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Best Oral Presentation Award Finalist – Allied Health/Health Sciences/Paramedical Disciplines

Between the Rod and Reason: A Study on Asian Parental Disciplinary Methods and Child Emotional/Behavioural Outcomes

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Introduction

In contemporary, Western-based, literature, Asian parenting is frequently described as authoritarian.[1] Authoritarian parents value control and expect children to conform to a standard set of conduct and punish them for violating it.[2] Hence, it has been a common notion that authoritarian, and therefore, Asian parents are in favor of physical punishment to gain compliance.

Research on the effect of physical punishment on child outcomes yielded mix findings. It has been reported that frequent physical punishment is associated with negative child outcomes, such as emotional disorders, while child compliance as a result of punishment has been regarded as a desirable outcome.[3]

Most of these studies have been conducted with Western populations. From the classic writing of Whiting and Whiting[4] on the *Children of Six Cultures,* it is evident that child-rearing practices and child development vary across cultures. Hence, this calls for a need for more research on the nature of Asian parenting and child outcomes in the Asian context, which has been scanty.

In the present study, we sought to understand Asian parental disciplinary practices and their associations with child behaviours. We first examined the most frequent form of disciplinary practice used by parents of children seen at a child mental health facility in Singapore; disciplinary practices were operationalised as: Reasoning with the child, caning the child or a combination of both practices. Caning was taken to be a form of punitive parenting practice in this context. The association between these disciplinary practices and child emotional/behavioural outcomes was also investigated.

Materials and Methods

Participants were 230 Singapore parents (63 males and 167 females; aged 23 to 52 years old, M = 40.7 years; SD = 4.4 years) whose children (173 boys and 57 girls; aged 4 to 12 years old, M = 8.7 years; SD = 1.8 years) were attending the Child Guidance Clinic. Parents reported how they would react when their child has done something wrong, and scored their child's behaviour in the Child Behaviour Checklist (CBCL/4-18).[5] The CBCL/4-18 comprises of eight syndrome scores (namely, Withdrawn, Somatic Complaints, Anxious/Depressed, Social Problems, Thought Problems, Attention Problems, Delinquent Behaviour and Aggressive Behavior), 2 syndrome group scores (namely, Internalising/Emotional-based problems and Externalising/Behavioural-based problems) and a total score.

Results

From Figure 1, it could be seen that most parents do not involve caning as a form of disciplinary practice. 68.3% of the parents would employ reasoning, while only 31.7% of the parents would involve caning, when disciplining their children.

One-way ANOVAs showed that (1) compared to parents who used caning as a disciplinary method (with or without reasoning), parents who do not cane their children at all reported significantly the least externalizing problems (including attention problems, delinquent behaviour, aggressive behaviour) in their children, and (2) compared to parents who used either reasoning or caning solely as their respective disciplinary method, parents who used a combination of reasoning and caning reported the least internalizing problems (including withdrawn syndrome, somatic complaints, anxiety/ depression) in their children, though the findings did not reach statistical significance (Table 1).

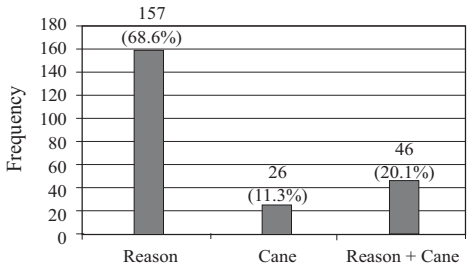

Fig. 1. Frequency of disciplinary practices.

Table 1. Mean Scores of CBCL Across Types of Disciplinary Practices

	Disciplinary practices				
	Reason	Cane	Reason + Cane	F	*P*
Withdrawn	3.94 (3.43)	4.92 (3.62)	3.38 (3.08)	1.74	ns
Somatic complaints	2.41 (2.80)	3.23 (2.79)	2.09 (2.65)	1.45	ns
Anxious/depressed	5.64 (5.15)	7.85 (5.99)	5.17 (4.47)	2.51	ns
Social problems	4.29 (3.27)	5.50 (3.06)	4.49 (2.64)	1.66	ns
Thought problems	2.37 (2.52)	2.77 (2.44)	2.36 (2.01)	0.32	ns
Attention problems	7.89 (4.69)	10.31 (3.42)	9.26 (3.72)	4.43	<0.05
Delinquent behaviour	2.48 (2.49)	4.15 (2.94)	3.47 (2.40)	6.53	<0.01
Aggressive behaviour	10.39 (7.42)	14.81 (7.87)	13.62 (8.09)	5.95	<0.01
Internalising problems	11.56 (9.23)	15.58 (10.06)	10.30 (7.66)	2.98	ns
Externalising problems	12.87 (9.48)	18.96 (10.24)	17.09 (9.81)	6.71	<0.01
Total problems	44.17 (27.35)	59.27 (29.08)	49.06 (24.21)	3.69	<0.05

ns: not significant
* Values in parentheses denote standard deviations

Discussion

Contrary to prevailing belief, caning was not the most commonly

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used form of disciplinary practice engaged by Asian parents; reasoning was found to be used most frequently in the present study, suggesting that Asian parents might not be as punitive as suggested by previous research on Asian parenting (with punitive being strictly defined as caning in the present context).

Past research showed that physical punishment tends to predict higher rates of externalizing problems.[6] This pattern of association was observed in the present study as well. This could be explained using the learning theory, in which children learned from the parents' caning that violence is a solution to interpersonal conflict. Over time, these children become aggressive too.

Interestingly, our finding further revealed that reasoning alone does not seem to be the best disciplinary practice across context, as parents who used a combination of reasoning and caning reported the least internalizing problems in their children, although this result did not reach statistical significance.

Conclusions

Our data shed some light on the effects of physical punishment on child behaviour and mental health in the Singaporean context, but this remains a complex issue. A significant lesson learnt is that different parental disciplinary practices might be related to different aspects of child well-being, and a clinical implication is that depending on the type of problems displayed by children, parental disciplinary methods must be adjusted accordingly.

The present study contained some limitations. By using a clinical sample, external validity is low. Furthermore, referral bias might have affected the informant ratings. In future studies, a non-referred sample like a community sample should be used. In addition, it may be useful to have a child self-rating form, which we did not have in the present study.

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Best Oral Presentation Award Finalist – Allied Health/Health Sciences/Paramedical Disciplines

EXTENDED ABSTRACTS

Anthropometric Indices as Screening Tools for Cardiovascular Risk Factors in Singaporeans: Receiver Operating Characteristic Curves Analysis

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Introduction

Obesity is an independent risk factor for cardiovascular diseases (CVD), and is strongly associated with risk factors like hypertension, dyslipidaemia and diabetes. From the public health perspective, it is important to define cut-off values for obesity indices such as body mass index (BMI), waist to hip ratio (WHR), and waist to stature ratio (WSR) to achieve effective screening. The World Health Organization (WHO) has recommended WHRs of 1.0 and 0.85 as obesity cut-off points for males and females, and BMI of $\geq$25kg/m^2 and $\geq$30kg/m^2, for overweight and obesity respectively.[1] In Asia, many studies have indicated the need to revise these values.[2-6] In Singapore, Deurenberg-Yap et al[5] demonstrated that for the same BMI, Singaporean adults displayed higher body fat percentages than Europeans. In another study, CVD risks were found to increase at lower BMI and WHR levels.[6] In these studies, however, no specific BMI and WHR cut-off values were proposed. Moreover, no cut-off values for WSR were recommended by the WHO, although it has been suggested that WSR may be the most appropriate screening tool.[2,3] Therefore, the purpose of this study was to delineate cut-off points of BMI, WHR, WSR in Singaporeans based on 3 CVD risk factors (hypertension, diabetes and dyslipidaemia).

Materials and Methods

The subjects were 730 hospital employees (61% Chinese, 27% Malays and 12% Indians), who underwent health screening after an overnight fast. Waist and hip circumferences (measured to nearest 0.1cm) were measured midway between the last rib and iliac crest, and over the greater trochanters, respectively. WHR and WSR were then calculated, while BMI was calculated as weight (kg) divided by height squared (m^2). Blood pressure (BP) was measured using a digital sphygmomanometer. Serum levels of total cholesterol (TC), high-density lipoprotein (HDL)-cholesterol triglycerides (TG), and blood glucose concentration were measured using enzymatic methods.

Hypertension was defined as systolic BP $\geq$140 mmHg and/or diastolic BP $\geq$90 mmHg. Dyslipidaemia was defined as TC $\geq$ 6.2 mmol/L, and/or elevated TC/HDL ratio $\geq$4.4, and/or TG $\geq$1.4 mmol/L. Type 2 diabetes mellitus was defined as fasting blood glucose $\geq$7.0 mmol/L. "At risk" was defined as having at least one risk factor.

Statistical Analysis

Using SPSS for Windows (v9.01), Receiver Operating Characteristic (ROC) curves were plotted for each index, and the optimal cut-off value was one that yielded the largest sum of sensitivity and specificity. Area

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Table 1. Anthropometric Indices and Cardiovascular Risk Factors by Gender and Ethnic Group (mean ± SD)

Variables	Males (n = 164)			Females (n = 566)		
	Chinese (n = 104)	Malay (n = 42)	Indian (n = 18)	Chinese (n = 337)	Malay (n = 157)	Indian (n = 72)
Age (y)	40.7 ± 12.0	42.2 ± 9.7	39.2 ± 11.1	39.0 ± 12.0	35.2 ± 12.0	39.4 ± 11.4
Weight (kg)	69 ± 12.8	70 ± 12.4	77 ± 17.9*	55.1 ± 8.9	60 ± 12.4*	63 ± 13.8*
Height (cm)	168.4 ± 6.9	165.9 ± 6.5	167.7 ± 9.9	157 ± 6.3	155 ± 6.2	155 ± 5.6
Waist (cm)	84.1 ± 10.1	84.6 ± 10.4	91.9 ± 13.3*	72.0 ± 8.5	76.0 ± 5.7*	81.0 ± 13.0*
Hip (cm)	96.8 ± 7.7	96.5 ± 7.6	101.9 ± 11.1*	93.2 ± 8.0	97.1 ± 9.9*	100.7 ± 11.6*
BMI (kg/m^2)	24.3 ± 4.2	25.4 ± 4.2	27.5 ± 6.8*	22.2 ± 5.5	24.8 ± 5.2*	26.2 ± 5.4*
WHR	0.87 ± 0.05	0.87 ± 0.05	0.90 ± 0.05*	0.77 ± 0.05	0.78 ± 0.06*	0.80 ± 0.07*
WSR	0.50 ± 0.06	0.51 ± 0.07	0.55 ± 0.08*	0.46 ± 0.06	0.49 ± 0.07*	0.52 ± 0.08*
TC (mmol/L)	5.5 ± 0.93	6.1 ± 1.1*	5.7 ± 1.4	5.5 ± 1.0	5.4 ± 0.9	5.6 ± 0.9
HDL (mmol/L)	1.5 ± 0.33	1.3 ± 0.31*	1.2 ± 0.30*	1.9 ± 0.43	1.7 ± 0.39*	1.5 ± 0.42*
LDL (mmol/L)	3.3 ± 1.0	3.8 ± 1.1*	3.5 ± 1.5	3.2 ± 0.9	3.2 ± 0.9	3.6 ± 0.8*
TG (mmol/L)	1.4 ± 0.89	2.0 ± 1.5*	2.0 ± 2.5*	1.1 ± 0.74	1.1 ± 0.72	1.2 ± 0.56*
TC/HDL	3.9 ± 1.0	4.8 ± 1.5*	5.0 ± 2.0*	3.0 ± 0.8	3.4 ± 0.9*	3.9 ± 1.2*
SBP (mmHg)	126.9 ± 14.4	126.1 ± 14.8	122.7 ± 12.1	115.3 ± 14.8	117.0 ± 15.1	117.7 ± 15.5
DBP (mmHg)	80.1 ± 10.3	82.0 ± 11.2	77.7 ± 9.5	74.1 ± 9.3	75.0 ± 9.3	75.7 ± 9.7
Glucose (mmol/L)	5.4 ± 0.95	5.9 ± 2.3	5.1 ± 0.49	5.1 ± 0.73	5.4 ± 2.1*	5.6 ± 1.6*
Hypertension (%)	24.0	19.0	16.7	10.2	10.2	11.1
Dyslipidaemia (%)	49.0	81.0*	66.7*	32.3	35.7	45.8
Diabetes (%)	4.8	11.9	0	3.3	6.4	9.7
Risk (%)	56.7	81.0*	66.7*	37.4	39.5	55.6*

BMI: body mass index; DBP: diastolic blood pressure; glucose, fasting plasma glucose; HDL: high-density lipoprotein cholesterol; Hip: hip circumference; LDL: low-density lipoprotein cholesterol; Risk: at least one CVD risk factor (hypertension or diabetes or dyslipidaemia); SBP: systolic blood pressure; TC: total cholesterol; TG: fasting triglycerides; Waist: waist circumference; WHR: waist-to-hip ratio; WSR: waist-to-stature ratio
*Significant differences in means between ethnic groups (age-adjusted for all variables, Chinese as reference category).

Table 2. Optimal Cut-off Values, Sensitivity, Specificity, and Area Under Curve of Various Anthropometric Indices to Predict Hypertension, Type II Diabetes Mellitus, Dyslipidaemia Based on ROC Analysis

Indices	CVDRisk Factor	Males (n = 164)				Females (n = 566)			
		Cut-off	Sens.(%)	Spec.(%)	AUC(95%CI)	Cut-off	Sens.(%)	Spec.(%)	AUC(95%CI)
BMI (kg/m^2)	Hypertension	24.8	64.5	63.5	0.67 (0.57-0.76)	23.4	83.3	61.3	0.76 (0.70-0.80)
	Dyslipidaemia	24.2	68.5	61.7	0.69 (0.60-0.77)	23.9	55.0	71.7	0.66 (0.61-0.71)
	Diabetes	24.1	90.0	44.5	0.67 (0.50-0.83)	23.2	96.4	57.4	0.80 (0.74-0.86)
	Risk	24.2	67.4	64.3	0.72 (0.63-0.80)	23.6	59.6	73.1	0.69 (0.65-0.74)
WHR	Hypertension	0.89	69.4	64.8	0.67 (0.56-0.77)	0.80	65.0	74.3	0.74 (0.68-0.80)
	Dyslipidaemia	0.86	73.2	53.7	0.64 (0.55-0.73)	0.80	54.0	74.7	0.67 (0.62-0.71)
	Diabetes	0.85	90.0	30.5	0.69 (0.55-0.84)	0.82	71.4	79.4	0.81 (0.74-0.89)
	Risk	0.86	72.2	57.1	0.67 (0.58-0.76)	0.80	54.4	77.5	0.69 (0.65-0.74)
WSR	Hypertension	0.48	91.7	39.1	0.67 (0.58-0.77)	0.51	68.3	77.9	0.79 (0.73-0.84)
	Dyslipidaemia	0.50	63.9	68.7	0.69 (0.60-0.77)	0.48	62.6	71.7	0.70 (0.65-0.75)
	Diabetes	0.50	80.0	48.1	0.61 (0.44-0.79)	0.50	92.8	68.4	0.84 (0.78-0.89)
	Risk	0.48	80.5	57.1	0.72 (0.63-0.80)	0.48	64.5	76.0	0.73 (0.69-0.78)

AUC: area under curves; BMI: body mass index; Risk: at least one CVD risk factor (hypertension or diabetes or dyslipidaemia); Sens: Sensitivity; Spec: Specificity; WHR: waist-to-hip ratio; WSR: waist-to-stature ratio

under the curve (AUC) and forward multiple regression analysis were applied to determine the best index. Adjustment was made for age, ethnicity, smoking status, and physical activity levels. Alpha level was set at 0.05 (2-tailed).

Results

The subjects' anthropometric and metabolic characteristics are shown in Table 1. The cut-off values of the indices are listed in Table 2. As an overall estimation, the cut-off values of BMI, WHR and WSR in predicting at least one risk factor were 24.2 kg/m^2, 0.86 and 0.48 in men, and 23.6 kg/m^2, 0.80 and 0.48 respectively in women. Including ties, the AUC of WSR was highest for all 3 risk factors in females (0.79 for hypertension, 0.70 for dyslipidaemia, 0.84 for diabetes mellitus), and 2 risk factors in males (0.67 for hypertension, 0.69 dyslipidaemia). As an overall estimation, WSR had the largest AUC for the presence of at least one CVD risk factor in both males and females (0.72 and 0.73 respectively).

All 3 indices were significantly inter-correlated (P <0.001). In particular, strong and positive correlations were found between WSR and the other 2 indices (r = 0.89 and 0.79 with BMI and WHR respectively in males, 0.85 with BMI in females). Results of regression analysis revealed that WSR was independently associated with all risk factors in females, and dyslipidaemia in males, while BMI was associated with hypertension and diabetes in males.

Discussion

Our study indicated that the cut-off values of BMI and WHR are lower than those recommended by the WHO[1]. Applying the WHO's BMI cut-off points for overweight and obesity, the specificity of BMI in predicting risk factors was more than 80%, but the sensitivity was less than 50%. The WHO recommends WHRs of 1.0 and 0.85 for males and females respectively, resulting in a high specificity (>90%) and a low attendant sensitivity (<15%) in our study. Taken together, these results further indicate that the cut-off values by WHO are inappropriately high for Singaporeans.

Few studies have examined the association between CVD risk factors and obesity indices at various cut-off values in Singaporeans. In Deurenberg-Yap's study,[6] it was found that at low categories of BMI (22-24kg/m^2) and WHR (0.80-0.85 for women, 0.90-0.95 for

men), the odds ratios (ranging from 1.97 to 4.38) for having at least one risk factor were significantly higher compared to the reference category. Using ROC analysis, our results are consistent with that of Deurenberg-Yap's,[6] and extend their findings to include specific BMI cut-off values at 24.2 and 23.6 kg/m^2, WHR cut-off values at 0.86 and 0.80, and WSR cut-off values of 0.48 for men and women respectively.

In relation to predicting at least one risk factor, WSR was the only index with AUC greater than 0.70 (0.72 and 0.73 in men and women, respectively). Using regression analysis, WSR was selected as the main predictor for both sexes. Moreover, the cut-off value of 0.48 was the same for both genders. Taken together, these results indicate that WSR may the most appropriate screening tool.

Comparing our results with that of other studies performed in Asia, the cut-off values in relation to each risk factor were comparable with those obtained in Japanese men,[4] Hong Kong Chinese[2] and Taiwanese.[3] However, our results were apparently higher than values obtained from Japanese women. In our study and that of others,[2,3] the cut-off point was one that yielded the largest sum of sensitivity and specificity. In contrast, Ito et al[4] determined cut-off values at a point where sensitivity equated to specificity. Notwithstanding the methodological differences, we believe our study has provided convergent evidence to support recent revisions of obesity indices to represent over-weight for Asians.

Conclusion

The cut-off points of anthropometric indices in Singaporean adults were lower than the WHO criteria, but were in agreement with those reported for Asians.[2-4] The WSR may be the best screening tool, and such a conclusion awaits further verification in prospective or longitudinal epidemiological studies on morbidity and mortality.

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EXTENDED ABSTRACTS

Best Oral Presentation Award Finalist – Allied Health/Health Sciences/Paramedical Disciplines

CC3/TIP30 Expression was Strongly Associated with HER-2/*NEU* Status in Breast Cancer

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Introduction

Cancer metastasis is a complicated process in tumour development. It involves up- and down-regulation of genes in malignant tumour cells. A human gene, *CC3*, has been identified as a novel metastasis suppressor gene of variant small-cell lung carcinoma (v-SCLC).[1] This gene is identical to the transcription cofactor, *TIP30*, which can bind to the human immunodeficiency virus (HIV) Tat protein and initiates Tat-activated transcription.[2] Overexpression of the *CC3/TIP30* gene resulted in suppression of the metastatic potential of v-SCLC cells in SCID-hu mice and of mouse melanoma. *CC3/TIP30* also inhibited angiogenic properties of tumour cells by inducing changes in the RNA level of angiogenic modulators.[3] This led to the proposal that *CC3/TIP30* functions as a metastasis suppressor gene, linking the control of apoptosis to metastasis. However, its expression profile in clinical specimens, especially in breast cancer, has not been reported.

In this study, we investigated the differential expression of *CC3/TIP30* in breast tumour and matched normal tissues. We observed that overexpression of *CC3/TIP30* was mostly found in HER-2/*neu* positive breast carcinoma. Our results may indicate the potential involvement of *CC3/TIP30* in the enhanced breast tumour differentiation, proliferation and invasion caused by the HER-2/*neu* proto-oncogene.

Materials and Methods

Total RNA was extracted from 43 pairs of frozen breast tumour/normal tissues with previously characterized HER-2/*neu* status.[4] The relative levels of *CC3/TIP30* and β-actin were determined by semi-quantitative RT-PCR and reverse-phase protein array. The latter was performed to ascertain whether *CC3/TIP30* was differentially expressed at protein level in the two subtypes of breast cancer. Immunohistochemical staining of sections from a breast cancer tissue microarray containing 97 breast tumours and matched normal

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tissues[5] were carried out to study the expression of the *CC3/TIP30* protein and its potential association with various clinicopathological parameters. Full length *CC3/TIP30* cDNA was amplified and sequenced for mutation detection. SPSS v12.0 software and Fisher's exact test were used for statistical analysis. A value of $P < 0.05$ was considered statistically significant.

Results

RT-PCR. *CC3/TIP30* was successfully amplified in 39 pairs of tissues and its relative expression levels were normalized with β-actin. The mean value of relative expression in these 43 cases was defined as 0.17 (95%CI: 0.11-0.24) for all the matched normal tissues (N = 39), 0.57 (95%CI: 0.41-0.73) for HER-2/*neu* negative tumour tissues (N = 29) and 1.08 (95%CI: 0.91-1.25) for the HER-2/*neu* positive tumour tissues (N = 10). In Figure 1 (panel I), the overall relative expression level of *CC3/TIP30* in the cohort of HER-2/*neu* positive tumours was significantly higher than that in the HER-2/*neu* negative tumours and normal tissues ($P = 0.023$).

Reverse-phase protein array. Total proteins from 10 HER-2/*neu* positive and 20 -negative tumour tissues and 20 normal tissues were spotted onto the PVDF membrane and the relative expression of *CC3/TIP30* to β-actin were analyzed. As shown in Figure 1 (panel II), *CC3/TIP30* was significantly overexpressed in HER-2/*neu* positive breast tumours ($P = 0.016$).

Immunohistochemical staining of breast tissue microarray. No staining was detected in normal tissues. In the tumour tissues, *CC3/TIP30* was stained only in cytoplasm (Fig. 2). Only 68 cases with *CC3/TIP30* staining status were available for analysis. In the 17 HER-2/*neu* positive tumours, 7 cases were stained strongly, 5 moderately and 5 weakly, whereas in 51 HER-2/*neu* negative tumours, 7 cases were strongly stained, 16 moderately and 28 weakly and/or no staining ($P = 0.027$). There were no significant associations with ER, PR, lymph node and tumour grade and size.

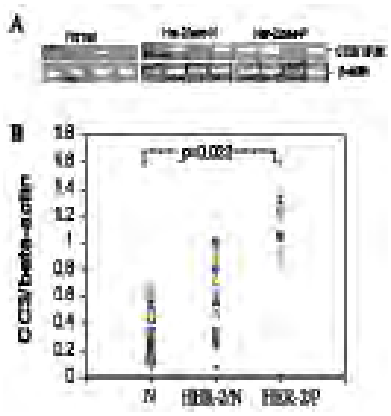
Full length cDNA sequencing. To understand whether the abnormal expression of *CC3/TIP30* was caused by mutations, full length cDNA from 5 cases with high expression and 5 cases with low expression level of *CC3/TIP30* and 8 normal cases were amplified and sequenced. Four single nucleotide polymorphisms in the *CC3* coding region (listed in the SNP database) were excluded when comparing the sequence with *CC3/TIP30* cDNA. Of these 18 cases, no insertion, deletion and single bp mutations which cause amino acid substitutions were found in the coding region.

Discussion

In this study, we observed that *CC3/TIP30* is mostly overexpressed in HER-2/*neu* positive breast carcinomas at both transcription and translation levels. To our knowledge, this is the first finding that shows *CC3/TIP30* expression associated with HER-2/*neu* status in breast cancer. In contrast to published results, our findings may implicate that in breast carcinoma, *CC3/TIP30* has different functions in primary and metastatic tumour cells or that its functions could be tumour-dependent or that its cellular functions could depend on the balance between the regulated pro- and anti-apoptotic molecules.

Our present data demonstrated a possible association of *CC3/TIP30* with the HER-2/*neu* oncogene or its downstream signal pathway. HER-2/*neu* is an established adverse prognostic factor in breast cancer and is strongly associated with a more aggressive phenotype HER-2/*neu* activation also initiates signaling cascades

I



II

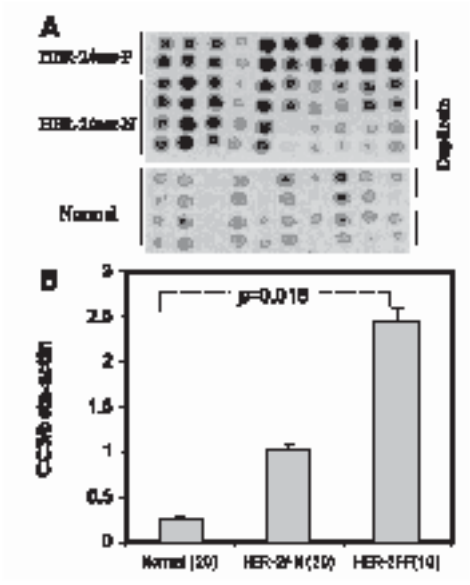
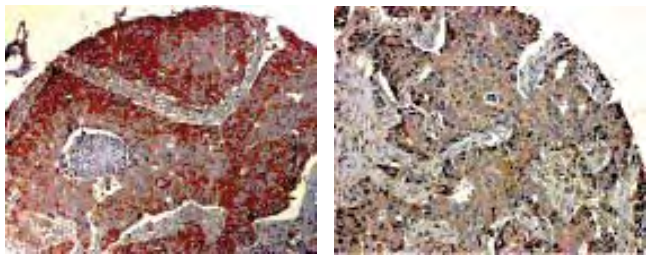
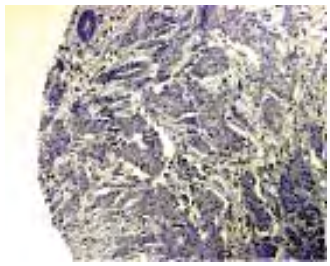


Fig. 1. Reverse transcription (RT)-PCR (Panel I) and Reverse phase protein array (Panel II) of CC3/TIP30.
(Panel I) A representative amplification of CC3/TIP30 *(top) and expression level of* CC3/TIP30 *in normal and tumour tissues.*
(Panel II) Signal detection of CC3 *protein (top) and relative to β-actin.*



(A) *(B)*



(C)

Fig. 2. Immunohistochemistry staining of CC3/TIP30 *in breast cancer tissue microarray showing strong staining (A), moderate staining (B) and negative staining (C) respectively. (Original magnification x200)*

EXTENDED ABSTRACTS

including the MAPK and PI3K/AKT pathways that are essential for cell proliferation and differentiation. The finding that *CC3* was overexpressed in the TGF-b1-treated T84 cells implied the possible function of CC3 in cell differentiation. However, the biological function of *CC3/TIP30* and its connection with HER-2/*neu*-related signal pathways in breast cancer remain elusive.

Based on the immunohistochemical staining, we observed that *CC3/TIP30* was present only in the cytoplasm. We did not find any cases showing nuclear staining. To understand whether the cytoplasmic localization of CC3 was due to the site mutation at codon position 106 (R to H) as reported [6], we amplified and sequenced the full cDNA of *CC3/TIP30*. No mutations, insertions and deletions were found in these limited specimens, hence more breast carcinoma cases may need to be screened.

Conclusion

Our data showed that *CC3/TIP30* expression is strongly associated with HER-2/*neu* oncogene in breast cancer. Cytoplasmic localization of *CC3/TIP30* may indicate the functional variance of *CC3/TIP30* in breast cancer with SCLC and other tumour types. The identification of differentially expressed gene and/or protein profiles initiated by *CC3/TIP30* in breast cancer cell lines will unravel, at least in part, the molecular events that may provide novel cross-talk between the signal networks and contribute to a better understanding of *CC3/TIP30*-mediated cell behavior. The association between increased expression of *CC3/TIP30* and HER-2/*neu* positivity presents an interesting paradox.

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Best Oral Presentation Award Finalist – General Practice

A KAP Survey of Evidence-Based Medicine and Clinical Practice Guidelines Among Primary Care Doctors in Singapore

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EXTENDED ABSTRACTS

Introduction

Primary healthcare doctors working in the National Healthcare Group Polyclinics and also private General Practitioners who are affiliated to the National Healthcare Group (NHG) have been using a NHG Clinical Practice Guidelines (CPGs) in the form of a flipchart to guide them on the management of the common diseases encountered in the primary health care setting. The content of this flipchart were adapted mainly from MOH's clinical practice guidelines and other sources of evidence based medicine. Clinical practice guidelines have proven to be useful in the practice of evidence based medicine in primary healthcare.[1]

This flipchart was developed to guide the busy primary healthcare doctors to practice evidence-based medicine in a practical way, to ensure their patients have the most appropriate quality of care, based on the latest evidence available.

This was the first survey on the flipchart since it has been published by NHG in 2002. The result of this survey will help the organisation in revising the flipchart to be more user friendly, if necessary.

The objective of this study was to determine the usefulness of these Clinical Practice Guidelines in the primary healthcare doctors' clinical practice and their attitude towards evidence-based medicine.

Materials and Methods

There were 2 separate surveys carried out for this study. The first survey was to all 130 NHG Polyclinics doctors. The self-administered survey forms were distributed to all the doctors though the doctors-in-charge of the nine NHG polyclinics. Response to the survey was voluntary and anonymous. Each clinic collected all the completed survey forms and sent back to the researchers at the end of the survey week.

Concurrently, a second survey was carried out among the GPs who were under the NHG Partners programme. As the researchers required about 100 respondents from the GPs, a randomized list of GPs was generated for this selection process. This was followed by a telephone-administered interview using the same survey form.

The reason to use a telephone administer interview for the GPs instead of a mailed survey form like to the polyclinic doctors was to ensure the response rate to be high and comparable to the polyclinic doctors. This was because based from previous experience; the response rate for self-administered mailed surveys among the GPs was low and thus increased the risk of response bias in the result.

A trained staff conducted the telephone interviews over a period of 2 weeks. The response from the GPs was also purely voluntary and anonymous. If a GP could not contactable for three times on different occasions, it would be deemed as "non-respondent".

At the end of the study period, all questionnaires were centrally collected and audited for completeness and consistency, so that discrepancies and incomplete responses were corrected immediately.

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Responses elicited were encoded and data entered in a database. Scores were assigned after data entry. Statistical package like SPSS was used for univariate, bivariate and regression analysis.

The surveys did not involve any patient's personal information from the doctors. As such, there was no ethical issue to the patients and the doctors. This study merely to obtain feedback on the usefulness of this clinical practice guideline flipchart and to assess the level of evidence-based medicine practiced by the primary healthcare doctors. Approval from the institution's ethics board had also been obtained.

Results

A total of 114 GPs and 74 polyclinic doctors responded to the survey, making the response rate to be 45.4% and 56.9%, respectively. 35.1% of the GPs and 44.8% of the polyclinic doctors have postgraduate qualifications. The respondents work experience varies widely from those who had graduated with basic medical degree more than 40 years ago to those who had just graduated in the last few years.

95% of the polyclinic doctors found the flipcharts useful, compared to only 54.1% of the GPs (P <0.05) (Table 1). Both groups agreed that the use of evidence-based medicine improved patient care in their clinical practice (median score of 8 from a scale of 1 to 10 for both groups, 10 being total agreement).

Those who have favorable attitude towards clinical practice guidelines (score 6 and above out of 10) used the flipchart more often than those who have less favorable attitude. Work experience has no correlation to the use of this flipchart.

Table 1. Percentage of Doctors Who Found the Flipcharts Useful

	Number of doctors	Number who found the flipcharts useful (%)	P
Polyclinic doctors	75	71 (95%)	P <0.05
General practitioners	114	47 (54%)	
Total	189	118	

Discussion

The response rate of about 50% in both surveys was acceptable and comparable to similar surveys conducted previously among primary healthcare doctors in Singapore. The issue of selection biased was minimized with this high response rate.

Although both groups of doctors agreed that the use of evidence-based medicine were important in their clinical practice, GPs in the private practice found the NHG flipchart less useful than their counterparts working in the polyclinics. This could be due to the polyclinic doctors were subjected to regular clinical audits and the flipchart was a useful tool to help them manage patients according to the audit criteria. In a study conducted in UK, it was also found that doctors who were subjected to clinical audits would use clinical practice guidelines more often than those who were not.[2]

Those doctors who have favorable attitude towards the use of clinical practice guidelines were more willing to use the flipchart than those who do not. Hence, it is important to educate the doctors on the importance and usefulness of clinical practice guidelines to improve the use of this flipchart, which would translate into better care for the patients. Alternate forms of dissemination of these evidence based medicine information like through e-mail could also be considered in the future.[3]

Acknowledgements

The authors would like to thank A/Prof Shanta Emmanuel, CEO, NHG Polyclinics for allowing them to conduct this survey among the polyclinic doctors and also to NHG Partners for providing a list of GPs for the researchers to interview.

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Best Oral Presentation Award Finalist – General Practice

Attitudes and Perceptions of Physicians to Genetic Testing and Establishment of Genetic Database

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Introduction

The goals of the Human Genome Project are to identify all the genes in human genome and determine the sequence of the 3 billion chemical base pairs that make up the human DNA. Researchers worldwide are now using this data to devise creative applications in an expanding array of fields. However, it has also brought much disquiet and controversies; individuals and society are now beginning to confront complex ethical and policy issues which, include concepts of anonymity, banking of DNA samples, and the commercialization of genes.[1]

The medical profession in most societies is entrusted with the task of looking after the health of its people and safeguarding the interests of patients and the successful implementation of a health initiative (both clinical and research) depends on the support of the medical

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profession. The objectives of this study were to determine the attitudes and perceptions among physicians in Singapore towards genetic testing and the establishment of genetic databanks.

Materials and Methods

Four thousand medical practitioners who were on the mailing list of the Singapore Medical Council were mailed a questionnaire and a covering letter explaining the purpose of the study. They were asked to fill the self–reporting questionnaire adapted from one developed by Hietala et al[2] and to mail it back to the research institute. This questionnaire consists of 18 self-rating Likert type statements on the following topics (A) attitudes towards gene testing, (B) attitudes towards genetic databanks, and (C) reasons for or against genetic testing. Data from the answered questionnaires was collected and analysed using SPSS v.10 software. Frequency distribution was tested using the c^2 test.

Results

Of the 4000 physicians, 554 (14%) responded. The sociodemographic factors of the respondents are shown in Table 1.

Attitudes Towards Gene Testing

While 84 % of the respondents agree that genetic testing should be made available to those who wish to have information about disease gene they carry, only 20% felt that gene testing should be performed on new- born babies. The majority disagreed with the statement that genetic testing should not be performed at all.

Attitudes Towards Genetic Databanks

Majority of the respondents agreed that genetic banks should be established for all genetic diseases and that they would also personally contribute their genetic material for the establishment of a genetic bank if they or their relatives were suffering from the disease. Most were concerned that the investigators or institutes would derive financial benefits from the genetic bank. Over half of the respondents (67%) agree that the data should be completely anonymised, but more (88%) were of the opinion that donors should be given access to the results of the genotyping.

Table 1. Characteristics of the Group Surveyed for Attitudes and Perceptions to Genetic Database and Genetic Testing (n = 554)

		n	%
Sex	Female	169	30.5
	Male	385	69.5
Age (y)	20-30	136	24.5
	31-40	179	32.3
	41-50	98	17.7
	51-60	87	15.7
	61 and above	52	9.4
Ethnicity	Chinese	506	91.3
	Malay	8	1.4
	Indian	30	5.4
	Others	10	1.8
Religion	Buddhism	55	9.9
	Hinduism	17	3.1
	Islam	13	2.3
	Taoism	3	0.5
	Christianity	330	59.6
	Others	59	10.6
	Atheist	77	13.9
Medical discipline	Surgery	65	11.7
	Psychiatry	26	4.7
	General practitioners	336	60.6
	Obstetrics and gynaecology	21	3.8
	Paediatrics	23	4.2
	Others	6	1.1

Reasons For or Against Genetic Testing

While most (89%) of the respondents felt that people have the right to know about the genes that affect their health as well as that of their children, the majority have concerns about the ethical and legal aspects of genetic testing and genetic banks (Table 2). Eighty-three percent felt that knowledge may lead to discrimination against disease gene carriers, 94% felt that patient's insurance would be jeopardized and 88% were concerned that unexpected family relationships may show up. Just over half (56%) felt that society would save on the cost of treatments as a result of genetic testing.

Discussion

The current survey indicates that the physicians in Singapore have a positive attitude towards genetic testing. The autonomy of the person and the right to self-determination is something which the majority of physicians uphold. The vast majority of respondents was also in favour of establishing a genetic bank and indicated that they would personally donate their own samples. This is not surprising in the case of the physicians practicing in Singapore as the various local pathology departments have long stored tissues samples from autopsies and biopsies, and their training and clinical experience are probably important in influencing their positive perception of such facilities. However, most doctors favour anonymization, expressing concerns about the ramification of personal clinical data being made known to other parties which ranged from fears of discrimination from insurance companies and others, and breakup of relationships. While the majority felt that data should be completely anonymized, they also felt that donors should have access to the results of any

Table 2. Reasons For or Against Genetic Testing

	n	%
Society would save on the costs of treatment of diseases form genetic testing		
Agree	308	55.6
Disagree	138	24.9
Do not know	106	19.1
People have the right to know about their genes which can affect their health and lives		
Agree	496	89.5
Disagree	31	5.6
Do not know	24	4.3
There are more significant public health care problems that must be taken care of first		
Agree	382	69.0
Disagree	104	18.8
Do not know	66	11.9
Knowledge of results may lead to discrimination against disease gene carriers		
Agree	461	83.2
Disagree	66	11.9
Do not know	26	4.7
Patients' insurance coverage could be jeopardized		
Agree	520	93.9
Disagree	12	2.2
Do not know	22	4.0
The genetic material may be used for other tests without the knowledge of the person concerned		
Agree	406	73.3
Disagree	115	20.8
Do not know	32	5.8
The results may end up in the hands of 'outsiders'		
Agree	448	80.9
Disagree	49	8.8
Do not know	55	9.9

subsequent genotyping. The latter would be impossible if there is complete anonymization where the DNA is stripped of its identifiers so that it could not be traced back to the individual person. This apparent contradictory attitude may be due to the inadequacies of the questionnaire which did not explore the degrees of protection that are available to protect the privacy of the donor (for example, the protection of the individual identifying data by independent third parties). This study demonstrates that the physicians in Singapore on the whole favour genetic testing but are concerned about the ethics of such research activities. The continued education and dissemination of information for physicians is important especially with the rapid advance in scientific knowledge which at times outpaces the existing societal ethical and legal norms. It has been suggested that the education of health professionals should also focus on societal, ethical, religious and political issues.[3] A mechanism must in put in place to ensure this as well as to provide a just system for genetic research where the interests of the community is protected without stifling vital research and the development of the biotechnology industry.

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Best Oral Presentation Award Finalist – Medicine/Paediatrics (Clinical Based)

Psychometric Properties of a New Systemic Lupus Erythematosus-Specific Quality-of-Life Instrument (SLEQOL)

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Introduction

Systemic lupus erythematosus (SLE), a chronic autoimmune illness that usually begins in early adulthood, affects multiple organ systems and may be associated with considerable morbidity and mortality. The onset of complications and response to treatment are generally unpredictable. Thus, SLE impacts the quality of life (QOL) in unique ways and merely measuring the objective outcomes of morbidity and mortality is insufficient.[1] General health instruments have been used to assess QOL in SLE, but disease-specific scales may show greater responsiveness and content validity. We have developed such an instrument (SLEQOL) and we now present its psychometric properties.

Materials and Methods

We developed SLEQOL from scratch. Briefly, a team of Rheumatologists and Nurse Clinicians generated the initial list of 51 QOL items in English. The draft questionnaire was administered to 100 patients. Their responses were subjected to Rasch model and factor analysis and expert review to reduce the item number. SLEQOL was assembled from the reduced list of 40 items, which were divided into 6 subsections.

SLEQOL was administered to a cohort of SLE patients. Data was captured systematically during every study visit. In addition, the SLE Disease Activity Index (SLEDAI), Systemic Lupus Activity Measure (SLAM), Rheumatology Attitudes Index (RAI), MOS 36-Item Short-Form Health Survey (SF-36) were recorded. Patients with recent disease onset within three years were interviewed every three months.

The factors were extracted by principal component analysis and subjected to varimax rotation. We analysed the data with the SPSS Professional Statistics 8.0. We performed Rasch model analysis with BIGSTEPS, a program available at www.winsteps.com. Test-retest reliability was evaluated with the intra-class correlation coefficient (ICC). There is no single best responsiveness statistic for assessing HRQL instruments, so we used four responsiveness statistics to assess the evaluative property of SLEQOL and SF-36 with regards to SLE: Liang's relative efficacy (RE),[2] Liang's standardized response mean (SRM),[3] Kazis' effect size (ES)[4] and Guyatt's coefficient (GC).[5]

Results

In this cohort of 275 patients, there were 249 females and 26 males, with a mean age was 40.1 ± 13.4 years. The mean age of diagnosis was 31.6 ± 15.2 years. Prospective data at 3-month intervals were obtained from 95 patients and test-retest data from 35.

Face and Content Validity

By inviting 16 Rheumatologists and Rheumatology Nurses and 100 patients to contribute to the initial 51 items, we believe that there had been reasonable face and content validities. The correlation matrix of the SLEQOL against the SF-36, RAI and its helplessness subscale, and the lupus activity scores (Table 1) demonstrates that was minimal association between the SLEQOL and SF-36, SLEDAI or SLAM.

Internal Consistency

Overall, SLEQOL has Cronbach's alpha of 0.95. Cronbach's

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Table 1. The Cross-sectional Correlation Between the SLEQOL (and its Subsections) and SF-36, Rheumatology Attitudes Index (and its Helplessness Index) and the 2 SLE Activity Indices SLEDAI and SLAM

		SLEQOL						
		Summary score	Subsection 1	Subsection 2	Subsection 3	Subsection 4	Subsection 5	Subsection 6
SF-36	Physical functioning	0.112	0.234	0.069	0.066	0.066	0.055	0.042
	Role physical	0.140	0.139	0.106	0.129	0.087	0.067	0.044
	Bodily pain	0.171	0.171	0.098	0.159	0.084	0.090	0.084
	General health	0.127	0.090	0.058	0.112	0.063	0.102	0.080
	Vitality	0.140	0.059	0.087	0.134	0.043	0.127	0.082
	Social functioning	0.107	0.069	0.087	0.059	0.042	0.108	0.055
	Role emotional	0.061	0.056	0.029	0.045	0.030	0.076	0.030
	Mental health	0.125	0.034	0.071	0.096	0.045	0.171	0.082
Rheumatology Attitudes Index		0.081	0.042	0.016	0.068	0.041	0.053	0.110
Helplessness subscale		0.115	0.053	0.048	0.078	0.046	0.099	0.119
SLEDAI		0.022	0.003	0.018	0.025	0.013	0.010	0.011
SLAM		0.018	0.016	0.010	0.010	0.029	0.007	0.009

Table 2. Rasch Analysis of the 40-item SLEQOL Based on Data Provided by 275 SLE Patients. The Items are Ranked by the Degree of Difficulty, with the Easiest at the Top of the Table

No.	Item	Item Measure	INFIT mean squares	INFIT zstd	OUTFIT mean squares	OUTFIT zstd
2	Shopping	1.01	1.66	2.4	0.74	-0.9
4	Marketing	0.92	1.4	1.6	0.76	-0.9
39	Fears bad news from doctor	0.71	1.59	2.7	1.61	2
40	Consuming more alcohol or tobacco	0.51	1.04	0.3	0.78	-1
3	Turning taps on and off	0.46	1.17	1.1	1.31	1.3
1	Walking outdoors on level ground	0.41	1.06	0.4	0.86	-0.7
32	Wishing others did not know that I have SLE	0.36	1.18	1.2	0.87	-0.6
20	Itchy skin	0.3	1.24	1.6	1.12	0.6
11	Sports	0.25	1.01	0	0.79	-1.1
34	Low self-esteem	0.24	1.04	0.3	0.88	-0.6
24	Fear of needles	0.15	0.89	-1	0.96	-0.2
16	Poor memory	0.14	0.74	-2.4	0.74	-1.5
21	Sore mouth	0.12	1.1	0.9	0.88	-0.7
25	Dietary restrictions	0.12	1.11	1	1.14	0.7
23	Joint pain and swelling	0.08	1.64	4.8	2.4	5.7
33	Friends and colleagues made fun of me	0.05	0.91	-0.9	0.73	-1.7
12	Sex	0	0.86	-1.4	0.72	-1.8
31	Anxiety	-0.01	1.39	3.3	1.77	3.7
9	Missed work or school	-0.03	1.11	1	0.84	-1
27	Inconvenience of frequent clinic visits	-0.04	0.74	-2.8	0.63	-2.6
8	Career or education interference	-0.06	1.09	0.9	0.91	-0.5
7	Work and school performance affected	-0.08	0.9	-1	0.79	-1.4
6	Three km	-0.1	1	0	0.96	-0.2
19	Concentration	-0.1	1.14	1.4	1.4	2.2
26	Inconvenience of daily medications	-0.11	1.05	0.5	1.24	1.4
10	Difficult relationship with friends and relations	-0.11	0.98	-0.2	1.72	3.7
30	Depression	-0.13	0.67	-3.9	0.63	-2.7
29	Feeling low	-0.18	0.67	-4	0.71	-2.1
5	Bathing and drying	-0.18	1.29	2.8	2.23	6
28	Self-consciousness	-0.23	0.66	-4.3	0.63	-2.9
36	Concern about being financial burden to the family	-0.28	1.22	2.3	1.09	0.6
18	Fatigue	-0.31	0.58	-5.7	0.6	-3.4
15	Loss of income	-0.31	0.78	-2.7	0.83	-1.3
22	Sore, painful or stinging skin	-0.32	1.03	0.4	0.93	-0.5
14	Sun	-0.38	1.14	1.5	1.19	1.3
17	Loss appetite	-0.46	0.66	-4.6	0.85	-1.2
13	Social activities	-0.57	1.42	4.5	1.81	5.3
38	Side effects of medicines	-0.58	1.39	4.2	1.33	2.4
37	Medicines don't work	-0.6	1.24	2.7	1.28	2.1
35	Embarrassment	-0.65	1.2	2.2	1.1	0.8

alpha was 0.85 for subsection 1, 0.90 for subsection 2, 0.89 for subsection 3, 0.76 for subsection 4, 0.93 for subsection 5 and 0.86 for subsection 6.

The items in SLEQOL resolve into 8 domains on factor analysis: social and occupational activities, mood and self-image, physical functioning, physical symptoms, self-esteem and the unpredictability of the illness and its response to treatment.

Floor and Ceiling Effect

The ceiling or floor effect occurs when patients perceive that their condition has improved or deteriorated, respectively, beyond what a QOL questionnaire can measure. The floor effect was more substantial than the ceiling effect in SLEQOL, while the reverse was true for SF-36 (data not shown).

Rasch Analysis of SLEQOL

The Rasch model analysis of SLEQOL is shown in Table 2. The most difficult items for the patients, in decreasing order, were embarrassment, worry that medications do not work, worry about side-effects of medicines and difficulty with social activities. The easiest ones were, in decreasing order, shopping, marketing and fear of receiving bad news from doctors.

Table 3. Responsiveness of the SLEQOL and SF-36. The 4 Responsiveness Statistics Showed Similar Results

Responsiveness statistic		SLEQOL							SF-36							
		Summary score	Subsection 1	Subsection 2	Subsection 3	Subsection 4	Subsection 5	Subsection 6	Physical Functioning	Role Physical	Bodily Pain	General Health	Vitality	Social Functioning	Role Emotional	Mental Health
Liang's standardised response mean	Overall QOL improved	0.44	0.31	0.51	0.59	0.23	0.37	0.23	0.00	0.11	0.13	0.31	0.11	0.21	0.13	0.12
	Overall QOL unchanged	0.21	0.13	0.27	0.00	0.05	0.07	0.16	0.29	-0.08	-0.08	-0.10	0.01	0.02	0.02	-0.01
Kazis' effect size	Overall QOL improved	0.33	0.29	0.39	0.48	0.25	0.33	0.19	0.00	0.11	0.12	0.26	0.11	0.20	0.14	0.12
	Overall QOL unchanged	0.15	0.12	0.19	0.00	0.04	0.07	0.17	0.20	-0.08	-0.07	-0.09	0.01	0.01	0.02	-0.01
Guyatt's coefficient	Overall QOL improved	0.37	0.31	0.43	0.57	0.22	0.35	0.22	0.00	0.12	0.12	0.26	0.11	0.18	0.15	0.12
	Overall QOL unchanged	0.15	0.12	0.19	0.00	0.04	0.07	0.17	0.20	-0.08	-0.07	-0.09	0.01	0.01	0.02	-0.01
Liang's relative efficacy (excluding the SLEQOL subsections)		1.00	-	-	-	-	-	-	0.04	0.03	0.12	0.32	0.03	0.06	0.06	0.01
Liang's relative efficacy		0.97	0.17	0.73	1.00	0.20	0.54	0.24	0.03	0.03	0.11	0.31	0.03	0.06	0.06	0.01

Test-retest Reliability

Thirty-five patients with stable disease were interviewed twice in two weeks to determine the test-retest reliability. The ICC was 0.86 for the summary score. For subsections 1 to 6, the ICCs were 0.72, 0.56, 0.74, 0.66, 0.68 and 0.82 respectively.

Responsiveness

All patients who completed the SLEQOL in the subsequent visits beyond the baseline were asked to rate their global change in QOL using a scale of integers from –7 to +7. In all, 119 data pairs from 95 patients were available for analysis. All the 4 responsiveness statistics show that the SLEQOL is more sensitive than any of the 8 domains of the SF-36 (Table 3). Subsections 3, 4 and 5 of the SLEQOL were the best indicators of change of QOL, superior to the summary score and all the domains of the SF-36. They were very sensitive to change in the QOL and they did not vary when the patients rated their QOL as unaltered.

Conclusion

We have developed a new 40-item SLEQOL in English and showed that it is valid for use in SLE patients in Singapore. It is internally consistent and has reasonable test-retest reliability and floor and ceiling effect. It offered better content validity and responsiveness to change than the SF-36.

Acknowledgements

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Perinatal Depressive Disorders in Singaporean Women and Their Partners

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Introduction

Depression is a leading cause of disease burden in women aged 15 to 44 years in both developed and developing countries,[1] affecting up to 13% of women[2] and often having onset during the reproductive years. Apart from the adverse consequences for the women involved, maternal depression has been shown to have profound effects on the relationship between mother and child and on the child's emotional, behavioral and cognitive development.[1]

The prevalence of postnatal depression in Singapore is unknown,[3] although studies in Asian populations have suggested that it is no longer as uncommon as previously reported.[4] Our aim was to study the prevalence of depressive disorders in women and their partners in Singapore, as well various psychosocial variables associated with perinatal depression.

Materials and Methods

Five hundred fifty-nine women and 308 men were recruited antenatally from obstetric clinics at National University Hospital between July 2002 and July 2004. Following baseline evaluation, subjects were followed up at 6 weeks postnatally.

Evaluation for depressive disorders used a two-stage design, with women and men scoring above cut-offs on the Edinburgh Postnatal Depression Scale (EPDS)[5] going on to a Structured Clinical Interview for DSM-IV (SCID-IV). The 10-item, self-report EPDS has been well-validated in several languages for use as a screening tool for both antenatal and postnatal depression and is easy to administer. The mood disorders that were specifically looked for in this sample were that of major depressive disorder (MDD), minor depressive disorder and dysthymia.

We also examined risk factors that were commonly reported in previous studies of postnatal depression. Various domains of risk factors included:

(a) demographic and socio-economic factors such as age, marital status, number, age and gender of other children, educational level, employment status, and household income, whether living with extended family or not
(b) medical and psychiatric history: past depressive episodes, previous miscarriage, previous induced abortion, obstetric complications
(c) interpersonal relationships: marital and sexual satisfaction, ratings of emotional and practical support (also known as instrumental support) by various family members such as one's partner, parents, parents-in-law as well as by domestic helpers
(d) sociocultural factors such as perceived potential conflicts with other family members, confinement practices, as well as miscellaneous associations such as breastfeeding at 6-weeks postnatally.

Statistical analysis was done using SPSS version 12.0 for Windows OS). Univariate analysis for independent variables was performed using independent Student's *t*-test for continuous variables and chi-square tests for dichotomous and categorical variables. Risk ratios were calculated with 95% confidence intervals.

Logistic regression analysis was used to estimate the univariate odds ratios and their corresponding 95% confidence intervals. Multiple logistic regression was used to explore the important independent associations between variables and mood disorders after adjustment for confounders.

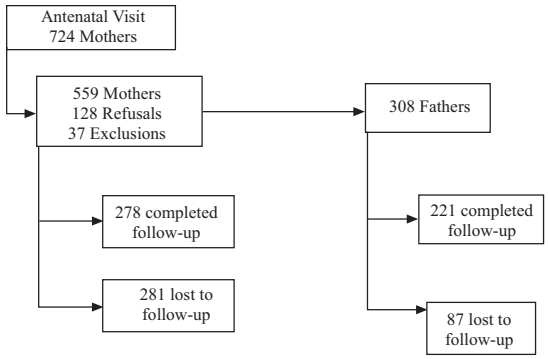

Fig. 1. Summary flowchart of patients who were recruited antenatally, refusals, exclusions and patients lost to follow-up at 6 weeks.

Results

Limited information about the refusals was obtained from their patient registration data; however, comparison with baseline data from the 559 patients who agreed to participate in the study showed that the 2 groups appeared to be comparable in terms of age and ethnic make-up.

Statistical analysis done on the mothers who dropped out at the 6-week postnatal interview showed that the 2 groups did not differ significantly from each other in all the studied variables except for ethnic group.

12.2% of women and 4.55% of men were diagnosed as having a depressive illness antenatally. This fell to 6.8% of women and 1.81%

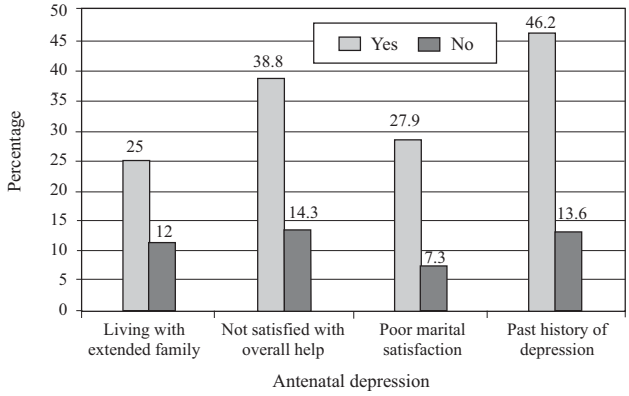

Fig. 2. Significant predictors of depressive disorders in antenatal women using logistic regression modelling.

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of men at 6 weeks postnatally. When considering major depressive disorder alone, the percentage of mothers suffering from MDD stayed fairly stable at 4.29% and 4.32% respectively. The percentage of fathers suffering from MDD was too small for meaningful analysis. Owing to the high drop-out rates, one must be careful not to over-interpret the results. However, given that the baseline characteristics of follow-ups and drop-outs did not differ, it is striking to note the drop in minor depressive disorder rates but not major depressive disorder rates across delivery.

Significant factors associated with antenatal depression in women included marital dissatisfaction, dissatisfaction with overall help given, past history of depression and living with one's own parents.

Factors assessed antenatally which were not statistically significant were: the gender of the baby, young age (less than 25 years old), ethnic group, whether the patient was undergoing confinement or not, past history of abortion or miscarriage, employment status, educational level, household income and practical help scores.

At 6 weeks postnatally, multiple logistic regression analysis showed that the women who suffered from depressive disorders were more likely to have poor overall help, to have poor marital satisfaction, to have brought their child to the doctor for 3 or more non-routine visits and to have lower practical help scores than those who were not depressed.

Breastfeeding status (either full or supplemented) at 6 weeks postnatally was not significantly associated with depressive disorder in our sample.

Factors associated with depressive disorders in partners included whether they thought their wives were likely to have significant conflict with relatives over childcare. Other factors did not reach statistical significance owing to the suboptimal sample size of partners with depressive disorders.

Conclusions

Perinatal depressive disorders are not uncommon in Singaporean women. Though there is a drop in their prevalence at 6 weeks postnatally, a significant minority of women continues to suffer from depressive disorders, and prevalence rates of major depressive disorder remain constant both antenatally and postnatally. Psychosocial factors particularly associated with depressive disorder included marital dissatisfaction and dissatisfaction with overall help.

Screening and follow-up for depression in at-risk women are recommended to ensure early detection and treatment of this debilitating condition. Given the loss to follow-up in postnatal populations, screening could be targeted at antenatal women and in women who bring their infants for 3 or more non-routine clinic visits during the postpartum period.

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Best Oral Presentation Award Finalist – Medicine/Paediatrics (Clinical Based)

Improved Outcome with Intensive Chemotherapy in Paediatric Acute Myeloid Leukaemia

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Introduction

Acute myeloid leukaemia (AML) accounts for 20% of childhood acute leukaemia.[1] As recently as the 1990s, 2-year disease-free survival from AML in Singapore was only 30%.[2]

Since September 1996, the United Kingdom Medical Research Council's 10th AML trial (UKAML-10) protocol has been adopted in Singapore. UKAML-10 is a highly intensive, near-myeloablative regimen designed to improve the previously dismal results of AML therapy for both children and adults. Although superiority of the UKAML-10 protocol in developed countries can be demonstrated by comparing published data,[3-4] its value in countries with more limited resources and its toxicity profile have yet to be documented.

We review our institutional experience with UKAML-10 and previous regimens in a consecutive series of children with AML. Comparing their survival and toxicity profiles will ascertain if survival benefits accrued after switching protocol outweigh the risks.

Materials and Methods

Patients

Characteristics of the 34 patients treated between April 1988 and December 2003 are summarised in Table 1.

Diagnosis of Leukaemia

This was established with standard methodology.

Treatment

Prior to September 1996, 10 children received the POG-8498 protocol and nine received other regimens.

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Table 1. Patient Characteristics

Parameter	No. of children	% of children
Gender		
Male	21	62
Female	13	38
Age (years)		
<2	12	35
2-7	14	41
>7	8	24
Type of AML		
De novo	31	91
Secondary	0	0
MDS-AML	3	9
FAB subtype		
M0/M1	0	0
M2	7	21
M3	2	6
M4	7	21
M5	4	12
M6	0	0
M7	13	38
unclassifiable	1	3
Extramedullary disease		
None	30	88
CNS	0	0
Chloroma	4	12
White cell count ($x10^9$/L)		
<10	15	44
10-29	10	29
30-99	6	18
>100	3	9
Cytogenetic group		
Favourable – t(8;21), t(15;17), inv(16)	8	24
Intermediate – all other cytogenetics	14	53
Adverse – monosomy 5/7, del(5q), 3q abnormalities, complex karyotype	4	12
Unknown	4	12
Down syndrome		
Yes	7	21
No	27	79
Chemotherapy protocol		
UKAML-10	14	41
Others	20	59

AML: acute myeloid leukaemia; CNS: central nervous system; UKAML: United Kingdom Medical Research Council 10th AML Trial

Of 15 children treated from September 1996 onwards, 14 received UKAML-10 (four courses in all: two remission induction courses with ADE [cytarabine, daunorubicin, etoposide] given for 10, 3 and 5 days and 8, 3 and 5 days for course 1 and 2 respectively; 2 consolidation courses consisting of MACE [amsacrine, cytarabine, etoposide] and MidAC [mitoxantrone, cytarabine]). One received another protocol.

Definitions

Complete remission (CR) was defined by a normocellular bone marrow aspirate containing <5% blasts and normal maturation of other marrow elements.

Overall survival (OS) is time from diagnosis to death; event-free survival (EFS) is time from diagnosis to first event (relapse, death in CR or death without CR); disease-free survival (DFS) is time from CR to any event (relapse or death in CR).

Statistical methods

Remission rates were compared using chi-squared tests. Survival data were computed using Kaplan-Meier analysis. Prognosticators analysed included: presenting white blood cell count (WBC); cytogenetics; French-American British (FAB) subtype; achievement of CR after course 1; age; and glutathione s-transferase (GST) T1/M1 genotype. The Cox proportional hazard model was used to adjust for influence of protocol on survival. Surviving patients were censored at 31 May 2004, when follow-up was up-to-date for all children.

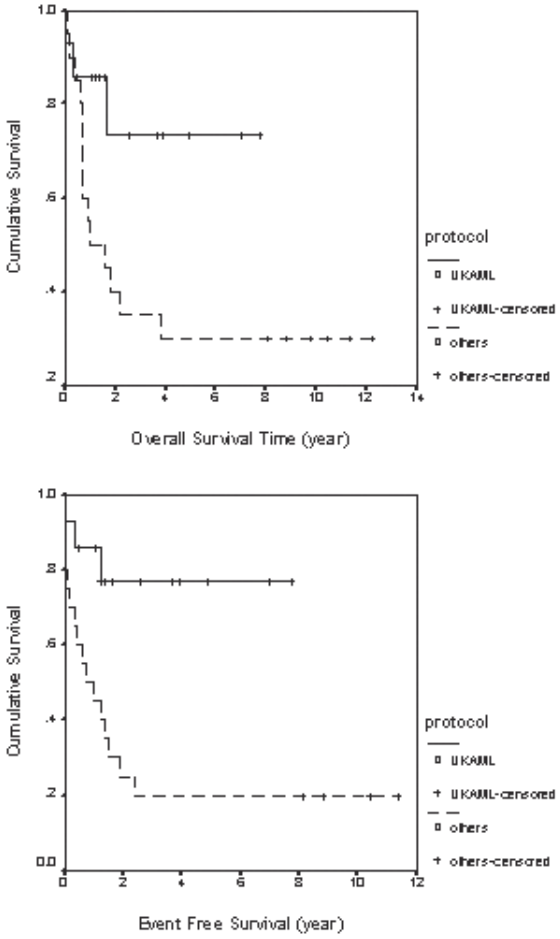

Fig. 1. Overall survival and event-free survival for UKAML-10 vs other protocols.

Results

Outcomes

At time of analysis, 17 were dead from disease, and 17 patients alive among whom 2 relapsed. Median follow-up was 1.6 years (range, 0-12.3).

UKAML-10-treated patients had significantly better 2-year OS, EFS and DFS (73.5% *vs* 40.0%, 77.1% *vs* 25.0%, 83.1% *vs* 38.5%; $P = 0.039$, $P = 0.005$, $P = 0.023$ respectively) [Fig. 1] and were likelier to achieve CR than non-UKAML patients (92.9% *vs* 65.0%, $P = 0.102$, odds ratio = 7.0). Among patients achieving CR, UKAML-10-treated patients were significantly likelier to achieve CR after only 1 cycle of chemotherapy (84.6% *v* 38.5%, $P=0.016$, odds ratio=8.8).

Toxicity of Chemotherapy

Haematologic toxicity: There was no significant difference between UKAML-10 and other protocols in median duration of hospitalisation for neutropaenic fever, duration of neutropaenia or thrombocytopaenia ($P = 0.920$, $P = 0.724$, $P = 0.479$ respectively; Mann-Whitney U test).

Non-haematologic toxicities: These were graded by current NCI criteria. UKAML-10 and other protocols did not differ much in gastrointestinal toxicity, cardiotoxicity, hepatotoxicity, and nephrotoxicity. Four non-UKAML patients died from sepsis, versus none from the UKAML-10 group.

Prognostic Factors

Presenting WBC: WBC>30x10^9/L predicted a lower OS (P = 0.009) and EFS (P = 0.01), independent of protocol.

Cytogenetics: Adverse cytogenetics predicted for poorer OS (P = 0.023) and EFS (P = 0.014) compared to intermediate risk cytogenetics, adjusting for protocol.

Other prognosticators: FAB subtype, achievement of CR after course 1, and age at presentation did not show any significance in 2-year OS, EFS or DFS. GST genotypes are discussed below.

Cost

Median cost of chemotherapy was S$4,026 (range, 2013-8168) for UKAML-10 patients and S$1624 (range, 809-6929) for non-UKAML patients. The disparity (P = 0.003) was due to higher doses of existing drugs, such as daunorubicin and etoposide, as well as the introduction of newer, more expensive drugs such as amsacrine and mitoxantrone, in UKAML-10.

Discussion

The intensity of UKAML-10 chemotherapy is greater than in other protocols, in terms of the duration and dosage of individual courses and total number of courses given. This appears to translate into a significant survival advantage. Given the lower relapse rate and proportion of deaths in relapse for UKAML-10, it is likelier that improved survival is primarily due to the protocol itself rather than advances in supportive care.

Interestingly, the more intensive UKAML-10 chemotherapy did not result in greater haematologic toxicity. Although this was probably confounded by increased G-CSF use, the latter is unlikely to affect overall outcome as meta-analysis shows that G-CSF only shortens duration of neutropaenia without reducing overall mortality.[5]

Regarding cardiotoxicity, resting fractional shortening (FS) on echocardiography is known to be inversely related to cumulative anthracycline dose (rate of decline 1.2%/100mg/m^2).[6] Three patients (9% of total) experienced a 10% drop in FS after a 250mg/m^2 anthracycline dose (corresponding to a 4%/100mg/m^2 rate of decline), possibly reflecting greater susceptibility to anthracycline cardiotoxicity in the Singapore population.

In the original UKAML-10 trial, lower presenting WBC, favourable cytogenetics, M5 FAB subtype and younger age predicted for better OS and EFS. Only the first 2 were significantly associated with survival in our study.

Patients with GST wild-type and less intensive chemotherapy (ie. non-UKAML protocols) showed a trend towards lower survival, compared to GST-null patients. This may have implications for treatment stratification; GST-positive children (efficient metabolisers of chemotherapeutics) will require more intensive induction therapy to ensure a durable remission.

Although overall cost of managing each patient was not analysed, UKAML-10 is probably much cheaper despite the high cost upfront, as we no longer resort to more expensive modalities like bone marrow transplantation to salvage relapse patients.

Conclusion

The quantum leap in survival from childhood AML over the past decade validates UKAML-10 as an effective chemotherapy regimen. Future studies should dissect individual components of UKAML-10 to elucidate their relative efficacy and fine-tune therapy. For a disease once considered uniformly fatal, the synergy of treatment optimisation and translational research heralds a much improved prognosis.

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Best Oral Presentation Award Finalist – Medicine/Paediatrics (Laboratory Based)

Effects of Inhibitors of the Tyrosine Signalling Cascade on Antigen Challenge of Guinea Pig Airways *in vitro*

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Introduction

Mast cell has been implicated to play a pivotal role in asthma because mast cell degranulation induced by the cross-linking of the high-affinity IgE receptors (FcεRI) releases a panel of inflammatory mediators such as histamine, leukotrienes, and cytokines which can initiate, coordinate, and perpetuate the allergic inflammatory responses. Cumulative evidence obtained from rat basophilic leukaemia cell line (RBL-2H3) and bone marrow-derived mast cells shows that activation of src-related kinase Lyn and 72-kDa Syk tyrosine kinase is the earliest detectable signalling response to FceRI cross-linking. This is followed by the activation of downstream signalling molecules including phospholipase Cγ, protein kinase C, phosphatidylinositol-3-kinase (PI3K), and mitogen-activated protein kinase (MAPK), which eventually leads to mast cell degranulation (Fig. 1).

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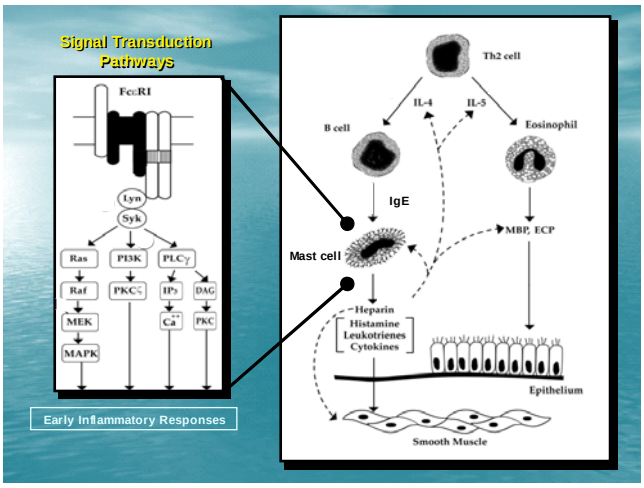

Fig. 1. Schematic diagram showing the immunopathogenesis of asthma.

In mammalian systems, three major groups of MAPK have been identified: p42/44 MAPK (also referred to as extracellular signal-regulated kinase 2 (ERK2) and ERK1, respectively), p38 MAPK and c-Jun NH_2-terminal kinase (JNK). It has been shown that IgE-mediated FcεRI cross-linking in various mast cell cultures caused activation of all three MAPKs. However, as the time-course of JNK activation did not precede that of mediator release, it was believed that only p42/44 MAPK and p38 MAPK were potentially involved in IgE-induced mast cell degranulation. Because mast cell degranulation is the hallmark of immediate-type hypersensitivity reaction, which is also the major mechanism for a variety of allergic diseases such as bronchial asthma, it is imperative to examine the effects of protein tyrosine kinase inhibitors on an *in vitro* model of allergic asthma.

The present study compared the effects of 4 tyrosine kinase inhibitors: Piceatannol, a Syk-selective tyrosine kinase inhibitor; U0126 [1,4-diamino-2,3-dicyano-1,4-bis(aminophenylthio) butadiene], a selective p42/p44 MAPK kinase inhibitor and p38 MAPK inhibitors PD169316 [4-(4-fluorophenyl)-2-(4-nitrophenyl)-5-(4-pyridyl)-1H-imidazole] and SB220025 [5-(2-amino-4-pyrimidinyl)-4-(4-fluorophenyl)-1-(4-piperidinlyl)imidazole] in an *in vitro* model of allergic asthma.

Materials and Methods

Guinea pigs were sensitised by intra-peritoneal injections of ovalbumin (OVA). They were subsequently sacrificed and bronchial rings were contracted to 60 mM KCl, and this contraction was defined as the maximum tissue response to which all subsequent anaphylactic contractions were compared. To evaluate the role of tyrosine kinase in mediating anaphylactic bronchial smooth muscle contraction, the inhibitors were each pre-incubated with bronchial rings 30 min before exposure to ovalbumin. To examine potential direct receptor antagonistic effects of the inhibitors, we also studied bronchial contraction directly induced by histamine, LTD_4, or KCl in the presence and absence of the inhibitors. To study the potential direct smooth muscle relaxant effects, the inhibitors were individually added at peak ovalbumin-induced anaphylactic bronchial contractions, with 1 μM salbutamol used as a positive control.

To determine the inhibitory effects on antigen-induced release of histamine and peptidoleukotrienes, each of these inhibitors was pre-incubated for 30 min before ovalbumin challenge. To determine if these inhibitors have any direct inhibitory effects on the *de novo* synthesis of peptidoleukotrienes such as inhibition of 5-lipooxygenase activity, 70 μM exogenous arachidonic acid was added to lung fragments alone or with ovalbumin challenge for 10 min in the presence and absence of these inhibitors. Diffusates were then collected and stored at –70°C until assay. Histamine and peptidoleukotrieness release from lung samples in response to ovalbumin was determined using an enzyme-linked immuno-sorbent assay (ELISA). Optical density was determined using a microplate reader (Tecan, Austria) at 450 nm. Samples were assayed in duplicate.

Results

Pre-treatment with piceatannol and U0126 produced minor reduction in peak ovalbumin-induced bronchial contraction but markedly facilitated relaxation of the anaphylactically contracted bronchi. SB 220025 and PD 169316 did not suppress ovalbumin-induced peak bronchial contraction or facilitate its relaxation.

Piceatannol and U0126 did not inhibit bronchial contractions induced by KCl, histamine or leukotriene D4-induced bronchial contraction. Correspondingly, U0126 produced slight reduction in ovalbumin-induced release of histamine but significant inhibition on the release of peptidoleukotrienes from lung fragments. Piceatannol, at 30 μM and above, significantly (P <0.05) prevented ovalbumin-induced release of both histamine and peptidoleukotrienes from lung fragments.

Exogenous arachidonic acid-induced release of peptidoleukotrienes was not blocked by U0126. Piceatannol did not inhibit exogenous arachidonic acid-induced release of peptidoleukotrienes from lung fragments.

SB220025 and PD169316 failed to show any inhibitory effect on ovalbumin-induced release of histamine and peptidoleukotrienes. Our findings indicate that inhibition of Syk tyrosine kinase and p42/44 MAPK kinase, but not p38 MAPK, significantly reduced allergen-induced release of mediators leading to rapid relaxation of anaphylactic bronchial contraction.

Discussion

Inhibition of Syk by piceatannol, a widely reported Syk-selective inhibitor, has been shown to inhibit FcεRI-mediated histamine and serotonin release from isolated mast cells and basophils. Piceatannol has been shown to potently inhibit the activity of Syk at concentrations that have little or no effect on Lyn, Fyn and cyclooxygenase. Our findings show that inhibiton of Syk by piceatannol can block mast cell degranulation in airways in vitro, consistent with the findings observed in isolated mast cells, and a substantial reduction in histamine release is required in order to significantly attenuate the peak anaphylactic bronchial contraction. Results of the mediators release study showed that piceatannol did not block exogenous arachidonic acid-induced release of peptidoleukotrienes from lung fragments, suggesting that the inhibitor does not have direct effect on 5-lipooxygenase activity. Therefore the substantial reduction in peptidoleukotrienes release by piceatannol via Syk inhibition is likely linked to the rapid relaxation of the ovalbumin-induced anaphylactic bronchial contraction.

Our work with U0126 shows that it did not substantially inhibit the amplitude of ovalbumin-induced bronchial contraction. This observation corroborates well with a slight 20% inhibition of ovalbumin-induced histamine release from lung fragments by U0126. This suggests that p42/44 MAPK plays only a minor role in IgE-mediated histamine release from mast cells. In contrast, U0126 significantly facilitated relaxation of the anaphylactically contracted bronchi in a concentration-dependent manner. Since U0126 did not affect direct histamine-, LTD_4- or KCl-induced bronchial contraction, the rapid relaxant effect is not mediated by non-specific receptor antagonism or voltage-dependent calcium channel blockade. Instead,

U0126-mediated bronchial relaxation is likely associated with the substantial suppression of ovalbumin-induced release of peptidoleukotrienes as shown in the mediator release studies in lung fragments.

Inhibitors of the p38 MAPK such as SB239063 and SB220025 have been shown to reduce inflammatory cytokine production and eosinophil infiltration into the lungs in animal models of asthma, and inhibited angiogenesis in murine model of rheumatoid arthritis. Although IgE cross-linking on mast cells has been shown to activate p38 MAPK, inhibition of p38 MAPK by SB203580 failed to block the release of histamine, arachidonic acid and peptidoleukotrienes from cultured mast cells. In line with the cell culture findings, our results show that both SB220025 and PD169316 failed to inhibit ovalbumin-induced bronchial contraction and the release of histamine and peptidoleukotrienes from lung fragments. These findings together with our *in vitro* results clearly confirm that p38 MAPK does not play a role in early asthmatic response upon mast cell degranulation.

The detailed signaling mechanisms mediating FcεRI-induced mast cell degranulation have been unraveled and a signal transduction-based approach offers a specific strategy to block mast cell activation. Our present findings show that Syk and p42/44 MAPK inhibitors, but not p38 MAPK inhibitors, facilitated relaxation of constricted airways by preventing antigen-induced release of mediators, indicating that they may have therapeutic potential for asthma.

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Best Oral Presentation Award Finalist – Medicine/Paediatrics (Laboratory Based)

Serum Transferrin Receptor Levels in the Normal Population and Subjects With Iron Deficiency and Thalassaemia Trait

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Introduction

Traditional biochemical markers used in iron deficiency diagnosis have pitfalls, especially in the presence of inflammation. Serum transferrin receptor level (sTfR) is helpful as it is unaffected by inflammation. However, disease states like hemolytic anemias and ineffective erythropoiesis can confound the results. We aimed to study how demographic factors affect sTfR and how thalassaemia trait and iron deficiency interact to affect sTfR.

Materials and Methods

From May 1999 till December 2001, blood sent for thalassaemia screening from patients in Tan Tock Seng Hospital were concurrently checked for sTfR, serum iron, serum transferrin (or total iron binding capacity (TIBC)) and serum ferritin. The subjects' age, sex and the closest full blood count (FBC) were recorded. As control, 140 health screening participants were checked for sTfR, serum ferritin and FBC.

sTfR levels were quantitated using N Latex sTfR assay (Dade Behring, Liederbach, Germany) on a BN100 nephelometer (Dade Behring, Liederbach, Germany). Transferrin was similarly measured by an immunonephelometric assay. Ferritin was measured using a microparticle enzyme immunoassay on the Abbott AxSym system. Iron and TIBC was measured using the FerroZine photometric method on Roche/Hitachi analysers.

A parallel study analysing the accuracy of iron parameters in diagnosing iron deficiency in tertiary hospital patients found that an optimal ferritin level of <60 μg/L or a transferrin saturation level of <7% has a positive predictive value of 94%. Iron deficiency diagnosis for study subjects is based on similar criteria. Iron deficiency among controls was diagnosed only if ferritin <30 μg/L, based on normal reference ranges.

Full blood count was measured on the Gen.S™ automated analyser (Coulter Corporation, Miami, Florida). Thalassaemia screening included analysis by haemoglobin electrophoresis in alkaline pH on cellulose acetate plates (Helena Laboratories, Beaumont, Texas) and HbA_2 quantitatation by high performance liquid chromatography using the Variant™ hemoglobin testing system (Bio-Rad Laboratories, California). HbA_2 levels between 3.8% and 8% with appropriate red cell indices were considered diagnostic of β thalassaemia trait. Samples demonstrating occasional HbH inclusion bodies using Methylene Blue were classified as a thalassaemia trait (mainly 2 gene deletions).

Statistical analyses (correlation coefficient and Student's *t*-test) were done using Microsoft™ Excel. Significance testing of the correlation coefficient was done using an online calculator from Vassar College.

Results

Controls

Among 107 controls (61 males, 46 females) with normal ferritin levels, the mean sTfR level (±2 SD) is 1.20 ± 0.47mg/L. There is no

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difference in mean sTfR levels between males (1.21 mg/L) and females (1.18 mg/L) (P = 0.41), even though ferritin levels is significantly higher among males. A weak negative correlation exists between age and sTfR levels (r = -0.21, P = 0.02), stronger among males (r = -0.26, P = 0.02) than females (r=-0.12, P = 0.21).

Thirty-three controls had subclinical iron deficiency (normal hemoglobin and mean corpuscular volume, low ferritin levels). Their mean sTfR level (1.39 mg/L) was significantly higher than that of the above 107 controls (P = 0.003). Nonetheless, the large overlap in sTfR levels between these two groups precludes its use in iron deficiency screening.

Subjects

There were 432 subjects with different combinations of α,β-thalassaemia traits and iron deficiency (Table 1). Subjects with isolated iron-deficiency and isolated β-thalassaemia trait have significantly higher sTfR levels than subjects with no pathology (P <0.001). This difference was not found amongst subjects with isolated a-thalassaemia trait.

Table 1. Distribution of Subjects with Iron Deficiency/Thalassaemia Trait

	n	Mean sTfR (mg/L)	Mean ferritin (μg/L)
No pathology (non iron deficient, non thalassaemia trait)	23	1.54	384
Isolated α-thalassaemia trait	100	1.48	230
Isolated β-thalassaemia trait	112	2.06	377
Isolated iron deficiency	116	4.64	10.5
Ferritin ≥10 μg/L	36	3.82	24.8
Ferritin ≥15 μg/L	26	3.74	30.0
Combined α-thalassaemia trait and iron deficiency	56	2.80	22.6
Combined β-thalassaemia trait and iron deficiency	25	2.57	30.1
Total	432		

Subjects with combined thalassaemia trait and iron deficiency have significantly lower sTfR levels than subjects with isolated iron deficiency (P <0.001). This might be partly due to the significantly greater degree of iron deficiency, as measured by ferritin, amongst isolated iron deficient subjects (P <0.001). Adjustment for comparability was done to the ferritin levels by considering isolated iron deficient subjects with ferritin levels ≥10 μg/L or ≥15 μg/L in the comparison with combined α-thalassaemia/ iron deficiency or β-thalassaemia/ iron deficiency respectively. Re-analysis showed that the differences in the sTfR levels persisted (P = 0.02 for both comparisons).

Discussion

In this study, we analysed how physiological factors influence sTfR levels. One published study measured sTfR in 56 normal adults and found a slight but insignificant reduction of sTfR with age.[1] Our study of 107 normal controls showed a weak but significant negative correlation between age and sTfR levels which is not due to variations in ferritin levels. Whether this weak negative correlation between sTfR levels and age contributes to the physiological decline in hemoglobin levels with age requires further investigation.

Among subjects, iron deficiency significantly raises the sTfR levels, as shown in other studies.[2,3] A Greek study showed that β thalassaemia trait raises sTfR levels in proportion to the degree of ineffective erythropoiesis.[4] We also showed that β thalassaemia trait

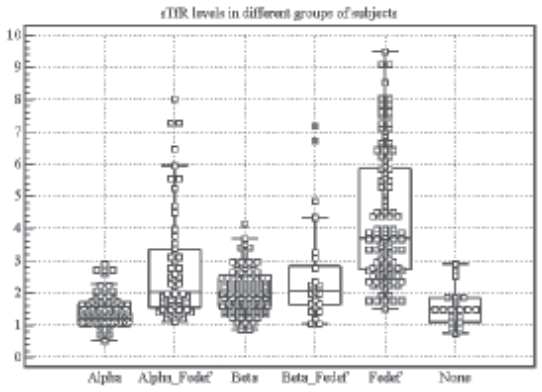

Fig. 1. sTfR levels in different groups of subjects. 3 outliers in Fe def group not included.

raises sTfR levels but correlation of sTfR levels with the degree of ineffective erythropoiesis was not found (results not shown). In our study, α thalassaemia trait does not raise sTfR levels. This is at variance with South Pacific island study where sTfR levels were found to increase in proportion to the number of α globin gene mutations.[5] Nevertheless, this study was done in children and applicability to an adult inpatient population is uncertain.

We demonstrated that both thalassaemia traits attenuate the erythron's transferrin receptor upregulating response to the absence of iron. The mechanism behind this phenomenon is unknown but it is possible that the statistical adjustment made in our study is not reflected biologically. Whether this will translate into a clinically reduced response to iron therapy in patients with concurrent thalassaemia trait and iron deficiency needs further investigation.

The utility of sTfR as a single marker in the diagnosis of iron deficiency can be predicted in Figure 1. The significant overlap, especially between iron deficient subjects and non-iron deficient subjects with thalassaemia trait will reduce accuracy of this marker.

Conclusion

Age negatively correlates with sTfR among males. β-thalassaemia trait but not α-thalassaemia trait causes a rise in sTfR levels. Thalassaemia traits attenuate the increase in sTfR caused by iron deficiency. This will affect the accuracy of sTfR in iron deficiency diagnosis in this group of patients.

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Escherichia coli-asparaginase (Elspar) is Superior to *Erwinia*-asparaginase (Erwinase) in Childhood Acute Lymphoblastic Leukaemia (ALL) Induction – An Early Response Study Using Minimal Residual Disease (MRD) Markers

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Introduction

L-asparaginase hydrolyses L-asparagine, causing its depletion in the blood. It is one of the most important drugs responsible for the highly successful therapy of childhood acute lymphoblastic leukaemia (ALL). Malignant lymphoblasts, unlike normal cells, are unable to synthesise asparagine, hence its depletion leads to lymphoblast apoptosis.[1,2]

L-asparaginase in clinical use is derived from either *Escherichia coli* (Elspar) or *Erwinia chrysanthemi* (Erwinase). Its adverse effects, including coagulopathy, hepatotoxicity and pancreatitis,[3] result primarily from the effect of asparagine depletion on normal protein synthesis. Different sources of asparaginase differ in pharmacodynamics and pharmacokinetics, with Elspar believed to be more potent and longer-acting, while Erwinase causes fewer allergies and toxicities.[1,2] Initial widespread switches from Elspar to Erwinase were blamed for poorer results in some groups; however, direct proof of the inferiority of Erwinase has not been demonstrated.

Sensitive molecular techniques allow detection of minimal residual disease (MRD) levels as low as 1 leukaemia cell in a background of 10,000 normal cells, compared to 5 in 100 cells with conventional microscopy, allowing accurate following of the kinetics of early leukaemia cell kill.[4] We utilised these techniques to compare the efficacy of Elspar versus Erwinase in killing lymphoblasts *in vivo* in childhood ALL patients.

Materials and Methods

Patients and Samples

Using MRD as an indicator of treatment response, we compared the induction regimens of 2 childhood ALL trials: NUH (1992-1996) and MA-SPORE (2002-ongoing). The NUH protocol used either Erwinase or Elspar 10,000 IU/m^2 twice a week, while the MA-SPORE uses Elspar 7500 IU/m^2 bi-weekly. Dosages of other drugs were similar.

A total of 110 newly diagnosed precursor-B ALL patients from NUH (n = 45) and MA-SPORE (n = 65) protocols with data available regarding the L-asparaginase source were included; 21 were on Erwinase, 89 on Elspar. The median age was 5.3 years (range, 0.36 to 13.8), the median presenting total white count (TWC) 11.5x10^9/L (range, 1.08 to 729). DNA was extracted from bone marrow mononuclear cells (MNC).

Minimal Residual Disease Determination

Antigen receptor gene rearrangements were analysed by PCR as described.[4] Sequences were compared against the human germline (http://www.ncbi.nlm.gov/BLAST/) and patient-specific primers constructed.

Southern Blot (SB): Patient DNA was amplified by PCR, transferred onto a nylon membrane and hybridised with the specific radio-labelled oligoprobe.

RQ-PCR: The LightCycler (Roche Diagnostics) was used, with patient-specific primers and Taqman probes.

In both methods, the remission sample was compared against a standard dilution of the diagnostic sample, with water and MNC as negative controls.[3,5]

Patients were stratified into High Risk (HR: $\geq 10^{-2}$); Intermediate Risk (IR: 10^{-2}-10^{-4}); and Standard Risk (SR: $\leq 10^{-4}$) according to level of MRD at week 5.

Toxicity Data

Data regarding adverse effects were collected from casenotes. Toxicity was graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (http://ctep.cancer.gov/reporting/ctc.html).

Results

SB And RQ-PCR Correlation

Blinded comparison between SB and RQ-PCR gave 100% (11/11) correlation between the 2 methods of MRD detection, with both reaching at least 10^{-4} sensitivity.

Patient Composition

The composition of the 2 groups were identical; specifically, 71% of both Erwinase and Elspar-treated patients are low risk by NCI criteria, thus there is unlikely to be a selection bias (Table 1).

MRD Risk Stratification

Samples at both diagnosis and end of induction were available for 62% (13/21) of Erwinase-treated patients and 84% (75/89) of Elspar-treated patients, allowing MRD determination for an overall 80%.

Significantly more Erwinase-treated patients were MRD-stratified into high risk compared to Elspar-treated patients (31%, 4/13 vs 8%, 6/75, P = 0.037; OR = 5.11, 95% CI, 1.3-20.6). Within the Elspar group, stratification of NUH or MA-SPORE patients was not significantly different (HR: 21%, 3/14 vs 5%, 3/58, P = 0.13), showing that the better response of Elspar patients was unlikely to be due to chronological difference in treatment. Standard risk patients made up only 31% (4/13) of Erwinase-treated patients, compared to 55% (41/75) of Elspar-treated patients (Table 1). The smaller proportion of high-risk patients reflects the greater clearance of lymphoblasts with Elspar, demonstrating its greater efficacy.

Toxicity

Toxicity information was available for 96% of patients (Erwinase: 20/21; Elspar: 87/90). The number of patients experiencing grades 3 to 5 toxicities was similar (20%, 4/20 vs 24%, 21/86). Interestingly,

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Table 1. Patient Characteristics and Risk Stratification by L-asparaginase Formulation

	Erwinase (n = 21)	Elspar (n = 89)	Overall (n = 110)
Age (y)	0.67-7.86 Median = 3.66	0.36-13.83 Median = 4.09	0.36-13.83 Median = 3.88
Presenting TWC x10^9/L	1.08-729 Median = 10.6	1.20-447 Median = 11.7	1.08-447 Median = 11.5
NCI criteria	(n = 21)	(n = 89)	(n = 110)
Low risk	71% (15)	71% (63)	71% (78)
High risk	29% (6)	29% (26)	29% (32)
MRD	(n = 13)	(n = 75)	(n = 88)
Standard risk	31% (4)	55% (41)	44% (45)
Intermediate risk	38% (5)	37% (28)	30% (33)
High risk	31% (4)	8% (6)	9% (10)

MRD: minimal residual disease; NCI: National Cancer Institute

Table 2. Toxicity During Induction

Adverse effect (n= ?)	Erwinase (n = 20)	Elspar (n = 86)
Overall grade 3-5	20% (4)	24% (21)
Overall grade 4	0%	8% (7)
≥Grade 3 infection	20% (4)	12% (10)
≥Grade 3 hepatotoxicity	0%	6% (5)
Diabetes mellitus	0%	3% (3)
Coagulopathy (e.g. thrombosis, stroke, seizure)	0%	7% (6)
Induction deaths	5% (1)- Infection	2% (2)- Infection, GI bleeding

GI: gastrointestinal

all 4 cases of severe toxicity in Erwinase patients were infection-related, with none experiencing asparaginase-related effects. On the other hand, Elspar patients had a similar rate of infections (12%, 10/86) but, in addition, there was a 3% to 8% incidence of severe hepatotoxicity, diabetes secondary to pancreatitis, coagulopathy and neuropathy (Table 2). There were no asparaginase-related deaths in Erwinase patients but 1 Elspar patient died of gastrointestinal bleeding.

Discussion

This is the first study that utilises MRD to *directly* compare the efficacy of *E. coli*-asparaginase with *Erwinia*-asparaginase. MRD measures the effectiveness of therapy *in vivo*, and patients on Erwinase were 5 times more likely to be stratified into high risk at the end of induction, indicating poorer response. As other induction drugs and patient characteristics were comparable, any difference can be attributed to the different formulations of L-asparaginase used.

The lower efficacy of Erwinase, using the current schedule, is borne out by the poorer outcome of Erwinase-treated patients – 5-year EFS of 62% (13/21) versus 75% (18/24) for Elspar. This is consistent with results from a randomised trial showing 6-year EFS of 60% for *Erwinia*-asparaginase versus 73% for *E. coli*-asparaginase.[3]

However, fewer patients on Erwinase experienced severe asparaginase-related toxicities like thrombosis, hepatitis and diabetes. The lower efficacy and toxicity of Erwinase can be explained by its shorter half-life and lower activity level *in vivo* compared to Elspar.[1,2]

Conclusion

This study provides evidence by MRD that Elspar is significantly superior to Erwinase in childhood ALL induction. The greater efficacy of Elspar is accompanied by more severe toxicity, but this is balanced by better treatment response and improved outcome. *E. coli*-asparaginase should be considered the first-line of L-asparaginase therapy for childhood ALL, with *Erwinia*-asparaginase reserved for patients allergic to *E. coli*-asparaginase. Furthermore, we have convincingly shown that MRD can be a powerful tool to compare the efficacy of chemotherapeutic agents in remission induction and treatment of childhood ALL. This can provide faster information early in therapy compared to the conventional relapse outcome 4 to 6 years later.

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EXTENDED ABSTRACTS

Best Oral Presentation Award Finalist – Nursing

Determinants of Patient's Willingness Towards Participation in Clinical Drug Trial in a Psychiatric Setting

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Introduction

More than 82% of clinical research trials have been delayed because there are not enough volunteers.[1] Several studies have shown that there are many factors that determine and influence the decision made by patient.[2-4] When disease strikes, many people seek a cure through experiment drug in clinical trials: patient pursues trial as a primary path to state-of-art care as a hope after exhausting all conventional treatments and remedies.[1] Thus, it is significant to find

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out if patients, particularly psychiatric patients who need long-term medications will resort to clinical drug trial as a hope of treating their illness.

Materials and Methods

Specific Aims

The purpose of this study was to elucidate and analyse factors that determine the patients' willingness towards participation in clinical drug trial in a psychiatric setting. The information will help us to understand the concerns of the patient for participating in a clinical drug trial.

Methods

This study took on a non-experimental descriptive study design. The scales and questionnaires that were administered in the study are drug attitude inventory-10, rating scale for side effects, SF-12 health questionnaires and self-reporting survey. These scales and questionnaires were administered to 100 patients in a psychiatrist hospital setting as outpatients. Socio-demographic factors, attitudes towards current medication, presence or absence of side-effects, general health of the patient and the self-reporting survey were evaluated. These factors were analysed to see if there was any correlation to willingness in fostering patient's participation in a clinical drug trial.

Statistical Analysis

All analyses were performed using Statistical Package for Social Science 11.5. Statistical significance was set at $P < 0.05$.

Results

Socio-Demographics

A total of 100 patients were recruited in the study. Table 1 shows the demographic details of the patients. Overall, there was no strong interest in participating in the clinical drug trial in any of the demographic groups shown. Out of the 46 participants who expressed interest in clinical drug trial, only 7 (35%) had previously been involved in clinical drug trial. Out of the 54 participants who were not interested in clinical drug trial, 13 (65%) of the participants had participated in a clinical drug trial before.

Scales/Questionnaires

Performing a factor analysis on the 13 self-reported questionnaires, 5 factors were obtained (explaining 69.17% of the variance): benefit factor, trial-related factor, medical factor, satisfaction factor and future treatment and interpersonal factor.

Univariate analysis showed that RSSE, benefit, trial-related and, future treatment and interpersonal factors influenced participation. Those whose scores ranged from 4 to 10 in the rating for side effects were 5.5 (95% CI, 1.10-27.0) times more likely to participate compared with those whose scores ranging from 0 to 3 ($P = 0.041$, Table 2). The median rating was also significantly higher (means that the participant has higher side effects) for those who had interest in participating (Table 2).

Those who scored a higher level of agreement that their interest in clinical drug trial participation was due to benefit, future treatment and interpersonal factors were more likely to participate in a trial ($P < 0.001$).

A logistic regression was performed with all the socio-demographic variables and the scales/questionnaires. The following significant predictors for participation were obtained: Chinese were more likely to be interested to participate compared to non-Chinese (OR = 67.9; 95% CI, 1.4 to 3296; $P = 0.033$). Those whose duration of illness was 1 to 6 years were more likely to be interested to participate compared to those whose illness were more than duration of 13 years (OR = 12831; 95% CI, 11.1-15000; $P = 0.009$). Those who took <3 medications were more likely to be interested to participate compared to those who took ≥3 medications (OR = 34.9; 95% CI, 2.0-614.9; $P = 0.015$). Those whose side effects rating scores ranged from 4 to 10 were more likely to be interested to participate compared to those whose scores ranged from 0 to 3 (OR = 3347.3; 95% CI, 6.6-5000; $P = 0.011$). Those who scored higher level of agreement that their interest in trial participation was due to future and interpersonal factors were more likely to have interest in the participation (OR = 17.2; 95% CI, 2.7-111.2; $P = 0.003$).

Table 1. Demographic Results of Patients' Interest in Clinical Drug Trial Participation

	Interest in clinical drug trial participation			No. of participants
	Yes (n = 46)	No (n = 54)	*P*	n = 100
Age (y)				
18-28	7 (50.0%)	7 (50.0%)		14
29-39	11 (42.3%)	15 (57.7%)		26
40-50	18 (46.2%)	21 (53.8%)	0.982	39
51-61	9 (50.0%)	9 (50.0%)		18
≥62	1 (33.3%)	2 (66.7%)		3
Gender				
Male	20 (55.6%)	16 (44.4%)	0.15	36
Female	26 (40.6%)	38 (59.4%)		64
Marital status				
Single	33 (47.8%)	36 (52.2%)		69
Married	13 (43.3%)	17 (56.7%)	0.906	30
Divorced	0 (0%)	1 (100%)		1
Race				
Chinese	41 (48.2%)	44 (51.8%)		85
Malay	4 (66.7%)	2 (33.3%)	0.091	6
Indian	1 (12.5%)	7 (87.5%)		8
Others	0 (0%)	1 (100%)		1
Religion				
Christianity	15 (38.5%)	24 (61.5%)		39
Buddhism	15 (60.0%)	10 (40.0%)		25
Hinduism	1 (20.0%)	4 (80.0%)	0.222	5
Islam	4 (57.1%)	3 (42.9%)		7
Taoism	4 (30.8%)	9 (69.2%)		13
Others	7 (63.6%)	4 (36.4%)		11
Education level				
No education	2 (66.7%)	1 (33.3%)		3
Primary	8 (53.3%)	7 (46.7%)	0.640	15
Secondary	23 (47.9%)	25 (52.1%)		48
Tertiary	13 (38.2%)	21 (61.8%)		34
Household income				
Below S$500	14 (48.3%)	15 (51.7%)		29
S$501-S$1000	12 (48.0%)	13 (52.0%)	0.985	25
S$1001-S$2000	13 (43.3%)	17 (56.7%)		30
S$2001-S$3500	3 (50.0%)	3 (50.0%)		6
≥S$3501	4 (40.0%)	6 (60.0%)		10
Duration of illness (y)				
1-6	18 (64.3%)	10 (35.7%)		28
7-12	13 (41.9%)	18 (58.1%)	0.067	31
≥13	15 (36.6%)	26 (63.4%)		41
Previously involved in any clinical drug trial				
Yes	7 (35.0%)	13 (65.0%)	0.27	20
No	39 (48.8%)	41 (51.3%)		80
Number of medications				
≤2	24 (48.0%)	26 (52.0%)	0.688	50
≥3	22 (44.0%)	28 (56.0%)		50
Number of hospitalisation				
No hospitalisation	5 (45.5%)	6 (54.5%)		11
1-3 times	21 (42.0%)	29 (58.0%)	0.681	50
4-6 times	11 (45.8%)	13 (54.2%)		24
≥7 times	9 (60.0%)	6 (40.0%)		15

Discussion

The results showed that only 46% of the participants expressed interest in participating in a clinical drug trial. Those who scored higher rating for side effects and those who had illness between 1 and

Table 2. The Scale and Questionnaire Results of the Patient's Interest in Clinical Drug Trial Participant

	Interest in clinical drug trial participation		
	Yes (n = 46)	No (n = 54)	*P*
Rating for side effects			
0-3	38 (42.2%)	52 (57.8%)	
4-10	8 (80.0%)	2 (20.0%)	0.041
Rating for side effects			
Mean (SD)	1.52 (2.12)	0.57 (1.21)	
Range	0 to 10	0 to 6	0.005
Median	1.0	0	
Drug Attitude Inventory (DAI)			
Non-compliant	5 (45.5%)	6 (54.5%)	0.974
Compliant	40 (46.0%)	47 (54.0%)	
SF12 (QOL)			
Physical functioning			
Mean (SD)	5.0 (1.2)	4.9 (1.2)	
Range	2.0 to 6.0	2.0 to 6.0	0.516
Median	6.0	5.0	
Role physical			
Mean (SD)	3.7 (0.7)	3.5 (0.9)	
Range	2.0 to 4.0	2.0 to 4.0	0.169
Median	4.0	4.0	
Role emotional			
Mean (SD)	3.7 (0.7)	3.5 (0.9)	
Range	2.0 to 4.0	2.0 to 4.0	0.299
Median	4.0	4.0	
General health			
Excellent	0 (0%)	2 (100%)	
Very Good	11 (40.7%)	16 (59.3%)	
Good	21 (44.7%)	26 (55.3%)	0.365
Fair	12 (54.5%)	10 (45.5%)	
Poor	2 (100%)	0 (0%)	

6 years were more likely to participate in the clinical drug trial. Thus, the degree or severity of the side effects on the patients' current medication and the stage of their mental illness such as those who are newly diagnosed or not in the chronic stage contribute and therefore, determine their interest in clinical drug trial participation.

In performing factor analysis on the self-reporting survey, high scores on trial-related and, future treatment and interpersonal are factors for encouraging participation in clinical drug trial.

Conclusion

This study showed that very few patients are interested in clinical drug trials. The findings of the study showed significant differences on the factors, which can be explored, in a greater depth such as the trial-related and interpersonal factors. The information that we have elucidated from the study will help us to further assess patient's attitude and to understand patient's reasons for not participating in a clinical drug trial. The personnel who will be conducting the trial perhaps can address this.

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EXTENDED ABSTRACTS

Best Oral Presentation Award Finalist – Nursing

Algorithm to Achieve Prescribed Dialysis Adequacy Targets for Non-compliant Children on Automated Peritoneal Dialysis (APD)

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Introduction

Automated peritoneal dialysis (APD) is the dialysis modality of choice in children with end stage renal disease (ESRD).[1] It is thus important to optimise APD prescriptions in young patients in order to achieve dialysis adequacy targets and in turn, improve clinical outcome. There is evidence that higher total weekly urea clearance (Kt/Vurea) and creatinine clearance (CCr) values are associated with lower morbidity and mortality rates.[2] Hence, it is current clinical practice to use computerized kinetic modeling of Kt/Vurea and CCr as a tool for modifying APD prescriptions, in order to achieve such dialysis adequacy targets.[3,4]

However, non-compliance is a major factor in failing to achieve dialysis adequacy targets, particularly in adolescents.[5,6] To compensate for shortfalls due to non-compliance, clinicians often advise non-compliant patients to carry out 'extra dialysis' on non-working days by increasing the number of hours or cycles of dialysis or the dialysate glucose concentration. The clinical outcomes of such 'compensatory dialysis' has never been studied. The study aimed firstly at designing a technique to compute dialysis adequacy in the non-compliant patient, and subsequently to develop an algorithm to calculate the daily prescription adjustments required to compensate for any shortfalls in preceding days,

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such that dialysis adequacy targets are met even in the non-compliant patient.

Materials and Methods

A total of 14 patients undergoing APD, who are currently on chronic peritoneal dialysis in the pediatric renal replacement program at the Shaw-NKF Children's Kidney Centre in Singapore, were recruited into the study on a voluntarily basis. The patients were from 3 to 25 years of age and were on APD for a mean of 2.7 ± 2.4 (range, 0.5 to 8.1) years. No patients had peritonitis during the study or 1 year prior to entering the study.

Using predicted Kt/Vurea and CCr data generated by PD Adequest® 2.0 program, secondary models were derived by multivariate regression as functions of the prescription parameters (coefficient of determination r^2 >0.99). An algorithm was formulated, based on these secondary models, to compute the monthly average of the daily Kt/Vurea and CCr values (i.e. computed Kt/Vurea and CCr). The study comprised 3 phases of 1 month duration each. In phase I, patients were compliant with the APD prescription. In phase II, patients were non-compliant for at least 3 days per week. In phase III, an adjustment program was written for the APD prescription so that daily changes could be made to compensate for shortfalls due to non-compliance. Data from the APD cycler were recorded on the HomeChoice PRO™ System.

Results

In phase I (compliant phase), the algorithm-computed Kt/Vurea and CCr, had excellent agreement with the Adequest-predicted parameters (intraclass correlation r_i = 0.99, Bland-Altman coefficient of clinical agreement C_{CA} = 98%). In phase II (non-compliant phase), the algorithm-computed Kt/Vurea (1.7 ± 0.5) and CCr (42.4 ± 15.6) were significantly lower than Adequest-predicted Kt/Vurea (1.8 ± 0.6) and CCr parameters (44.4 ± 16.9) (P = 0.001). In phase III, using the daily adjustment program to compensate for shortfalls, both the algorithm-computed Kt/Vurea (Fig. 1) and CCr (Fig. 2) had good agreement with the Adequest-predicted parameters (r_i = 0.98 and 0.99 respectively and C_{CA} = 100% for both).

Discussion

It was firstly important to validate the use of PD ADEQUEST 2.0 in our population. In phase I of our study, the ADEQUEST-predicted Kt/Vurea and CCr values correlated well with the actual measured values, with an intraclass correlation coefficient of 0.72 and 0.77 respectively. Our results in pediatric patients were similar to that reported by Warady et al,[4] where the concordance correlation between ADEQUEST-predicted and measured results were 0.70 for total weekly Kt/Vurea and 0.77 for total weekly CCr. Hence, PD ADEQUEST 2.0 accurately predicts total Kt/Vurea and CCr adequacy targets in our sample population. In addition, the strong correlation between ADEQUEST-predicted and algorithm-computed Kt/Vurea and CCr values in phase I demonstrated that the algorithm had been accurately derived from the predicted values generated by PD ADEQUEST 2.0.

In phase II, patients were non-compliant. The algorithm-computed Kt/Vurea and CCr values in phase II were significantly lower than the ADEQUEST-predicted targets. This demonstrates the algorithm's sensitivity to daily changes and shortfalls in the dialysis regimen due to non-compliance.

In phase III, despite intermittent non-compliance, adjustments to the daily dialysis regimen could be computed by the algorithm. Patients were able to carry out these recommended 'extra dialysis' adjustments such that the computed values, which had been lowered due to non-compliance, could attain the ADEQUEST-predicted

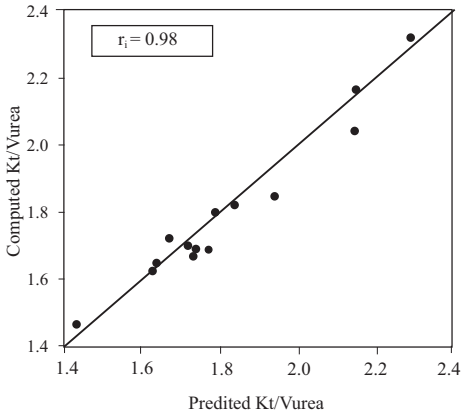

Fig. 1. Plot of Phase III algorithm-computed against ADEQUEST-predicted Kt/Vurea values with intraclass correlation coefficient (r_i).

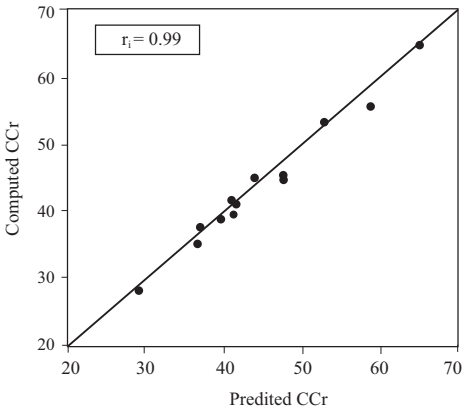

Fig. 2. Plot of Phase III algorithm-computed against ADEQUEST-predicted or CCr values with intraclass correlation coefficient (r_i).

targets. In other words, the patient has been fully compensated for shortfalls. This is clearly shown by the good agreement between the algorithm-computed values and ADEQUEST-predicted targets in this phase.

For all 3 phases, the measured and algorithm-computed Kt/Vurea and CCr values correlated reasonably well. However, the algorithm-computed values are sensitive to changes in the daily dialysis regimen over a certain period of time, while the measured results represent clearance from the previous night's dialysis only. As the 2 methods of measuring adequacy have a different basis, the significance of comparing computed and measured values is questionable.

The concept of using the computed average Kt/Vurea and CCr values instead of actual measured results to assess adequacy in patients is new. Thus, further research is necessary to compare the clinical outcomes of fully compensated non-compliant dialysis patients (using the algorithm) with consistently noncompliant patients and consistently compliant patients. This would determine whether 'compensatory dialysis' can provide adequate clearance of uremic toxins over the long term. The algorithm, which calculates the optimal dialysis dose to fully compensate noncompliant patients, can be used to facilitate such research.

Conclusion

In conclusion, the results presented here show that our algorithm computes adequacy Kt/Vurea and CCr values, taking into consideration changes in the daily regimen over a one month period.

It was also clinically possible to use the algorithm to calculate the necessary 'compensatory' prescription such that predicted target Kt/Vurea and CCr were achievable using the algorithm for adjustment of the daily APD prescription, despite shortfalls due to non-compliance. Further research is necessary to investigate the long-term outcome of non-compliant patients, who are compensated for shortfalls using the algorithm.

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Best Oral Presentation Award Finalist – Nursing

Health Screening Outcomes that Lead to Health Seeking Behaviours

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EXTENDED ABSTRACTS

Introduction

Health screening is an essential component of disease management in the community. There is little controversy that early detection and treatment of risk factors or diseases such as diabetes, hypertension and renal disease, which can save lives and minimize potential complication and unnecessary morbidity.[1]

In 1997, the National Kidney Foundation (NKF) launched a nation-wide community health-screening programme. It is a community-based screening programme, which targets detection of diseases such as diabetes, hypertension and high blood cholesterol in asymptomatic people aged 18 and above. These 3 conditions, if poorly managed, could eventually lead to heart disease, stroke and end stage renal failure (ESRF). This programme also includes a nurse-based educational component that emphasizes healthy living and the need for regular screening (referral to the NKF prevention centres and affiliated physicians for recheck of abnormal values).

During the health-screening session, the following procedures are done:

1. Weight and height measurement
2. Random blood glucose and random total cholesterol
3. Urine test (consist of testing any presence of blood, protein, glucose, ketones, and pH)
4. Waist-hip ratio measurement
5. Blood pressure measurement
6. Counselling (providing advice on participants' abnormalities)

To date, more than 1.1 million screening episodes have been conducted island-wide; few studies have examined whether participants with screen-detected abnormalities attend confirmatory evaluation.[2,3] We sought to determine the health-seeking behaviour among health-screening participants with abnormal test results, and compare the characteristics of participants who sought and did not seek confirmatory evaluation. We also attempted to identify potential risk factors for inappropriate behavior.

Materials and Methods

A sample of health screening attendees with raised blood glucose level (≥140 mg/dL), total blood cholesterol level (≥200 mg/dL), blood pressure reading (≥140/90 mm Hg), proteinuria and haematuria screened during the period from June 2003 to March 2004 were identified from our patient database (sample size = 1255). Demographic data of the attendees were collected for age, ethnic group, gender and educational status. Nurses conducted standardised follow-up telephone interviews with the respondents. Each respondent was asked whether they had gone for confirmatory tests with a physician pertaining to the screening abnormality that was detected by the nurses at the health screening session.

A maximum of 2 calls were made before a subject was excluded from the study. Respondents were grouped according to whether they had gone for confirmatory evaluation. Demographic characteristics were compared among the 2 groups. The Chi-square test was used for statistical analysis.

Results

One thousand, one hundred and forty-seven participants, with complete data, were included in the study. The response rate was 49.5% (568 respondents). Five hundred and seventy-nine participants (non-respondents) could not be contacted. However, demographic characteristics such as ethnicity, gender, age and educational status among respondents and non-respondents were similar. Two hundred and seventy-eight (48%) of the 568 respondents had visited a physician to do confirmatory tests, after being told to recheck their abnormal results.

Respondents who did not seek confirmatory evaluations had higher educational status compared to those who did (P = 0.005). There was no difference in gender, age, and ethnicity among respondents who attended and those who did not attend confirmatory evaluation with their physicians (Table 1). Respondents who have raised blood glucose (60.9%), raised blood pressure (60.2%) and haematuria (60.2%) were more likely to seek confirmatory tests (Table 2).

Discussion

The appropriate health-seeking behaviour of seeing a doctor for confirmation and management of screen-detected abnormalities in our participants were relatively high (48.9%, 278/568). In comparison

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Table 1

Characteristics	Consulted doctor (%)	Did not consult doctor (%)	*P*
Gender			
Male	139 (46.6%)	159 (53.4%)	0.249
Female	139 (51.5%)	131 (48.5%)	
Age (y)			
18-39	166 (49.8%)	167 (50.2%)	0.123
40-59	83 (44.4%)	104 (55.6%)	
≥60	29 (60.4%)	19 (39.6%)	
Race			
Chinese	252 (49.8%)	254 (50.2%)	0.106
Malay	10 (29.4%)	24 (70.6%)	
Indian	13 (56.5%)	10 (43.5%)	
Others	3 (60%)	2 (40%)	
Language			
English	182 (48.7%)	192 (51.3%)	0.799
Chinese	87 (50.3%)	86 (49.7%)	
English & Chinese	9 (42.9%)	12 (57.1%)	

Table 2

Characteristics	Consulted doctor (%)	Did not consult doctor (%)	*P*
Abnormalities			
Blood glucose	78 (60.9%)	50 (39.1%)	0.000
Blood pressure	56 (60.2%)	37 (39.8%)	
Blood in the urine	59 (60.2%)	39 (39.8%)	
Cholesterol	46 (37.4%)	77 (62.6%)	
Protein in the urine	39 (31.0%)	87 (69.0%)	
Education			
Below tertiary	181 (53.9%)	155 (46.1%)	0.05
Tertiary and above	97 (41.8%)	135 (58.2%)	

to the "Check Your Health" Community Health Screening Programme, only 15.4% (1530/9934) of the participants have attended a follow-up recheck with a physician.[4] The higher adherence rate may be due to the sufficient time given over the counselling station for each participant, which results in a better understanding of the importance of follow-up actions with a physician in regards to their abnormalities.

Raised blood glucose, raised blood pressure and haematuria were the abnormalities most likely to result in consultation for our participants. Fontana et al[5] have also reported increased consultations with physicians for patients with detected raised blood pressure during their primary health screening. Higher educated people are also less likely to seek confirmatory evaluation.

As treatment for diabetes, hypertension and high blood cholesterol level is crucial to prevent related complications such as end-stage renal disease, the NKF sends follow-up letters to those with abnormal results to remind them to seek further confirmatory tests with their doctors. As a result of the survey, the foundation has also commenced telephone follow-ups to reinforce the need for future evaluation. Its 2 prevention centres have also expanded their services to help participants confirm any abnormalities.

Conclusion

Health screening is a vital component of healthy living. Follow-up action is essential to ensure that participants with screened detected abnormality seek appropriate medical advice.

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Best Oral Presentation Award Finalist – Surgery/Obstetrics & Gynaecology/Dentistry/ Ophthalmology (Clinical Based) – Ethicon Surgical Book Prize (Clinical)

Long-term Survival Following Liver Resection for Colorectal Metastases – An Adelaide Experience

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Introduction

The aim of this study was to assess patient outcomes as well as the factors influencing patient and disease free survival in a cohort of patients treated over the last decade for surgically resectable liver metastases from colorectal primaries. Kaplan-Meier survival statistics and Cox regression were used to analyse factors that affected survival.

Materials and Methods

This was a retrospective study of prospectively collected data over the last 10 years, from February 1992 till April 2003. Data were taken from patients' operation reports and charts, and also direct follow-up with the respective general practitioners, referring physicians, oncologists and surgeons. Mortality was double checked with the cancer registry. The last follow-up death recorded was 21 April 2003.

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All cases were assessed by CT and MRI prior to surgery, with the use of routine PET scan in the past 3 years to exclude extra hepatic disease. It was the unit's policy to apply minimal resection to achieve tumour clearance. Routine intraoperative ultrasound was used. Parenchymal transection was achieved via a variety of techniques, more recently with CUSA and harmonic shears, with the pedicles being stapled.

Results

All patients who were operated in this centre were entered into the study. A total of 72 patients were treated over the period from February 1992 till April 2003. There were 53 male and 19 female patients. The median age was 63.8 (range, 31.7 to 81.7) years, with a mean of 63.2 years. The median follow-up period was 3.5 (range, 0.1 to 11.2) years, with a mean of 4.22 years. At the end of the study, there were 27 deaths and 45 patients who were alive, with 29 being disease free. Clear margins in this study were defined as those with margins microscopically free of tumour.

The results of the pathological resection margins, original colonic staging and dominant metastatic size are shown below:

- Resection margin
  Involved (or <1 mm) 4 (5%)
  <2 mm 7 (10%)
  Clear margin 61 (85%)
- Colonic staging
  A = 5 (7.7%)
  B = 21 (32.3%)
  C = 39 (60%)
  D = 5 (7.7%)
- Dominant metastasis size
  Median 45 mm (range, 6 to 140 mm)
  Mean 49 mm

Complications

There were no operative or hospital mortality. There were 7 bile leaks, 4 of which needed percutaneous drainage, with the leak sealing off. Two needed ERCP stenting, also with subsequent sealing of the leak and stent removal.

There were 7 chest infections, 1 prolonged small bowel ileus, which resolved with conservative management, and 1 patient who had a haematoma around the surgical site, which was drained percutaneously.

The Kaplan Meier curve in Figure 1 illustrates the overall patient survival and the patient disease free survival over a period of 5 years. The institution achieved a 1-year patient survival rate of 95% and a 5-year survival rate of 45%. The disease free survival rate was 71% in 1 year and 29% in 5 years.

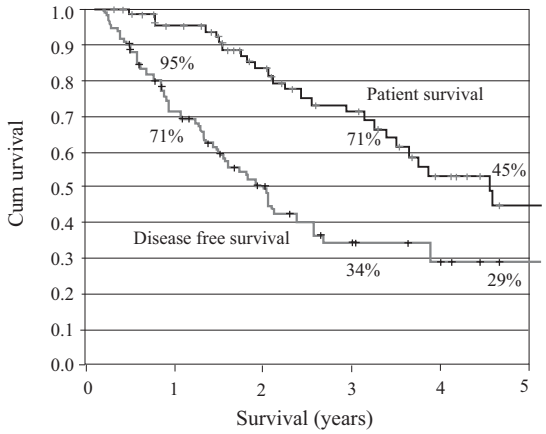

Fig. 1. Patient vs disease free survival.

A univariate analysis of various determinants which may affect patient overall survival revealed that age >70 years was significant with a *P* value of <0.002, and tumours >3 cm approached significance with a *P* value of 0.055. Sex, the type of resection, location of disease, Duke's staging of the colorectal tumour, the number of tumours, the resection margins, chemotherapy and the interval between colonic and liver resection were not significant.

With regards to disease free survival, age >70 years and bilobar disease proved significant in affecting recurrence of disease, with a *P* value of <0.004 and <0.01, respectively. Sex, the type of resection, Duke's staging of the colorectal tumour, the number of tumours, the resection margins, chemotherapy and the interval between colonic and liver resection were not significant in affecting recurrence.

Outcomes

Of the 72 patients treated, 45 patients were alive at the end of the study, with 29 among them disease free. Of the 43 who had recurrence of the disease, 26 (67%) had disease which included the liver, with 15 (38%) who had only liver recurrence. Fourteen had disease which included the lung (36%) and 3 had recurrent disease in other sites in the abdomen. Thirteen (33%) had disease recur in more than 1 site.

Five-year Patients' Survival

Author	Institution	Year	No.	5-year survival (%)
Foster	Multi	1978	170	20
Adson	Mayo Clinic	1984	141	25
Hughes	Multi	1988	859	33
Iwatsuki	Pittsburg/Colorado	1988	90	36
Fong	MSKCC	1997	456	38
Choti	John Hopkins	2002	226	40
Current series	Flinders + Ashford Med Centres	2003	72	45

Five-year Disease-free Survival

Author	Institution	Year	Number	5-year survival (%)
Harmon	Virginia Mason	1999	110	28
Choti	John Hopkins	2002	226	20
Kokudo	Cancer Institute Hospital, Tokyo	2002	102	29.7
Current series	Flinders + Ashford Med Centre	2003	72	29

Our institution had very comparable rates to current reported standards, with a 45% overall 5-year survival and a 29% 5-year disease free survival.

Conclusion

The patient 3- and 5-year survival after curative resection for colorectal metastasis was 71% and 45%, respectively. Twenty-nine per cent survived without recurrence at 5 years. Ninety-five per cent of cases had clear margins. Patient's age >70 years is associated with poorer patient survival, and metastatic size >3cm has shown a trend towards poorer survival, with a *P* value of 0.055. Anatomical resection in this study did not confer any advantage over non-anatomical resection. Bilobar disease was significant in affecting the recurrence of disease.

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Best Oral Presentation Award Finalist – Surgery/Obstetrics & Gynaecology/Dentistry/ Ophthalmology (Clinical Based) – Ethicon Surgical Book Prize (Clinical)

Rapid Prenatal Diagnosis by AmnioPCR and AmnioFISH: Routine Testing for Down's Syndrome (Trisomy 21) and Sex Chromosome Trisomies, but Targeted Testing for Edward's (Trisomy 18) and Patau's Syndromes (Trisomy 13)

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Introduction

Prenatal diagnosis of chromosomal abnormality using conventional metaphase karyotyping requires up to three weeks for the full diagnosis to be released. This delay results in significant parental anxiety. Amniotic fluid polymerase chain reaction (Amnio-PCR) and fluorescence *in situ* hybridisation (Amnio-FISH) are new, but expensive, molecular genetic techniques allowing rapid prenatal detection of fetal aneuploidy within 24 to 72 hours. Amnio-PCR involves identification and amplification of small-tandem-repeat (STR) markers located on the specific chromosome to identify the presence or absence of a chromosome. In Amnio-FISH, a chromosome is recognised by being labelled with a fluorescence probe. The efficacy of these techniques and the reduction of parental anxiety have been well-documented.[1-3] Given a choice, most patients and healthcare providers would request rapid tests followed by a full conventional karyotype.[4] The main limitation of these tests is the cost incurred by the individual and the healthcare system. The challenge is to find an economical way to provide these tests for a broader population.

Aneuploidies involving chromosomes 13, 18, 21, X and Y account for more than 80% of significant chromosomal abnormalities.[3] Therefore, prenatal samples are routinely analysed by PCR or FISH for all these 5 chromosomes in most centres, while awaiting the karyotype. Few fetuses with trisomy 13 or 18 have no ultrasound abnormalities.[3] We hypothesised that if we test for trisomy 21 and sex chromosome trisomies routinely, but for trisomy 13 or 18 only if ultrasound abnormalities were present, we would rapidly detect more than 90% of these fetal aneuploidies.

Materials and Methods

From January 1992 to April 2004, 14,091 antenatal diagnostic procedures were performed in our department, out of which 262 cases of trisomy 13, 18, 21 and sex chromosome abnormalities were diagnosed. The referral criteria for prenatal karyotyping included advanced maternal age, positive screening tests, abnormal ultrasonographic findings, previous child with chromosomal abnormality and parental chromosomal rearrangement.

The data were obtained via our electronic database and clinical notes. The presence or absence of ultrasound abnormalities was analysed for each case. The number and the type of abnormalities were also reviewed for each patient. The ultrasonographic and Amnio-FISH features of fetuses with chromosomal aneuploidies are shown in Figure 1.

Kruskal-Wallis and Mann-Whitney tests were used for analysis of these data.

Results

Distinct ultrasonographic abnormalities were seen in 76.7% of trisomy 18 (n = 46) and 90.0% of trisomy 13 (n = 18) fetuses. Only 23.3% of trisomy 18 (n = 14) and 10.0% of trisomy 13 (n = 2) fetuses had no detectable ultrasound abnormalities. In contrast, only 58.2% of trisomy 21 fetuses (n = 71) had structural abnormalities, whereas 41.8% of them (n = 51) had no detectable ultrasound abnormalities.

For sex chromosome trisomies (XXX, XXY, XYY), only 6.5% (n = 2) had structural abnormalities, but 96.6% (n = 28) monosomy X fetuses had abnormalities. The ultrasound evaluation of 93.5% (n = 29) of fetuses with sex chromosome trisomies and 3.4% (n = 1) of monosomy X fetuses were normal. However, as an entity, 50% of these fetuses with sex chromosome abnormalities had no ultrasonographic abnormalities.

This difference in the presence or absence of structural abnormalities in these cases of fetal aneuoploidies is statistically significant ($\chi^2 = 68.5$; df = 4; $P < 0.001$). If rapid detection for trisomy 21 and sex chromosome abnormalities were performed routinely, whereas Amnio-PCR /Amnio-FISH for trisomy 13 and 18 were performed only if indicated by the presence of abnormalities, 93.9% of these aneuploidies would be identified (z = 3.2; $P = 0.001$) (Fig. 2).

Our data showed that routine testing for trisomy 21 is recommended, as a significant percentage of abnormal fetuses would otherwise be missed with the rapid tests. However, for trisomy 13 and 18, the majority of the fetuses have ultrasound abnormalities. In fact, the majority of these fetuses have multiple rather than single abnormalities. It would therefore be more cost-effective if Amnio-PCR/Amnio-FISH were done for chromosomes 13 and 18 only if indicated by abnormal ultrasound findings.

For sex chromosome abnormalities, our study showed clearly that most of the fetuses with sex chromosome trisomies have no structural

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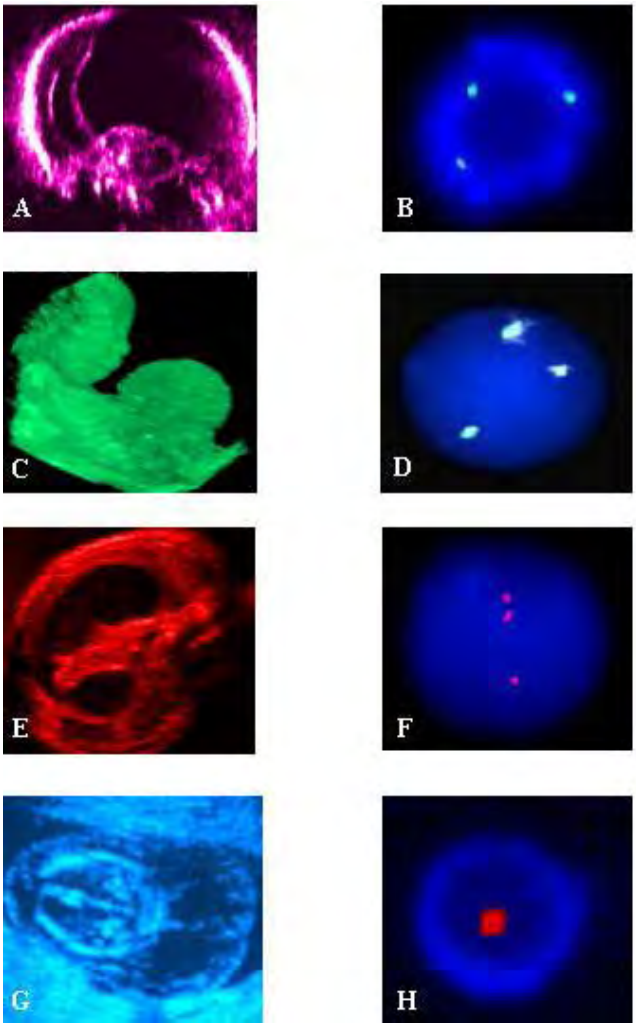

Fig. 1. Ultrasonographic and Amnio-FISH features of fetuses with chromosomal aneuplodies. (A) Holoprosencephaly in Trisomy 13. (B) Amnio-FISH showed 3 copies of chromosome 13. (C) Exomphalos in Trisomy 18. (D) Amnio-FISH revealed 3 copies of chromosome 18. (D) Double bubble sign characteristic of duodenal atresia in Trisomy 21. (E) Amnio-FISH showed 3 copies of chromosome 21. (F) Cystic hygroma is a characteristic feature of Turner's syndrome (XO). (G) Amnio-FISH showed a single copy of chromosome X.

abnormalities. However, almost all the monosomy X fetuses have distinct ultrasonographic abnormalities. The analysis of sex chromosome abnormalities as an entity suggested that rapid testing for chromosomes X and Y is still recommended as half of them appear ultrasonographically normal.

Discussion

We have shown that a significant proportion of fetuses with trisomy 21 and sex chromosome abnormalities had no structural abnormalities but most of trisomy 13 and 18 fetuses have ultrasonographic abnormalities. The findings suggest that routine testing should be done for chromosomes 21, X and Y, but targeted testing can be performed for chromosomes 13 and 18, when prenatal samples are sent for Amnio-PCR/Amnio-FISH.

As prenatal ultrasound technology advances, especially with the introduction of 3 or 4 dimensional ultrasound machines, the sensitivity

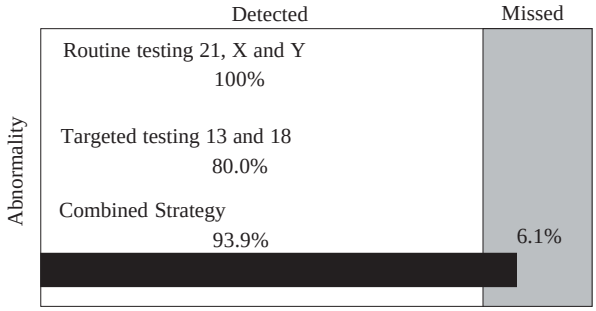

Fig. 2. Routine rapid testing for chromosomes 21, X and Y (100% detection rate) and targeted testing for chromosomes 13 and 18 (80% detection rate) allows almost 94% rapid detection of these aneuploidies (z = 3.2; P = *0.001).*

of detecting abnormalities will continue to improve. This will further reduce the number of cases where abnormalities are missed on ultrasound evaluation. It will therefore make the strategy of performing rapid tests based on ultrasound indications even more accurate.

If the cost issue can be alleviated, the healthcare providers will be able to provide rapid results for a broader patient population. Implementation of this service could lead to rapid diagnosis of abnormalities and early reassurance for women with normal results. Earlier clinical decision could also be made for patients with abnormal results. The American College of Medical Genetics and most authors agreed that clinical decision can be made if the positive PCR/FISH results are associated with ultrasound findings compatible with the aneuploidy detected by the rapid prenatal tests.[4-6] Evans et al[6] had shown in a series of over 300 high-risk patients that there was 100% concordance between ultrasonographic predictions of aneuploidy, and confirmation with FISH results.

Our work showed that, when performing Amnio-PCR/Amnio-FISH, routine testing for trisomy 21 and sex chromosome abnormalities but targeted testing for trisomies 13 and 18 based upon ultrasound evidence of abnormality is scientifically sound. Costing less, and allowing rapid detection of almost 94% of significant fetal aneuploidies, this novel approach will be readily accepted by couples-at-risk. This strategy can therefore allow rapid prenatal tests for a wider, even nationwide population.

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Best Oral Presentation Award Finalist – Surgery/Obstetrics & Gynaecology/Dentistry/ Ophthalmology (Clinical Based) – Ethicon Surgical Book Prize (Clinical)

Reconstruction of the Ocular Surface by Transplantation of a Serum free Cultivated Conjunctival Tissue equivalent

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Introduction

Damage to the conjunctiva may arise from various diseases, such as pterygia, conjunctival tumours, chemical injury or surgical trauma. Treatment of these disorders, by surgical removal of large areas of conjunctiva results in subconjunctival scarring and fibrosis, and may be complicated by granuloma formation, cicatricial fornix shortening, symblepharon formation, or ocular motility restriction.[1] The use of free conjunctival autografts to repair these surgical defects is accompanied by iatrogenic injury to the donor site, and may further complicate the management of patients with extensive or bilateral ocular surface disorders.

The use of bioengineered conjunctival equivalents represents a novel approach to replace conjunctiva, without causing iatrogenic injury associated with the harvesting of large autografts. It is particularly useful in situations where the normal conjunctiva is deficient either from disease or scarring. Conventional culture methods for ocular surface epithelial cells require the use of bovine serum and murine 3T3 feeder cells, with the attendant risks of transmission of zoonotic infection (e.g. bovine spongioform encephalitis) and xenograft rejection.[2-5] The use of serum-free media is therefore significantly advantageous, as it eliminates the need for serum and feeder cells, thereby reducing these risks.

We describe the development of serum-free derived autologous conjunctival tissue-equivalents and report the novel use of these cultivated conjunctival equivalents for ocular surface transplantation and reconstruction.

Materials and Methods

We conducted a prospective clinical trial involving autologous cultivated conjunctival transplantation for the treatment of ocular surface diseases. This study was approved by the Institutional Review Board and written informed consent was obtained for all patients.

Development of a Conjunctival Tissue-Equivalent

Preparation of human amniotic membrane (HAM) substrates. HAMs were obtained from healthy mothers who had undergone cesarean sections. The HAMs were cleaned and placed basement–membrane side up on a nitrocellulose support. They were then incubated with Dispase II 1.2 U/mL to remove any amniotic epithelial cells.

Ex vivo expansion of conjunctival epithelial cells. Superior forniceal conjunctival biopsies were obtained from healthy donors undergoing routine pterygium or cataract surgery. The conjunctival tissues were cut into 0.5 mm pieces, and cultivated on the HAM under serum-free conditions, at 37°C, 5%CO_2 and 95% air, with media change carried out every 2 days. Upon reaching confluence, cells were exposed to differentiating medium to promote stratification. Two weeks later, a confluent stratified epithelial sheet was obtained over the HAM substrate.

The conjunctival equivalents were analysed by light and electron microscopy. The expression of keratins K4 and 19, and MUC5AC goblet cell mucin, was determined by immunohistochemistry.

Ocular Surface Transplantation

All patients underwent a superior forniceal conjunctival biopsy, measuring 1 mm x 3 mm in size. The conjunctival epithelium was carefully dissected free from the underlying tenons, cut into 0.5 mm pieces, and inoculated onto the basement-membrane side of the HAM in serum-free media, using the methods described above.

Transplantation of cultivated conjunctival equivalents. Definitive surgery 2 weeks later involved excision of the diseased conjunctiva and transplantation of the conjunctival equivalent. The area of diseased conjunctiva was first excised. The conjunctival equivalent was then cut in size to match the surgical defect and sutured in place with interrupted 10/0 vicryl sutures. Tobradex eyedrops were administered for 1 month following surgery.

Postoperatively, these patients were monitored with serial slit-lamp examinations and fluorescein staining to access the epithelial integrity. Main outcome measures included maintenance of conjunctival epithelialisation, integrity of the graft, resolution of the disease, and presence of complications. All patients were followed up for a minimum of 6 months.

Results

Twenty-six patients with various ocular surface diseases requiring conjunctival transplantation and reconstruction were treated. The mean age of patients was 48.3 $\pm$ 12.1 years (range, 9 to 72 years). There were 13 males and 13 females. Fourteen left eyes and 12 right eyes were operated on. The mean follow-up period was 12.9 $\pm$ 4.3 months (range, 8 to 18 months).

A stratified conjunctival epithelial sheet was formed on the HAMs. Following transplantation, complete epithelialisation was confirmed by the absence of fluorescein staining within 72 hours. Patients were noted to have less conjunctival inflammation, as compared to conventional autograft surgery. A good functional and cosmetic result was achieved in all eyes. A successful outcome, as defined as resolution of the disease, maintenance of conjunctival epithelialisation and maintenance of graft integrity was obtained in all patients. There were no cases with significant subconjunctival scarring, symblepheron formation, eyelid cicatrisation, or ocular motility restriction. No significant complications were noted during the follow-up period and the ocular surface with surviving transplanted epithelia remained stable and healthy.

Ultrastructural examination of the conjunctival equivalents revealed

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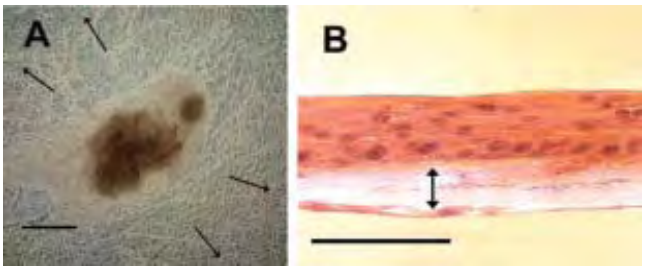

Fig. 1. Human conjunctival epithelial cells cultivated on a HAM substrate. (A), epithelial cell outgrowth from explant culture on day 3, as observed under a phase contrast microscope. (B), light microscopy demonstrating a stratified conjunctival epithelial sheet on HAM (HAM is indicated by the double-headed arrow). (A) Bar = 500 μm, (B) Bar = 63 μm.

apical microvilli and a basal lamina with hemidesmosomes. Immunostaining confirmed the expression of cytokeratins 4 and 19, and MUC5AC mucin.

Discussion

We describe the safe and effective use of autologous cultivated conjunctival transplantation for the treatment of ocular surface disease. This treatment modality results in almost immediate epithelialization of the ocular surface, thereby allowing earlier ocular rehabilitation, a reduction in postoperative inflammation, and a rapid return of the protective and supportive function provided by the conjunctiva.

Small conjunctival biopsies were taken from the fornix, the site enriched in conjunctiva stem cells.[6] A significant advantage is the minimal damage to the normal conjunctival surface, as compared to conventional surgery where large pieces of normal conjunctiva may need to be harvested. The use of autologous tissue minimizes any risk of immunologic rejection and negates the use of immunosuppressive therapy. In addition, the use of amniotic membranes has been shown to promote wound healing and reduce scarring.

Previous studies on epithelial cell culture have required the use of animal serum and feeder cells, with the attendant risks of zoonotic infection and xenograft rejection.[2-5] The elimination of bovine serum and feeder cells from our culture system has the advantage that it reduces the risks associated with the use of animal material for clinical transplantation.

Transplantation of autologous serum-free cultivated conjunctival equivalents may provide a novel method for treating a wide range of ocular surface disorders where the normal conjunctiva is damaged or deficient. This is particularly useful in diseases requiring extensive conjunctival excision, bilateral diseases, severe ocular surface disease with conjunctival contracture, or when preservation of the conjunctiva is required for future glaucoma surgery. These findings bring us one step closer towards the development of a safe and effective xenobiotic-free bioengineered tissue-equivalent for clinical transplantation.

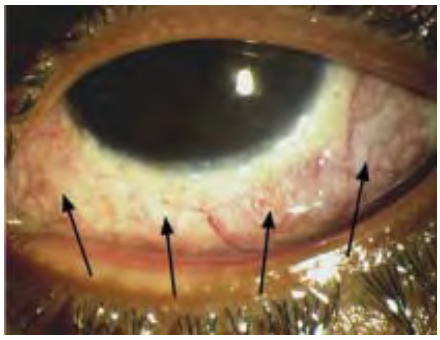

Fig. 2. Day 3 postoperative appearance, demonstrating restoration of the ocular surface following cultivated conjunctival transplantation. The transplanted conjunctival tissue-equivalent shown here has resurfaced the entire inferior bulbar conjunctival surface (indicated by arrows), following a large surgical excision of diseased tissue.

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Best Oral Presentation Award Finalist – Surgery/Obstetrics & Gynaecology/Dentistry/ Ophthalmology (Laboratory Based) – Ethicon Surgical Book Prize (Laboratory)

The Expression of Insulin-like Growth Factor-I in Periodontal Healing Following Tooth Replantation

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Introduction

Tooth avulsion represents the most severe form of dental injuries and is known to inflict extensive damages to the pulp and periodontium. While necrotic pulp resulting from severance of apical neurovasculature could be dealt with by optimal timely endodontic therapy, it is the re-establishment of the vital intact periodontal ligament (PDL) that is crucial for maintaining a stable tooth/bone interface and essential for tooth retention.

Attempts to regenerate functional periodontium after tooth replantation following undesirable prolonged extra-oral duration/ condition, which could potentially lead to tooth loss, had not been met with success. The mechanism underlying functional healing following replantation of avulsed teeth with severely damaged periodontium had not been fully elucidated.[1]

Recent evidence has implicated growth factors in the wound repair and regenerative processes. Insulin-like Growth Factor (IGF) has been shown to play biologic roles in periodontal disease model.[2] Nevertheless, there is no known study to date evaluating the immunohistochemical profiles of IGF-I potentially modulating the periodontal healing following tooth replantation.

Aim

The aim of this study was to characterise the early temporal expression of IGF-I in the normal periodontium and the healing periodontal tissues following immediate and delayed replantation.

Materials and Methods

Experimental Procedures

The animal surgical experiments were performed in accordance with the International Guiding Principles for Animal Research. Under general anaesthesia, periodontally healthy and caries-free mature teeth from 6 adult mongrel dogs (20 kg) were endodontically treated to prevent inflammatory root resorption of pulp origin. The hemisected premolar roots were used as separate units.

Experimental Samples

The experimental samples consisted of 64 randomly distributed roots in the control group and 2 treatment groups. The roots in the non-experimental control group (serving as a baseline) were not extracted. The roots in the immediate replantation (representing optimal healing) and in the delayed-replantation (representing adverse healing) treatment groups were extracted simulating avulsion injury and atraumatically replanted into the sockets immediately and after 1-hour bench-drying, respectively.

Specimen Preparation

The jaw blocks containing the replanted roots and the surrounding periodontal tissues were harvested according to the representative healing phases at ½-, 2-, 3- and 4-day periods. The specimens were fixed, decalcified, processed for embedment in paraffin wax, step-serially cross-sectioned at 5-µm thickness, 100-µm intervals and prepared for immunohistochemistry according to the standard protocols. The sections were stained with primary antibody, rabbit polyclonal antihuman IGF-I (ab9572, Abcam Ltd, Cambridge, UK) at the concentration of 1:50 polyclonal IGF-I antibody with 10% PBS at 4°C. The Xam-mounted slides were then subjected to immuno-histomorphometric assay modified from the established histomorphometric evaluation method.[3]

Immuno-histomorphometric Assay

The specimens were evaluated using a light microscope (Nikon Optiphot II, Tokyo, Japan) at x40 and x400, with images projected onto a monitor (JVC, Yokohama, Japan) superimposed on a four 45° angle radii grid. With the center of the grid coinciding with the center of the root canal in the labiolingual axis, the 3 periodontal structures (cementum, PDL and bone) at the 8 intersection points along the root circumference were evaluated by 2 independent prior-calibrated examiners.

Statistical Analysis

The percentage of immunopositive cell counts and the mean extracellular intensity score, based on a pre-formulated visual analog scale (range, 0 to 3) (Fig. 1), were computed. Further analysis using Kruskal-Wallis and Mann-Whitney U tests with P value set at ≤ 0.05 were used to compare each periodontal structure over the 4 time points; the 3 periodontal structures at each time point within each experimental group; as well as the non-experimental group and the 2 treatment groups at each time point.

Results

The results are summarised in Figure 2. In bone, the lowest percentage cell count in the immediate replantation group at day 2 (89.05 ± 5.10%) was significantly lower than at day 4 and the non-experimental group ($P = 0.00$). There were also significant differences in percentage of cell counts between PDL and bone at day 2 ($P \leq 0.01$).

IGF-I was least expressed in bone extracellular matrix in delayed replantation group at day ½ (1.29 ± 0.17) compared to day 3 (1.95 ± 0.43) and day 4 (1.72 ± 0.31) as well as the controls (1.66 ± 0.17) ($P < 0.01$). The extracellular bone intensity scores in the immediate replantation group at day ½ (1.57 ± 0.23) and day 3 (1.42 ± 0.37) were significantly different from that of delayed replantation group (P =0.029; 0.008).

Discussion

The 100% IGF-I-positive cell counts and the mild to moderate immuno-stained extracellular matrix in all 3 periodontal structures suggested that IGF-I is stored in the cementum, PDL and bone under the physiologic condition.

It was observed that, cementum, being avascular mineralised connective tissue, appeared to play less significant role, compared to the vascularised bone and PDL, in the modulation of periodontal healing following tooth replantation.

The findings that the percentage of immunopositive-PDL cells and

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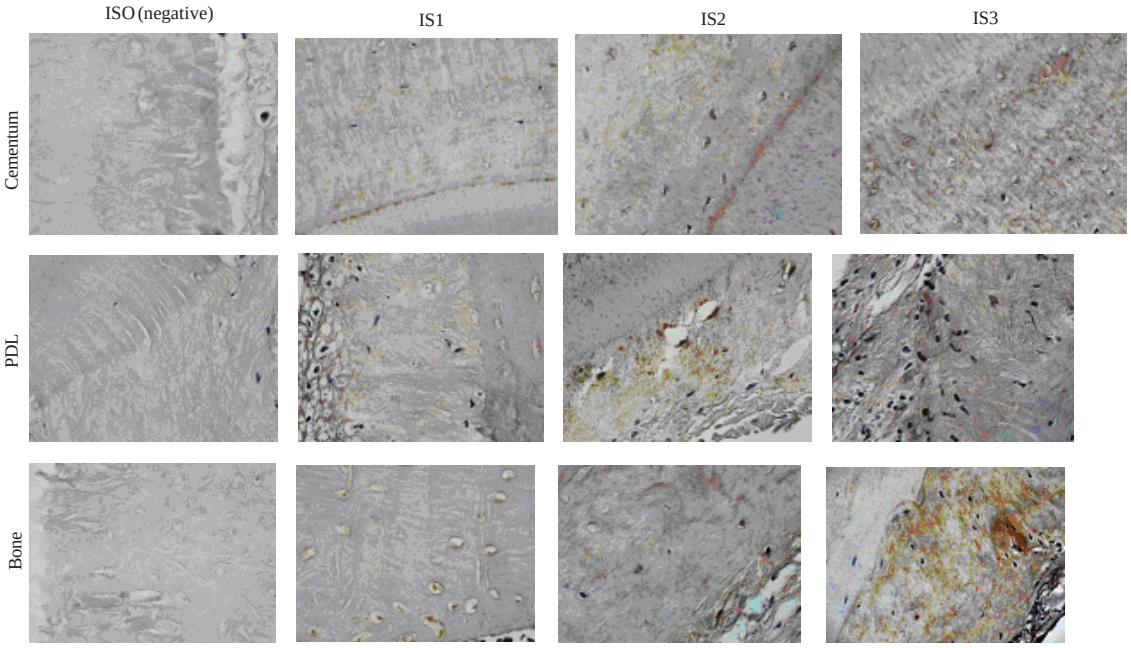

Fig. 1. Visual analog scale of intensity scores for IGF-I: Intensity Score (IS) 0 (negative), IS 1 (mild), IS 2 (moderate) and IS 3 (intense) for cementum, periodontal ligament and bone. Immunohistochemical staining with DAB chromogen, counterstained with haematoxylin.

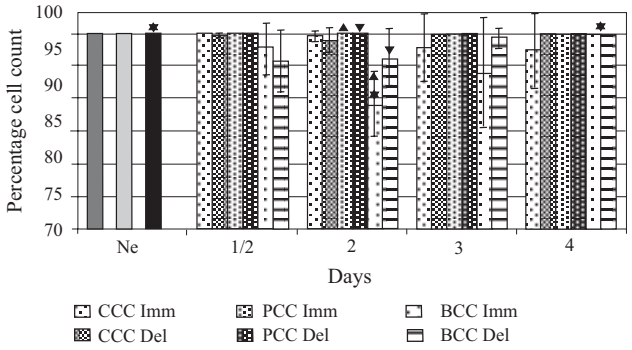

Fig. 2a. Comparison of percentage of cell counts in cementum, periodontal ligament and bone between immediate and delayed replantation groups at different observation times.

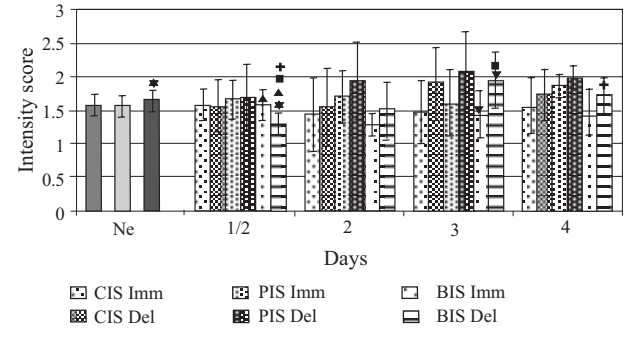

Fig. 2b. Comparison of intensity scores in cementum, periodontal ligament and bone between immediate and delayed replantation groups at different observation times.

the extracellular intensity in the 2 replantation groups at all observation timing were consistently near to the physiologic baseline, despite direct injuries incurred within PDL during the extraction/avulsion, substantiated the inherently high PDL turnover rate and the mitogenicity of IGF-I for PDL fibroblasts.[2]

With the ongoing bone remodelling, the decrease from the physiologic baseline in the percentage of IGF-I-positive-bone cells at day 2 and the subsequent normalisation at day 4 implicated the participation of paravascular progenitor cells within 48 hours.[4] The significant decrease in extracellular intensity at day ½ compared to the physiologic baselines and to day 3 and day 4 delayed replantation group may be due to stimulation of osteoblastic proliferation, inducing noncollagenous proteins such as bone sialoprotein and osteopontin[5] in the extracellular matrix.

In the delayed replantation group, the blood clot formed in the 1-hour socket could have served as a biological barrier to IGF-I rich plasma.[6] This might explain the initial significantly low IGF-I expression in bone matrix at day ½ with the subsequent up-regulation at day 3, signalled by intensified inflammatory response perpetuated by the 1-hour desiccated necrotic PDL cells as the clot gradually replaced by granulation tissue. High IGF-expression correlated well with the area of injury where demand for cellular repair and regeneration is.

Conclusion

The early temporal and spatial IGF-I expression profile appears to be implicated in periodontal healing following tooth replantation.

Acknowledgement

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Best Oral Presentation Award Finalist – Surgery/Obstetrics & Gynaecology/Dentistry/ Ophthalmology (Laboratory Based) – Ethicon Surgical Book Prize (Laboratory)

Early Detection of Epithelial Ovarian Cancer Using a Proteomics-based Protein-Profiling Approach Combined with a Novel Selection Strategy

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Introduction

Ovarian cancer (OC) is the fifth most common cancer among women. The American Cancer Society estimates that about 25,580 new cases of OC will be diagnosed in the US during 2004. About 85% of OCs are epithelial ovarian carcinomas (EOC). The current survival of patients with EOC is low (5-year survival = 30%). The strategy that can help these patients is early diagnosis (Stage 1, 5-year survival = 90%).

The presence or abundance of specific molecules (biomarkers) in a biological fluid signals disease.[1,2] The conventional approach relies on detection and monitoring of solitary markers in diagnostics and is not always reliable. The standard screening tool for EOC, the CA125, lacks sensitivity and specificity.[3] Recently, newer classes of biomarkers derived from mass spectrometry analysis of low molecular weight proteome have shown improved results in early detection of EOC .[4]

We hypothesise that early EOC has a distinctive plasma/serum protein-profile signature (PPS) that allows it to be distinguished from normal individuals, and from patients with benign ovarian cysts (BOC) and late EOC. To support this, we have developed 2 diagnostic processes based on PPS that allow 100% accurate diagnosis of these 4 categories of patients without any misdiagnosis.

Material and Methods

A total of 129 plasma and 109 serum samples were analysed: early EOC (7,13), healthy controls (35,33), BOC (49,49), late EOC (18,34). The first number in brackets corresponds to serum samples, the second to plasma samples. Proteins from plasma and serum samples were analysed using surface-enhanced laser desorption/ ionisation time-of-flight mass spectrometry (SELDI-TOF MS). Different chip surface chemistries (NP20, IMAC, SAX2, WCX2, H4) were tested and the hydrophobic H4 chip was selected as proteins detected on the H4 chip were the most numerous when compared to other chips in the selected molecular weight range of 0 to 20 kDa. The samples were processed according to the recommended Ciphergen procedures.

After spectra have been obtained, they were processed and analysed as explained in the following section on Bioinformatics Analysis.

Bioinformatics Analysis

We developed a new method to correctly diagnose patients with the 4 categories based on serum and plasma protein spectra (PS). The method relied on combinations of proteomic patterns (PPs) determined from PS. Each spectrum contained a total of 22,500 data points. A baseline correction was made to reduce the noise in spectra. Essentially, the feature selection was based on selecting those PPs that were the least mutually correlated and whose combination produced the best separation between the considered classes based on linear discriminant models. For training, a very small fraction of available data was used (Table 1) while the remaining data was used for blind testing. We attempted to use as few PPs as possible while ensuring accurate diagnosis.

Results

We established hierarchical diagnosis strategies, as shown in Fig. 1, which resulted in 100% accuracy (sensitivity = 100%, specificity = 100%) in diagnosis of cases from both the training data and the test data used as blind tests. Diagnostic processes are illustrated in Figure 1. Three diagnosis steps were required for serum or plasma PS to produce correct diagnosis of all cases (early EOC, late EOC, benign, normal). The diagnostic processes are illustrated in Figure 1 and Table 1. In plasma based PS, cancer cases were first separated from non-cancer cases. In the cancer cases, early-stage EOC cases were separated from the late-stage EOC. Similarly for non-cancer cases, benign cases were separated from normal, thus allowing complete diagnosis of patients into the 4 categories. The process was applied to serum based PS as per Figure 1. PPs from serum and plasma constituents of blood showed distinct appearance of proteins for each of the 4 categories. Majority of the proteins were identified in the region between 7 and 12 kDa. These were used in the

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Table 1. Diagnosis Results Produced on the Training and Test Data for Serum Based Proteomic Spectra and for Plasma Based Proteomic Spectra

Serum based proteomic spectra						
Non-cancer		Cancer			Model type	Features
Normal	Benign	Early	Late	Unclear		
35	49	7	18	7		
4		8			Training set 1 (cancer + benign vs. normal)	9
31		68			Test 1 (cancers + benign vs. normal)	9
	10		3		Training set 2 (cancer vs. benign)	12
	39		22		Test 2 (cancer vs. benign)	12
		2	2		Training set 3 (early vs. late)	2
		5	16		Test 3 (early vs. late)	2
Plasma based proteomic spectra						
Non-cancer		Cancer			Model type	Features
Normal	Benign	Early	Late	Unclear		
33	49	13	34	7		
10		5			Training set 1 (cancer vs. non-cancer)	8
72		42			Test 1 (cancer vs. non-cancer)	8
		2	8		Training set 2 (early vs. late)	8
		11	26		Test 2 (early vs. late)	8
3	5				Training set 3 (benign vs. normal)	12
30	44				Test 3 (benign vs. normal)	12

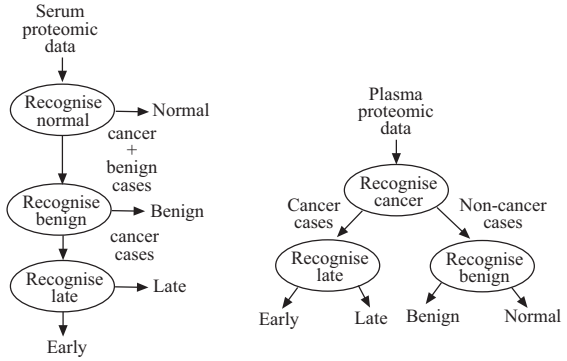

Fig. 1 Illustration of diagnostic processes in cases when diagnosis is made based on serum or plasma spectra. Different diagnostic procedures are applied for data from serum and from plasma. Diagnostic models in both cases are applied 3 times in order to diagnose all possible cases. However, diagnostic models are developed for different groups of patients and used different proteomic patterns in the diagnosis decision. The final goal is to diagnose patients with the correct category out of 4 (early EOC, late EOC, benign, normal). The nodes in the diagnosis processes depicted represent points where decisions are made. The text next to branches indicates which cases have been distinguished by the diagnostic process.

generation of diagnostic models (DMs) which are illustrated in the case of plasma data (Table 1). To separate cancer from non-cancer cases, 10 non-cancer and 5 cancer cases were randomly selected, and DM trained to separate them. The number of selected PPs was increased from 1 to 8, until perfect diagnosis was achieved. Two cases from early EOC and 8 cases from late EOC were used to train DM to separate these cases and required 8 PPs. Analogously, we used 3 normal and 5 benign cases to train DM to separate benign from normal cases with 12 PPs. The resultant DMs and the diagnostic process produced perfect diagnostic results on both the training and test data without any misdiagnosis.

Discussion

Our goal is not only to apply these biomarkers for the early diagnosis of EOC (benign or malignant), but also to distinguish benign from malignant disease. The novelty of this approach is the minimum number of features required to discriminate different stages of cancer.

Previous reports were not based on the clinically tested data as done in our study based on several supporting evidences.[5] Our approach is more of diagnosis type and not of screening as was focus of previous studies.

We have used both serum and plasma proteins for the first time to discriminate different classes and stages of EOC. However, we were not able to identify a biomarker that correlated in size to CA125. We speculate that CA125 may not bind to the H4 or other chips used in our studies or that its large size is not conducive to ionisation and flight. Majority of the markers identified were found in the low molecular weight range in the region between 7.5 kDa and 10 kDa, similar to previous reports.[6]

In conclusion, we have discovered a novel selection strategy based on protein profiling technology that could significantly improve the early detection of EOC.

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Best Oral Presentation Award Finalist – Surgery/Obstetrics & Gynaecology/Dentistry/ Ophthalmology (Laboratory Based) – Ethicon Surgical Book Prize (Laboratory)

Non-invasive Prenatal Diagnosis of Fetal Gender Using Real-time Polymerase Chain Reaction Amplification of *SRY* in Maternal Plasma

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Introduction

In Singapore, 1 in 5 pregnancies occur in mothers >35 years old and genetic diseases are common. Without prenatal diagnosis, 1 in 50 babies are born with serious physical or mental handicap, and as many as 1 in 30 with some form of congenital malformation. Current methods for the diagnosis of aneuploidy and monogenic disorders require invasive testing by amniocentesis, chorion villus biopsy or fetal blood sampling. These tests carry a procedure-related risk of miscarriage of 1% to 4% that is unacceptable to many couples. Non-invasive methods of prenatal diagnosis are desired to both patients and clinicians. A novel approach for non-invasive prenatal diagnosis is the use of cell-free fetal DNA in the serum or plasma of pregnant women. The presence of fetal DNA in maternal plasma was first demonstrated by Lo et al in 1997.[1] He showed that fetal DNA constitutes a mean 3.4% (range, 0.39% to 11.9%) of the total plasma DNA in the first trimester and a mean of 6.2% (range, 2.33% to 11.4%) in the third trimester.[2] This would mean that fetal genetic material can be obtained in first trimester maternal plasma for genetic analysis. We successfully isolated cell-free fetal DNA from maternal plasma in as early as 8 gestational weeks. Using real-time polymerase chain reaction (PCR), we were able to amplify and quantify the isolated fetal DNA. Fetal gender is also determined with 100% accuracy using this novel non-invasive prenatal diagnosis technique.

Materials and Methods

Sample Collection and DNA Isolation

Sample collection for research was approved by the Institutional Review Board. Two millilitres of maternal peripheral blood were collected in EDTA tubes after written informed consent from 23 mothers undergoing amniocentesis, ultrasound or termination of pregnancy (TOP) between 6^{+5} to 36^{+4} gestational weeks. Trophoblast tissues were collected from TOP for molecular genetic analysis of fetal gender. All blood samples were processed within 2 hours of sampling. The peripheral blood was centrifuged at 2760 rpm for 10 minutes and at 13,000 rpm for another 10 minutes to obtain the plasma fraction for DNA isolation. DNA was isolated from 800 µL of plasma using the High Pure PCR Template Preparation Kit according to the manufacturer's recommendations (Roche Diagnostics GmbH, Germany).

Quantitative Measurement and Fetal Gender Determination from DNA Isolated from Maternal Plasma using Real-time Quantitative PCR

Real-time PCR analysis was performed by the use of a PE Applied Biosystems 7000 Sequence Detector. *β-globin*, a chromosome 11 locus, was used as the endogenous control for the quantitative measurement of total DNA. The Y chromosome *SRY* (testis-determining factor) locus was used for the quantitation of fetal DNA and fetal gender determination. The sequence of primers and probe combinations were described by Lo et al.[2] Positive controls for male gender determination were commercial male genomic DNA while commercial female genomic DNA were used as the negative controls. The commercial male genomic DNA was serially diluted 5-fold and set as standards to generate the standard curve for absolute quantitation. Each sample and standard was run in triplicates with both sample and standards running in parallel.

Fluorescence in situ Hybridisation (FISH) of Trophoblast Tissues for Fetal Gender

In all cases of TOP, FISH of trophoblast cells was used to confirm fetal gender. We used centromeric enumeration CEP® X/Y probe (Vysis Inc, Downers Grove, Illinois, USA) to label the sex chromosomes for fetal gender analysis.

Results

Qualitative Analysis

Of the 23 maternal plasma samples analysed, 13 male and 10 female fetuses were identified by real-time PCR amplifications of *SRY* and *β-globin.* Endogenous controls (*β-globin*) amplified in all reactions (100%) and *SRY* was amplified in 13 maternal plasma samples. For samples obtained from mothers undergoing amniocentesis, fetal genders were confirmed by cytogenetic karyotypes. Ultrasound results were confirmed at birth. FISH of trophoblast cells obtained from TOP confirmed fetal gender. In all cases (100%), fetal gender was concordant between *SRY* amplification and known fetal gender (chi-square 23.0; Fisher's exact $P < 0.001$).

Quantitative Analysis

The conversion factor of 6.6 pg of DNA per cell was used, for expression of results as genome equivalents (GE). Total DNA concentrations in maternal plasma (measured by *β-globin* amplifications) ranged from 355.4 to 4642.4 GE/mL (median = 1653.0 GE/mL). Fetal DNA concentrations determined from *SRY* amplifications ranged from 3.04 to 169.00 GE/mL (median = 10.97 GE/mL).

Sensitivities of SRY and β-globin Assays

The lowest serial dilution of commercial male genomic DNA is 1.5 GE/mL, which is amplified and detected by both *SRY* and *β-globin* real-time PCR assays in all runs.

Standard Curves

Regression coefficients for all runs were at -0.99 (ideal -1.00). For *SRY* standard curves, the mean ± SD slope was -3.55 ± 0.16 with the mean ± SD intercept of 38.11 ± 0.49. For *β-globin* standard curves, the mean ± SD slope was -3.52 ± 0.13 with the mean ± SD intercept of 37.13 ± 0.78.

Discussion

In this study, we had shown: (1) 100% accuracy in fetal gender determination using maternal plasma in 3 trimesters (Table 1); (2) that cell-free fetal DNA in maternal plasma can be quantified in as early as 8^{+2} gestational weeks (Table 1); (3) that fetal DNA concentration in maternal plasma increased as pregnancy progressed

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Table 1. Determination of Fetal Gender from Maternal Plasma

Sample	Gestational weeks	Real-time PCR results	Confirmatory results
1	6+5	Female	XX
2	7+6	Female	XX
3	8+2	Male	XY
4	8+5	Female	XX
5	8+5	Male	XY
6	9+0	Male	XY
7	9+3	Female	XX
8	16+4	Male	XY
9	21+5	Female	XX
10	21+6	Female	XX
11	22+0	Male	XY
12	24+0	Male	XY
13	32+0	Female	XX
14	32+0	Male	XY
15	32+0	Male	XY
16	33+1	Male	XY
17	34+1	Male	XY
18	35+0	Male	XY
19	35+4	Female	XX
20	36+0	Female	XX
21	36+0	Male	XY
22	36+2	Male	XY
23	36+4	Female	XX

Out of 23 maternal plasma samples (gestational weeks ranged from 6^{+5} to 36^{+4}), 13 are determined as male while 10 are determined as female fetuses. Real-time PCR results are 100% concordant with confirmatory results obtained from conventional karyotype, fluorescence in situ hybridisation and at birth.

(from a median of 11.1 GE/mL in the first trimester to a median of 84.8 GE/mL in the third trimester) (Table 2); (4) with a detection limit of 1.5 GE/mL, the sensitivities of our *SRY* and *β-globin* real-time PCR assays are high. The lowest fetal DNA concentration that we had quantified from maternal plasma was 3.04 GE/mL (Table 2).

Cell-free fetal DNA in maternal plasma provides an alternative source of fetal genetic material without the need for invasive procedures. As demonstrated in this study, this source of fetal DNA can be used for fetal gender determination. Other genetic analysis that is feasible with this non-invasive prenatal diagnosis technique includes assessment of sex-linked disorders, the detection of unique

Table 2. Relationship of Gestational Age with Fetal DNA and Total DNA Concentrations in Maternal Plasma

	SRY concentration (GE/mL)		
	1st trimester	2nd trimester	3rd trimester
Range	11.0-26.2	3.0-129.2	67.4-121.6
Mean	16.1	56.8	100.1
Median	11.1	38.0	84.8
	β-globin concentration (GE/mL)		
	1st trimester	2nd trimester	3rd trimester
Range	355.4-2544.1	722.4-2443.4	802.8-4642.4
Mean	1413.73	1424.3	2352.3
Median	1476.67	1171.2	2315.2

GE/mL: genome equivalents per millilitres of maternal plasma

gene sequences such as RhD locus to determine fetomaternal blood group incompatibility[3], and the detection of dominantly inherited, paternally derived mutations for single gene disorders diagnosis.[4,5] Advances in technology had also allowed researchers to expand the genetic analysis of fetal DNA in maternal plasma to include more mutations.[6]

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