and breakthroughs in medical fields. We also saw a few emerging health related crisis occurring to human mankind, due to global warming effect and war refugee mobilization. Reflecting back, JUMMEC too, has gone through some ups and downs this year. We have worked hard to gather the most relevant and interesting articles for you, and to keep our promise to ensure speedy submission and review process for each issue. We are indeed, very lucky, to have good administrative support and more importantly, to have your interest and contribution throughout this year. In this last issue of the year (Volume 18), it is my pleasure to introduce you a few more interesting articles from researchers in the field of Health and Translational Research.

Treatment for melasma, a commonly acquired hyperpigmentary disorder, remains a challenge. Amini F and colleagues reported two cases of treatment of refractory dermal melasma using autologous platelet rich plasma injection. This approach, while still experimental at stage, has shown to be effective without much clinical complications in one of the patients. More of such case reports are necessary to gather isolated evidence occurring in various clinical settings to make better informed decision in similar cases in the future. Another such example is an article by Makmor T and team, who described a detailed survey of quality of life on living donors who donated their kidney to University of Malaya Medical Center between 1991 and 2012. Such data collection on quality of life of donors and its association with the frequency of post-transplantation follow-up visits can give significant impact to how care should be emphasized to both patients and donors.

In this issue, we also have a rather comprehensive review on the physiology and cellular biology of tendon, and the rationale behind the conventional tendon repair methods. Tan SL *et al* also discussed some important aspects about the use of tissue regeneration and engineering methodologies as future therapies for tendon repair. For those who are into parasitology and immunology, you will not be disappointed by a review by Lau YL *et al* on the evaluation and immunogenicity of a blood-stage antigen carried by human *Plasmodium sp*, the major carrier of malaria. There are many ways one can improve delivery of effective healthcare – one of which is the use of appropriate information and communication technologies (ICT). If you are among the hospital management or interested in ICT, you should take a look at an article by Bulgiba A, who described the use of united theory of acceptance and use of technology in Saudi Arabian hospital setting.

As you may already aware, JUMMEC caters for all fields related to medical healthcare, whether you are a tech-geek or a bug puzzle solver. We especially welcome articles on studies and subjects of local and regional interests. With support from Medical Faculty and University of Malaya, we hope to continue provide you good quality published material, without article-processing charges. Till we meet next year, JUMMEC editorial board would like to wish you a happy holidays and Happy New Year 2016!

Ivy Chung
Managing Editor (Volume 18 Number 2)

RESPONSE TO INTRADERMAL AUTOLOGOUS PLATELET RICH PLASMA INJECTION IN REFRACTORY DERMAL MELASMA: REPORT OF TWO CASES

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ABSTRACT

Refractory dermal melasma is resistant to conventional treatment. Platelet rich plasma (PRP) may help to reduce the pigmentation of melasma. We present a case report on the clinical outcome of 2 patients with melasma, given PRP, as an adjunct therapy. PRP was administered at a monthly interval for 2 sessions in combination with a monthly Q-switched Nd Yag laser treatment and topical alpha arbutin application. A modified melasma area and severity index (MASI) was evaluated by two dermatologists who were blinded. At the follow up on the 3rd months, the MASI score was reduced by mean 33.5% for case 1 and 20% for case 2. There were no clinical complications for case 1. However recurrence of melasma was noted in case 2 by a worsening of the MASI score mean to 53% at the sixth months follow up. In conclusion, intradermal PRP injection as an adjunct to the conventional treatment of melasma presented with differing results in two cases.

Keywords: *Alpha arbutin, Melasma, Platelet rich plasma, PRP, Q-switched Nd Yag laser*

Introduction

Melasma is a chronic pigmentary skin disease. It is diagnosed by the detection of brown or gray patches on sun exposed areas of the face including the forehead, nose, and over the malar and mandibular region (1). It is commonly seen in women especially those with a darker skin tone with Fitzpatrick photo types III through VI. Melasma can be histologically classified into three types, according to the deposition of the hyperactive melanocytes, as the epidermal, dermal or a mixed type involving both the epidermal and dermal layers. The prevalence of melasma in the general population varies according to geographical location and race. Prevalence of 8.8% in Hispanic women, 40% in South East Asian women and 20% in men have been reported (2,3).

The exact pathogenesis of melasma is not fully understood. Two theories have been reported in regards to the hyperpigmentation of melasma, an increased synthesis of melanin by melanocytes resulting in a melanocytic hyperpigmentation, or an increase in the number of melanocytes, forming a melanocytic hyperpigmentation (4). According to the study by Kang et al. (5) there were no significant differences between the number of Langerhans cells, collagen and basement membrane appearance in normal and melasma skin. But melanocytes obtained from melasma skin contained more cytoplasmic organelles such as dendrites, mitochondria, Golgi bodies, and rough endoplasmic reticulum compared to the perilesional normal skin. The study suggested that in melasma affected skin, the number of melanocytes were not increased but were biologically hyperactive and produced more melanin.

The risk factors associated with melasma are ultra violet (UV) radiation, pregnancy and hormonal therapies with oral contraceptive pill and with, thyroid hormone supplements, phototoxic drugs and anticonvulsant medications (1, 6-7).

Some studies have shown that the exposure to UV radiation over the cutaneous vasculature and hormonal changes are risk factors for melasma (5, 8-11).

Management of melasma is challenging for both the physicians and the patient. Treatment are mainly designed to target key factors in the synthesis of melanin (12). First line treatment target pigment production by using a broad spectrum sunscreen in combination with topical agents such as keratinocyte turnover stimulator (retinoids), tyrosinase inhibitor (hydroquinone, azelaic acid, arbutin), melanosome transfer inhibitor (retinoids, soybean trypsin inhibitor), and chelation of copper (kojic acid, ascorbic acid). Second line treatment are chemical based peeling such as glycolic acid peels starting at 30% for every 4-6 weeks (13-14). If the outcome of the second line treatments is not satisfactory then the third line treatment would be considered which includes light based and fractional laser therapies. Recently the sub-thermolytic Q-switched Nd Yag laser therapy has been widely used because of its efficacy and safety even in Asian patients with darker skin tone (15). Although sub-thermolytic Q-switched 1064nm Nd Yag laser therapy has shown promising results, this treatment would require multiple sessions on a weekly basis, which would burden the patients (16, 17). In addition, there are some reports of confetti-like hypopigmentation occurring after multiple treatment sessions (18, 19). In general, epidermal type melasma respond well to these conventional treatment (20). However, many of the melasma patients who present at the clinic have mixed type melasma which is difficult to treat due to its recurring nature and post-inflammatory pigmentation alteration after treatment (16, 21).

A pilot study on Korean subjects demonstrated an improvement in the melanin index of patients treated with PRP in combination with ablative fractional photothermolysis (22). As there is no randomized controlled study using PRP therapy for treating melasma, we undertook a pilot study to evaluate the effectiveness of PRP in treating melasma.

Case Presentation

Subject 1: She was a 42 year old female with a Fitzpatrick skin type IV. She had no known medical illness, did not take any oral contraceptive pill (OCP) and was not undergoing hormone replacement therapy (HRT). There was no significant aesthetic and surgical history. She came with the complaint of having a few patches of brown pigmentation on both her cheeks for about 10 years, but worsening for the past few years. Clinical examination showed that a few patches of brown pigment macule on both malar and temporal area were suggestive of a mixed melasma. Three to four small bluish brown macules on her cheeks might be Hori nevus. Initially she was given a depigmenting agent Pro-heal Serum (IS Clinic Products) which contains topical alpha arbutin for a twice daily application and she was also started on a 1064nm Q-switched Nd Yag laser (Conbio RevLite) at 10Hz, spot size of 8mm, at the fluence of 2.0-2.3J/cm2 for two passes then spot size of 6mm at 3.0-3.6J/cm2 for 2 to 4 passes ~~in~~ at a monthly interval from June 2012 to November 2012. However her melasma pigmentation was not improving and an intradermal injection of PRP as adjunctive therapy was added and started in December 2012 and administered at a monthly interval.

Subject 2*:* She was a 51 year old female with a Fitzpatrick skin type V. She had no known medical illness and was not on any OCP, HRT, anti-convulsant or thyroid medications. There was no significant aesthetic and surgical history. She was concerned over the large patches of brown pigmentation on both her cheeks for about 15 years. A clinical assessment showed that there were irregular brown pigment macules over both malar areas with telangiectasia suggestive of a mixed melasma. She had been treated with laser at another centre with no improvement. She was given topical Pro-heal Serum (IS Clinic Products) which contains alpha arbutin for a twice daily application, and she was started on 1064nm Q-switched Nd Yag laser (Conbio RevLite) at 10Hz, spot size of 8mm at the fluence of 1.8-2.1J/cm2 for 2 passes then spot size of 6mm at 2.8-3.1J/cm2 for 2 to 4 passes at a monthly interval from June 2013 to September 2013. In view of her concern of dry skin with fine lines and telangiectasia, an intradermal injection of PRP was offered for the pigmentation area as an adjunctive therapy, together with the Q-switched laser session at a monthly interval from June 2013.

In this case study, assessment of efficacy of treatment and the severity of the melisma was done using a modified melasma area and severity index (MASI) scoring (Figure 1) by two blinded dermatologists.

Subject 1 showed a remarkable improvement of the MASI score after a single session of PRP therapy (Figure 2). The mean percentage of MASI score improvement was about 33.5% at the first follow up on the third month (Appendix 1). A further improvement was noted with a reduction of MASI mean score to about 26.4% at the follow up on the sixth month. Subject 2 also showed improvements of the severity of melasma with the mean reduction of MASI score by approximately 20%. However, the rebound hyperpigmentation was noted by two blinded dermatologists in this case during 6 months follow up, with the mean increase of MASI score for about 53% (Figure 3). Details of MASI scoring for each patient from two assessors can be found in appendix 1. A comparison of mean MASI scores given by 2 assessors for case 1 & 2 is depicted in Figure 4.

The side effects and complications observed in this case study were minimal and tolerable to the subjects. Both subjects experienced mild erythema, oedema and small needle prick mark bruises for the first few days after the PRP therapy. The erythema and oedema were resolved within one to two days. The bruises lasted slightly longer, for about 4 to 5 days as if the injections of the PRP had injured some of the small blood vessels.

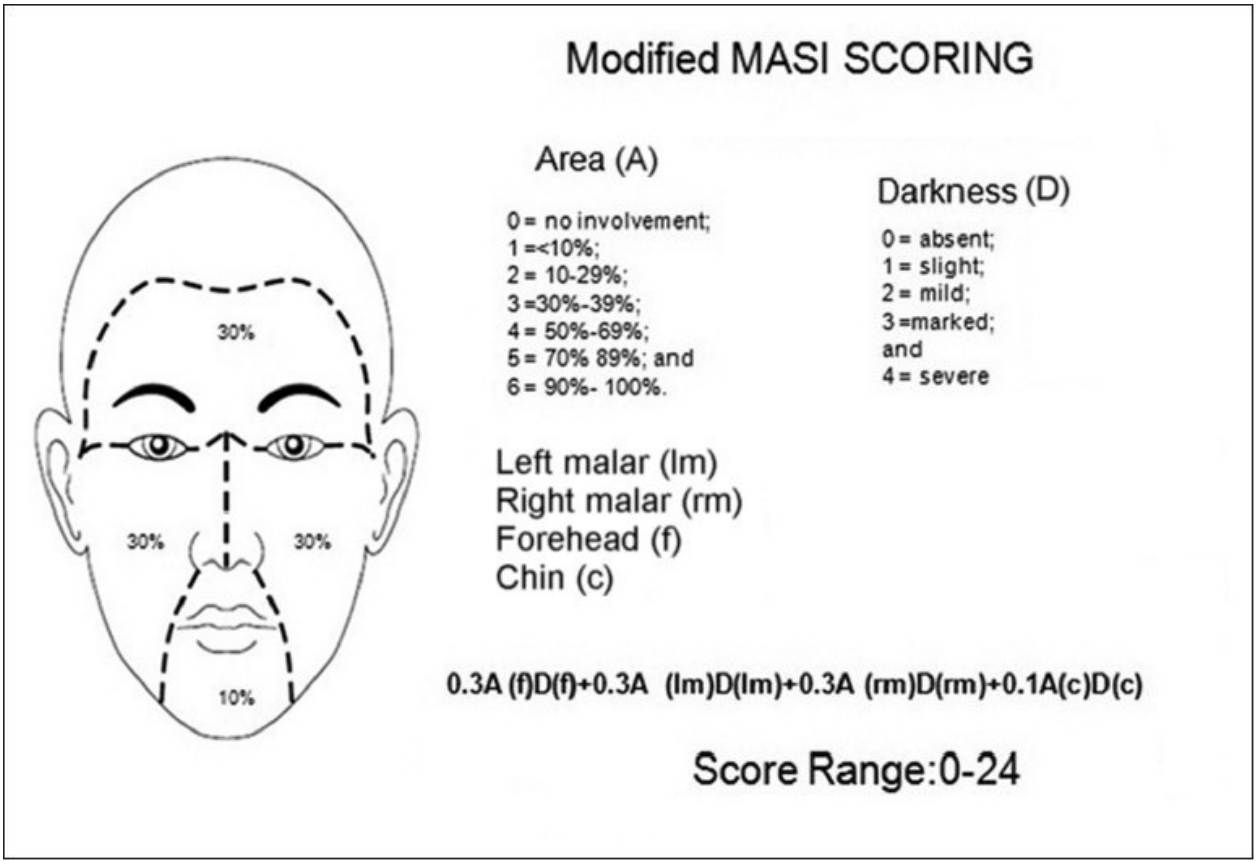

Figure 1: Melasma Area and Severity Index (MASI) (Adapted from Kimbrough-Green et al. (23)

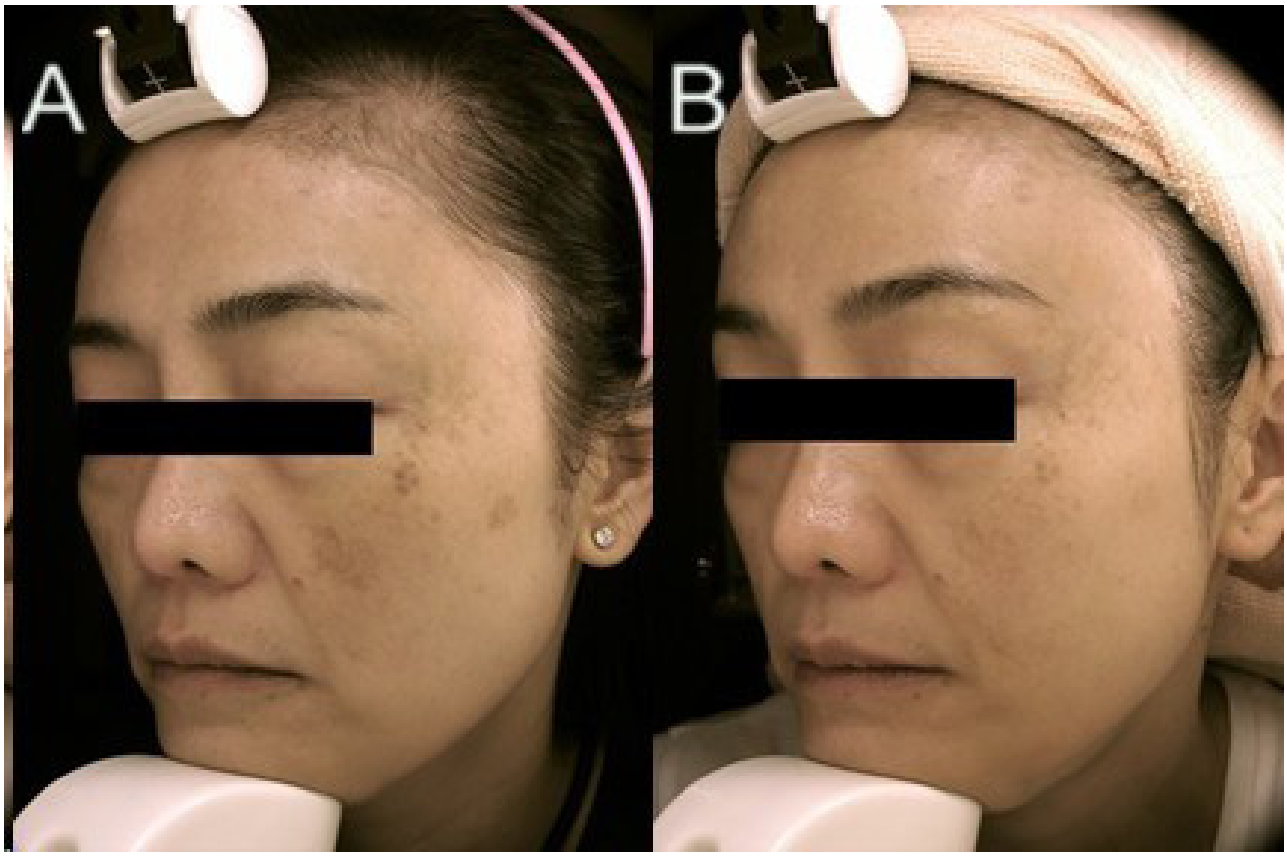

Figure 2: Clinical photograph of case 1 at the baseline (A) and after 6 months follow-up (B)

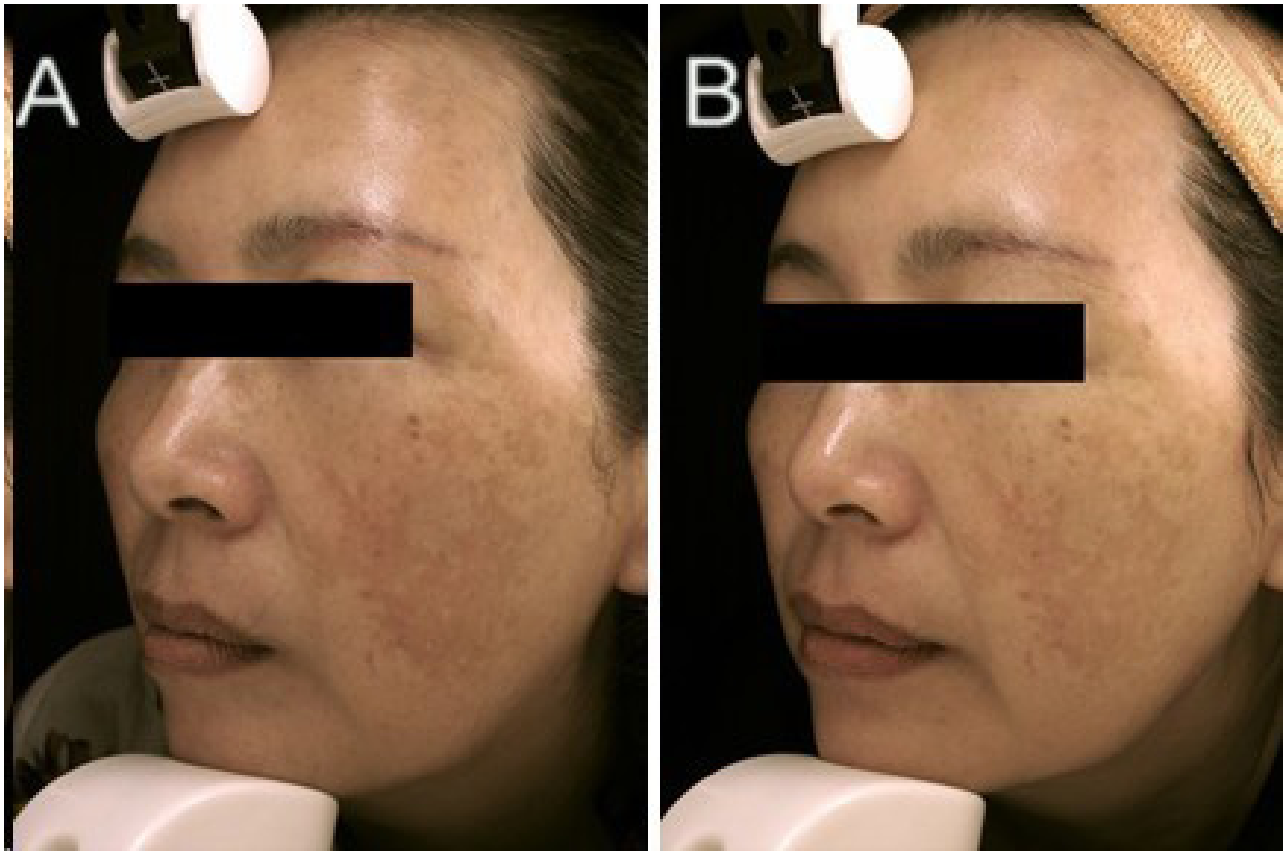

Figure 3: Clinical photograph of case 2 at the baseline (A) and after 6 months follow-up (B)

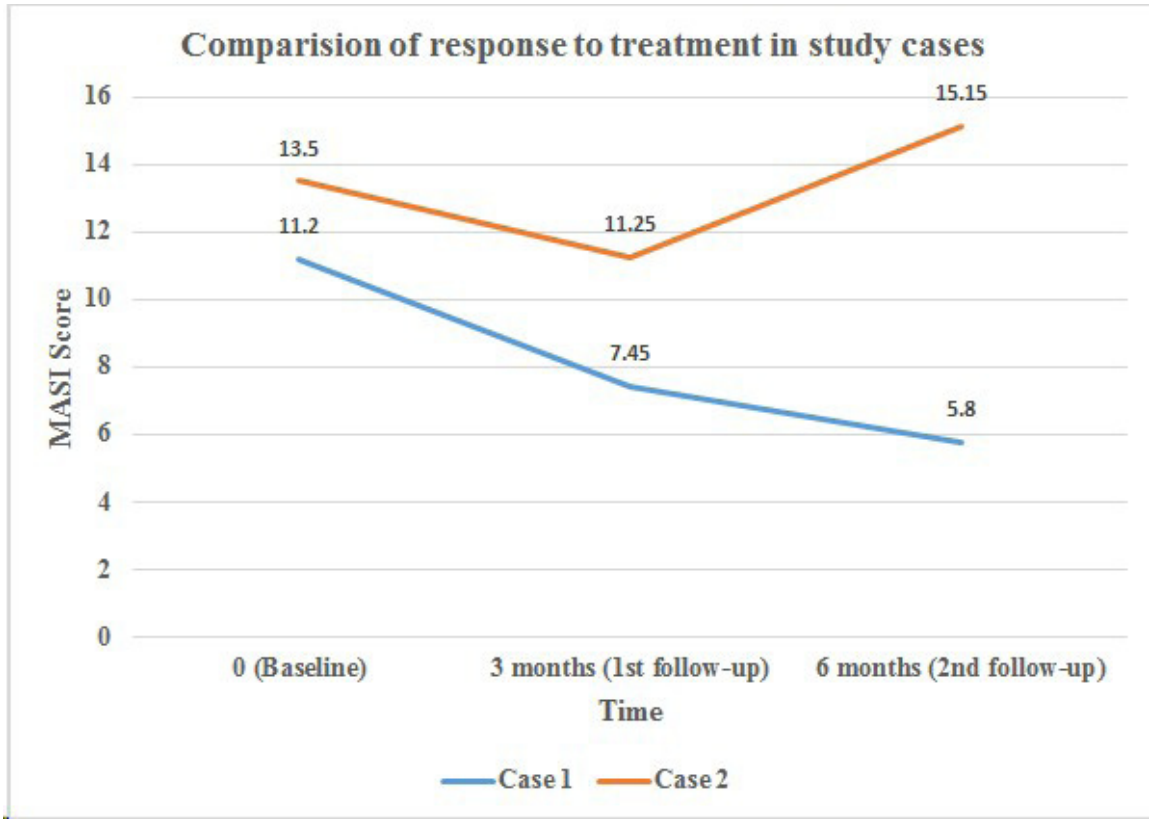

Figure 4: Comparative graph depicting the changes in MASI score in case 1 and 2

Discussion

PRP was used in this study as adjunct to the conventional therapies of melasma because PRP has been reported to reduce the pigmentation when it used to treat other skin conditions. For instances, application of PRP was observed to reduce the incidence of post-inflammatory hyperpigmentation; an effect which might be due to PRP exerting a healing of the basement membrane by laminin, collagen IV, and tenascin stimulated by the TGF-b (24, 25) found in the PRP.

In this case study, two cases showed different degrees of improvement in MASI score using the same therapy regime by identical operator. In case one, the improvement observed was remarkable, after treatment as well as at the follow up on the 3rd and the 6th months only minor degree of improvement was observed in case two after treatment, and in case 2, at the 6 months follow up, a rebound of hyperpigmentation was noted. Recurrence of melasma is common even after successful treatment (26).

The divergent response of the two patients to an identical therapy in this study, could be due to a number of factors. Less improvement observed in case 2 could be due to a higher Fitzpatrick skin type and a more severe mixed type melasma with telangiectasia, as has been noted in an earlier report (18). Improvement of the telangiectasia and erythema was noted by the subject herself after the first session of PRP therapy. These might be due to the underlying modulation of angiogenesis by the orchestration of the growth factors released during the PRP therapy. This mechanism is similar to wound healings and would seem to promote appropriate angiogenesis without inducing excessive blood vessels formation.

In addition to its known function of coagulation, platelet may also play a role in the field of aesthetic medicine, for skin rejuvenation. When the platelets are activated by thrombin, calcium or collagen, degranulation of the intracellular alpha-granules of the platelets lead to the release of growth factors such as platelet derived growth factor (PDGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and transforming growth factor-beta (TGF-β) which stimulate the proliferation of fibroblast and epidermal cell, promote angiogenesis and induce collagen synthesis (27).

The improvement of pigmentation after fractional photothermolysis and PRP therapy in one of the cases in this study may be attributed to the release of TGF-β which is known to decrease melanogenesis (25, 28). TGF-β1 is a cytokine involved in cell differentiation, proliferation and apoptosis. There are studies demonstrating that TGF-β1 inhibits melanogenesis by the down-regulation of the microphthalmia associated transcription factor (MITF) promoter activity and inhibits the expression of paired-box homeotic gene (PAX 3), which at the protein level, it reduces the production of tyrosinase, tyrosinase-related protein 1, tyrosinase-related protein 2 and microphthalmia associated transcription factor (MITF) (29-31). Therefore it is postulated that TGF-β1, one of the main cytokines released from platelet rich plasma, would help to reduce the pigmentation of melasma.

The side effects and complications observed in this case study were minimal and tolerable to the subjects with the careful application of local anaesthetic cream, a meticulous injection techniques and an application of the cooling mask or cold compression. At the 6th months follow up, the recurrence or rebound of melasma for subjects 2 was high as the change of the mean MASI score was 53%, and this was higher than results from other studies (20, 32). This might be due to the loss of inhibitory effects of melanogenesis by PRP therapy with the transient release of platelet growth factor.

Conclusion

Melasma is common among Asian females and it is resistant to conventional therapies. Our study shows that PRP may serve as a source of different growth factors to reduce the pigmentation, in some cases, acting synergistically with conventional therapy. In this case study, minimal side effects were observed with the intradermal PRP injection, with little pain, erythema, oedema and bruises. This case study can serve as a pilot study for PRP injection, as adjunct to other conventional therapy. It will be more beneficial to the melasma patients if multi-centre studies can be done in with a larger sample size with a control group to evaluate the effect of PRP injection in melasma patients with different skin types.

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THE IMPORTANCE OF LONG-TERM FOLLOW-UP VISITS FOR KIDNEY DONORS

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ABSTRACT

Background: This paper examined the importance and influence of post-transplantation follow-up visits on the quality-of-life (QoL) of living kidney donors in Malaysia.

Methods: Based on data collected from 80 living kidney donors, the relationship between QoL and the frequency of follow-up visits was examined. QoL was measured using standard SF-8 questions to capture its different dimensions.

Results: Donors in the 1991–1998 donation cohort have low QoL, especially in the domains of physical and vitality, compared with the other two cohorts (1999–2005 and 2006–2012). The mean scores showed that donors who never went for any follow-up activities visits experience low QoL in most of the categories, particularly those related to physical activities, implying the importance of follow-up activities visits in influencing the donors' QoL. Lower QoL was recorded for respondents that never received post-transplant treatment.

Conclusion: Although this study found no serious post-transplant QoL issues in Malaysia, it is still important to set up a donor registry and provide free and mandatory follow-up visits for all donors in order to adequately monitor their health.

Keywords: *kidney donation, living donors, Malaysia, quality of life, sociology*

Introduction

Developments in organ-transplant technology are of less significance if there is a negligible supply of organs. In Malaysia, although 15,489 people were on the waiting list for organ transplantation in July 2012 (1), the deceased donation rate was only 0.64 per million people (2). The short supply of transplant organs from deceased donors necessitates alternative methods of harvesting organs, and living donation seems to be a better option than other possible alternatives. Although living donation is acknowledged as a harmless procedure and donors are generally assumed to be safe physically and mentally post-transplant (3-10), it is worth noting that such a surgical procedure is certainly not risk-free. As of December 2012, there were 1,894 functioning transplanted kidneys in Malaysia, and all were sourced from related donors (11).

Although Malaysia's National Organ, Tissue and Cell Transplantation Policy of 2007, under Article 2.5 (Aim of the Policy) and 2.4 (General Statement of the Policy), stipulated that living donors must attend post-transplantation follow-up health monitoring visits and that the government would attend to their welfare (12), the implementation of this policy remains almost nonexistent. The first living donation took place in 1975 (12), but only from November 2012 were free medical services provided to living donors (10), and even then services were only provided to Malaysians who donated their organs from November 2012 onwards

(13). Furthermore, the absence of a living-donor registry makes monitoring difficult.

Studies conducted in both developed (3,14-18) and developing countries (19-21) confirmed that donors enjoy better Quality of Life (QoL) than does the rest of the population. In addition, studies using the SF-36 questionnaire have confirmed that donors not only enjoy better QoL compared with control groups, but also that the majority of donors are willing to donate again, if possible. A systematic review of studies involving 5,139 donors in 19 countries confirmed these norms (7), except in the case of Iran (19). The study in Iran suggested the opposite result, with donors suffering lower QoL on all eight domains of the SF-36 compared to the control group.

However, most of the above studies based their conclusions on small sample sizes, as their authors readily admit. Besides, the method of using only a sample of donors, thus neglecting the rest of the donors, raises the question of whether an inclusive, long-term study of donor QoL might support the same results. For instance, while an earlier study of donors' QoL in Norway indicated that donors enjoy a better QoL than a control group (14), a more recent and robust study in that same country produced the opposite result, in which donors had a lower QoL in the physical and psychological domains (15). Furthermore, another robust study of donors' morbidity found surgical mortality of 3.1 out of 1000 donors. Such studies can only be properly conducted in jurisdictions with a living-donor registry where registration is mandated. The absence of a living-donor registry in many countries, including Malaysia, makes it difficult to trace all donors.

In Malaysia there is evidence that living kidney donors enjoy better QoL than do their healthy counterparts from the general public (20). However, to the best of our knowledge, no study has yet investigated donors' QoL differences based on the time of donation and frequency of follow-up attendance. Hence, this study investigated donors' QoL from these two dimensions.

Subjects and Methods

A QoL survey was administered to living donors who had donated their kidneys between 1991 and 2012 at the University of Malaya Medical Center (UMMC), Kuala Lumpur, Malaysia. Two enumerators (one from the UMMC and another, independent third party) were instructed to contact all 178 living donors recorded (until June 2012) at the medical center. Of these, they managed to contact 111 donors (or their relatives) currently living in Malaysia via telephone call, which resulted in 80 donors agreeing to participate in the study. Of the 98 non-respondent donors, 67 were unreachable, 11 were too busy, 10 refused to participate, five were abroad, two had follow-ups at other hospitals, two were deceased, and one was chronically ill. We focus on donors who donated before November 2012 in order to reduce bias resulting from the previously mentioned implementation of free medical facilities for organ donors.

SF-8 QoL survey questions were used to make it easier for the respondents to complete questions about other information, such as follow-up activity. The SF-8 measures eight domains, as follows: (1) physical functioning; (2) role limitations due to physical health; (3) role limitations due to emotional problems; (4) energy/fatigue; (5) emotional well-being; (6) social functioning; (7) pain; and (8) general health. We prepared the questionnaire in three different languages (Malay, English, and Mandarin).

First, we analyzed donors' overall QoL based on the eight QoL domains, comparing them with a gender- and age-matched, healthy control group (individuals identified as having systolic blood pressure of 140 mmHg and diastolic blood pressure below 90 mmHg at the time of survey, no history of medical problems, and not being treated with drugs for hypertension) with income higher than RM 3,000 (700 USD).

In this paper, we only compare donors' QoL by different cohorts of time since donation, followed by comparing their QoL based on their frequencies of follow-up attendance. Discussion of the comparison between the donor and control group has been published elsewhere (20). We converted the scores for each QoL category from a Likert-type scale to percentages, such that the higher the percentage, the lower the QoL. Based on a tabulation of the donors, we divided the donors into three donation cohorts: (1) 1991–1998; (2) 1999–2005; and (3) 2006–2012.

This study received ethics approval from the Medical Ethics Committee of UMMC on 19th July 2012 (MEC Ref. No: 932.23).

Results

The overall results for donors' QoL are shown in Figure 1, along with the QoL domains and mean scores for all donors. On average, donors scored vitality and general health the highest, indicating that donors' QoL was the lowest in these categories.

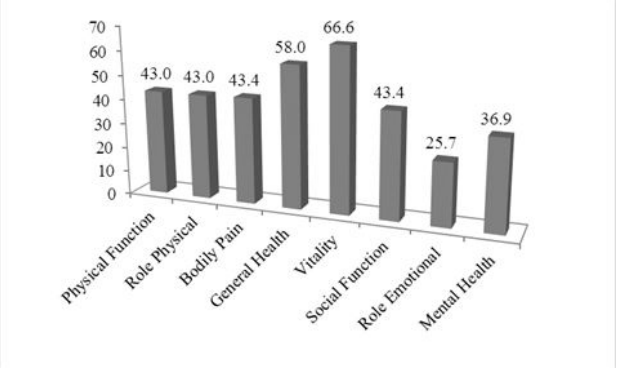

Figure 1: Respondents' overall QoL.

Basic background information on the 80 respondent donors follows. Women outnumbered men (51/80), and

Chinese outnumbered other ethnicities (50/80; Malay=16 and Indian=14). A total of 64 participants were married, while 13 were single and three were widowed/divorced. Regarding education, 46 had finished at least a secondary level of education, 26 had finished primary level, and eight have had tertiary-level education.

Table 1: Respondents' background information at the time of donation and at the time of survey administration.

Variable	At the time of donation		At the time of survey	
Age	No.	%	No.	%
40 and below	31	(38.8%)	15	(18.8%)
41 to 55	33	(41.2%)	32	(40.0%)
56 and above	16	(20.0%)	33	(41.2%)
Employment status				
Homemaker	21	(26.3%)	16	(20.0%)
Unemployed	15	(18.8%)	10	(12.5%)
Self-employed	14	(17.5%)	16	(20.0%)
Public-sector employee	9	(11.3%)	12	(15.0%)
Private-sector employee	15	(18.8%)	19	(23.8%)
Other	6	(7.5%)	7	(8.8%)
Donor's monthly income				
None	36	(45.0%)	25	(31.3%)
Less than RM3000 (700 USD)[2]	39	(48.8%)	43	(53.8%)
RM3000 (700 USD) and above	5	(6.3%)	12	(15.0%)
No-income: donor's supporter[1]				
Supported mainly by the family	23	(71.9%)	19	(79.2%)
Supported mainly by the recipient	1	(3.1%)	0	(0.0%)
Supported mainly by own savings	5	(15.6%)	3	(12.5%)
Other	3	(9.4%)	2	(8.3%)

1 Based on valid percentage. There are 4 missing cases at time of donation and 1 missing case at time of survey.
2 Based on current exchange rate 1USD=RM4.29 (October 2015)

The information in Table 1 clearly indicates that a large portion of respondents have low incomes. The number of donors without income nonetheless decreased between the time of donation and the administration of the survey, even though many donors had reached the age of 40 and above by the time the survey was administered. Donors without income were not only supported by their families, but also by other means such as through the support of the organ recipient, own savings and others.

Next, based on the time since donation, we divided the donors into three categories: (1) 1991–1998; (2) 1999–2005; and (3) 2006–2012. The mean age for each cohort was 56.8, 54.8, and 47.9, respectively. Table 2 clearly illustrates that those in the first cohort suffered low QoL. Their mean scores, as shown in Table 2, depict that donors who underwent transplantation between 1991 and 1998 experienced the worst QoL in all categories except role emotional and mental health (Figure 2).

Table 2: Respondents' QoL by domain, based on time-since-donation cohort (%).

Domain	1991 – 1998	1999 – 2005	2006 – 2012
Physical function	45.6	45.5	41.5
Role physical	45.6	45.5	41.5
Bodily pain	40	40	38
General health	58.9	55.5	57
Vitality	58.9	55.5	57
Social function	41.1	30	35
Role emotional	23.3	23.8	23.4
Mental health	34.9	38.3	36.9

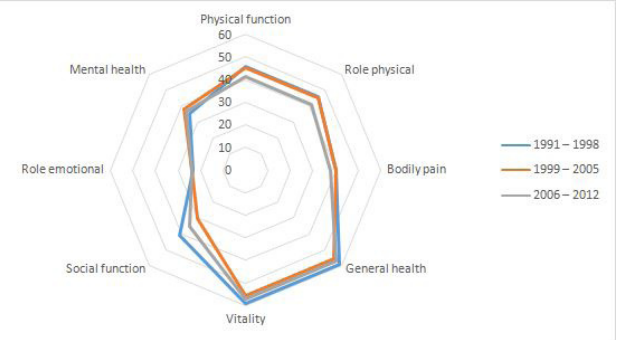

Figure 2: Donors' QoL based on time since donation. The figure compares QoL of the donors by donation cohorts. The closer the lines are to the center of the radar chart, the better is those donors' QoL. In general, the line for donors from the 2009–2012 cohort always lies inside the lines representing the other two cohorts. Donors from 1991–1998 scored lowest on the social, vitality, and general health dimensions.

Donors' QoL was also assessed based on their follow-up visit frequencies. Donors' follow-up activities in terms of percentages were as follows: 31.25% of respondents had regular follow-up visits (n=25); 62.5% (n=50) had one visit in several years; and only 6.25% (n=5) had never had any follow-up visits. The mean scores for each of the QoL categories are presented in Table 3 and suggest a significant difference in three of the QoL categories: (1) physical function; (2) role physical; and (3) vitality.

The mean scores show that donors who never went for any follow-up visits experience low QoL in most categories, particularly those related to physical activities, implying the importance of follow-up visits in influencing donors' QoL (Figure 3).

Table 3: Respondents' QoL by domain, based on frequency of follow-up attendance (%)

Quality of Life	Regular	Non-regular	Never
Physical function	40.7	44.4	72
Role physical	40.7	44.4	72
Bodily pain	37.5	43.3	40
General health	58.2	56.7	44
Vitality	67.4	61.1	76
Social function	34.4	41.1	20
Role emotional	22.2	26.8	26.4
Mental health	37.3	33.9	41.6

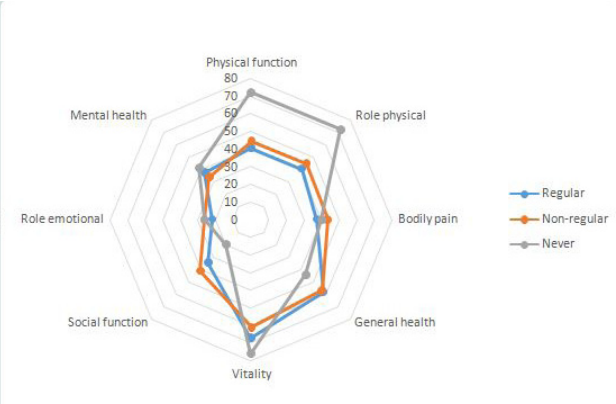

Figure 3: Donors' QoL based on follow-up frequencies. The figure compares QoL of the donors by frequency of follow-up visits. The closer the lines are to the center, the better is those donors' QoL. Donors who committed to regular visits scored better, on average, as compared to non-regular and non-visitor groups, except in terms of general health. Those who never attended any follow-up visits experienced poor QoL, especially on the physical and role physical dimensions.

Discussion

Most relevant literature provides evidence that kidney donors' QoL is not lower than their counterparts from the general public or from healthy individuals who would be eligible for kidney donation (3-10). A recent and large study in Norway introduced the surprising suggestion that in the long run, approximately 10 years after kidney donation, all-cause mortality increases among living kidney donors compared to healthy eligible-donor controls (24). The results of our time-grouped study of kidney donors are in line with this finding, revealing that the older the donor, the lower the QoL, with particularly lower general heath, vitality, and social function (Figure 2).

There is evidence that long-term follow-up appointments lower donors' morbidity risk (25). The findings of this study add to this evidence. Thus, serious calls have been made by donors and policymakers regarding the importance of follow-up visits post-transplantation (26). In fact, some developed countries have established special donor clinics to cater to the physical and psychological needs of donors and also to provide consultation services for them. The above findings urge Malaysia towards serious efforts to establish a national donor registry with mandated follow-up attendance, since this is the only method to ensure that the health of living donors is monitored. The government should provide all living donors with free medical services (at all levels, including secondary and tertiary), regardless of their date of donation, in order to guarantee that living donors—who are the majority of donors—attend their follow-up visits. The literature reveals that eligible donors may not donate due to their fear of facing financial problems after donation (27-28), so we expect that providing free follow-up care would tend to increase the number of living donations.

Conclusion

This study revealed two important findings. Firstly, living kidney donors' QoL was found to decline over the long run. Secondly, living donors with higher commitments to post-donation follow-up visits enjoy better QoL than those who never or infrequently attend follow-ups. Hence, this study suggests that officials should take the initiative to establish a living-donor registry and to make follow-up attendance mandatory and free of charge for all donors. These changes would enhance donors' QoL.

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A MINI REVIEW ON THE BASIC KNOWLEDGE ON TENDON: REVISITING THE NORMAL & INJURED TENDON

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ABSTRACT

Tendon is a dense connective tissue that connects muscle to bone. Tendon can adapt to mechanical forces passing across it, through a reciprocal relationship between its cellular components (tenocytes and tenoblasts) and the extracellular matrix (ECM). In early development, the formation of scleraxis-expressing tendon progenitor population in the sclerotome is induced by a fibroblast growth factor signal secreted by the myotome. Tendon injury has been defined as a loss of cells or ECM caused by trauma. It represents a failure of cells and matrix adaptation to mechanical loading. Injury initiates attempts of tendon to repair itself, which has been defined as replacement of damaged or lost cells and ECM by new cells or new matrices. Tendon healing generally consists of four different phases: the inflammatory, proliferation, differentiation and remodelling phases. Clinically, tendons are repaired with a variety of surgical techniques, which show various degrees of success. In order to improve the conventional tendon repair methods, current tendon tissue engineering aims to investigate a repair method which can restore tissue defects with living cells, or cell based therapy. Advances in tissue engineering techniques would potentially yield to a cell-based product that could regenerate functional tendon tissue.

Keywords: *Cell based therapy, cell differentiation, expression profile, orthopaedics, stem cell biology, tendon tissue engineering*

STRUCTURE AND FUNCTION OF NORMAL TENDON

A. Tendon, Tenocyte and Tendon Extracellular Matrix

Tendon is dense connective tissue which connects muscle to bone and allows transmission of forces generated by muscle to bone, resulting in joint movement. It is living tissue with mechanical adaptation ability that allows it to respond to mechanical forces (eg. high tensional loading). This is achieved through changes in the metabolism as well as its structural and mechanical properties (1-3). These critical biological and biomechanical roles of tendon are played through a reciprocal relationship between its two main components, i.e. cells and extracellular matrix (ECM) (Table 1).

The overall cell content in tendon tissue is low (20%). Tenocytes and tenoblasts are the two main cell types which coexist in tendon. Both of these cells are of mesenchymal origin and they constitute about 90-95% of the cellular component of tendons (4). Tenoblasts are immature tendon cells. They are spindle-shaped and have numerous cytoplasmic organelles. The high organelle content reflects their high metabolic activity. As they mature, tenoblasts become elongated and transformed into tenocytes. Tenocytes have lower nucleus-to-cytoplasm ratio than tenoblasts. These cells lie between the collagen fibers along

Table 1: Structural compositions of tendon (1,7).

Component	Total (%)
I. Cellular materials	**20**
i. Tenocytes and tenoblasts	90-95
ii. Others (Chondrocytes, synovial cells and vascular cells)	5-10
II. Extracellular matrix (ECM)	**80**
i. Water	60-80
ii. Dry mass	20-40
a. Collagen	75-85
Type-I	95-99
Type-III and V	1-5
Others (Type II, VI, IX, X and XI)	Trace amount
b. Ground substance (Proteoglycan, glycoproteins and etc.)	15-25

the long axis of the tendon (5). The remaining 5-10% of the cellular elements of tendon consists of chondrocytes at the bone attachment and insertion sites (6), synovial cells of the tendon sheath, and vascular cells, including capillary endothelial cells and smooth muscle cells of arterioles (7). Recently, several studies have shown that multipotent tendon stem cells/tendon progenitor cells (TSC/TPC) also exist in human and animal tendon tissues (8-10). Nevertheless, it remains unclear whether the TSC/TPC are the same population of cells as the tenoblast. It is also unclear whether the tenoblast is a committed tenogenic progenitor cell and whether these cells are different from TSC/TPC. At this point there are no known cell markers to differentiate between the tenocyte, tenoblast and TSC/TPC.

In normal tendon, the tenocyte synthesizes a wide range of ECM proteins in a well-ordered structure. Among the most abundant of these proteins is type-I collagen. This protein is organized in a parallel arrangement providing a distinct hierarchical structure, which ultimately forms the tendon (Figure 1). The tenocyte secretes soluble trihelical tropocollagen that is assembled and cross-linked in parallel fibrillar arrays. Higher-order organization of these arrays is provided by the endotenon, which appears as a loose connective tissue layer that envelopes collagen fibrils to form tendon fascicles. Fascicles in turn are bundled together by the epitenon, a layer contiguous with the endotenon through which the microvasculature traverses and provides nutrients (11,12). This multi-unit hierarchical structure aligns fiber bundles parallel with the long axis of the tendon and affords the tendon high tensile strength (1).

Normal tendon ECM is composed largely of collagen (predominantly type-I collagen, COL-I[1]), which provides structural integrity and mechanical strength (13). A small amount of ground substances (Table 1) is not only important in fibrillogenesis but also provides tendon its high resistance behaviour to compressive and tensile forces (14). COL-I constitutes about 60% of the dry mass of the tendon and about 95% of the total collagen in tendon (15). The remaining 5% consists of type III and V collagens. In a normal tendon, type III collagen (COL-III) is mainly located in the endotenon and epitenon (16,17). The ratio of COL-I to COL-III has been previously used as indicators of the tenogenic characteristics in tendon tissues and tenocyte cultures (18,19). Other collagen (types II, VI, IX, X and XI) are present in trace amount in tendons (6). The ground substance of the tendon ECM network surrounding the collagen and tenocytes is composed of proteoglycans and several other small molecules (7). The proteoglycan content in a tendon (dry mass) is relatively lower than other musculoskeletal tissue (14). The content varies at different sites of the tendon and is dependant on the mechanical loading conditions, eg. tension vs. compression (20-22) [~6% in the compression region and ~0.2% in the tensional region]. A summary of the types of proteoglycans present in tendon is presented in Table 2. Although normal mechanical function of tendon depends on the precise alignment of collagen fibrils, it is proteoglycans that regulate collagen fibrillogenesis. This is achieved via the interaction between the positively-charged groups of collagen fibers and the negatively-charged groups of the glycosaminoglycans (GAGs) in a proteoglycan molecule (14). This, indirectly affects a tendon's functionality. Members of the small-leucine-rich proteoglycan (SLRP) family (eg. decorin, biglycan, fibromodulin and lumican) bind to collagen fibrils and actively participate in fibrillogenesis (23). Depletion of biglycan and fibromodulin affects the TSP/TPC differentiation and impairs tendon formation *in vivo* (8). Other proteins, such as adhesive glycoproteins (eg. fibronectin and thrombospondin) are involved in binding the tenocytes to the collagen fibers

[1] Please note that the abbreviation for the gene is given in italics and the abbreviation for the protein expressed by the gene is given in capital letters.

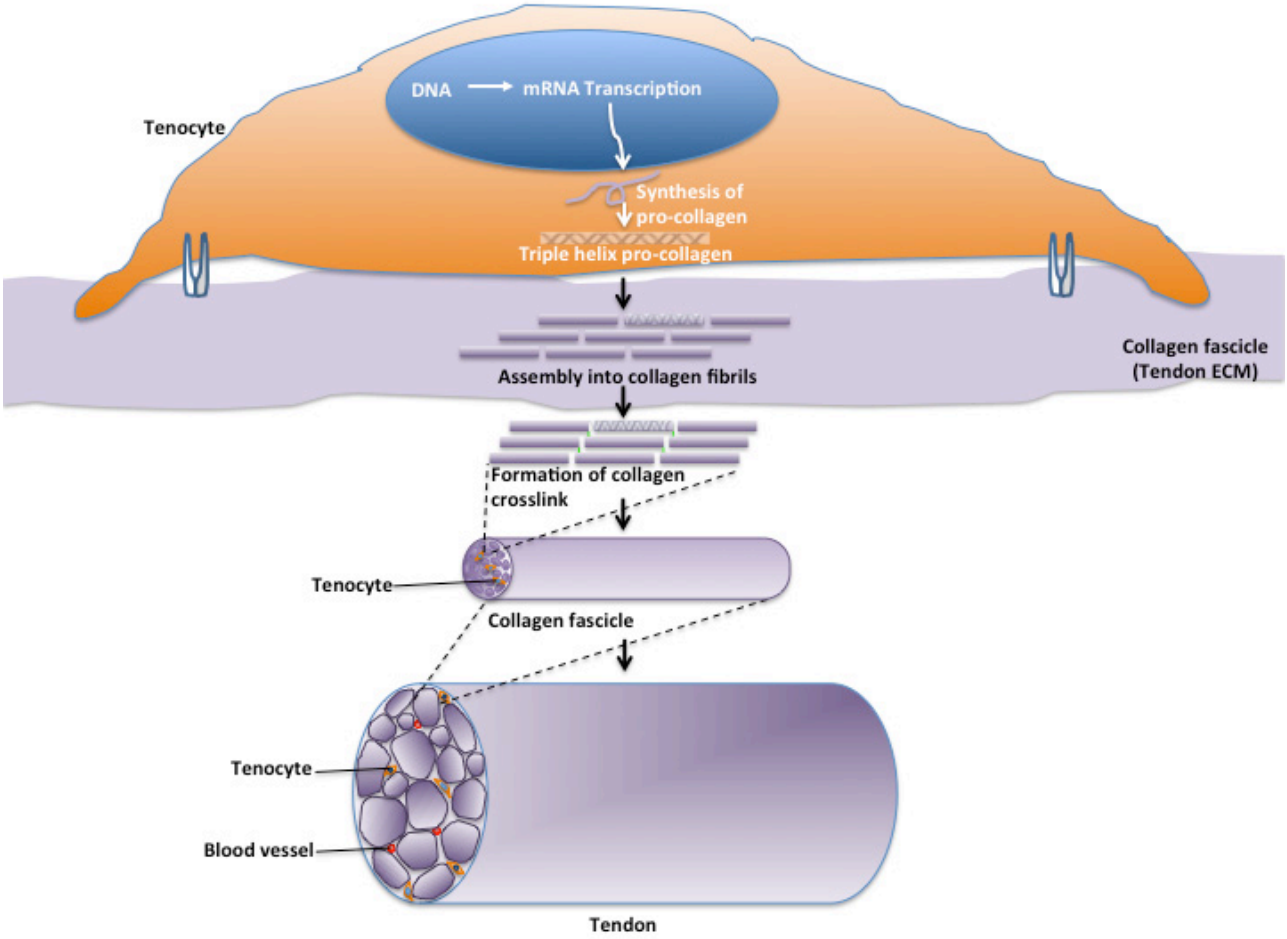

Figure 1: Schematic diagram of hierarchical structure of tendon (68).

The fibril is the smallest tendon structural unit; it consists largely of rod-like collagen molecules aligned end-to-end in a quarter staggered arrays. Fibers form the next level of tendon structure. Fibers are composed of collagen fibrils and are bound by endotenons. Fiber bundles form fascicles, and bundles of fascicles are enclosed by the epitenon. Tendons are also surrounded by a third layer of connective tissue called paratenon (not shown in this figure).

(24). These, are important in the repair and regeneration process in tendon (25-27). Apart from these ECM proteins, several polypeptide factors are important in regulating the expression of specific genes that are commonly found in tendons and the expression of these genes influences the ECM metabolism and subsequently modulates the composition and organization of the tendon ECM (Table 3).

Early Tendon Development

The formation of the musculoskeletal system from the somatic mesoderm requires the coordinated development of muscle, cartilage and tendon lineages. In the early somite development, muscle and cartilage emerge from two distinct compartments, the myotome and the sclerotome. This is in response to signals secreted from the surrounding tissues. As the somite matures, the tendon lineage is established within the dorsolateral sclerotome (or syndetome, the fourth somitic compartment (28)), which is adjacent to and beneath the myotome. The formation of a scleraxis (*Scx*)-expressing tendon progenitor (TP) population in the sclerotome is induced by a fibroblast growth factor (FGF) signal secreted from the myotome.

The FGF transcription effectors (*Pea3* and *Erm*) are necessary for TP marker *Scx* expression in the somite to be expressed (29,30). The domain of *Scx* expression, or the location of the syndetome, is dependent on the combined conditions of the restricted expression pattern of *Pea3* and *Erm* within the anterior and posterior sclerotome, and the distances that FGFs secreted from the center of the myotome are able to travel. Brent and colleagues (2005) also suggested that the early myotome regulatory factors, *Myf5* and *Myod1* (previously known as *MyoD*) expressions are required for FGF protein expression in the myotome, which in turn is required for the induction

Table 2: Summary of most abundant tendon proteoglycans.

Class	Designation	Role in Tendon
SLRP	Decorin	Binds to fibrillar collagen, inhibits collagen fibrillogenesis, binds TGF, and EGF (70).
	Biglycan	Binds to fibrillar collagen, absent in avian species (23).
	Fibromodulin	Binds to type I collagen, facilitates formation of mature large collagen fibrils, modulation of tendon strength (71).
	Lumican	Binds to type I collagen, inhibits size of collagen fibrils, modulation of tendon strength (71).
Modular (lectican)	Aggrecan	Linked to hyaluronan, provides resiliency, low levels in tensional parts of tendon, high levels in compressed regions, particularly in fibrocartilage (72).
	Versican	Linked to hyaluronan, low levels in tensional parts of tendon, somewhat higher levels in compressed regions, increases viscoelasticity, maintains cell shape (73).

Table 3: Genes involved in tendon development and repair (Adapted from James *et al*., 2008) (74).

Gene	Function in development, repair or tissue regeneration
Scleraxis (*Scx*)	Molecular regulator of tenocyte differentiation (75) and activate the *Col-1a1* gene in tendon fibroblast (76).
Tenomodulin (*Tnmd*)	A regulator of cell proliferation, differentiation and collagen fibril maturation (77).
Tenascin C (*Tnc*)	A mechano-responsive modulator of matrix formation expressed in high tensional loading tissue such as tendons and ligaments (78). An ECM protein that is evident during embryonic and tendon development (79).
Collagen I (*Col-I*)	Mature and highly organized collagen fibrils that allows tendon to withstand high tensional loading (76).
Collagen III (*Col-III*)	Early ECM collagen in wound repair (19,80).
Decorin (*Dcn*) and aggrecan (*Acan*)	Proteoglycan interactions modulating collagen fibril orientation and alignment (81).
Smad8	Tenocyte differentiation, phenotype modulation and intracellular signaling (82).

of TP markers. In addition, they suggested that tendon and cartilage lineages arising from the sclerotome appear to be alternative and mutually exclusive, where the loss of chondrocyte differentiation results in an expanded somitic TP population. This causes the *Sox9*-expressing mesenchymal condensations to begin expressing tendon markers. It worth noting that when the differentiation of one cell fate is blocked, the other is adopted (30).

In contrast to the differentiation of axial tendons, that of the cartilages or tendons of the appendicular skeleton arises *in situ*. The initiation of tendon differentiation in the appendicular skeleton does not seem to require the presence of muscle (31). Nevertheless, the maintenance of distal tendons does require interaction with muscle because in the absence of muscle these tendons gradually degenerate (31). Based on the observation of *Scx* expression in the subectodermal location of the appendicular skeleton, it has been postulated that ectodermal signals might play a role in the occurrence of *Scx*-expressing TPs (32). However, the signals that initiate the expression of *Scx* in the appendicular skeleton remain unknown.

In addition to FGF signaling for inducing sclerotomal cells to become tendon progenitor cells (TPC), transforming growth factor - β (TGFβ) signaling is also a potent inducer of *Scx* both in organ culture and in cultured cells (33). This is said to be essential for the maintenance of the early TPC and has been suggested to mediate the recruitment of additional tendon cells by the adjacent muscles and

cartilage condensations. This recruitment is to establish the connections of tendon primordia with these tissues, and it is an essential event for the subsequent differentiation and growth of mature tendons (33). In coordinating the cartilage and tendon differentiation in the developing limb mesenchyme, TGF-interacting factor, *Tgif1*, has been identified as one of the potential candidates which modulates the TGFβ signaling from chondrogenesis to fibrogenesis, and its expression pattern in the limb marks the developing tendons (34). This reprogramming of TGFβ signaling provokes down-regulation of *Sox9* and aggrecan and up-regulation of *Scx* and tenomodulin through the Smad pathway (34). A recent review on the musculoskeletal assembly in the vertebrate embryo postulated that the induction and differentiation of TPCs occur in three distinct stages (Figure 2): induction, organization as well as aggregation and differentiation (35). In brief, the differentiation of tendon in the somite depends upon a combination of both activating and repressing signals from the other compartments of the somite.

However, little is known about other TGF-β family members, in particular the bone morphogenetic protein

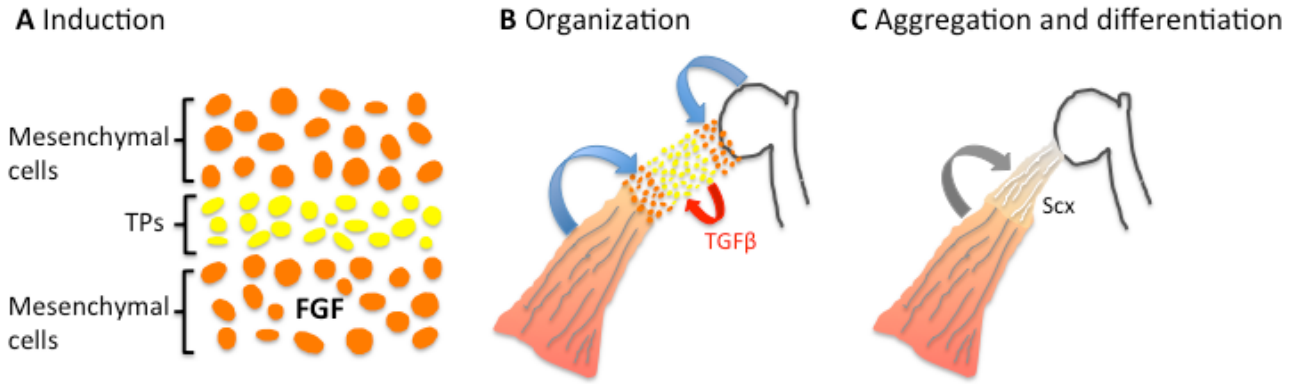

Figure 2: The three main stages and regulators of tendon induction and differentiation in vertebrate embryos (Adapted from Schweitzer *et al*. 2010) (35).

The *Scx*-expressing tendon progenitors (TPs) are represented in yellow and mesenchymal cells in orange to show the different stages of tendon induction and differentiation.

(a) Induction. The initial induction of *Scx*-expressing TPs is associated with FGF signaling, and the myotome in somites is the only identified source to date. In somites and digits, the progenitors are induced at or near their functional position between the myogenic and skeletogenic cells, but in the early limb bud and branchial arches the site of progenitor induction is not related to their final destination.

(b) Organization. At this stage, TPs throughout the embryonic body organize as loose cellular aggregations between the differentiating muscle and skeletal tissues. This transition depends on TGFβ signaling, which mediates the recruitment of additional TPs by the muscle and cartilage tissue to position and integrate the TPs with their interacting musculoskeletal tissues (blue arrows). In addition, TGFβ ligands expressed by the TPs are likely to contribute to the maintenance of the tenoblastic identity of the TPs (red arrow).

(c) Aggregation and differentiation. By E13.5, the TPs condense and organize into structurally distinct tendons that connect to the muscle and cartilage. In some, but not all tendons, tenocyte differentiation depends on *Scx* function. In most tissues, tendon differentiation depends on the presence of muscle (arrow), but the extensor and flexor tendons that extend into the autopod differentiate as structurally distinct tendons even in the absence of muscles.

(BMP) family members in musculoskeletal development. BMP5 is expressed in precise domains in the developing muscle masses and in the autopodial tendons. In the limb mesoderm, Smad and MAPK pathways act synergistically in the BMP pathway controlling limb development (36). Other BMP family members include growth and differentiation factor (GDF) isomers such as GDF5, -6 and -7 (also known as BMP 14, 13 and 12) have also been implicated in tendon development and healing (37-39). Mice deficient GDF5, -6 or -7 exhibit tendon ultrastructural, biological and/or biochemical abnormalities (38,39), whereas exogenous delivery of these factors causes ectopic tendon formation (40). In addition, as one of the earliest known markers of joint formation (37,41), GDF5 dysregulation is strongly linked to various musculoskeletal malformations. GDF5 expression/activity is important in controlling different stages of skeletogenesis, in particular chondrogenesis in a GDF5 dose-dependent manner (42). In cartilage development, GDF5 signaling has a characteristic development pattern in pre-cartilage condensations and in the developing cartilaginous joints (37). Mutations in either GDF5 or its receptor BMP receptor 1B (BMPR1B) lead to similar skeletal malformation phenotypes, indicating that in chondrogenesis, GDF5 signaling seems to be exclusively

mediated through BMPR-1B (43). Many developmental processes, including limb skeletogenesis, also require the segregation of signaling molecules into gradient or the functional compartmentalization of one cell type from another to generate information for differentiation and morphogenesis. Although GDF5 has functional roles in both tendon and cartilage development, it remains unclear whether GDF5 plays a role similar to that of FGF. It may be the case that tendon and cartilage lineages develop in an alternative and mutually exclusive manner through functional compartmentalization processes.

TENDON DAMAGE AND REPAIR MECHANISM

A. Tendon Injury

Tendon injuries, specifically at the shoulder, are a common cause of morbidity and contribute a significant health burden to society. It is defined as a loss of cells or ECM caused by trauma (44). Injury represents a failure of cell and matrix adaptation to a mechanical loading, in excess of the tolerance level, which can be repetitive or prolonged. In these circumstances, there is an inadequate response from the cells or tissues to the mechanical loading applied. In other words, tendon is injured when it is exposed to forces that damage it. Tendon injury at the shoulder can be as the result from forces that cause elongation of the tendon tissue extending into the micro- and macro-failure region. Under physiological circumstances, tendons function in the toe and linear region of the stress-strain curve. Repeated and prolonged load application has been shown to alter the stress-strain curve of the tendon tissue, where tendon injury may result from repeated loading into what would normally be the higher linear region of that curve (1). Rapid unloading has also been associated with tendon injury. Sudden force release is suggested to break interfibrillar adhesion because of shearing force within the tendon (7). In addition to forces that are too big for the tissue to withstand, tendon can also be injured when “normal” forces are applied. This occurrence can be seen in genetic disorders, aging, vascular changes, endocrine influences, nutritional deficiencies, inactivity, immobilization and exercise (45).

The cellular events in ruptured tendon (i.e. rotator cuff tendon) are closely related to the composition and integrity of ECM structure (21,46,47). Tendon ECM transmits mechanical loads, stores and dissipates loading-induced elastic energy. Mechanical deformation in the ECM can transmit forces through tendon cell actin cytoskeleton and cause the remodeling of the actin cytoskeleton (48,49). The cytoskeleton remodeling in turn controls the cell shape, affects cell motility and mediates various cellular functions including DNA and protein synthesis (50). Tendon cells sense mechanical force and convert them into biochemical signals via mechanotransduction mechanisms that ultimately lead to the physiological adaptiveness of tissue or conversely result in pathological changes.

B. Normal Repair Mechanism

Tendon injury will initiate attempts of tissue repair, which has been defined as replacement of damaged or lost cells and ECM by new cells or new matrices (44). In the natural healing process, tendon repair can be divided into different phases (Figure 3). Generally, it consists of an inflammatory phase, proliferation phase, differentiation phase and remodelling phase. In brief, the healing process starts with a hematoma, platelet activation and invasion of cells that form a granuloma. Inflammation after injury protects the body by eliminating and diluting harmful agents, preventing further injury, supplying large quantities of oxygen and nutrients needed for repair, and allowing the entry of clotting agents. Inflammation is triggered by several chemical mediators such as histamines, kinins, prostaglandins, complement, and lymphokines (51).

During the repair process, the clot formed during inflammation is transformed into granulation tissue. The circulating monocytes then differentiate into macrophages after entering the extravascular space. These macrophages are capable of digesting and removing the clot while providing a continuing source of growth factors, chemoattractants, and proteolytic enzymes as needed for tenocyte activation (44). The macrophage-derived growth factor and TGFβ cause the proliferation of tenoblasts originated in the epitenon (52). As tenoblasts infiltrate the wound, blood vessels are formed and facilitate RBC to carry oxygen and nutrients to the developing tissue. Tenoblasts rapidly produce COL-III, which is characterized by smaller fibrils lacking cross-links, which means that the tissue will be lacking tensile strength. At the later stage of this phase, the tenoblasts shift to produce COL-I. Initially, no cross-link occurs between the tropocollagen molecules. This facilitates the enzymatic breakdown and reorganization in the repaired tendon. Cross-links start to develop at 6-14 days post injury increasing tensile strength to the area of injury. At approximately 48 hours to 8 weeks post-injury, the disorganized collagen fibril deposition lies parallel to tensile forces within the tissue.

In the maturation and remodelling phase, cellularity and synthetic activity decreases in the tendon. However, the collagen production has been shown to be 15 times of normal tendon. The granulation tissue is supplanted by new collagen synthesis and deposition, as well as by remodelling myofibroblasts (which derived from the tenoblast that migrated from the edge of wound) that contract the matrix along the axis of the tendon. The ECM becomes more organized at this stage. Wound healing cells and their matrix exist in a dynamic reciprocity whereby cells deposit new matrix and that the matrix modulates gene expression and cell-matrix receptors (53). Through cell-cell and cell-matrix interactions, collagen fibrils align with tenocytes and join end-to-end with other fibrils in the wound and at the margin via covalent crosslinks (2). Most cells (endothelial cells, macrophages and myofibroblasts) then enter apoptosis (programmed cell death), the ECM

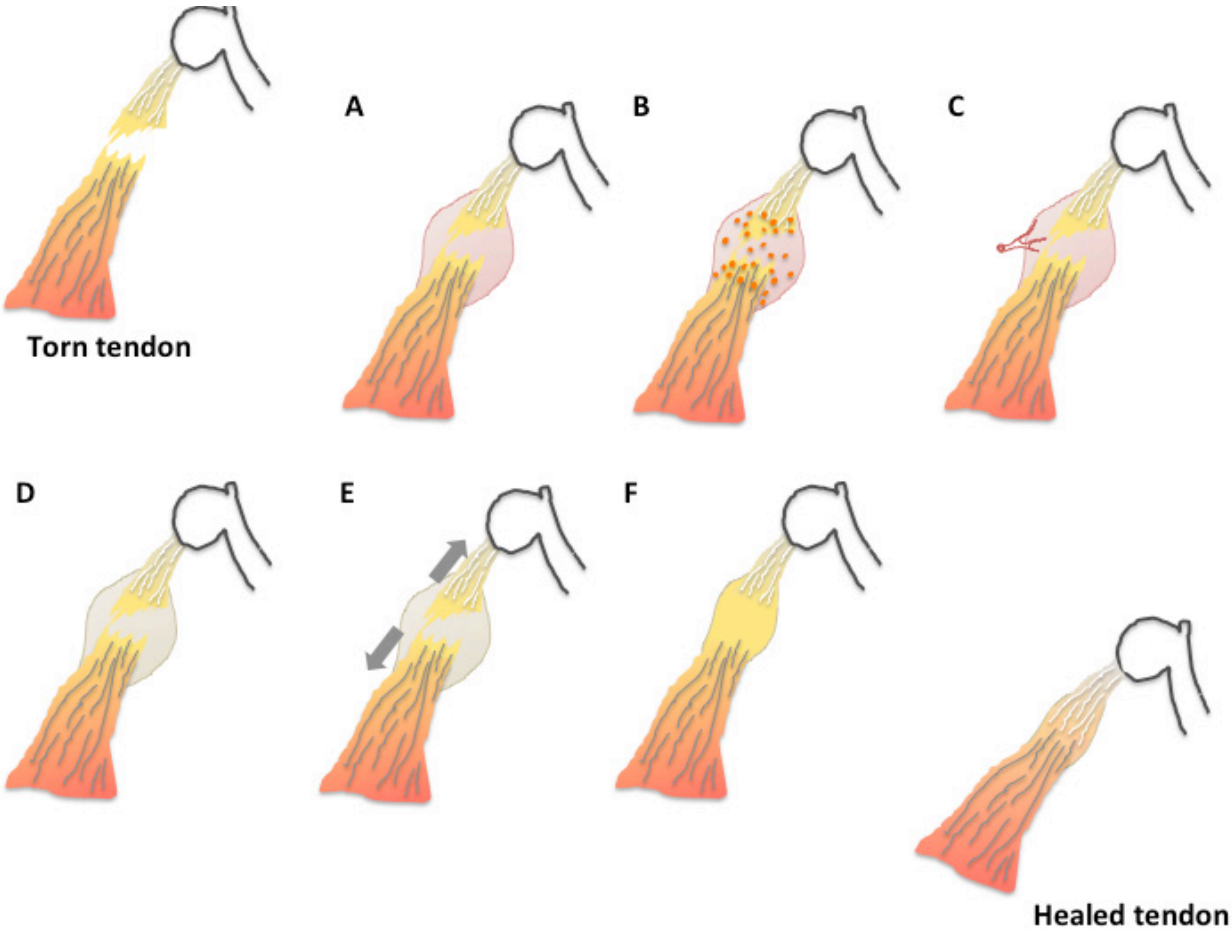

Figure 3: Schematic diagram of tendon repair (69).

(a) Haematoma with platelet activation (inflammatory phase).

(b) Invasion of cells and proliferation of paratenon (proliferation phase).

(c) Vascular and neuronal ingrowth.

(d) Loose collageneous callus formation (differentiation phase).

(e) Mechanical stimulation.

(f) Maturation and remodeling (remodelling phase).

thereby undergoes a transition from a highly cellular granulation tissue to a less densely populated scar tissue (53). Consequently, tendons usually heal with fibrosis and scar tissue, which may regain only 70-80% of their original structural and biomechanical integrity for as long as one-year-post injury. The healed tendon (with suboptimal tensile strength) is prone to reinjury, resulting in lifestyle changes with activity restriction. Poor vascularization (54) and histopathological changes (55) have been suggested as factors contributing to the resulting tendon thickening, fibrosis and being less resistant to tensile stress compared to its preinjured state. The origin of the cells responsible for repairing an injured tendon is controversial. Two mechanisms have been postulated: intrinsic and extrinsic. The former postulates that fibroblast populations come from the endotenon and epitenon, whereas the latter postulates that inflammatory cells and fibroblasts migrate in from surrounding tissues (56). However, a recent report suggested that intrinsic repair may require a progenitor class with predominant tendon marker expression, while extrinsic repair may involve a progenitor class recruited from perivascular cells of the peritenon (57). Tendon TSC/TPC decreases with age and alludes to its association with the age-related reduction in tendon repair as seen in rotator cuff tears (58). Molecular mechanisms controlling these events, either via tenocytes, tenoblast or/and TSC/TPC, and whether a fully differentiated replacement tendon forms at these sites remains largely unclear. The understanding of molecular mechanism in tendon development could assist us in better understanding of tendon etiology and repair.

C. Surgical Repair and Cell Based Therapy in Tendon Healing

Clinically, tendons are repaired or reconstructed using a variety of traditional and innovative methods or surgical techniques that vary with tendon location. These techniques, usually with application of tendon grafts (Table 4), demonstrate various degrees of success. In the light of current shortcomings of tendon repair, the current focus in tissue engineering research is to investigate a repair method which can restore the tissue defects with living cells, or a cell based therapy. A number of cell sources have been suggested, however each cell type demonstrate its own advantages and shortcomings as summarized in Table 5.

Table 4: The advantages and disadvantages of various type of tendon augmentation grafts.

Graft Type	Source	Advantages	Disadvantages
Autograft	Human	No disease transmission risk. No storage required. No preservation problem.	Donor site complication (83,84). Limited availability.
Allograft	Human	No donor site complications. Availability.	Immunogenicity problem (85-87). High risk of disease transmission (87). Required proper storage or preservation (88).
Xenograft	Animal	As with allograft above.	As with allograft above. Ethical issue, i.e. inappropriate animal source such as porcine derived tissue graft.
Prosthesis	Human or animal	As with allograft above.	Low mechanical properties (often result in failure of surgery). Non-specific new tissue induction ability. Induce inflammatory response and rejection (89).
Synthetic	Chemical compounds	Stronger mechanical strength and consistency in quality (89).	Low biocompatibility. Induce inflammatory response and rejection (89).

Table 5: A summary of cell therapy of different cell origins (Adapted from Obaid *et al*. 2010) (90).

Cell Type	Source	Advantages	Disadvantages	Study model (Reference)
Mesenchymal stem cells (MSCs)	Bone marrow-derived	Multilineage potential. Hypoimmunogenecity. Increase rate of tendon healing and maturation. Improve biomechanical and histologic properties of the tendon.	Cannot control differentiation into undesired tissue lineage such as bone, cartilage and muscle. Cell population diminished with age.	Rabbit (91-94) and Mice (94)
	Adipose tissue-derived	Widely available. Simple to obtain. No morbidity to donor site.	As with bone marrow derived MSCs above. Limited application in tendon therapy.	Equine (95,96)
	Synovium-derived	May promote bone-tendon regeneration.	As with bone marrow derived MSCs above.	Nil (97)
	Muscle-derived	As with bone marrow derived MSCs above.	As with bone marrow derived MSCs above. Limited evidence in tendon therapy.	Nil (98)
Fibroblasts	Skin	Great potential in tendon engineering and tendon repair. Widely available. Relatively noninvasive method for cell harvesting. No significant effect to the donor site. Potential source of cells for storage.	Differentiated cells. Uncertainty about behavior in tendon environment. Unsubstantiated repair process. Qualitative repair.	Human (99)
Tendon progenitor/ stem cells	Tendon	Can develop into tendon like tissue.	Morbidity to donor site. No tenocyte markers. No human studies.	Rat (100)

Table 6: A summary of cell therapy outcomes in clinical trials.

Cell Type	Source	Pathology	Patients/Follow-up	Findings
Mesenchymal stem cells (MSCs)	Bone marrow-derived mononuclear cells (64)	1. Complete rotator cuff tears	1. 14 patients (9 women and 5 men, mean age 59.2 years). 2. Mean preoperative UCLA score was 12±3 3. Follow-up at 12 month. 4. A pilot study (cohort study).	1. UCLA score after 12-month follow-up period was 31±3.2). 2. MRI showed tendon integrity in all cases (14/14).
	Bone marrow derived connective tissue progenitor cells (CTPs)(67)	1. Rotator cuff tears	1. 23 patients. 2. Cohort Study.	1. There was no statistical significant difference in: (a) Single Assessment Numeric Evaluation score (CTPs, 86.3 +/- 10.5; control, 83.6 +/- 15.1; p =0.54). (b) Range of motion measures (postoperative external rotation: CTPs, 65.0°±20.4°; control, 62.5 °±17.1°; p =0.67). (c) Postoperative forward elevation (CTPs, 163.0°±30.6°; control, 145.7°±41.4°; p =0.12). (d) Postoperative strength measures between groups (median, 5; range, 4-5 in the CTPs group compared with median, 5; range, 4-5 in the control group; $p > 0.05$).
Fibroblasts	Skin derived tenocyte-like cells (63)	1. Refractory patellar tendinopathy	1. 46 tendinopathy patients (60 patellar tendons). 2. Follow-up at 6 months. 3. Randomized control trial, level of evidence 1.	1. Victorian Institute of Sport Assessment (VISA) score improved from 44±15 before treatment to 75±17 at 6 months post-operative. 2. One patient had a late rupture and progressed to surgery.
	Skin derived tenocyte-like cells (65)	1. Clinical diagnosed refractory lateral epicondylitis.	1. 12 patients (7 women and 5 men). 2. Follow-up at 6 weeks, 3 months and 6 months. 3. Prospective clinical pilot study.	1. The median PRTEE score decreased from 78 before the procedure to 47 at 6 weeks, 35 at 3 months and 12 at 6 months after the procedure (p<0.05). 2. One patient proceeded to surgery after failure of treatment at the end of 3 months.
Tenocyte	Tendon derived tenocyte (66)	1. Rotator cuff tendon injury.	1. A 20-year-old gymnast presented with 12 months of increasing pain during gymnastics being unable to perform most skills. 2. Case study with one year follow-up.	1. At one year after autologous tenocyte implantation, the patient reported substantial improvement of clinical symptoms. 2. Pretreatment and follow-up MRIs scored independently by two musculoskeletal radiologists reported improvement in the tendinopathy and healing on the partial-thickness tear.

Cell based therapy seeks to enhance tissue repair by providing a cell and/or biological scaffold to a repair site in an attempt to elicit a healing response. In order to achieve this, investigators have seeded differentiated cells (mature cells or tenocytes) (59) and undifferentiated cells (mesenchymal stem cells) (60) on scaffolds to develop tissue engineered constructs. Various stimulations, either chemical (using growth factors and cytokines) (61) or mechanical (by stretching) (62), which can mimic the nature of normal tendon *in vivo* environment have been used to enhance the properties of the constructs. Advances in tendon tissue engineering approaches potentially yield a cell-based product that can markedly advance the repair of this soft tissue. Preclinical studies have shown the potential for cellular therapies to increase the tenocyte cell numbers and regenerate rather than repair tendon tissue (Table 5). To date, only 5 clinical studies of cell based therapy in tendons have been reported (Table 6). Of the five human studies reported, only one was randomized control trial, which showed the skin-derived tenocyte-like cells has a better potential than the autologous plasma to improve pain and function in patellar tendinopathy (63). Cohort studies showed that the bone marrow-derived mononuclear cells (64) and skin derived tenocyte-like cells (65) have a potential to improve rotator cuff tear and in lateral epicondylitis respectively. One case study using the ultrasound-guided autologous tenocyte implantation (ATI) showed an improve partial-thickness tear in a gymnast, who was able to return to national-level competition post-ATI (66). Nevertheless, one cohort study reported no significant improvement in the rotator cuff tear treated with bone marrow derived connective tissue progenitor cells (67).

Although current evidence shows that stem cells and tenocytes or tenocyte-like cells can have a positive effect on tendon healing, it remains to be elucidated whether the transplanted cells can help to produce tissue similar to the preinjury state. Questions remain whether tendon development events would re-occur and regenerate tendon tissue, when these cells (stem cells or TP cells) were transplanted to the defect site? In the course of cell-based therapy, would the implanted cells (stem cells, TP cells, tenoblast and tenocytes) together orchestrate cellular events of tendon regeneration? A better understanding in the cellular events involved in tendon development, differentiation and repair is needed in order to lead us to better outcomes for treating tendon injury. The use of adjuncts such as molecular signaling, mechanical stimulation, and other augmentation devices can potentially enhance stem cell therapy in the future.

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IMMUNOGENICITY OF THE MEROZOITE SURFACE PROTEIN-1 (MSP-1) OF HUMAN *PLASMODIUM* SP.

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ABSTRACT

Malaria is a major cause of mortality and morbidity globally. Great efforts have been made in the prevention and the elimination of malaria, especially in controlling the malaria vector, the mosquito. Another promising approach would be the development of malaria vaccines. Malaria vaccine studies can be focused on the pre-erythrocytic-stage antigens and the blood-stage antigens, and on the transmission blocking agents targeting the malaria gametocytes. The blood-stage antigens are the leading candidates in malaria vaccine development, as the blood-stage parasites are responsible for causing symptomatic malaria. Human acquired immunity largely targets on blood-stage antigens. This review focuses on one of the most extensively studied blood-stage antigen, the merozoite surface protein-1 (MSP-1), specifically on its evaluation and immunogenicity in rodents and primate models, and its safety and immunogenicity in human clinical trials.

Keywords: *merozoite surface protein, phase trials, Plasmodium, protective immune responses, vaccination*

Background

Malaria remains as one of the important infectious diseases which lead to high global mortality and morbidity annually. According to the World Malaria Report in 2013 (1), there are approximately 207 million clinical cases of malaria each year resulting in 670,000 deaths. About 2.5 billion people, approximately 40% of the world's population, are at risk. There are five common *Plasmodium* sp. that naturally infect humans: *P. falciparum, P. vivax, P. malariae, P. ovale*, and *P. knowlesi*. More than 90% of the global malaria mortality cases occur in sub-Saharan Africa as the majority of the infections in that area is caused by the highly malignant species *P. falciparum* (2, 3). Most of the victims are children under 5 years old. *Plasmodium vivax* has been described as the benign form of malaria, yet many case reports showed that vivax malaria remains responsible for severe clinical manifestations and mortality in vivax-endemic areas (4). Knowlesi-infection is widely distributed in South East Asian countries (5-9) and could lead to hyperparasitemia in a short period as *P. knowlesi* has the fastest life cycle of 24 hours among the human *Plasmodium* sp. (10). There are limited literature reviews available for *P. malariae* and *P. Ovale,* and this could possibly be due to their low prevalence and milder clinical manifestations compared to the other human *Plasmodium* sp.

The life cycle of *Plasmodium* consists of an exoerythrocytic cycle which takes place in the liver, an erythrocytic cycle in the blood circulation and a sporogonic cycle in the mosquito vector. In the erythrocytic cycle, the steps involved are the release of merozoites from the infected hepatocytes, the invasion of merozoites into the erythrocytes, the development and maturation of trophozoites in the erythrocytes, and the release of new merozoites with the rupture of the erythrocytes. This blood-stage life cycle repeats every 24 to 72 hours, with the period dependent on the species of *Plasmodium*. Clinical illness only occurs in this stage. After repeated exposures, naturally acquired immunity against malaria could develop, predominantly targeting the blood-stage parasites (11). In immunization studies using animal models, a protective immune response is elicited when animals were immunized with blood-stage antigens, particularly merozoite antigens (12, 13). Therefore merozoite proteins, either located within the apical organelles or on the merozoite surface, are the leading blood-stage vaccine candidates. To date, the vaccine studies in human trials have been carried out with merozoite surface protein-1 (MSP-1), merozoite surface protein-2, (MSP-2) (14), merozoite surface protein-3 (MSP-3) (15), apical membrane antigen-1 (AMA-1) (16, 17), erythrocyte-binding antigens-175 (EBA-175) (18) , glutamate-rich protein (GLURP) (19), serine repeat antigen

(SERA) (20), circumsporozoite protein (CSP) (21), and sporozoite surface protein (SSP). Among these candidates, merozoite surface protein-1 (MSP-1) is one of the most extensively studied.

MSP-1 is a high molecular mass protein, of ~185 to 225 kDa which fixes at the merozoite surface membrane of the *Plasmodium* parasite via the glycosyl-phosphatidyl-inositol (GPI) anchor (22, 23). MSP-1 undergoes two steps of processing by proteases and cleaves into a number of fragments. The first processing occurs during the rupture of schizonts, when the merozoites differentiate and are released from an infected erythrocyte. The MSP-1 precursor polypeptide cleaves into four major fragments of different sizes: 83 kDa (MSP-1_{83}), 30 kDa (MSP-1_{30}), 38 kDa (MSP-1_{38}), and 42 kDa (MSP-1_{42}) (24). During the invasion of merozoites into the new erythrocytes, the second processing further cleaves MSP-1_{42} into two fragments, 33 kDa (MSP- 1_{33}) and 19 kDa (MSP-1_{19}) (25). The MSP-1_{33}, corresponding to the N-terminal region of MSP-1_{42}, sheds from the surface in a fully soluble form (26, 27), whereas membrane-bound MSP-1_{19} remains anchored to the merozoite membrane by the GPI tail attached to the C-terminal residue and will be carried into a new red blood cell (26, 28).

Malaria infected individuals who have significantly higher IgG level against N-terminus of MSP-1 are usually asymptomatic compared to the subjects who have acute malaria, demonstrating that anti-MSP-1 antibodies are associated with clinical malarial protection and a reduction of an infection risk (29, 30). Studies have also shown that anti-MSP-1_{42}, anti-MSP-1_{33} and anti-MSP-1_{19} could be detected in most of the malaria infected samples, indicating that MSP-1 is one of the immunodominant antigens that could be useful in malaria vaccine development or seroepidemiological screening (31-35). Antibodies directed against MSP-1_{19} and MSP-1_{42} can interrupt merozoite invasions, and may also inhibit MSP-1 processing (36-39). MSP-1_{42} and MSP-1_{19} are the two fragments that have been most studied, especially in *P. falciparum*. MSP-1_{19} is responsible for humoral immunity responses and a considerable number of studies show that anti-MSP-1_{19} antibody plays a role in the protection from symptomatic disease. Children with a naturally acquired immune response to *Plasmodium* MSP-1_{19} are significantly associated with a resistance towards malaria infection and clinical manifestations (40), while pregnant women with anti-MSP-1_{19} antibodies are protected against placental infection and infection in infants (41).

This paper aims to provide an overview on the immunogenicity of MSP-1 and its proteolytic fragments, particularly MSP-1_{42} and MSP-1_{19}. The main issues to be discussed include cell mediated immune response, humoral immune response, protective effects of these fragments in rodent and primate models and human trials. The potential and limitation of MSP-1 as a malaria vaccine candidate will also be discussed.

Immunogenicity of MSP-1 in rodent models

Before a target antigen could be used in vaccine development, immunization study with animal models is a crucial step to validate the immunogenicity and immunoprotectivity of the target antigen. Rodent models are often chosen as the preliminary animal model due to the ease of handling. In MSP-1_{42} and MSP-1_{19} immunization studies using rodent models, animals were found to be variably protected during a challenge with live *Plasmodium* parasites. Immunogenicity studies by Dutta *et al.* (42) in a mice model showed that *Escherichia coli* (*E. coli*)-expressed *P. vivax* MSP-1_{42} induced specific antibody production and lymphocyte proliferation. Protective cytokines, interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-10 (IL-10) and interferon-gamma (IFN-γ), were detected in the immunized mice. A similar study was carried out on *E. coli*-expressed *P. falciparum* MSP-$1_{42,}$ and the rabbit-raised anti-MSP-1_{42} antibodies significantly inhibited the merozoite invasion, while the mice which were passively immunized with anti-MSP-1_{42} IgG were protected during a challenge with *P. berghei*/*P. falciparum* chimeric line that expresses *P. falciparum* MSP-1_{19} (43). Cheong *et al.* (32) also demonstrated that mice immunized with *E. coli*-expressed *P. knowlesi* MSP-1_{42} exhibited significantly higher levels of IFN-γ, IL-2, IL-4, IL-10 compared to the negative control mice. It is important to take note that MSP-1_{42} exists in nature as two distinct major allelic forms, and immune response produced towards this protein may be greatly affected. Co immunization with *E. coli*-expressed *P. falciparum* and *P. vivax* MSP-1_{19} stimulated specific antibody responses against both antigens and the elicited humoral response lasted up to one year after immunization (44). Sachdeva *et al.* (45) also found that both *E. coli*-expressed *P. vivax* MSP-1_{42} and MSP-1_{19} induced specific antibody responses and T-cell responses with six different adjuvants in immunized mice, and high levels of immunoglobulin G1 (IgG1), IL-4, interleukin-5 (IL-5) and IFN-γ were detected. Parween *et al*. (46) showed that the immunogenicity of recombinant *P. falciparum* MSP-1_{19} and *P. vivax* MSP-1_{19} was strongly enhanced when the recombinant proteins were coated on Gold Nanoparticles formulated with alum. The raised anti-PfMSP-1_{19} antibodies could inhibit an *in vitro* merozoite invasion. As opposed to MSP-1_{42}, MSP-1_{19} is highly conserved but may not provide as much immune response as the longer MSP-1_{42}.

Immunogenicity of MSP-1 in primate models

Immunization studies in primate models are believed to better resemble the regulation of human immune responses. Numerous immunization studies of MSP-1_{42} and MSP-1_{19} in primate models demonstrated encouraging results, as most of the MSP-1_{42} and/or MSP-1_{19}-immunized non-human primates were significantly protected when challenged with live malaria parasites. For instance, Rogers and co-workers immunized *Macaca mulatta* with DNA plasmids encoding four *P. knowlesi* antigens including MSP-1_{42}, and they found that a few of the immunized macaques

were sterilely protected, while the mean parasitemia in the other macaques was significantly lower than the control macaques during a challenge with *P. knowlesi* sporozoites (47, 48). A significantly lower parasitemic level was detected in the rhesus monkeys immunized with *E. coli*-expressed *P. vivax* MSP-1_{42} compared to the negative control group upon a challenge with *P. cynomolgi, a P. vivax*-closely related *Plasmodium* sp., blood stage parasites (49, 50). On the other hand, *A. nancymai* vaccinated with *E. coli*-expressed *P. falciparum* MSP-1_{42} was highly protected during a lethal *P. falciparum* challenge (51-53), and the protective effect was stronger than the baculovirus-expressed *P. falciparum* MSP-1_{42} (54). Moreover, specific antibodies and antigen-specific T-cell responses with the production of IFN-γ were also detected in *M. mulatta* which were immunized with DNA plasmid encoding *P. falciparum* MSP-1_{42} (55). Before a human trial, pre-clinical analysis on *P. falciparum* MSP-1_{42} formulated with adjuvant AS02A or alum was performed in *M. mulatta* macaques and both vaccines were shown to be safe and highly immunogenic (56). MSP-1_{42} formulated with other adjuvants AS01B, AS05 and AS08 were also tested in rhesus. All these formulations were found to be safe and immunogenic. AS01B formulation induced a strong Th1 response compared to AS02A which induced a balanced Th1/Th2 response (57). Partial protection was detected in *Saimiri boliviensis* monkeys upon immunization with yeast-expressed *P. vivax* MSP-1_{19} (58, 59). On the other hand, vaccination of yeast-expressed *P. falciparum* MSP-1_{19} also conferred protection against a lethal challenge of *P. falciparum* in *Aotus vociferans* monkeys, and the raised anti-MSP-1_{19} antibodies could inhibit the secondary processing of MSP-1_{42} (60). Efficacy of baculovirus-infected insect cells-expressed *P. falciparum* MSP-1_{42} and *Saccharomyces cerevisiae* (*S. cerevisiae*)-expressed *P. falciparum* MSP-1_{19} were compared in an *A. nancymai* monkey model. A significantly higher protection level was observed in insect cells-expressed *P. falciparum* MSP-1_{42}-vaccinated group compared to *S. cerevisiae*-expressed *P. falciparum* MSP-1_{19}-vaccinated group, and the protection was associated with corresponding antibody levels in the immunized monkeys (61).

MSP-1 in human trials

Besides animal models, efficacy of MSP-1, including MSP-1_{42} and MSP-1_{19} fragments, as a vaccine candidate has also been tested in human trials. Most of the efforts for the development of malaria vaccines and human trials are still focused on *P. falciparum*. Results indicated that MSP-1 formulated with different adjuvants conferred different levels of protection in human clinical trials. Sheehy *et al.* (62) evaluated the *P. falciparum* MSP-1 in a Phase Ia clinical trial and induction of exceptionally strong T-cell responses was detected. *P. falciparum* MSP-1 was tested together with MSP-2 and ring-infected erythrocyte surface antigen (RESA) as a three-component blood-stage vaccine, formulated with Montanide ISA720, in ten male adults (63) and 120 children (64) in a malaria endemic area of Papua New Guinea. The vaccine was found to be safe for use in an already immune population, and MSP-1 was shown to be the most immunogenic molecule in that vaccine cocktail, as good cellular response with an increased level of IFN-γ and an increase in geometric mean antibody titres against MSP-1 was detected.

The C-terminal MSP-1_{42} has also been tested in human trials besides that of the full length MSP-1. Phase I human vaccine studies by using *P. falciparum* MSP-1_{42} formulated with adjuvant AS02A (FMP1/AS02A) have been carried out in USA with 15 adults. The vaccine was shown to be safe and created minimum reactogenicity with no severe adverse effects in all subjects. A high titre of parasite-reactive anti-MSP-1 antibodies was induced and 80% of immunized-subject sera reached the minimum functional inhibitory level of 15% inhibition in parasite growth inhibition assay (65). The same vaccine was evaluated in the falciparum-malaria endemic areas, including western Kenya (66) and Mali (67), with 40 adult volunteers in a Phase I trial. The safety and tolerability levels of FMP1/AS02A were high, and the vaccine was highly immunogenic and a statistically significant antibody response was detected. Another Phase I trial was conducted in 135 Kenyan children of ages 12 years to 47 months, and the induced-immune response was dosage-dependent (68). However, a Phase II trial with 400 Kenyan children indicated that FMP1/AS02A may not be a promising candidate for monovalent malaria vaccine, with an overall vaccine efficacy of 5.1% only (69).

Besides AS02A, MSP-1_{42} was tested with a few other different adjuvants. The adjuvant Alhydrogel was formulated to *Plasmodium falciparum* MSP-1_{42}, with both 3D7 and FVO alleles, and evaluated in 60 volunteers in USA. The results showed that although the cytokines IFN-γ, IL-2, IL-5, IL-10 and IL-13 were detected in the vaccinated-volunteers, addition of other immunostimulants to both vaccines were needed as the raised anti-MSP-1_{42} antibodies were insufficient to inhibit parasite growth up to protection level (70, 71). Ellis *et al.* (72) mixed the FVO and 3D7 of *E. coli*-expressed *P. falciparum* MSP-$1_{42,}$ formulated with Alhydrogel and novel adjuvant CPG 7909, in order to induce immune responses that recognized the major antigenic polymorphisms. A Phase I trial was carried out in 60 healthy adults. A high safety profile was demonstrated and the encouraging result showed that the sera of MSP-1_{42}/CPG 7909-immunized volunteers had an average of 14%, ranging from 3% to 32%, inhibition activity in the parasite growth inhibition assay. Recently, two Phase I clinical studies on MSP-1_{42} (FVO) formulated with adjuvant AS01 were conducted in 26 adults in USA and 30 adults in Kenya (73). The sera of only a few vaccinated-volunteers significantly inhibited parasite growth *in vitro*. However, the raised-antibodies in USA volunteers exhibited better cross-reactivity to heterologous MSP-1 alleles than the previously tested MSP-1_{42} (3D7) vaccine.

P. falciparum MSP-1_{19} was combined with domain 3 of apical membrane antigen 1 (AMA1) as a chimeric protein (PfCP-2.9) in human vaccine studies (74, 75). This chimeric

vaccine was formulated with adjuvant Montanide ISA720 and tested in 52 healthy adults (76) and 70 healthy Chinese adults (77), separately. The results demonstrated tolerability and immunogenicity of the formulation, yet optimization evaluations are needed to reduce the reactogenicity. No functional activity against the parasite was observed. Human trials with MSP-1 of other human *Plasmodium* sp. have not been carried out (78, 79).

Conclusion

Development of malaria vaccines by using MSP-1 encounters numerous challenges. Knowledge about the real functions of this antigen and its interactive mechanisms with human host cells are limited. The approaches of vaccine development to target on functionally important domains/epitopes on MSP-1 are difficult. Human malaria *P. falciparum* and *P. vivax* cannot infect mice. Therefore, vaccine studies of human *Plasmodium* MSP-1 in non-human models , especially in rodent models, are unable to completely represent the safety, efficacy and immunogenicity of the vaccine targets in human, as there are distinct differences between rodents and human in the immunity regulation and pathogenic responses towards malaria. Most of the non-human primate vaccine studies on MSP-1 alone indicated that only a small percentage of monkeys was protected (58, 59, 61), either partially or completely, during a challenge of live *Plasmodium*, while in human vaccine trials, although specific antibodies have been induced, yet the titres were insufficient to neutralize the parasites *in vitro*. Hence, immunostimulants are needed in order to induce a higher level of protective immune responses. The low level of protection elicited by this single antigen vaccine is an impetus to develop multi-antigen vaccines. Nonetheless, the highly immunogenic MSP-1 should remain as one of the potential candidates for blood-stage malaria vaccine design (32, 35, 66, 67). Further investigations and evaluation are needed.

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USE OF THE UNITED THEORY OF ACCEPTANCE AND USE OF TECHNOLOGY MODEL TO STUDY INFORMATION COMMUNICATION TECHNOLOGY- ADOPTION IN FIVE SAUDI ARABIAN PRIVATE HOSPITALS

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ABSTRACT

We conducted a multi-institutional case study to identify the issues associated with the adoption of information and communication technology (ICT) in five private care hospitals in the Kingdom of Saudi Arabia. We conducted interviews with 37 respondents primarily comprising IT professionals.

We found that there were three determinants of behavioural intentions in this case study: organisation objectives, facilitating conditions and social influence where there are no effects of performance expectancy or effort expectancy. In all five cases, none of the moderators (age, gender, experience and voluntariness) in the original united theory of acceptance and use of technology model were considered critically important by IT professionals. In the present paper, all qualitative elements such as themes, patterns and overarching in the data were analysed to reach a conclusion. In addition, the various perspectives of using ICT are discussed.

Keywords: *Effort Expectancy, Information and Communication Technology (ICT), Performance Expectancy, Social Influence, Turnover and Talent Acquisition, Vendor Credibility.*

Introduction

Information and communication technology (ICT) is viewed as an effective tool to improve the quality of health services. A number of studies (1-3) explain how adoption of ICT in healthcare organisations, particularly hospitals, successfully supports solutions for improved services of the organisation. Nevertheless, many of these studies were conducted in non-Arab settings with economies that can be described as matured. Thus, some of the existing and contemporary challenges that could lead to a better understanding of ICT adoption in private hospitals in developing economies may not be sufficiently covered in the literature.

Developing countries such as India and China have also made significant contributions to the development of this technology (4, 5). Many developing countries have started implementing measures to improve their national information infrastructure and create an environment conducive to ICT growth as a tool to advance human development (6). In Saudi Arabia, expenditure on IT services witnessed an annual growth rate of 45.7%, i.e. up to 7.9 billion riyals, in 2012 (6). However, Altuwaijiri (7) observed that the Saudi healthcare sector has not yet attained significant levels of advancement in the field of ICT; in fact, its use had created technology gaps between clinical professionals and data managers. The aim of this study was to explore the adoption of ICT in five private hospitals in Saudi Arabia according to the unified theory of acceptance and use of technology (UTAUT) model. Such phenomenon of technology adoption in various contexts and settings has been examined and explored through

different theories of technology adoption such as the theory of reasoned action (8), technology acceptance model (9), theory of planned behaviour (9), diffusion of innovation theory (10) and UTAUT (11).

The Unified Theory of Acceptance and Use of Technology (UTAUT) model (11) was a result of integrating eight theoretical models (Figure 1). It has been successfully employed in many technology adoption studies (12). UTAUT has been tested in different countries such as the USA (13), study used UTAUT to examine Hospital Information Technology (HIT) adoption in a small medical office. The results of the study reveal that a major factor affecting HIT adoption in a specialty practice was the physician's need to have regulations to protect the privacy and increase the security of patient data. UTAUT has been used in different types of healthcare organisations (14,15). According to Ivanov (14), individual attitudes and habits are significant factors affecting the establishment of new technology in healthcare practices (14).

Methods

Study design

In order to understand the crux of the issues of ICT adoption in Saudi, we conducted a multi-institutional case study. The case study method was chosen on the basis of the aims of this study, which were to discover qualitative elements such as themes, patterns in five private hospitals, the Specialized Medical Center Hospital, the Sulaiman Al-Habib Medical Group, the Al-Muwasat Hospital, the Saudi German Hospitals Group and Kingdom Hospital. The five private hospitals were categorised according to the length of time they have been in operation. Based on the year in which the hospital was established, the hospital was considered either newly established/young or experienced/mature.

These hospitals were chosen for this study because they are the biggest private hospitals in Saudi Arabia. For this study, hospitals established after the year 2000 were recognised as newly established or young. Conversely, the hospitals founded and established before the year 2000 were classified as experienced or mature hospitals.

The newly established hospital group comprised the Kingdom Hospital, Specialized Medical Center Hospital and Sulaiman Al-Habib Medical Group, whereas experienced and mature hospitals group comprised the Al-Muwasat Hospital and Saudi German Hospitals Group. (Table 1).

Data collection

This study was conducted using qualitative research methods, specifically the interview method. The interview method enabled the researchers to understand issues in a deep manner that could not have been possibly achieved using quantitative measures. Henceforth, the researchers made enquiries and elicited opinions on issues and factors influencing the adoption of ICT in these hospitals. This approach helped the researchers develop in-depth understanding on unique social and behavioural patterns as well as specific contextual elements within the organisational settings that influence respective ICT adoptions. The respondents were asked to answer three questions: How mature are the situational and interactional workflow conditions in your hospital? What are the factors affecting the adoption of information and communication technology in your hospital? What aspects or challenges will encourage or discourage from achieving full information and communication technology utilisation in healthcare?

The required data were gathered by interviewing 37 respondents who were selected according to their experiences, knowledge and educational attainment. The participants comprised IT personnel and administrative staff of these hospitals. Each interviewee was briefed on the information concerning the goals of the study and the purpose of conducting the interviews.

All interviews were conducted in Arabic. Each interview session lasted from 50 minutes to one hour. With the permission of the interviewees, most sessions were audiotaped. The recorded interviews were then transcribed and analysed to determine the issues and factors influencing the adoption of ICT specified as critical by each individual. The three interview questions were designed as open-ended questions developed from literature review and were related to the various aspects of factors that affect the adoption of ICT in healthcare organisations.

To analyse the interviews, the tapes were transcribed verbatim to paper by the researcher to ensure that all the information was intact. The researcher carefully read and re-read each transcript (at least five times) to identify the appropriate nodes or themes that would be used for the analysis.

The interviewers posed many issues and questions related to the ICT field. In order to examine the collected data, each theme was individually analysed, and a general conclusion was reached. These data have been summarised at the end of this paper.

Case Description

For this study, a newly established hospital is defined by hospital founded and established after year 2000. Conversely, those hospitals founded and established before the year 2000 were classified as experienced and matured hospitals (Table 1).

Results and Discussion

Demographic Distribution of Study Participants

A total of 37 respondents, comprising 31 (84%) foreign nationals and six Saudi nationals (16%), participated in the study.

The educational attainments of the respondents were as follows: 8% (N = 3) were PhD holders, 40% (N = 15) had

Table 1:

Branches	Operating year	Hospital name	
-	2000	Kingdom Hospital	Newly established Hospitals
-	2001	Specialized Medical Center Hospital	
8	2003	Sulaiman Al-Habib Medical Group	
6	1975	Al-Muwasat Hospital	Experienced and Mature Hospitals
6	1988	Saudi German Group	

master's degrees, 45% (N = 17) had bachelor's degree, whereas 5% (N = 2) were diploma holders. The majority of the respondents, with a combined percent of 85%, have either bachelor's or master's degrees. Twenty-four (65%) respondents were male, whereas 13 (35%) were female.

Typical sectoral approaches to ICT adoption

On the basis of the research questions and corresponding responses elicited, the major factors contributing to and acting as barriers to ICT adoption were identified. It was found that the variables tended to facilitate and shape various expectancies.

- **Perceived usefulness**

Along with the general external variables that prevail, IT departments appear to perceive ICT as their immediate means of confronting core issues related to, but not limited to, the scope of a planned action, service coverage, data management and security and meeting business demands. Despite differences in emphases by their respective management, IT professionals would look up to ICT as a potential solution for coping with increasing patient demands for quality healthcare, with little regard for sector features. However, the typical clinical setting in the Kingdom of Saudi Arabia (KSA) is still characterised by manual data collection processes. Some examples are admission and discharge activities as well as the writing of prescriptions and clinical reports that are more prevalent in physicians' clinics and nurse stations.

The results of this study suggest that the typical sectoral orientation of a hospital can greatly shape and influence its ICT infrastructural requirements. Following the above discussions, the main sectoral orientation of the hospitals was included in the study (Figure 1).

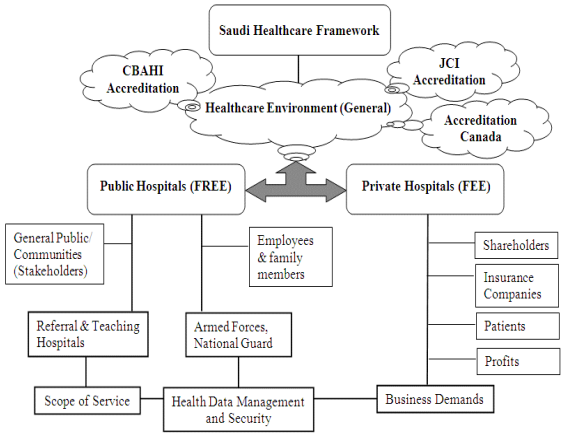

Figure 1: Typical Sectoral Approaches to ICT Adoption

- **Framework of UTAUT**

Because this study was undertaken at an institutional level and included IT professionals, a pre-conceived notion that views ICT as a solution was expected. Relative to this notion, the intention to use ICT was already a profound concept or a known approach to the problems referred to IT departments. With regard to the respondents of this study, the use of ICT was almost always indispensable, and in these individuals, behavioural intentions (BIs) to use ICT are already present at the onset of objective-setting or problem-solving.

In the UTAUT model (figure 2), there are four constructs that act as determinants of BIs: (1) performance expectancy (PE), (2) effort expectancy (EE), (3) social influence (SI) and (4) facilitating conditions (FCs).

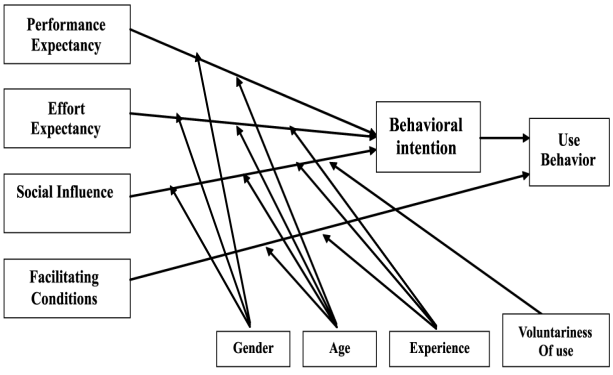

Figure 2: The UTAUT model

Nonetheless, just as UTAUT was originally developed to predict acceptance by non-IT professionals, the use of the UTAUT model by IT professionals yielded a hypothetical framework structured quite differently from the expectations in the original UTAUT context. This study can put forward a proposition suggesting that the four key moderating factors identified in the original UTAUT

model by [11] have little to no significance for employed IT professionals compared with that for non-IT professionals. One probable explanation is the homogenous features and work functions of the respondents (i.e. professions, levels of IT literacy and expected job performances under each one's departmental management and organisational culture).

- **Derived Analytical Model**

The development of this framework was based on the respondents' narratives The narrative accounts of the respondents indicated that decisions by the higher management, strategic objectives, pacing of IT infrastructure with overall hospital development (organisational functions), funding and release of budget (facilitating factors) and opinions and consensus within the department and across departments (SIs) have shaped their intentions and behaviours in the direction of factors such as which system to use, what to deploy and how to develop it (i.e. in-house or vendor-developed programmes and integration). Therefore, there were three known determinants that have direct influences on BIs: SI, organisational objectives (OOs) and FCs. The two moderating factors external to organisation, namely vendor's credibility (VC) and turnover and talent acquisition (TTA), converge with BIs at the PE and EE points. Instead of PE and EE directly influencing BIs, the latter joins VC and TTA in moderating the impacts of PE and EE, respectively, which consequently influence use behaviour. To illustrate, this can be given as follows: VC → PE → BIs and TTA → EE → BIs.

- **Determinants of Behavioural Intentions**

1. Organisational objectives

For this study, OOs were defined as the extent or degree to which they influence BIs to recommend and use a projected size or array of ICT infrastructure perceived by IT professionals as necessary to implement and achieve an envisioned objective. Depending on the expanse of the plan, i.e. whether it is long-term or short-term, a corresponding action is presumed to be implemented according to an ICT development scheme that possibly varies from one organisation to another.

Reasonably, IT managers are aware that the fundamental prerequisite for determining the size of ICT, whether it is a new installation or upgrade, is its utilisation, which should match their interim or long-term project needs as closely as possible.

2. Facilitating conditions

This study defined FCs as the extent to which an IT professional believes that an organisation renders support to facilitate ICT development and plan implementations. They can be understood as the perceived amount of support that IT professionals receive from the organisation to meet their ICT-related targets.

On the basis of the research questions, three variables were pre-qualified in this study as determinants of ICT adoption. Two pertain to internal (top management support and selection and procurement) variables and one to an external variable (price). Conversely, these may be translated under the model derived from UTAUT as conditions that facilitate ICT use.

- **Social Influence**

SI was applied in this study from a social perspective that is much wider than that originally defined in the UTAUT model. Far more than the degree of the user's perception about the significance of beliefs held by other people in terms of using a new technology (11), the context of SI used in the analysis refers by and large to social demands for quality healthcare services, quicker patient turnaround and faster insurance claims processing.

Qualitative Evaluation of Total BIs

Influence of business demands was a common feature for all five private hospitals involved in this study. Approximately 70% of the IT professionals interviewed explained how their departments assume the role of reactors according to growth, client demands and patient needs. The results showed that newer and older private hospitals demonstrated different approaches to ICT infrastructure development. One newer and two older hospitals had chosen a vendor-developed approach with built-in software and applications, whereas two newer ones had customised their own systems and software in-house.

A. Social Influence

With private hospitals, public pressure comes from paying patients and insurance companies that often demand quicker turnaround and faster claims processing. The major involvement of insurance companies as third party places pressure and influence on private hospitals to adopt certain ICT nomenclatures or structures matching third party requirements because of which conformity pressures would often necessitate ICT implementation outside the true healthcare constructs. Other than patients and companies, the top management, including the CEO, the COO and the like, may also influence the IT department for the use of certain specifications or programs that may not have been innovated or introduced previously by the IT department.

B. Facilitating conditions

As Sarosa (1) emphasised, the resources provided by the top management, including financial resources, IT infrastructure availability, training support and operational expenses, can altogether encourage ICT adoption in an organisation. Largely, private hospital managements support ICT development with assiduousness over expected outcomes and return of investments. This is particularly observed in the older hospitals, which consider some factors to be more important than ICT, such as medical equipment. Staff training support (i.e. funds for training) is also lacking in privately owned hospital establishments.

C. Organisational objectives

OOs in the private hospital sector are business-driven, with strong indications suggesting that the pace of ICT development is anchored on incumbent or short-term business demands. Conversely, private hospitals may have to wait for the opportunity to develop before they can move on to decide whether investments in ICT would be feasible.

Moderators of Expectancies

In all five cases included in this study, none of the moderators in the original UTAUT model were considered critically important by IT managers, colleagues or subordinates in the IT profession. Instead, it was evident that managers were more concerned about the effects of VC and TTA on PE and EE, respectively. The participants tackled the issue of moderating factors in the context of IT human resource management, where independent variables such as age, gender, experience and voluntariness of use were not considered as factors contributing to adoption constraints. Rather, they were considered more as variables that can be dealt with at human resource levels at pre-employment and personnel management (by department heads) during stages of employment. Therefore, every IT professional who is a member of the IT department works under the assumption of competence and performs his/her role and tasks. If any of these variables (age, gender, voluntariness of use and experience) negatively influence BIs of an individual IT professional, the department heads or immediate supervisors (IT managers) will have to assume their roles to deal with it internally. Besides, IT managers are held partly responsible for the performance of their department or team. Any deficiencies in subordinates, nonetheless, reflect upon the managerial capabilities of the IT managers.

Consequently, on the basis of the perspectives gathered from this study, IT managers have attributed tendencies of moderation to two external moderating factors: VC affecting PE (VC → PE) and TTF affecting EE (TTF → EE). Evidently, no internal factor or any moderator originating from within one's department was mentioned. Instead, this study found that PE was linked to VC very tightly, indicating strong dependence on the vendor's capability of sustaining technical support, even for periods longer than the terms originally stipulated in the contract (VC → sustained technical support → PE). Conversely, PE is mainly anchored to the vendor's reliability, reputation and good service track record, thus leading to the vendor's established credibility (VC = reliability + reputation + good service track record → PE). Therefore, if PE is linked to VC, then the degree of confidence as well as factors that contribute to the enhancement of an IT professional's performance in the surveyed hospitals are partly dependent on the vendor capabilities to extend technical support.

Because the VC → PE link had become more profoundly identified as a dependent and external variable, another external factor associated to show strong dependence in the hospitals was TTF. In this study, TTF was viewed to be critical to EE (TTF → EE). As with any organisation, when an employee who plays a vital role or responsibility leaves, the vacancy leaves a departing expertise that needs to be filled in a timely and competent manner. If the IT staff is unprepared, the level of ease that other users already have in working with the system will cause adverse adjustments or alterations. Unfortunately, the Saudi labour context and other mandatory policies concerning expatriate labour promote the recurrences of TTF across many sectors and industries.

A. Behavioural Intentions

While the configuration of the model is a modified version of the original UTAUT, the BI deserves also a modified description. The interview participants have indicated that their roles are often not limited only to system integration or computational program development; they should also deal with knowledge facilitation and skills transfer and development to all the users of IT across all departments. Therefore, BIs should be viewed from the perspective of not only user intentions but also the degree to which an IT professional is influenced by OOs, SI and FCs to take the lead in promoting positive use behaviour upon others.

B. Vendor's Credibility

This factor, which is external to the organisation, was found to be a critical component to ICT adoption because there were instances in KSA where some vendors abandoned their responsibilities in the midst of unmanageable difficulties (i.e. vendor terminates or breaches service contracts). Furthermore, whenever an expected performance fails, the organisation is at the greatest disadvantage. Several measures have been undertaken to ameliorate this problem, one of which is to seek intervention from the MOH or the Ministry of Commerce and Industry to blacklist erring contractors and vendors.

Vendor integrity is an important aspect while choosing a system. Numerous studies (9-11) provide evidence that sellers and buyers of IT systems can develop relationships before any sale, which in turn influence purchasing decisions at a later period.

Turnover and Talent Acquisition

In the same context as 'being around when computing efforts by others run tough', an IT personnel is expected to ease the adoption process by helping others manage through and get acquainted with the system. With a positive BI, the perception of difficulties will be diffused and efforts expectancy will be relatively eased. However, the labour structure in KSA can be a disincentive to effective EE promotion because of quick talent turnover and subsequent difficulty in acquiring an equally competent individual to replace a departing employee.

1. Lack of management support to provide training

The notion of temporary employment is a disincentive for hospital management to offer and invest in extensive

training programmes for foreign employees. Because the ratio of long-term employment is negligible, managements tend to withhold offers for advancements knowing that many of those who will receive training will leave shortly, and the original sponsors may no longer benefit over a longer term.

Assumingly, when a system switches users (from the previously trained user to a novice), certain performance expectations would be likewise affected by the emergence of gaps such as the relative lack of awareness by the new user. On the basis of the UTAUT model, awareness deficiencies delimit PE and breed negative perceptions towards the system from new users, thus forming barriers to ICT adoption.

2. Shortage of IT professionals

This is another challenge brought about by the non-permanence of foreign labour and shortages of domestic or local IT talents. This study has found that Saudi IT professionals employed in the private sector prefer to work in the public sector because it provides job security and high pay. Workers are always eager to catch that opportunity when made available (16).

Conclusions and Recommendations

The use of ICT across the health value chain is widely recognised to be of significant importance for lowering costs and improving services at all care levels in the entire KSA (3). The major institutions mandated by the KSA Government, namely MOH, the Communications and Information Technology Commission, the King Abdul-Aziz University (KAU) and the Central Board for Accreditation of Health Care Institution (CBAHI), have assumed their respective invaluable roles in promoting the use of ICT products and services for improving healthcare delivery.

However, the functional roles of these institutions remain limited to central roles in spearheading initiatives or disseminating good practices that do not generally carry a mandate to control, restrict or regulate ICT practices in healthcare facilities. Therefore, all healthcare facilities are exposed to identical sets of external factors, whereby the concept of autonomy or self-regulation in terms of ICT adoption still remains the standard guiding parameter. However, internal deficiencies to control lapses are noticeable, including poor data delivery, time lags, weak or inferior applications, overestimations and underutilisation.

Having applied the UTAUT model in departmental or collective contexts, the eventual outcome necessitated the modifications in the UTAUT configuration. The newly identified categories influencing BIs were found to be consistent and applicable to all hospital sectors even if specific definitions relevant to the meaning of each category are assigned. Although more research is needed, it appears that some viable solutions can be offered, or perhaps, some positive outcomes can be expected if BIs of IT professionals profoundly support their OOs and are supported by the organisations they serve. Lastly, the inclusion of BIs as an active interactive moderator of use behaviour presents an encouraging tone towards the potentials of cushioning negative impacts and relieving ICT professional users of the dilemma brought about by unreliable vendors and quick employee turnovers.

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