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*“Whoever goes with the wise becomes wise,
but the companion of fools suffers harm.”*

Anonymous Author

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Understanding Intra-tumour Heterogeneity—The Next Holy Grail of Cancer Therapeutics?

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Advances in strategies to characterise tumours in the last decade have uncovered distinct molecular subtypes with prognostic and predictive relevance in both solid tumours and haematological malignancies. While conventional chemotherapy remains the mainstay in cancer therapeutics, the recognition of distinct “actionable genotypes” has led to a new paradigm shift towards targeted therapy, both as primary or adjunct treatment with chemotherapy, to improve patient outcomes.

Inter- and Intra-tumour Heterogeneity

Inter-tumour heterogeneity (between patients) is a long recognised phenomenon, but more recently, there is revelation of a surprisingly high degree of tumour heterogeneity even within the same patient (intra-tumour heterogeneity), with marked diversity demonstrated both between different geographical locations of the same tumour (spatial heterogeneity) and between primary and metastatic tumour sites (temporal heterogeneity).¹ PET-CT imaging with radionucleotide-tagged estradiol have shown varying estrogen receptor distribution between primary and metastatic sites of breast cancer, highlighting the pitfall of relying on a single tumour biopsy to guide treatment choices. These observations also account for variable treatment response in different tumour sites often observed in the same patient.² Interestingly, while one expects metastatic lesions and primary tumours to share common ancestral lineage, whole-exome sequencing of tissue samples of brain metastases with matched primary tumour and normal tissue identified subpopulations in both primary and metastatic lesions that bear little resemblance to the common ancestor. This suggests continued, independent evolution of subclones in distinct sites within the same patient, possibly accounting for temporal and spatial tumour heterogeneity.³ Much of this heterogeneity exists even before treatment, but the exertion of selection pressure with anti-cancer therapy further encourages proliferation and evolution of tumour subpopulations that are resistant to initial therapy.

Clonal Evolution and Darwinian Selection

While anti-cancer therapy, in particular, targeted therapy often leads to dramatic responses in the initial stages, this “honeymoon period” is usually short-lived, with inevitable development of resistance and disease progression—but why so? Similar to the Darwinian theory, the clonal evolution model of tumour carcinogenesis first proposed by Nowell in 1976⁴ hypothesised that external factors like environment and therapy leads to selection pressure for clonal populations that are resistant to proliferate while susceptible populations perish. This bottle neck effect may manifest as a period of partial response to therapy, but quickly results in tumour progression once there is clonal outgrowth of resistant populations.⁵ In addition to resistant subpopulations present in tumours prior to systemic chemotherapy, the intrinsic genomic instability of tumour cells also leads to accelerated adaptive mechanisms that allow susceptible populations to develop new mutations, thus conferring acquired resistance. This is well documented in lung cancer patients with epidermal growth factor receptor (EGFR) mutations who are initially sensitive to EGFR tyrosine kinase inhibitors, but subsequently develop resistance through acquisition of a secondary mutation T790M.⁶ Further complicating this is the observation that secondary mutations can be highly heterogeneous, with the possibility of simultaneous development of 2 or more such mutations, as has been described in lung cancer patients with ALK translocation who develop resistance towards ALK inhibitor crizotinib.⁷ This drive of tumour cells to thrive despite anti-cancer therapy is an exhibition of “survival of the fittest” at its best.

Repeated Tumour Sampling to Characterise the Constantly Evolving Tumour

The presence of tumour heterogeneity poses great challenges to cancer therapeutics. What does this entail towards our pursuit of cancer eradication, and are we always going to be blindsided by its chameleon-like nature or can we develop a counter-strategy? With the understanding of

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constant change in clonal composition of tumour cells, it is prudent to revisit our usual practice of relying on a single tissue biopsy at diagnosis to determine all future courses of therapy. The fluidity in genetic composition suggests the need for constant surveillance of new sub-clones, and multiple biopsies during different time points at various tumour sites may be required for proper characterisation to aid rational drug choices. However, this is admittedly difficult in the clinical setting both in terms of accessibility to tumour sites and acceptability to patients. To overcome this problem, development of reliable, non-invasive techniques to monitor tumour burden becomes increasingly urgent. Circulating tumour cells (CTC) and DNA (ctDNA) have shown promise, with recent studies concluding that levels of ctDNA with targeted sequencing of crucial genes, such as *PIK3CA* and *TP53*, correlated with treatment response in breast cancer.⁸ Further studies are ongoing to investigate similar non-invasive methods to monitor emerging resistance patterns.

Therapeutic Implications of Intra-tumoural Heterogeneity and Clonal Evolution

The success of molecular therapy relies heavily on the presence of an oncogenic addiction pathway that when targeted, causes downstream cell death and thus tumour eradication. While tumour heterogeneity may seemingly be the nemesis of targeted therapy, increasing understanding of “branched evolution” of clonal populations may identify “trunk mutations” occurring early in pathogenesis that can be targeted simultaneously with a combination of drugs to prevent low frequency subpopulations from becoming resistant dominant populations later in the disease.⁹ Newer technologies like next generation sequencing that offer high sensitivity testing to detect minor subpopulation of cells could be crucial to rationalise such therapeutics.

Another approach towards eradication of residual disease is to consider surgical resection post-chemotherapy. This has been tested in advanced gastro-intestinal stromal tumours where patients with residual disease, believed to be subpopulations that are resistant to chemotherapy, underwent surgical resection. Patients with stable disease or limited progression on initial therapy had prolonged overall survival after surgical resection but limited advantage was gained in patients with generalised progression.¹⁰ This approach remains controversial but could warrant evaluation in selected patients.

The landscape of tumour heterogeneity is rapidly evolving and fast gaining recognition as an important issue that needs to be addressed in today’s climate of targeted therapy. While its impact on treatment response and resistance is still not well documented, it is possible that with the advent of newer genomic technologies and more intensive study on the longitudinal impact of targeted therapy in trials, we may still be able to overcome these limitations and gain trajectory towards personalised medicine. The first step will be to acknowledge and embrace that change is the only constant.

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The Use of Traditional Chinese Medicine Among Breast Cancer Patients: Implications for The Clinician

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Abstract

Introduction: Traditional Chinese Medicine or Traditional Complementary Medicine (TCM) is commonly used in our culture. There are several concerns regarding its use in patients undergoing conventional treatments for breast cancer. In this study, we aimed to evaluate the prevalence and pattern of TCM use among our breast cancer patients, and to identify patients who were most likely to choose TCM. **Materials and Methods:** A total of 300 patients on active follow-up with Breast Service at Tan Tock Seng Hospital were interviewed using a structured questionnaire. **Results:** A total of 35% (104 of 296) of patients reported using TCM. The majority of the patients were introduced to TCM by family and friends following the diagnosis of breast cancer. All except 3 patients continued with recommended conventional therapy although most did not inform their clinicians of TCM use. None of the patients reported any serious adverse events and 75% of them perceived a benefit from TCM use. Younger patients and those of Chinese ethnicity were more likely to use TCM ($P < 0.01$ and $P = 0.03$ respectively). There was no significant difference in the dialect group, religious beliefs and educational level between the 2 groups ($P > 0.05$). **Conclusion:** TCM use is common among our breast cancer patients, particularly the younger women. However, most patients do not inform their clinicians of TCM use while on recommended conventional therapies. It is therefore important for clinicians to initiate discussions regarding TCM use in order to be aware of potential unwanted drug interactions.

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Key words: Adjuvant therapy, Complementary medicine, Young breast cancer patients

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Introduction

Complementary and alternative medicine practices and products are often believed to be holistic and natural cures for many ailments. Traditional Chinese medicine (TCM) is one of the most commonly used forms of complementary medicine in our culture. Rather than treating just the disease condition alone, TCM philosophy strives to restore the balance between the yin and yang elements in the body in order to promote general well being. TCM is commonly used by those afflicted by cancer.¹⁻⁴ It is believed to boost the immune system and relieve cancer-related symptoms as well as the adverse effects associated with conventional cancer treatments.^{5,6} Some also believe TCM to be effective in preventing disease recurrence.

While TCM continues to be widely used in countries like China and Taiwan, it is thought to have become less popular locally. Over the years, Singaporeans have adopted

an increasingly westernised lifestyle. Likewise, the local healthcare system is heavily influenced by Western practices and places great emphasis on treatments that have proven scientific benefits. TCM has not been subjected to rigorous evaluation, and its benefits are suggested from small studies, which often have inadequate methodologies. Those better educated are thought more likely to opt for treatments that have been scientifically proven. It was therefore assumed that TCM use remains common only among the older and less educated, with most of the younger generation considering it akin to folk remedies. However, recent studies in Singapore found that TCM use is common among adult cancer patients.¹⁻³ In fact, studies have consistently found that those who use complementary treatments, including TCM, are more likely to be younger, better educated and of higher socioeconomic status.⁷

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Complementary and alternative medicine use is reportedly most common among women with breast cancer.^{8,9} Locally, discussions about management often involve only conventional treatments and TCM use is seldom mentioned. Many patients do not volunteer their use of TCM either because they feel that it is irrelevant, or because they believe their clinicians will disapprove. On the other hand, many clinicians are hesitant to endorse TCM use because they are unfamiliar with TCM, and certain herbs are known to interfere with conventional agents.^{10,11} Furthermore, TCM products are not regulated by the same standards applied to conventional Western medicines and instances of serious toxicities arising from contamination with undeclared pharmaceutical agents are not uncommon.^{12,13} In this study, we aim to determine how common TCM use is among our local breast cancer patients, and to identify those who are most likely to use TCM. This will allow us to better counsel and advise patients regarding concurrent TCM use while undergoing conventional therapy.

Materials and Methods

A total of 300 consecutive patients on follow-up with the Breast Service were recruited. All patients had been previously diagnosed with breast cancer and were on active follow-up. Two patients were excluded after they declined to participate in the study. The remaining 298 patients who consented to participate in this study were individually interviewed in-person by a trained interviewer using a structured questionnaire. This study had institutional ethics approval (DSRB B/08/072).

The questionnaire survey collected information pertaining to: (i) patients' biodata, including their age, ethnicity, dialect group, religion and highest education level, (ii) disease characteristics, including the mode of presentation, whether the lump was self-detected or an incidental finding and the tumour stage, and (iii) TCM use, including how they were introduced to TCM, whether TCM use was discussed with their doctor, whether TCM was used together with or instead of recommended conventional therapies, the perceived benefit from TCM use and the duration of use. In this study, TCM included medicinal herbs purchased over the counter from Chinese medicinal halls or prescribed by TCM practitioners, as well as formulations and decoctions with Chinese herbs as the main ingredients, including Chinese patent medicines (standardised herbal formulas) and Chinese herbal extracts.

In total, 296 patients were included in the final analysis. Two patients were excluded because the questionnaire survey was incomplete. Statistical analysis was performed using GraphPadPrism version 4 (GraphPad software Inc., San Diego CA). Correlation analyses were performed using the chi-square test. Comparisons of patient age and tumour

size between TCM users and non-users were performed using the Mann-Whitney U test. A 2-tailed *P* value test was used in all analyses and a *P* value of less than 0.05 was considered statistically significant.

Results

Patient and Tumour Characteristics

Characteristics of the 296 patients included in the study are detailed in Tables 1 and 2. Fifty-five of 296 patients (18.5%) were diagnosed with DCIS, 34.8% (103 of 296) with Stage I breast cancer, 30.7% (91 of 296) with Stage II disease, 9.5% (28 of 296) with Stage III disease and 2.0% (6 of 296) with Stage IV disease. Median patient age was 56 years (range, 33 to 87 years). Fifty-three percent of patients

Table 1. Patient Demographics

	Patients who took TCM (n = 104)	Patients who did not take TCM (n = 192)	<i>P</i> value
Median age (years)	53.0 (33.0 to 80.0)	57.0 (33.0 to 88.0)	0.0002
Ethnicity			0.17
Chinese	98	167	
Malay	5	18	
Indian	1	8	
Others	0	1	
Dialect group			0.13
Hokkien	40	52	
Cantonese	23	30	
Teochew	24	52	
Others	12	32	
Religion			0.05
Christian/Catholic	19	53	
Buddhist	62	95	
Muslim	6	18	
Hindu	0	6	
Others	17	21	
Education level			0.10
None	9	33	
Primary	29	60	
Secondary	47	66	
Higher	18	28	
Presentation			0.39
Self detected lump	63	125	
Incidental	41	67	

were Buddhists, reflecting the large Chinese majority (90%) within the study population. Christians and Catholics accounted for 24.3% (72 of 296) of the study population, and Muslims another 8.1% (24 of 296). The majority of patients had at least completed primary school education; only 14.2% of them (42 of 296) had not received any formal education. Most patients (188 of 296, 64%) had detected a breast lump themselves; others were incidentally noted to have a breast lump by a clinician. Median tumours size was 25 mm (5 to 100 mm); 8 patients had presented with T3 tumours and 12 presented with T4 tumours.

Table 2. Characteristics of Breast Tumours and TCM Usage

	Patients who took TCM	Patients who did not take TCM	P value
Median tumour size (mm)	30.0 (10.0 to 100.0)	25.0 (5.0 to 100.0)	0.17
T status			0.96
Tis	20	35	
T1	45	87	
T2	29	56	
T3	4	4	
T4	4	8	
Stage of disease			0.46
0	20	35	
I	31	72	
II	27	64	
III	14	14	
IV	1	5	
Introduced by:			
Friends and relatives	77		
TCM practitioner	18		
Media	4		
Self search	5		
Discussed with doctor			
Yes	29		
No	75		
Concurrent conventional medication			0.10
Yes	57	82	
No	46 (3 had refused hormonal treatment and taken TCM instead)	42	
Median duration of TCM use (month)	6 (0.5 to 156)		
Benefits from TCM			
Yes	77		
No	27		
Started before surgery	26		

Characteristics of TCM Users

One hundred and four patients (35.1%) reported using TCM. Median age of TCM users was 53 years (range, 33 to 80 years). All except 6 (94.2%) were Chinese, and most (59.6%) were Buddhists. Only 9 patients in this group had no formal education. More than half the patients had detected a breast lump themselves and had presented with early breast cancer. In total, 75% of patients (78 of 104) started on TCM only after being diagnosed with breast cancer, and had been introduced to it by family and friends. Eighteen patients had sought advice from TCM practitioners. It was observed that only 27.8% (29 of 104) of patients had informed their doctors of their use of TCM. Most (70.7%) had not volunteered the information as they had not been asked specifically about it; 11 patients were afraid of being reprimanded by their clinicians, and 2 patients felt that their clinicians had no understanding of TCM. Three patients chose to use TCM instead of prescribed hormonal therapy. Median duration of TCM use was 6 months (range, 0.5 to 156 months). Of note was that 75% of the patients (77 of 104) perceived a benefit from TCM and none of the 104 patients reported any significant adverse effects.

Comparison with Non-TCM Users

Patients who chose TCM were significantly younger than those who did not (53.0 years and 57.0 years respectively; $P < 0.01$). Chinese patients were 3 times more likely to use TCM than non-Chinese patients ($P = 0.03$, OR 2.64, 95% CI, 1.05 to 6.62). There was no significant difference in the dialect group, religious beliefs and education level between the 2 groups ($P > 0.05$). In addition, there was also no statistical difference in the mode of detection (whether self-detected or clinician-detected), tumour size or disease ($P > 0.05$).

Discussion

Complementary alternative medicine comprises a diverse group of therapies, including herbs and supplements, massage therapy, acupuncture and psychological therapies. We have chosen to focus on TCM in this study since it is the most commonly used form of complementary medicine in our local population.¹⁻³ Although largely unsubstantiated, there are reports to suggest that TCM inhibits cellular proliferation and modulates apoptotic responses in breast cancer cells in vitro.^{14,15} Beta-glucans, present in Shiitake mushrooms, were found to promote innate anti-tumour immunity in combination with targeted therapies such as trastuzumab and rituximab.^{16,17} Another study reported that polysaccharide krestin (PSK), an extract from the fungus *Coriolus versicolor*, conferred a survival advantage in colorectal cancer when used together with conventional chemotherapy.¹⁸

The patients' demographics and tumour characteristics in our study are comparable to the figures published in 'Trends of Female Breast Cancer in Singapore 2006 to 2010' by National Registry of Diseases Office, Singapore in July 2012.¹⁹ Distribution of ethnicity, age specific incidence rate and stage of disease at the point of diagnosis closely reflect the data published by the national registry. Majority of our patients are of Chinese ethnicity and most breast cancers were diagnosed at early stages of I and II as opposed to advanced stages.

In our study, 35% of our breast cancer patients reported using TCM. Younger women were more likely to use TCM and only 9% of TCM users had no formal education, compared to 17% of non-TCM users. Religious beliefs did not influence the decision to use TCM. This is in keeping with other studies which have found TCM use more common among the better educated and younger age group, and is in contrast to the common belief that it is the older and less educated who are less familiar and comfortable with conventional Western medicine who are more likely to use TCM.^{4,7} Younger women may view TCM as a form of natural remedy that provides a more holistic cure without the potential adverse effects commonly associated with many conventional treatments. Our study observed that the majority of patients had started using TCM after being diagnosed with breast cancer. Notably, most patients continued to receive recommended conventional treatments and did not use TCM as an alternative. It is likely that many women do not consider TCM as having similar treatment efficacy as conventional treatments, but rather use TCM to boost their immunity so as to better tolerate surgery and subsequent adjuvant treatments. While this is reassuring, the concurrent use of TCM can potentially complicate conventional treatments, especially since clinicians are generally unaware of its use. Certain medicinal herbs have anti-coagulant properties and can increase the risk of excessive oozing and haematoma formation following surgery; unexpected drug interactions can also result in reduced efficacy or increased toxicity of chemotherapeutic agents.^{10,11,20}

Our study highlighted that most women used TCM despite a paucity of strong scientific evidence supporting its benefits. Most of the women in our study were introduced to TCM by their family and friends. But in spite of inconclusive data regarding the anti-tumour properties of TCM, the majority perceived a beneficial effect from TCM use. Even if this is purely psychological, it should not be disregarded, as there are suggestions that a positive psychology can improve

survival.^{21,22} Psychological interventions introduced following the diagnosis of breast cancer to encourage a positive psychology appeared to reduce the risk of disease recurrence.²³ A positive psychology may exert a direct effect on tumour cell biology by modulating cortisol levels, although the exact mechanisms remain unclear.²⁴ Perhaps more important is the observation that none of the patients reported any significant adverse effects from the use of TCM, further strengthening the widely held belief that TCM is safe. This perceived lack of toxicity possibly contributes to the willingness to use TCM despite a lack of proven benefit. Most feel that even if TCM provides no benefit, it will not cause any harm. This may not be entirely untrue and it will be unwise to go to the extreme of disapproving of all TCM use during the course of conventional cancer treatment. Such a stance is unlikely to change existing attitudes towards TCM and may in fact strengthen commonly held perceptions that Western medicine and TCM are mutually exclusive and cause more patients to withhold information regarding concurrent TCM. A moderated approach is probably more appropriate, where TCM use is acceptable so long as it does not interfere with conventional therapies. Rather than ignoring TCM use, clinicians should perhaps not shy away from asking their patients directly about concomitant or intended TCM use. Although most clinicians will not be able to engage in an in-depth discussion about the benefits of TCM use or advice about potential undesirable interactions with concurrent treatments, such an approach allows the clinician to direct these women to established TCM practitioners who can offer reliable and appropriate advice. This will help guide those keen on TCM in making well-informed decisions.

Conclusion

Our study has shown that TCM use is relatively common among local breast cancer patients, particularly the younger women. Most patients perceive it to be beneficial and with little serious adverse effect despite no convincing evidence of its anti-tumour or immune-boosting effects. It is likely that many will continue to use TCM while undergoing conventional cancer treatments. A more open approach towards the use of TCM will serve not only to alert clinicians to potentially important drug interactions, but will also ensure that its benefits, even if only psychological, are not overlooked.

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Young Breast Cancer in a Specialised Breast Unit in Singapore: Clinical, Radiological and Pathological Factors

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Abstract

Introduction: Whilst only 5.5% to 7% of breast cancer occurs in women less than 40 years of age in the West, the incidence has been reported in up to 18% in Asian population. This study seeks to evaluate our unit's experience in breast cancer in young women. **Materials and Methods:** A retrospective review of our database identified women with newly diagnosed breast cancer from January 2006 to February 2011. Patient demographics, clinical presentation, imaging and pathological findings and treatment received were determined. **Results:** Out of a total of 1160 women with breast cancer, 135 (11.6%) were under 40 years of age and made up our study population. The most common presentation was a self-detected breast lump. Most patients did not have a positive family history. Mammography demonstrated abnormal findings in 78% of patients. Ultrasound was very sensitive in the evaluation of a breast lump and demonstrated abnormal findings in 95%. Out of 129 women, 40 (31%) underwent breast-conserving surgery of which 5 (12.5%) proceeded to mastectomy due to involved margins. Also, 89 out of 129 women (69%) underwent mastectomy of which 19 (21.3%) had immediate reconstruction. Of a total of 121 primary resections, 94% were invasive ductal carcinoma while 15.5% were pure ductal in-situ carcinomas. The majority (61.2%) showed high grade disease. **Conclusion:** Most young breast cancer patients present with a self-detected breast lump and do not have a positive family history. A strong clinical index of suspicion and appropriate breast imaging workup are useful for the early and accurate diagnosis of breast cancer in young women.

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Key words: Breast carcinoma, Breast imaging, Family history, Symptoms, Young age

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Introduction

Breast cancer is the most common cancer to afflict women in Singapore, accounting for almost 30% of all female cancers. The greatest incidence occurred in women aged between 45 and 49 years but has more recently showed a shift towards older women in their late 50's.¹ There was also a worrying upward trend in the number of breast cancer cases with a 25% higher incidence in the period from 1998 to 2002 compared to 1993 to 1997.²

There are few studies examining breast cancer in women under 40 years of age. While it has been reported that only 5.5% to 7% of breast cancers are detected in women younger than 40 years in the West,^{3,4} this group has been reported to account for 12% to 18% of breast cancers in Asian population with an increasing trend observed over the years.⁵⁻⁷

The purpose of this study is to evaluate our unit's experience in breast cancer occurring in young women under 40 years of age in a predominantly Chinese population in Singapore. The span of the study includes clinical, radiological, surgical and pathological factors.

Materials and Methods

Young breast cancer is defined as breast cancer occurring in women younger than 40 years of age at diagnosis. A retrospective, institutional review board-approved review of our centre's surgical database identified a total of 1160 women diagnosed with breast cancer from January 2006 to February 2011. Breast cancer diagnosis was made by either core needle or excisional biopsy of a breast lesion. Of these,

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135 women were under the age of 40 at the time of diagnosis, comprising 11.6% of all patients with breast cancer. Data including clinical findings, imaging studies and findings, and histo-pathological findings were recorded. Imaging findings were retrospectively reviewed by 2 radiologists and classified according to the Breast Imaging Reporting and Data System (BI-RADS) developed by the American College of Radiology.⁸

Results

Clinical Findings (Table 1)

The median patient age at breast cancer diagnosis was 36 years with an age range from 18 to 39 years. Family history was unknown in one patient as she was adopted. For the rest of the patients, a family history of breast cancer was positive in 19 out of 134 (14.1%) of patients. The most common clinical presentation was a self-detected symptomatic breast lump ($n = 109$; 80.7%). Other patients presented with a self-detected breast lump together with

another breast symptom (nipple discharge, pain, or nipple change; $n = 15$; 11.3%). A few patients presented with nipple discharge only ($n = 5$; 3.5%) and others had mastalgia ($n = 2$; 1.5%). Four patients were asymptomatic but had abnormalities detected on breast imaging, including 1 patient with a positive family history and who underwent mammographic screening before the age of 40. The other 3 patients had undergone routine breast ultrasound.

Five women had pregnancy-related breast cancer (3.5%) including 4 who were pregnant at the time of diagnosis and 1 who was lactating. Most of the women were Chinese ($n = 91$; 67.4%), 27 were Malay (20%), 3 were Indian (2.2%), 1 was Caucasian (0.7%) and 13 were of other races (9.6%).

Imaging Findings (Table 2)

Amongst the 135 women, 7 had no preoperative breast imaging, including 2 with advanced disease at presentation and 5 with incidental malignancy detected after excision of a breast lump. Twelve had preoperative breast imaging studies performed at another institution which were not available for review. The preoperative breast imaging studies for the remaining 116 women were reviewed.

Table 1. Clinical Findings

Clinical factors	Study population	
	Number (n)	Percentage (%)
Age (years)		
Mean	34	(6.6)
Median (range)	36	(18 to 39)
Symptom duration (weeks)		
Mean (SD)	13	(36.4)
Median (range)	4	(<1 to 384)
Symptoms		
Breast lump only	109	80.7
Breast lump and other breast symptom	15	11.3
Nipple discharge only	5	3.5
Breast pain only	2	1.5
Asymptomatic with incidental imaging findings	4	3.0
Pregnancy-associated cancer		
No	130	96.3
Yes	5	3.7
Ethnicity		
Chinese	91	67.4
Malay	27	20.0
Indian	3	2.2
Others	14	10.4
Family history		
No	115	85.2
Yes	19	14.1
Unknown	1	0.7

Table 2. Imaging Findings

Imaging findings	Study population	
	Number (n)	Percentage (%)
Mammography		
n = 100		
Mammographic density		
Extremely dense	68	68.0
Heterogeneously dense	15	15.0
Scattered fibroglandular densities	14	14.0
Mainly fat replaced	3	3.0
Mammographic findings		
Normal/Benign	22	22.0
Abnormal (total)	78	78.0
Opacity	40	40.0
Microcalcifications only	19	19.0
Opacity and microcalcifications	10	10.0
Others	9	9.0
Ultrasound		
n = 115		
Ultrasound findings		
Normal/Benign	6	5.2
Abnormal	109	94.8
MRI		
n = 14		
MRI findings		
Mass	13	92.9
Non-mass enhancement	1	7.1
Additional abnormal findings not seen on mammogram and ultrasound	9	64.3

Mammography

Mammography was performed for 101 of the 116 women. Fourteen patients only had a preoperative ultrasound, and mammogram could not be performed for one patient as the affected breast was too hard to compress. The mammogram films for one of the 101 patients could not be retrieved as they had been culled. The mammogram examinations for 100 patients were thus reviewed. Most patients had dense breasts on mammogram ($n = 83$; 83%), including 68 women (68%) with extremely dense and 15 women (15%) with heterogeneously dense parenchymal patterns respectively. Fourteen (14%) women showed scattered fibroglandular densities and 3 (3%) were fat replaced.

Mammogram was either normal or benign in 22 patients (22%), with the cancer appearing mammographically occult with no other findings in 16 patients (BIRADS 1; 16%) and mammographically occult with other benign findings in 6 patients (BIRADS 2; 6%).

Suspicious mammographic findings (BIRADS 4/5) were present in 78 patients (78%)

The most common suspicious mammographic abnormality

was an abnormal opacity, seen in 40 patients (Fig. 1). Nineteen patients had abnormal microcalcifications as a solitary finding and 10 patients had an opacity associated with microcalcifications. The other findings included asymmetric density only ($n = 4$), asymmetric density with microcalcifications ($n = 3$) and microcalcifications with distortion ($n = 1$). Stromal distortion alone was present in 1 patient.

Ultrasound

Preoperative breast ultrasound examination was performed in almost all women ($n = 115$; 99.1%), except for 1 patient who had advanced disease at presentation.

The majority of patients ($n = 109$; 94.5 %) demonstrated suspicious sonographic findings (BIRADS 4/5), with the size of the masses ranging from 0.4 to 11.2 cm (mean = 2.6 cm; median = 2.0 cm). In 94 patients with an accompanying mammogram, the latter was normal or benign in 18. Most of these 18 patients had extremely dense breast parenchyma ($n = 17$; Fig. 2) and one was heterogeneously dense.

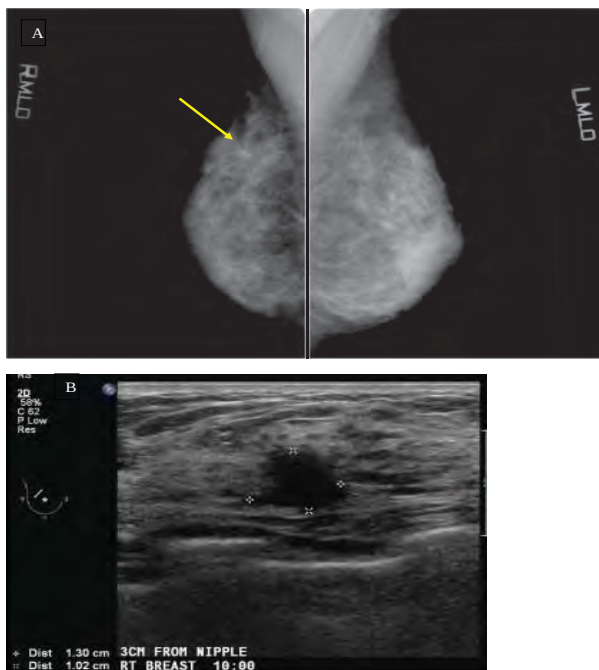


Fig. 1. A 34-year-old Malay lady with no family history who presented with right mastalgia. (A) Mammogram shows extremely dense parenchymal tissues and a spiculated opacity in the right upper breast (arrow). The left breast was normal. (B) Breast ultrasound shows an irregular hypoechoic nodule corresponding to the mammographic finding. Histology revealed invasive ductal carcinoma.



Fig. 2. A 38-year-old Chinese lady with no family history who presented with left sided breast lump. (A) Mammogram shows extremely dense parenchymal tissues with no abnormal finding. (B) Breast ultrasound shows a 2.2cm mass with slightly irregular margins and heterogeneous echotexture. Histology revealed invasive ductal carcinoma.

Ultrasound was normal in 2 patients. The first patient presented with a breast lump and mammogram showed an abnormal cluster of microcalcifications assigned a BIRADS 4 classification. She underwent stereotactic guided biopsy of the abnormal microcalcifications which yielded atypical cells. This was subsequently upgraded to DCIS after wide excision of the affected area (Fig. 3). The second patient presented with blood-stained nipple discharge. The accompanying mammogram was also normal; ductal carcinoma in-situ and mucinous carcinoma were found on micro-dochectomy.

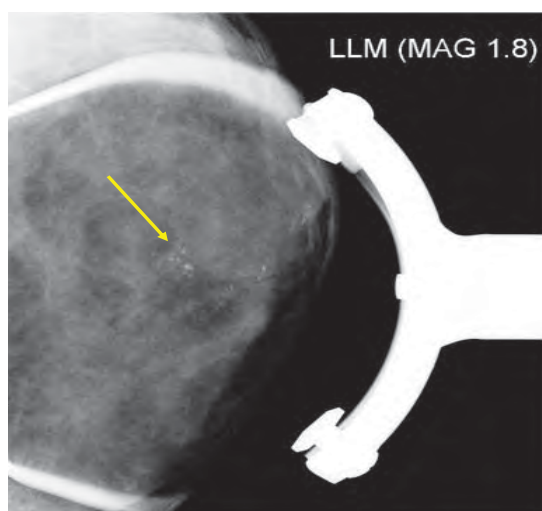


Fig. 3. A 35-year-old Chinese lady with no family history who presented with left breast lump. Magnification mammogram shows abnormal microcalcifications in a segmental distribution (arrow). Breast ultrasound was negative. Stereotactic mammographic guided biopsy of the microcalcifications revealed hyperplasia with moderate atypia. This was upgraded to ductal carcinoma-in-situ on wide excision.

Benign findings (BIRADS 2/3) were present in 4 patients. Of these, one patient did not have a mammogram and the other 3 had benign mammographic findings. The patient who only had an ultrasound had a 3.9 cm benign appearing solid mass. This was excised due to its size and a small incidental focus of DCIS was found involving a large fibroadenoma on pathology. Two other patients with mammogram and ultrasound had benign appearing solid nodules and cysts in both breasts. In one patient, open biopsy of an area of induration felt clinically revealed invasive lobular carcinoma. In the other patient, vacuum assisted percutaneous biopsy of an area of mastalgia showed ductal carcinoma in-situ (DCIS). The third patient presented with blood-stained nipple discharge. Ultrasound showed a dilated duct in the retro-areolar region and there were benign-appearing calcification on mammogram. DCIS was found on subsequent micro-dochectomy.

Magnetic Resonance Imaging (MRI)

Preoperative breast MRI was performed for 14 patients. Interpretation of the breast MRI study was accomplished using the ACR breast MRI reporting lexicon.⁸ Abnormal mass lesions were present in 13 patients and abnormal non-mass enhancement was present in 1 patient. MRI showed additional suspicious findings in 9 patients (64.3%) that were not detected on mammogram or US (Fig. 4). In 1 patient with lobular carcinoma, the tumour was seen only on MRI, being occult on both mammography and ultrasound. The rest of the 4 patients had no additional findings on MRI.

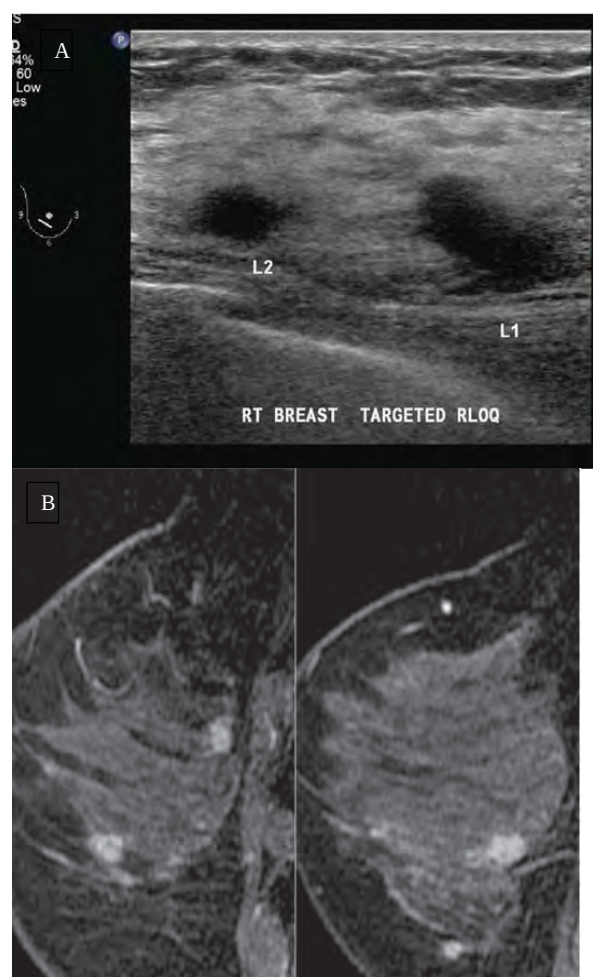


Fig. 4. A 35-year-old Malay lady with no family history who presented with a right breast lump. Mammogram showed extremely dense parenchymal tissues with no abnormal finding. (A) Breast ultrasound showed 2 hypoechoic nodules with ill-defined margins in the right lower outer quadrant. Biopsy of both nodules showed invasive ductal carcinoma. (B) Breast MRI showed multiple other suspicious enhancing nodules in the rest of the right breast. These were due to areas of solid and comedo DCIS on histology of the mastectomy specimen.

Surgical Management

Four (3%) women had metastatic disease at presentation and 2 (1.5%) defaulted treatment and follow-up after biopsy. These 6 women were excluded from further analysis; the remaining 129 patients underwent surgical treatment in our institution.

Forty out of 129 women (31%) underwent breast-conserving surgery (BCS) of which 5 (12.5%) proceeded to mastectomy due to involved margins. Eighty-nine out of 129 women (69%) underwent mastectomy of which 19 (21.3%) had immediate reconstruction.

Table 3. Histopathological Findings

Histopathological findings	Study population	
	Number	(%)
Primary surgical treatment	Total = 129	
Breast conservation	40	31
Mastectomy	70	54.3
Mastectomy with reconstruction	19	14.7
Tumour size (cm)		
Mean (SD)	2.8 (2.1)	
Median (range)	2.2 (1 to 10.0)	
Overall tumour size		
2.0 cm or less	67	51.9
More than 2.0 cm	62	48.1
Tumour histology type		
Pure DCIS	20	15.5
Invasive ductal carcinoma	94	72.9
Invasive lobular carcinoma	3	2.3
Invasive mucinous carcinoma	10	7.7
Metaplastic carcinoma	2	1.6
Histologic Grade		
Low	19	14.8
Intermediate	31	24.0
High	79	61.2
Nodal involvement (invasive cancers with operative histology n = 110)		
No	60	54.5
Yes	50	45.5
TNM Stage (n = 133 excluding loss to follow up)		
0	19	14.3
I	35	26.3
II	53	39.8
III	22	16.5
IV	4	3.0
Hormone receptor status (n = 133)		
ER negative	41	30.8
ER positive	92	69.2
CerbB2 overexpression (invasive cancers only, n = 118)		
No	83	70.3
Yes	35	29.7

Histo-pathological Findings (Table 3)

The median tumour size was 2.2 cm (range, 0.1 to 10.0 cm). Of them, 6.2% (8/129) received neo-adjuvant chemotherapy. Of a total of 121 primary resections, 94% were invasive ductal carcinoma with or without associated DCIS while 15.5% were pure DCIS. There were 2 cases of metaplastic adeno-squamous cancer. The majority (61.2%) showed high grade disease. A total of 45.5% of them had nodal involvement. Among these, 69.2%, 54.9% and 29.7% were oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER-2) positive respectively. Adjuvant chemotherapy was administered in 48.1% (62/129) of surgically treated patients with invasive disease.

Discussion

In Singapore, women 40 years of age and above are invited to undergo breast cancer screening under the BreastScreen Singapore programme. This programme has been shown to be effective in increasing breast cancer detection at an earlier stage and with more favourable tumour characteristics.⁹⁻¹¹ Younger women below the age of 40 do not qualify for and thus do not benefit from mammographic screening. As such, the vast majority of young breast cancer patients present with a breast symptom, most commonly a self-detected breast lump, as was seen in more than 90% of our patients, including 81% of them presenting with a breast lump alone, and 11% of them presenting with a breast lump and another accompanying breast symptom. This mirrors the findings of other studies.¹²⁻¹⁴

Unexpectedly, our study showed that a majority of these young women (86%) do not have a positive family history of breast cancer. This is contrary to the increased risk of breast cancer found in women above 40 years of age with a positive family history reported in a Singaporean population study.¹⁵ Only one patient in our study cohort had her breast cancer detected by early mammographic screening due to a positive family history. Our study also showed that a significant proportion of women were of the Malay race (20%). This high percentage is disproportionate to the percentage of resident Malays in Singapore (13.4%)¹⁶ and also contrary to a report in which Malays were found to have a low risk of cancer.¹⁷

A small proportion of women (3.5%) had pregnancy-related breast cancer (Fig. 5). Some women were pregnant at the time of diagnosis and others were lactating. These women generally presented with advanced disease possibly as a result of their symptoms being masked by pregnancy-related changes in the breasts. Due to the young age of these patients, the treatment of these patients can be complicated by additional fertility and psychosocial issues, requiring multi-disciplinary care and support.



Fig. 5. A 36-year-old Chinese lady with no family history who presented with a left breast lump in pregnancy. Breast ultrasound showed an irregular hypoechoic mass with echogenic foci within which may represent foci of microcalcification. No mammogram was done as she was pregnant. Histology revealed invasive ductal carcinoma.

Mammography was able to detect suspicious findings in slightly more than three quarters of patients, with an opacity being the most common abnormality. Mammography was however normal or benign in almost a quarter of patients. The relative insensitivity of mammography may be partly attributed to the presence of dense breast parenchyma, seen in more than 80% of patients in our study. This is not surprising, as increased breast density and young age are major factors associated with decreased mammographic sensitivity.¹⁸⁻²⁰ When mammogram was normal or benign, breast ultrasound found a suspicious finding in 86.4% (19 out of 22 patients).

When a breast lump is present, ultrasound is a very useful tool in its evaluation, with a sensitivity of around 95%. In almost 20% of patients, the accompanying mammogram was normal or benign. Again, this could be due to obscuration of an underlying mass by dense tissues on mammogram. Conversely, the mammogram may occasionally detect an abnormal finding in the presence of a normal ultrasound, as seen in one patient with abnormal calcification as a solitary imaging finding.

In the small number of cancers with normal or benign ultrasound findings ($n = 6$), one-third presented with bloody nipple discharge. Both patients also had mammogram with no abnormal findings. A third patient had abnormal mammographic findings which prompted a biopsy. Two other patients had normal or benign mammographic findings as well but clinical suspicion of a symptomatic area within the breast lead to the eventual diagnosis. One was due to an invasive lobular carcinoma; the other was found to have DCIS involving areas of fibrocystic change. The last patient had an incidental focus of DCIS involving

a large fibroadenoma.

Breast MRI has been shown to detect additional foci of occult cancer other than the index tumour in patients with a known breast malignancy, and is especially useful in the assessment of invasive lobular carcinomas.^{21,22} This was also seen in our study, where MRI detected additional suspicious findings in about 60% of patients. However, as only a small number of patients underwent preoperative breast MRI in our study, an in-depth evaluation and discussion on the use of breast MRI is beyond the scope of this article.

The high percentage of mastectomies in our cohort (73%) is a reflection of the relatively large size of the cancers at presentation. The median size of cancers is 2.2 cm which classifies them as stage T2 by virtue of their size alone. Moreover, nearly half had nodal involvement. Nearly 60% of women were at least stage II by TNM staging at presentation. Together with a general lack of family history in these women, these findings confirm the need for a high index of suspicion among clinicians in their management of breast lumps as well as greater breast cancer awareness among women. Histo-pathological data revealed that in excess of 90% of the cancers were invasive ductal carcinomas with a low rate of DCIS. This corresponds to the 85.5% rate of invasive ductal carcinomas reported by McAree et al¹³ and is consistent with the fact that our cohort is a non-screened population with a majority presenting with a breast lump. The distribution of ER positive and *cerbB2* over-expressed cancers are comparable to the general population.²³⁻²⁵

Our study was limited by its retrospective nature as well as the small number of patients in the study population in a single institution which may contribute to selection bias.

Conclusion

Breast cancer can afflict women younger than 40 years of age. As these occur before the commencement of breast screening, most patients present with a self-detected breast lump. A majority of patients do not have a positive family history of breast cancer, and the lack of a positive family history should not decrease the level of clinical suspicion. Ultrasound is a sensitive examination for the evaluation of a breast lump and can accurately detect suspicious findings. Mammography may sometimes be negative but in a small proportion detects abnormalities in the presence of a normal ultrasound study. The detection of breast cancer in young women under 40 years of age remains challenging due to a lack of routine breast screening and the presence of dense breast tissues. A strong clinical index of suspicion and appropriate breast imaging workup are useful for the early and accurate diagnosis of breast cancer in young women.

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Singapore Rhabdomyosarcoma (RMS) Experience: Shall We Change Our Practice?

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Abstract

Introduction: Although rhabdomyosarcoma (RMS) constitutes nearly 4% of all children diagnosed with cancer in the ethnically diverse small island city of Singapore, it is unknown how children with RMS fare. **Materials and Methods:** This study investigated 50 children with RMS from April 1993 to December 2010 from KK Women's and Children's Hospital (KKH) and National University Hospital (NUH). They were treated either as per Intergroup Rhabdomyosarcoma Study Group (IRSG) or Société Internationale Pédiatrique D'Oncologie (SIOP) regimens. **Results:** Median age of diagnosis was 5.1 years (range, 0.1 to 17.3 years) with a median follow-up of 3.3 years (range, 0.4 to 15.6 years). According to IRSG classification, 18 (36%) were staged as low-risk (LR); 19 (38%) were intermediate-risk (IR), 12 (24%) were high-risk (HR) and it was unknown in 1 patient. Twenty-nine (58%) were of embryonal subtype, 17 (34%) were alveolar and subclassification was not available in 4. The primary sites of tumour were: head and neck region (n = 22); genitourinary (n = 19); extremity (n = 10); and abdomen/retroperitoneal (n = 5). At the time of analysis, 80% were alive with no evidence of disease, 9 were dead of disease, and 2 were alive with disease. By disease risk group, the 5-year event-free survival (EFS) for LR group disease was 81.3% (95% CI, 62.0 to 100.0), IR group was 61.4% (95% CI, 32.3 to 90.4) and HR group was 25.0% (95% CI, 0.0 to 49.5) respectively ($P < 0.001$). The 5-year EFS for risk by chemotherapy received as per SIOP vs per IRSG revealed: LR 83.3% vs 75.0% ($P = 0.787$); IR 83.3% vs 43.8% ($P = 0.351$); HR 0.0% vs 42.9% ($P = 0.336$) respectively. Of 15 relapses (HR, n = 7), at median of 2 years, 4 of 6 patients treated as per SIOP regimen were dead of disease and 3 of 8 treated as per IRSG were alive. **Conclusion:** Radiation therapy (RT) can be avoided in LR classification although those in higher risk classification need RT to local and distant metastatic disease. The outcome of children with RMS in Singapore can be further improved by coming together as a cooperative group to provide the best total care. Improved communication, multidisciplinary team collaboration, standardisation of protocols and rigorous data collection are keys.

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Key words: Sarcoma, Survival, Treatment

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Singapore Rhabdomyosarcoma (RMS) Experience: Shall We Change Our Practice?

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Abstract

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Introduction

Rhabdomyosarcoma (RMS) constitutes nearly 4% of all children diagnosed with cancer in the ethnically diverse small island city of Singapore.¹ It is the most common primary soft tissue malignancy of childhood and one which is highly sensitive to chemotherapy and radiation therapy leading to excellent overall survivals.²⁻⁵ In KK Women's and Children's Hospital (KKH), majority of the children were treated according to the European Société Internationale Pédiatrique D'Oncologie (SIOP) regimen whilst in National University Hospital (NUH), children were treated as per the American Intergroup Rhabdomyosarcoma Study Group

(IRSG) regimen as described elsewhere.⁵⁻¹¹ The 2 regimens differ in the use of radiation therapy (RT) as a salvage treatment modality for relapsed disease in the former versus upfront radiation in all alveolar and higher risk disease in the latter. Several clinical and disease characteristics predict the outcome in children with RMS including age at diagnosis, stage, group, location of primary tumour and histology. According to the IRSG, staging is done in 3 basic steps: presurgical staging based on imaging studies, postsurgical grouping and finally risk group. Risk is divided into 3 prognostic groups, low-risk (LR), intermediate-risk

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Table 1. Intergroup Rhabdomyosarcoma Staging Group Risk Classification

IRSG Pre-Treatment Staging Classification					
Stage	Site	Tumour (T)	Size	Node (N)	Metastasis (M)
I	Orbit, head and neck (excluding parameningeal) GU: non-bladder/nonprostate	T1 or T2	a or b	N0, N1, or Nx	M0
II	Bladder/prostate, extremity, cranial, parameningeal, other (includes trunk, retroperitoneum, and so on)	T1 or T2	a	N0 or Nx	M0
III	Bladder/prostate, extremity, cranial, parameningeal, other (includes trunk, retroperitoneum, and so on)	T1 or T2	a b	N1 N0, N1, or Nx	M0 M0
IV	All	T1 or T2	a or b	N0 or N1	M1

Notes Tumour: T1, confined to anatomic site of origin, (a) ≤ 5 cm in diameter in size, (b) ≥ 5 cm in diameter in size; T2, extension and/or fixative to surrounding tissue; regional nodes: N0, regional nodes clinically not involved; N1, regional nodes clinically involved by neoplasm; Nx, clinical status of regional nodes unknown; metastasis: M0, no distant metastasis; M1, metastasis present

IRSG Surgical-Pathological Grouping Classification			IRSG Risk Classification	
Group 1	Localised disease, completely excised, no microscopic residual			Embryonal
A	Confined to site of origin, completely resected	Low		Group I, II
B	Infiltrating beyond site of origin, completely resected			Group III, stage I
Group 2	Total gross resection			Embryonal
A	Gross resection with evidence of microscopic local residual	Intermediate		Group III, stage 2, 3 (unfavourable sites)
B	Regional disease with involved lymph nodes, completely resected with no microscopic residual			Group IV age <10 years
C	Microscopic local and/or nodal residual			Alveolar
				Groups I-III
Group 3	Incomplete resection or biopsy with gross residual			
Group 4	Distant metastases	High		Group IV (excluding embryonal <10 years)

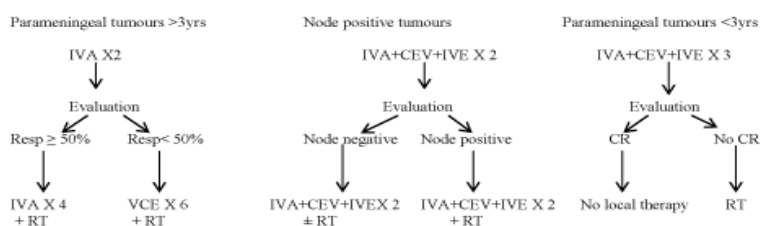
(IR), or high-risk (HR) subgroups based on estimated failure-free survival differences (Table 1).¹²

We set out to investigate the patient and tumour characteristics and the outcome based on the 2 varied regimens used in the care of these children diagnosed with this uncommon disease in Singapore.

Materials and Methods

This was a detailed retrospective chart review of all children and young adolescents diagnosed and treated with RMS in the Departments of Paediatric Haematology-Oncology, KKH and NUH. Data abstraction included patient demographics such as age at diagnosis, gender, race, disease characteristics, and types of treatments received. In addition to a detailed history and physical examination, staging evaluation included a number of tests. Primary tumour imaging was performed by magnetic resonance imaging (MRI) or computerised tomography (CT) scans. Metastatic disease workup included CT scan of the thorax, a bone scan, bilateral bone marrow and trephine biopsies and a lumbar puncture if the primary tumour was in a central nervous system location.

Presently, children diagnosed with RMS in Singapore are treated as per the SIOP regimen or as per the IRSG regimen depending on the treating physician's decision. The 2 regimens differ in the avoidance of RT in the former and its use as an upfront therapy in the latter. The approach by IRSG is to treat the systemic disease with a multimodality therapy, and to aim for maximum initial function sparing surgery. Local therapy, either surgical resection and/or RT is required for any disease visible by diagnostic imaging at primary or metastatic sites. RT is also given for any postoperative microscopic and gross residual disease and all alveolar subtype. All patients received chemotherapy whilst intensity, duration, and the number of drugs varied by IRSG classification risk factors (Fig. 1).⁷ Therapy spanning over 48 weeks is the standard of care. SIOP regimen's goal, on the other hand, is to avoid local therapy with radiation to diminish its late effects. Therapy duration varies from 3 to 6 months depending on whether the disease results in partial response (PR) or progressive disease (PD) at interim evaluation by imaging studies. If there were a complete response (CR), RT is omitted whilst RT will be given to those with PR or PD status after second-line chemotherapy (Fig. 1).⁹ CR is defined in our study as the

Treatment Schedule for Société Internationale d'Oncologie Pédiatrique (SIOP)

Abbreviations: IVA: ifosfamide, vincristine, actinomycin-D; CEV: carboplatin, epirubicin, vincristine; Resp: response; CR: complete remission; RT: radiation therapy; VCE: vincristine, carboplatin, etoposide; IVE: ifosfamide, vincristine, etoposide

Adapted from Stevens MC, et al. *J Clin Oncol* 2005;23:2618-28³

Treatment Roadmap for Intergroup Rhabdomyosarcoma Study Group (IRSG)**Intermediate Risk**

VAC Day 1 of weeks 0, 3, 6, 9, 12, 21, 24, 27, 30, 33
 V Day 1 of weeks 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 16, 17, 19, 20, 22, 23, 25, 26, 28, 29, 31, 32, 34, 35
 VC Day 1 of weeks 15, 18 during RT
 Evaluation weeks 12, 24, 35

Abbreviations: VAC: vincristine, actinomycin-D, cyclophosphamide; V: vincristine; VC: vincristine, cyclophosphamide; RT: radiation therapy

Adapted from Crist WM, et al. *J Clin Oncol* 2001;19:3091-102⁷

Fig. 1. The chemotherapy regimens.

disappearance of all tumour(s) with no evidence of disease. PR is defined as the following: $\geq 50\%$ decrease in the sum of the products of the maximum perpendicular diameters of all measurable lesions; there is no evidence of progression in any lesion; and no new lesions are found. Lastly, PD is defined as $\geq 25\%$ increase in the sum of the products of the maximum perpendicular diameters of measurable lesions at any involved site and/or the appearance of new lesions.

Statistical Analysis

All analyses were performed using SPSS15.0. Descriptive statistics for quantitative variables were presented as median (range) and n (%) for qualitative variables. The clinical primary endpoint of this study was adverse event free survival. This was defined as the time of entry into the study or time at diagnosis to the disease progression, occurrence of a metastatic disease, death from non-disease causes, development of a second malignant neoplasm, or last follow-up. Disease free survival and overall survivals were calculated using the method of Kaplan-Meier analysis.¹³ Log-rank test was performed to compare the disease free and overall survival for metastatic site, chemotherapy and amputation. Statistical significance was set at $P < 0.05$.

Results

Fifty patients with primary RMS are reported here. The median follow-up was 3.3 years (range, 0.4 to 15.6 years). The median age at diagnosis was 5.1 years (range, 0.1 to 17.3 years). Twenty-eight (56.0%) children were diagnosed

with RMS below the age of 6 years or younger with 15 (30.0%) of them being diagnosed under the age of 2 years. Head and neck primary was the most prevalent with a total of 19 patients. The common primary location in the head and neck was orbital in 5, non-orbital in 13 patients and 1 in the parameningeal region. The next most common site for the primary RMS was in the genitourinary tract with 17 patients; non-bladder in 8, bladder in 1, testes in 7, and prostate in 1. Extremities were the primary site in 8 patients; retroperitoneum in 3; spine in 2; and pancreas in 1. Histology as confirmed by biopsy of the primary tumour, was more common in embryonal subtype (E-RMS) in 29 (58.0%) patients; alveolar (A-RMS) in 17 (34.0%) and information was unable to be determined in 4 patients. Of the 17 patients with A-RMS, 9 were in the head and neck region (orbit = 3) and 3 involved the extremity. Of the 8 tumours in the extremity, 5 were embryonal and 3 were alveolar. According to the IRSG risk group, 18 (36.0%) were LR, 19 (38.0%) were IR, 12 (24.0%) were HR. Risk group was unknown in 1. Twenty-eight patients received chemotherapy as per the IRSG, 19 as per SIOP, 2 patients' treatment regimen was unknown and 1 received a combination of vincristine, etoposide, platinum, cyclophosphamide (VETOPEC)¹⁴ (Table 2).

Chemotherapy and Disease Response Status

In the study cohort, 28 (57.1%) patients were treated as per the IRSG regimen while 18 (36.7%) were as per the SIOP regimen and 3 were treated as per other regimens (Table 3). Of the 28 patients treated as per the IRSG regimen, 10

Table 2. Patient and Tumour Characteristics

	<u>Low-Risk (n = 18)</u>			<u>Intermediate-Risk (n = 19)</u>			<u>High-Risk (n = 12)</u>	
	IRSG (10)	SIOP (6)	Other (2)	IRSG (11)	SIOP (7)	Other (1)	IRSG (7)	SIOP (5)
Age (years)								
<1	1	2	1	1	2		1	1
1 to 4	5	1	1	2	3		1	2
5 to 9	1	2		6	1		1	
10 to 14	1			2	1	1	3	2
≥15 and above	2	1					1	
Histology								
Alveolar (17)	1			6	2	1	3	4
Embryonal (29)	7	6	1	5	4		3	1
Other/Unknown (5)	2	1					1	
Site Primary								
Orbit		1		2	1			
Head/Neck	3			4	2		1	3
Parameningeal				1				
GU B/P				3	3		1	
GU non B/P	4	5	1			1	1	
Extremity	1	1	1	1	1		1	1
Other	1						3	1
CR (39)	8	5	1	11	7		3	2
RT (21)								
Yes	1	1		9	4		3	3
Relapsed (15)								
Local	2	1		3	1		2	1
Metastatic (both)				0 (1)	0 (1)		1	3
Stats								
ANED (AWD)	9	6		9	6 (1)		4	0
DOD	1			2			3	4

ANED: Alive no evidence of disease; AWD: Alive with disease; B/P: Bladder and prostate; CR: Complete response; DOD: Dead of disease; GU: Genitourinary; IRSG: Intergroup Rhabdomyosarcoma Study Group; SIOP: Société Internationale d'Oncologie Pédiatrique

(35.7%) were in the LR group; 11 (39.3%) were in the IR group; and 7 (25.0%) were in the HR group. Complete response status as determined by CT or MRI imaging studies at the end of primary therapy was achieved in 8 of 10 (80.0%) in the LR group; all 11 (100.0%) in the IR group; and 3 of 7 (42.9%) in the HR group. Five (17.9%) of 28 patients treated as per the IRSG regimen achieved PR; 4 were from the HR group. In the 18 patients treated as per the SIOP regimen, 6 (33.3%) were in the LR group; 7 (38.9%) in the IR group; and 5 (27.8%) were in the HR group. CR status was achieved at the end of primary therapy in 5 (83.3%) in the LR group; all 7 (100.0%) in the IR group; and 2 (40.0%) in the HR group. Four of 18 (22.2%) patients treated as per the SIOP regimen achieved PR status; 3 were from the HR group (Table 3). The risk group of 1 patient treated as per SIOP regimen was unknown (no. 50). Every attempt was

made to adhere to the standard treatment protocols in all 50 patients. There were no reported treatment-related deaths.

Radiation Therapy

From the whole cohort of 50 patients, a total of 21 (42.0%) patients received chemotherapy and upfront RT. By risk group, 2 (11.1%) patients received both chemotherapy and upfront RT for their primary therapy in the LR group. In the 19 patients from the IR group, 13 (68.4%) received both chemotherapy and upfront RT and all achieved CR. In the 12 patients from the HR group, half of the patients received upfront RT and chemotherapy; 4 of whom achieved CR and 2 achieved PR at the end of primary therapy (Table 3). Of the 21 patients who received RT, 13 patients were treated as per the IRSG regimen and 8 patients were treated as

Table 3. Patient and Tumour Characteristics of All Patients by Risk Status

No.	Age	Histology	Site (primary)	Therapy (primary)	Relapse Site	Dx-FU (years)	Dx-R1 (years)	Status (primary)	Status Last FU	Therapy Relapse
Low-Risk										
1	4.1	A-RMS	extremity	IRSG		0.7		CR	ANED	
2	15.5	RMS	GU (testes)	IRSG		1.3		CR	ANED	
3	1.6	E-RMS	GU	IRSG	local	2.0	2.0	PR	DOD	Unk
4	6.3	E-RMS	retroperitoneum	IRSG+RT	Unk	2.4	2.1	Unk	ANED	Unk
5	12.1	E-RMS	HN (ear)	IRSG		2.6		CR	ANED	
6	1.6	E-RMS	GU	IRSG		5.9		CR	ANED	
7	2.4	E-RMS	HN	IRSG		7.0		CR	ANED	
8	17.1	E-RMS	GU (testes)	IRSG		8.4		CR	ANED	
9	2.8	RMS	HN (lip)	IRSG		8.8		CR	ANED	
10	0.5	E-RMS	extremity	IRSG		12.2		CR	ANED	
11	0.1	E-RMS	GU (testes)	SIOP		2.7		CR	ANED	
12	9.6	E-RMS	extremity	SIOP		3.9		CR	ANED	
13	3.5	E-RMS	GU	SIOP		4.7		CR	ANED	
14	15.4	E-RMS	GU (testes)	SIOP		4.7		CR	ANED	
15	8.5	E-RMS	HN (orbit)	SIOP+RT	local	8.8	1.4	PR	ANED	CE+V+
16	0.4	E-RMS	GU (prostate)	SIOP		9.6		CR	ANED	
17	0.3	E-RMS	extremity	Unk		4.4		CR	ANED	
18	4.5	RMS	GU (testes)	Other		10.0		CR	ANED	
Intermediate-Risk										
19	13.4	A-RMS	HN (orbit)	IRSG+RT		1.4		CR	ANED	
20	6.2	E-RMS	GU (bladder)	IRSG+RT		2.0		CR	ANED	
21	1.0	A-RMS	HN (NP)	IRSG+RT		2.7		CR	ANED	
22	3.9	A-RMS	extremity	IRSG+RT		2.9		CR	ANED	
23	0.9	A-RMS	HN (NP)	IRSG		4.0		CR	ANED	
24	5.7	A-RMS	HN (NP)	IRSG+RT	local	4.4	1.3	CR	DOD	IE+RT, VI*
25	8.4	A-RMS	HN	IRSG+RT	local	4.6	2.3	CR	ANED	S+GemDoce†
26	5.7	E-RMS	GU (pelvis)	IRSG+RT	local	5.3	4.4	CR	DOD	CE+V
27	12.3	E-RMS	HN (NP)	IRSG+RT		7.7		CR	ANED	
28	6.0	E-RMS	HN (orbit)	IRSG		8.2		CR	ANED	
29	7.0	E-RMS	HN (PM)	IRSG+RT		8.6		CR	ANED	
30	0.8	E-RMS	extremity	SIOP+RT		1.4		CR	ANED	
31	3.6	E-RMS	HN (pharynx)	SIOP+RT	local	1.6	1.5	CR	AWD	CarboplatinV
32	3.9	E-RMS	HN (ear)	SIOP		1.9		CR	ANED	
33	13.6	A-RMS	GU (perineum)	SIOP+RT		1.9		CR	ANED	
34	9.8	A-RMS	HN (orbit)	SIOP+RT		3.8		CR	ANED	
35	0.2	E-RMS	GU (pelvis)	SIOP		6.3		CR	ANED	
36	16.3	A-RMS	GU (testes)	VETOPEC	local	7.8	7.8	CR	AWD	None
37	3.3	E-RMS	GU (pelvis)	SIOP		15.6		CR	ANED	

Table 3. Patient and Tumour Characteristics of All Patients by Risk Status (Con't)

No.	Age	Histology	Site (primary)	Therapy (primary)	Relapse Site	Dx-FU (years)	Dx-R1 (years)	Status (primary)	Status Last FU	Therapy Relapse
High-Risk										
38	9.5	RMS	retroperitoneum	IRSG		0.4		PR	DOD	
39	0.8	E-RMS	pancreas	IRSG	local	0.8	0.8	PR	DOD	Unk
40	14.8	E-RMS	GU (pelvis)	IRSG	local	1.0	0.4	PR	DOD	VI*CT
41	12.5	E-RMS	HN (NP)	IRSG+RT	local	2.2	1.6	PR	ANED	Unk
42	1.6	A-RMS	extremity	IRSG		2.5		CR	ANED	
43	11.9	A-RMS	spine	IRSG+RT		2.5		CR	ANED	
44	17.3	A-RMS	GU (testes)	IRSG+RT		5.6		CR	ANED	
45	1.1	A-RMS	HN (parotid)	SIOP		0.5		PR	DOD	
46	12.4	A-RMS	HN (orbit, CNS)	SIOP+RT	brain	1.1	0.7	PR	DOD	VAC+RT, IVA
47	13.3	A-RMS	spine	SIOP+RT	leg	2.3	1.8	CR	DOD	E, VI*
48†	3.7	A-RMS	HN	SIOP+RT	thigh	2.6	2.0	CR	DOD	CE*V
49	0.8	E-RMS	extremity	SIOP	local, mets	6.4	0.8	PR	DOD	S
Unknown										
50	0.3	RMS	retroperitoneum	SIOP		1.4			CR	ANED

A: Actinomycin-D; ANED: Alive no evidence of disease; AWD: Alive with disease; A-RMS: Alveolar rhabdomyosarcoma; C: Cyclophosphamide; CNS: Central nervous system; CR: Complete response; Doce: Docetaxol; DOD: Dead of disease; Dx: Diagnosis; E+: Epirubicin; E: Etoposide; E-RMS: Embryonal rhabdomyosarcoma; FU: Follow-up; Gem: Gemcitabine; GU: Genitourinary; HN: Head and neck; I: Ifosfamide; I*: Irinotecan; IRSG: Intergroup Rhabdomyosarcoma Study group; Mets: Metastasis; NP: Nasopharynx; PM: Parameningeal; PR: Partial response; R1: First relapse; RT: Radiation therapy; SIOP: Société Internationale d'Oncologie Pédiatrique; S: Surgery; T: Teniposide; Unk: Unknown; V: Vincristine; VETOPEC: Vincristine, Etoposide, Platinum, Cyclophosphamide

†Autologous stem cell rescue.

‡Patient suffered second relapse in lungs and liver at 0.5 years from the first relapse.

per the SIOP regimen. In the 13 patients treated as per the IRSG regimen and upfront RT, a large majority, 9 (72.6%) were from the IR group while 3 (16.7%) were from the HR group. Almost all (11 out of 13) patients treated as per IRSG regimen and upfront RT achieved CR at the end of primary therapy. From those treated as per the SIOP regimen and upfront RT, 4 (50.0%) of 8 were in the IR group and 3 (37.5%) were in the HR group. Six of 8 in this group achieved CR with chemotherapy and upfront RT. In the study cohort, 2 patients received RT as an adjuvant therapy for their relapsed disease (nos. 25 and 46). The latter patient received RT for both primary and relapsed disease.

Relapse

Fifteen patients relapsed at a median of 6.3 years (range, 0.8 to 16.3 years) with a median follow-up of 2.4 years (range, 0.8 to 8.8 years). Amongst those with relapsed disease, histology of the primary tumour was embryonal in 9 and alveolar in 6 patients; disease risk group revealed 7 in HR, 5 in IR and 3 in LR groups. Head and neck primary (orbit, n = 2) was the most common primary site with 9

(60.0%) patients out of 15, followed by the genital urinary tract primary in 4. Initial chemotherapy regimen for the primary tumour in 8 out of the 15 relapsed patients was as per the IRSG regimen, 6 were as per the SIOP regimen and 1 was as per the VETOPEC¹⁴ regimen. Although 10 (66.7%) of 15 patients who relapsed received chemotherapy and upfront RT prior to relapse; only 2 received RT as an adjuvant therapy for their relapsed disease (nos. 24 and 46). The latter patient received both upfront and adjuvant RT for their primary tumour and relapsed disease. By risk group status, 2 of the patients in LR group with residual disease at the end of primary therapy relapsed locally, no. 3 and 15. The latter patient (no. 15), received upfront RT and was alive- no evidence of disease (ANED) at last follow-up whilst the former (no. 3), who did not receive upfront or adjuvant RT was dead of disease (DOD). Five patients relapsed locally in the IR group although all patients achieved CR at the end of primary therapy and 4 received upfront RT. Two of the patients were DOD. Seven patients relapsed in the HR group; there were 2 local relapses and 5 distant metastases inclusive of 1 who relapsed both locally

and with distant relapse (no. 49). Relapsed distant metastasis was only seen in those with HR group classification. Half of the relapsed patients in HR group achieved CR at the end of primary therapy while the remaining half achieved only PR. Nine (60.0%) patients in HR group with relapsed disease were DOD, 4 were ANED and 2 were alive with disease at last follow-up. Therapy for relapsed disease varied amongst all the patients and included various combinations of chemotherapy, surgery with or without RT. Patient and tumour characteristics are as outlined in Table 3.

Survival

The 5-year event-free survival (EFS) and overall survival (OS) for the cohort were 60.4% and 80.3% respectively. By disease risk group, the 5-year EFS for LR group disease was 81.3% (95% CI, 62.0 to 100.0), IR group was 61.4% (95% CI, 32.3 to 90.4) and HR group was 25.0% (95% CI, 0.0 to 49.5) respectively ($P < 0.001$) (Figs. 2 and 3). The 5-year EFS for disease risk classification by chemotherapy received as per SIOP vs per IRSG revealed: LR–83.3% vs 75.0% ($P = 0.787$); IR–83.3% vs 43.8% ($P = 0.351$); HR–0.0% vs 42.9% ($P = 0.336$) respectively (Figs. 4 and 5). Of 15 relapses ($n = 7$ HR), at a median of 2.0 years, 4 out of 5 patients treated as per SIOP regimen were DOD and 1 whose primary was orbital site was ANED. Three (37.5%) out of 8 treated as per IRSG and RT were alive at last follow-up. The 5-year EFS and OS for those with relapsed disease by chemotherapy received revealed 0% and 0% by SIOP and 50.0% and 44.4% by IRSG regimens respectively ($P = 0.23$, and $P = 0.37$) (Figs. 6 and 7).

Discussion

The incidence of RMS in Singapore is small but children diagnosed with the disease have a good chance of survival. High-risk disease classification treated as per IRSG regimen resulted in superior event-free survival than those treated as per SIOP regimen which is clinically important although not statistically significant ($P = 0.95$). Complete resolution of the disease by imaging studies can be achieved in a majority of the patients in all 3 risk groups at the end of primary therapy when treated with either as per IRSG or the SIOP regimens. RT cannot be avoided in those with higher risk classification RMS. Although relapsed disease can be salvaged in some after initial therapy using SIOP regimen, its impact on the family is not known. In our study, the main problem concerned the use of varied treatment protocols which makes analysis of current and future outcomes challenging.

A large majority of the children, 27 (54.0%) in this cohort were diagnosed with RMS under the age of 6 years whilst 14 were diagnosed between the ages of 10 to 17 years of

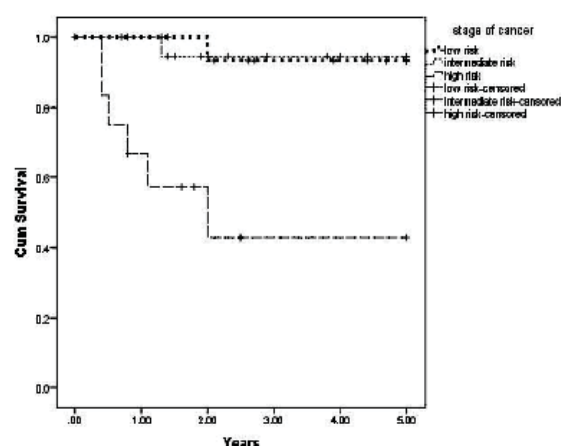


Fig. 2. Chart showing the event-free survival by disease risk status for cohort.

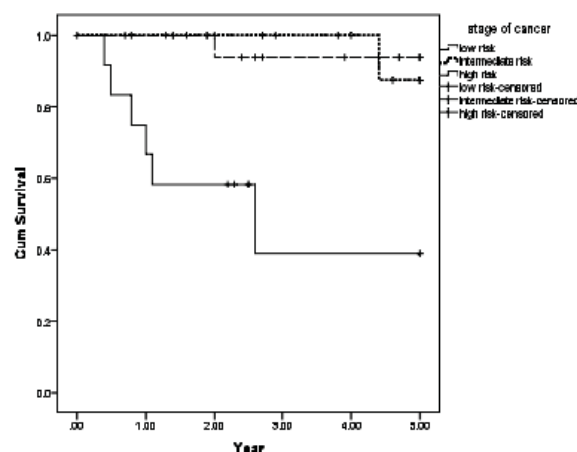


Fig. 3. Chart showing the overall survival by disease risk status for cohort.

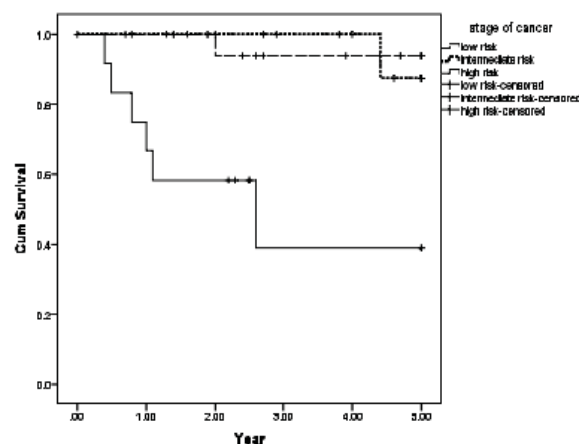


Fig. 4. Chart showing the event-free survival by chemo for high-risk patients.

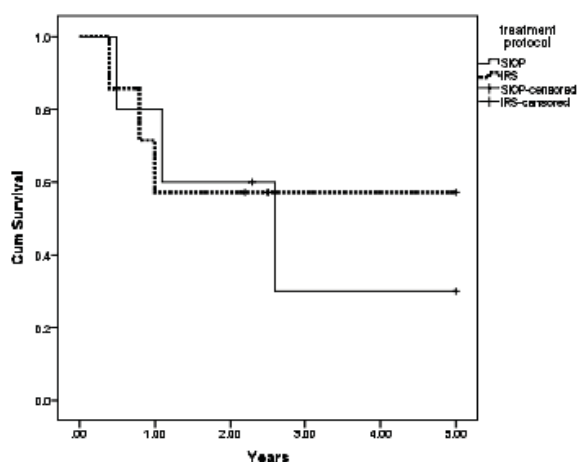


Fig. 5. Chart showing the overall survival by chemo for high-risk patients.

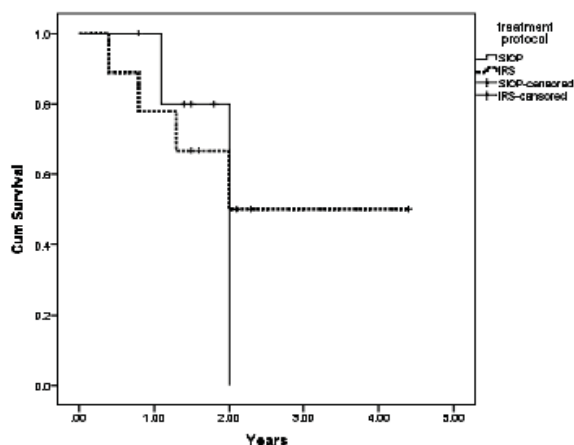


Fig. 6. Chart showing event-free survival for relapsed patients.

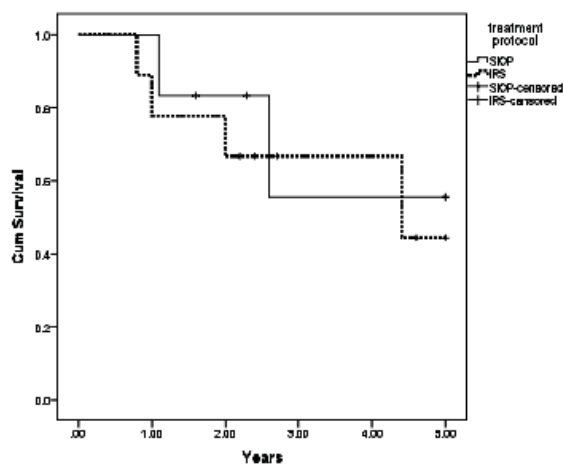


Fig. 7. Chart showing overall survival for relapsed patients.

age. Nine (18.0%) of the patients diagnosed with RMS were under the age of 1 year. Majority presented with head and neck: 19 (39.0%) primary, similar to our neighbour island city of Hong Kong.¹⁵ Histological subtype classification revealed that the embryonal RMS was more common. In our experience, embryonal subtype did better compared to those with alveolar subtype similar to international studies. However, in Hong Kong there was no difference in survival between those with embryonal versus alveolar tumours. Both our studies are limited by its retrospective nature and the small sample size. According to the IRSG risk group, majority of the children were in the IR group (38.0%). Surprisingly, a higher percentage, 24.0% of our patients presented with HR disease classification, whereas international studies approximate high-risk disease occurring at an average of 15.0%.^{6,16,17}

The 5-year EFS and OS for our cohort were higher than our neighbours with 60.4% and 80.3% respectively. In Hong Kong's experience, their 5-year EFS and OS rates for the entire cohort with a median follow-up of 3.4 years (range, 0.2 to 16.7 years), were 32.0 % (95% CI, 10.0% to 55.0%) and 49.0 % (26.0% to 73.0%) respectively. Not surprisingly, patients with high-risk or metastatic tumours fared worse in all the studies. However, those with high-risk disease treated as per IRSG regimen fared better in 5-year EFS than those treated as per the SIOP regimen, although statistically not significant. It appears that those in the LR and IR groups treated as per the SIOP regimen fared slightly better (10% more) although this was not statistically significant. Those in the IR group treated as per SIOP regimen could not avoid RT which may have helped achieve excellent local control contributing towards better survival. There were 15 relapses at a median of 2 years from diagnosis, 7 of who were with high-risk disease. Only 2 out of 6 treated as per SIOP regimen were salvageable and were alive at 0.1 and 7.4 years. The latter had an orbital primary, a favourable site at diagnosis. Although every possible attempt was made to perform a detailed chart review and treatment regimens were delivered according to standard protocols for all patients at both institutions, we were limited by the retrospective nature of our analysis and the small sample size.

RT can be avoided in LR classification although those in the higher risk group (IR and HR) should receive upfront RT to both local and metastatic disease sites. One of the main philosophies of the SIOP regimen is to avoid RT in an effort to eliminate its late effects by employing it as a salvage therapy in those with less than optimal response to initial chemotherapy or in those who relapsed. On the other hand, the approach by IRSG is for upfront RT in those with all alveolar histology or those in the IR or HR disease group. In our small cohort, only a handful of patients did not achieve CR at the end of primary therapy.

One patient in LR group treated as per the SIOP regimen did not achieve CR and required RT. In the IR group, a large majority, 5 (71.4%) of 7 patients treated as per the SIOP regimen could not avoid RT and all achieved CR by the end of therapy for primary disease. For those in the HR group, despite receiving upfront RT, achieving CR or PR status did not play a role in developing either local or metastatic RMS. A larger proportion of them suffered distant relapses and succumbed to the disease. For our close neighbours in Malaysia, the protocol adopted for children with RMS is as per the SIOP regimen. According to their publication from 1981, outcome has been poor, mainly due to advanced disease at presentation and abandonment to treatment.¹⁸ Apart from a few case series on RMS, there are no later follow-up reports from the country to determine whether our outcomes are similar.¹⁹⁻²¹ Nearby, Hong Kong's experience on 19 consecutive cases of RMS, each staged and treated according to the IRSG guidelines, found that their cohort of Chinese children did less favourably than that reported in international studies. They cited that their main reason was due to a high proportion of patients with metastatic disease, i.e. those with HR RMS.¹⁵ Although we have similarly high percentage of children with HR disease at the time of diagnosis, our patients fared better.

The outcome of children with RMS in Singapore can be further improved by coming together as a cooperative group to provide the best total care. The main challenge in our study concerned the use of varied treatment regimens or protocols. One possible explanation as to why 2 different regimens were used for treatment of this rather uncommon tumour is likely as a result of treating physician's preference and the location in which their subspecialty training was received. In Japan, a geographically larger country with a more complex medical set-up and numerous hospitals, they have successfully set up a cooperative Japan Rhabdomyosarcoma Study Group (JRSG). The aim of JRSG was to improve the outcome and to establish standardised therapy for children with RMS. This move resulted after retrospective studies performed as well as analysis from the Japanese Society for Paediatric Surgery found that the survival rates of each risk group, including those in LR group were lower than those in the US or Europe.^{22,23} Singapore is a rather small country with a resident population nearing 5 million. Incidence of childhood cancer is fortunately low with even lower rates of incidence of RMS. The use of a non-standardised disease classification system and varied treatment regimen do not allow us to consistently analyse and report our outcomes. Improved communication, together with a multidisciplinary team collaboration inclusive of paediatric pathologists, paediatric radiologists, paediatric sarcoma surgeons, paediatric radiation oncologists, paediatric oncology nurses, paediatric oncologists; and rigorous and robust data collection will be the key to success.

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Epidemiology of Oral Cancer Diagnosed at a Singapore Tertiary Healthcare Institution

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Abstract

Introduction: Oral and pharyngeal cancers grouped together are the sixth most common cancer seen worldwide. Oral cancers are, however, relatively not common in Singapore. There are few published epidemiological studies of oral cancers seen in Singapore. This article is a retrospective study of oral cancer incidence in a major tertiary institution in Singapore from 1991 to 2001. **Materials and Methods:** All oral cancers diagnosed from 1991 to 2001 were extracted from the register of histopathology results and case notes were reviewed. **Results:** In our study, it was found that oral malignancies preferentially affect older males. Chinese, being the predominant ethnicity, reports the highest incidence of oral malignancy. Squamous cell carcinoma was the most common oral malignancy with the tongue being the most commonly affected site. The majority of patients who sought treatment had complaints of swelling and were referred from restructured government hospitals and clinics. **Conclusion:** Dental professional should be aware of the common clinical presentation of oral cancers. As oral cancer is a disease of high morbidity and mortality, a concerted effort from the government and healthcare profession will be required to improve the outcome of the disease.

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Key words: Oral malignancies, Oral squamous cell carcinoma

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Introduction

Oral and pharyngeal cancers grouped together are the sixth most common cancer seen worldwide.¹ The annual estimated incidence is around 275,000 and 130,300 new cases of oral and pharyngeal cancers respectively.²

The most common type of head and neck cancer is squamous cell carcinoma.³ The most common site of malignancy in the oral cavity is the tongue in European and American populations and buccal mucosa in Asian populations.¹

The morbidity and mortality arising from the treatment of oral cancers are well documented. Current methods of treatment include surgery with neo-adjuvant radiotherapy with or without chemotherapy or in combination have resulted in good loco-regional control of the disease; the survival rate, however, has not improved much over the past decades especially in cases of regional metastasis. The overall survival rate in all stages of oral squamous cell

carcinoma was cited to be between 45% and 72%, and the survival rate is halved if cervical lymph node metastases are present.⁴

Oral cancers are, however, relatively not common in Singapore. There have been little data and few published epidemiological studies of oral cancers seen in Singapore. This paper aims to review previous oral cancers referred and diagnosed at the National Dental Centre over an 11-year period (1991 to 2001), to extract epidemiological data with respect to race, age at diagnosis, gender, types and sites of malignancies and also sources of referrals.

Materials and Methods

All cases of oral cancers diagnosed at our centre over an 11-year period between 1991 and 2001, were extracted from a register of histopathology results. Permission was granted from the local institutional review board to review

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the patients' case notes. The relevant data as detailed in Table 1 were retrieved.

Table 1. Histological Types of Oral Cancers Seen

Type of Cancer	Number of Cases Reported	Percentage
Cancer of epithelial origin		
Squamous cell carcinoma	134	77.5%
Spindle cell	4	2.3%
Salivary gland tumours		
Mucoepidermoid	9	5.2%
Adenocystic carcinoma	3	1.7%
Adenocarcinoma	4	2.3%
Unknown	1	0.6%
Other malignancies		
Clear cell	2	1.1%
Sarcoma	2	1.1%
Lymphoma	5	2.9%
Melanoma	2	1.1%
Osteosarcoma	2	1.1%
Origin of metastatic cancers		
Breast	1	0.6%
Liver	1	0.6%
Nasopharynx	1	0.6%
Gastrointestinal	1	0.6%
Unknown primary	1	0.6%
Total	173	100%

Inclusion Criteria

All oral cavity cancers were diagnosed based on anatomic sites as listed within the American Joint Committee on Cancer (AJCC) fifth edition of the Manual for Staging of Cancer.⁵ These sites include the tongue (ICD-02 topography codes C02.0-C02.9), gum (C03.0-03.9), floor of the mouth (C04.0-04.9), hard palate (C05.0, 05.8-05.9), cheek mucosa (C06.0), vestibule of the mouth (C06.1), retromolar area (C06.2), overlapping lesion of the mouth (C06.8) and lesion of the mouth not otherwise specified (NOS) (C06.9).

Exclusion Criteria

All cancers originating from the external lip and those involving the soft palate, uvula, tonsils, base of tongue are excluded from this study. These cancers are defined as not being within the oral cavity and also exhibit different local characterisation.

Histologic Type and Grade of Cancer

The histologic type and tumour grade of the identified oral cancer cases were grouped as follows:

1. Histologic type was grouped into 5 categories:
 - squamous cell carcinoma (ICD-0 2 morphologic codes M8052-8082)
 - verrucous carcinoma (M8051)
 - adenocarcinoma (M8140-8580)
 - lymphoma (M9590-9723)
 - Kaposi's sarcoma (M9140)
2. All tumours are graded using the World Health Organization (WHO) tumour grading system,⁶ categorised into 4 groups:
 - grade 1 (well differentiated)
 - grade 2 (moderately differentiated, moderately well differentiated, or intermediate differentiation)
 - grade 3 (poorly differentiated)
 - grade 4 (undifferentiated or anaplastic)

Results

A total of 173 cases of oral cancer were found. The gender and racial distribution are given in Figures 1 and 2.

Age at Diagnosis

Two patients (1.1%) were diagnosed with oral cancer before or at 20 years of age. Between the ages of 21 and 40 years, 14 patients (8.1%) were diagnosed with oral cancer. One-hundred and forty-six patients (84.4%) diagnosed with oral cancer were aged 41 years and above. The dates of birth of 11 patients (6.4%) were not clearly recorded. (Fig. 3).

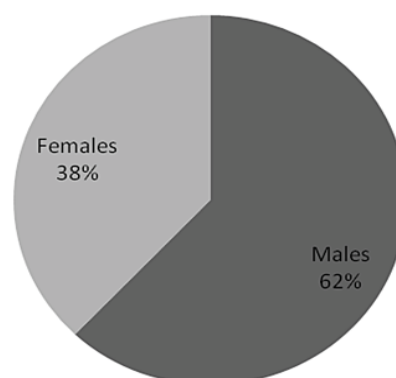


Fig. 1. Chart showing the gender distribution among the oral cancer cases (n = 173).

The youngest patient in our study that was diagnosed with an oral malignancy was 15 years old and the oldest was 94 years of age. The mean and median ages at diagnosis were 60 and 62 years of age respectively.

Types and Site of Cancer

Table 1 depicts the types and numbers of oral malignancies detected in this study. The most common oral malignancy was squamous cell carcinoma (77.5%), followed by salivary gland tumours (9.8%). Spindle cell carcinoma, a variant of the squamous cell carcinoma represented 2.3% of the study population and clear cell carcinoma was found in 1.1% of this cohort. Among the salivary gland tumours, mucoepidermoid carcinoma (5.2%) was the most common followed by adenocarcinoma (2.3%) and adenocystic carcinoma (1.7%).

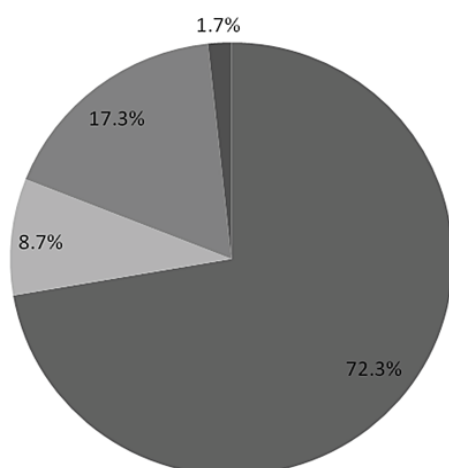


Fig. 2. Chart showing the racial distribution among the oral cancer cases (n = 173).

Sarcomas were found in 4 patients and this represents 2.2% of the study population, of which 2 (1.1%) were osteosarcomas. These sarcomas were found in the oral cavity after radiotherapy to the head and neck region.

Five cases of lymphomas were found in the study. These lymphomas were mainly B-cell lymphoma and it represents 2.9% of our population. Two cases of oral melanomas were noted (1.1%). Metastatic carcinomas from breast, liver, nasopharynx, gastrointestinal tract and unknown primary represented 3.0% of the population. The tumour grading for oral squamous cell carcinomas, according to WHO classification, is shown in Table 2.

Table 2. Distribution of Grading of Oral Squamous Cell Carcinoma

WHO Grading	Number of Subjects	Percentage	Histological Grading
Grade 1	35	26.1%	Well differentiated
Grade 2	82	61.2%	Moderately differentiate
Grade 3	9	6.7%	Poorly differentiated
Grade 4	2	1.5%	Anaplastic. Undifferentiated
Unknown	6	4.5%	
Total	134	100%	

WHO: World Health Organisation

Distribution of patients diagnosed with oral cancer

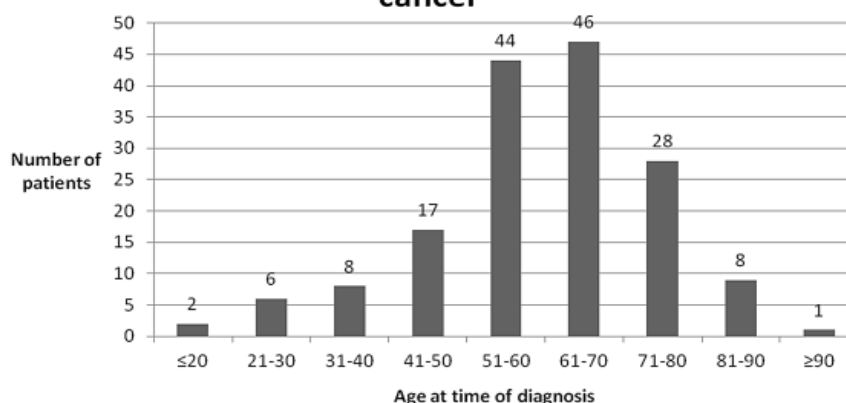


Fig. 3. Chart showing the age distribution among the oral cancer cases (n = 173).

The tongue was the most common site for oral squamous cell carcinomas. Other common areas for oral squamous cell carcinoma include gums, hard palate and cheek (Table 3). Other cancers and their location at time of biopsy are shown in Table 4. Lesions that were not stated included carcinomas in the fauces and chin.

Table 3. Incidence of Squamous Cell Carcinoma at Different Intraoral Sites

Sites of Squamous Cell Carcinoma	Number of Subjects	Percentage
Tongue	27	20.2%
Gums	20	14.9%
Floor of mouth	18	13.4%
Hard palate	28	20.9%
Cheek	17	12.7%
Vestibule	4	3.0%
Retromolar	9	6.7%
Overlapping lesion of mouth	5	3.7%
Intraosseous	1	0.8%
Not otherwise stated	5	3.7%
Total	134	100%

Presenting Complaint

The most common presenting complaint from the patients was swelling in the oral cavity. Other complaints included ulceration, pain from lesions or after dental treatment, and white lesions/patches in the oral cavity. A minority complained of “teeth falling off”, trismus, numbness or swollen lymph nodes (Table 5).

Referral Source

The primary source for oral cancer referrals is mainly from restructured hospitals and government polyclinics which made up close to half of all referrals. Private dental and medical clinics represented 13.8% and 3.4% respectively. A small number of patients (2.3%) noted oral changes themselves and presented without any referrals (Table 6).

Discussion

According to GLOBOCAN, cancers of the oral cavity and lips are the eleventh most common cancer reported globally with a crude rate of 3.9 and age standardised rate of 3.8 per 100,000.⁷ Incidence rates for oral cancer vary in men from 1 to 10 cases per 100,000 populations in many countries. The age standardised incidence rate of oral cancer in India for both genders is reported to be from 0.8 to 10.8 per 100,000 population. It was found that 33.5% of all oral and oropharyngeal cancer reported in the South

Table 4. Other Types of Oral Cancer Manifestation and Their Location

Type of Cancer	Location (Number of Cases)
Spindle cell	Palate (3) Overlapping lesion in the mouth (1)
Mucoepidermoid	Gum (2) Palate (6) Cheek (1)
Adenocystic carcinoma	Floor of mouth (1) Gum (1) Not otherwise stated (1)
Adeoncarcinoma	Floor of mouth (1) Palate (2)
Clear cell carcinoma	Intraosseous (1) Palate (1)
Sarcoma	Gum (1) Intraosseous (1)
Lymphoma	Palate (2) Cheek (2) Vestibule (1)
Melanoma	Overlapping lesion of mouth (2)
Osteosarcoma	Vestibule (1) Intraosseous (1)
Breast	Retromolar (1)
Liver	Gum (1)
Nasopharynx	Palate (1)
Unknown primary	Intraosseous (1)
Gastrointestinal	Retromolar (1)

Table 5. Presentation of Oral Malignancy

Complaints	Number of Subjects
Swelling ± pain	54
Pain	28
Ulcer	23
Leukoplakia	5
Others	8
Total	118

Table 6. Referral Sources

Referral Sources	Number of Cases	Percentage (%)
Self referral	4	2.3
Private dental clinic	24	13.8
Government institution	49	28.3
Polyclinic	37	21.4
Private medical	6	3.5
Not stated	53	30.6
Total	173	100

Asian country were mouth cancer and 43.0% were found in intraoral sites.⁸ Oral cancer is not one of the top 10 most common cancers seen in Singapore. Interestingly, “mouth” cancer was reported as the tenth cancer leading to death among Indians in Singapore from 1998 to 2002; 2.8 males and 2.1 females per 100,000 per year.⁹

This retrospective study showed a small number of patients ($n = 173$) diagnosed with oral cancer over a 11-year period which concurs with low incidence of oral cancer reported by National Cancer Registry in Singapore. This study showed oral cancer is more common among older males, a finding which is consistent with other studies.³ This could be related to the fact that males are more likely to be exposed to risk factors such as smoking and alcohol consumption. National surveys show significantly higher percentages of males who smoke and consume more alcohol than their female counterparts.^{10,11}

Ethnic Chinese formed the majority of this study cohort followed by Indians and Malays. This is in congruence with the population statistics of Singapore which consisted of the majority Chinese. However, this study showed that oral cancer is more common in Indians than in Malays. This is not in congruence with the population distribution. According to the Census of population in 2000, the distribution of ethnicity was 76.8% Chinese, 13.9% Malays, 7.9% Indians and 1.4% of other ethnicity.¹² From Figure 2, it can be seen that there is a greater proportion of patients of Indian ethnicity and of other races being diagnosed with oral cancer at 17.3% and 1.7% respectively in a country where they form only 9.3% of the entire population of Singapore. Our findings concur with statistics from National Cancer Registry of Peninsular Malaysia. With a population of 24.8 million, it made up of 54.3% Malay, 25.1% Chinese, 7.5% Indian and 13.1% others, the registry reported 149 per 100,000 Indian, 108 per 100,000 Malay and 74 per 100,000 Chinese were diagnosed with mouth and tongue cancer.¹³ Among the 3 ethnic groups diagnosed with oral and tongue cancer, 45% were Indians, 32.6% were Malays and 22.4% were Chinese.

A possible reason is that culturally, ethnic Malays tend not to indulge in alcohol, whereas other ethnicities do not have such a restriction. A national health survey in 1998 showed the prevalence of regular alcohol consumption in different ethnic groups was 3.6%, 0.6% and 2.7% for Chinese, Malays and Indians respectively.¹⁰

Contrarily, a significant percentage of Malays do smoke but we do not see a high percentage of Malays having oral cancer. The combination of smoking and alcohol consumption is known to be supermultiplicative in oral cancers.¹⁴ This might explain the discrepancy between the 2 ethnic groups. Moreover, betel nut or quid chewing could be other possible contributing risk factors among older Indian migrants.

In the Chinese population, the most common site for oral malignancy was the hard palate (23.5%), followed by lesion of the mouth not otherwise specified (NOS) (21.8%) and the tongue (17.6%). Similar to the Chinese population, the Malay population was found to have the highest incidence of oral malignancy at the hard palate (29.4%), and lesion of the mouth NOS (23.5%). The cheek and NOS were tied as the most common site (20.7%) in the Indian population.

Collectively, the highest incidence of oral malignancy in this study was the hard palate. This is then followed by tongue, gums, floor of the mouth and cheek mucosa. This is different from the Singapore Cancer Registry 1998-2002 report where the most common sites were tongue, palate (inclusive of hard palate, soft palate and uvula), cheek, floor of the mouth and gums.⁹ One of the reasons for the discrepancy might be that these patients could have problems with their dental prostheses and sought treatment in the National Dental Centre, assuming that it was a dental problem. This is especially true for patients with upper dentures, as these prostheses are fitted onto the hard palate. Any changes to the form of the palate will compromise the fit and function of the denture.

Comparatively, tongue lesions are not commonly viewed as a “dental” problem in the general population. Patients would have sought treatment with their family doctors instead of dentists if their function or speech were affected. This would result in a significant number of malignancies to be referred directly to the otolaryngologist or head and neck surgeons for management.

The most common oral malignancy seen in this study is squamous cell carcinoma and this concurs with most literature.³ However, in most studies, squamous cell carcinoma of the tongue ranked the top in terms of type and site of oral malignancy.¹⁵ This is also in alignment with the incidence data 1998 to 2002 in the Singapore Cancer Registry report where squamous cell carcinoma was found in 94.7% of all tongue cancers recorded in this period.⁹

In this study, squamous cell carcinoma of the tongue and palate both ranked the most common oral malignancy. Some have hypothesised that varying data regarding clinical presentation of oral squamous cell carcinoma may be explained by the type of epithelial origins, namely keratinised masticatory mucosa, non-keratinised lining mucosa and specialised mucosa of tongue. These 3 epithelia have significant differences in their development, structure and function.¹⁶ In our cohort, the prevalence of oral squamous cell carcinoma was about the same in all keratinised and non-keratinised oral epithelia and was higher than tongue epithelia.

Metastasis of malignant tumour to oral tissues is not common. Most reported in the English literature are case reports or series of cases. Those papers with large numbers

are usually reviews of many reports. Thus, it is not surprising that in our review of oral cancer over 11 years we only presented with 6 cases of metastasis to oral tissues. Three out of the 6 cases of metastasis were found to be the first sign of malignancy and the remaining half had known malignancy. Literature showed that the most common primary source of oral metastasis was from the lung for males and the breast for females.¹⁷ In this small cohort, there was 1 from lung and 1 from the breast. It would be interesting to gather more data on this to study if the pattern of metastasis concurs with other reports.

The most common symptoms from self-referred or referred patients were persistent swelling in the mouth. The other common presenting symptoms were pain and ulceration in the mouth. If odontogenic or infective source was ruled out as a causative factor, malignancy should be considered one of the differentials in the diagnosis of these symptoms. When malignancy is confirmed, depending on the type of histological diagnosis; it is critical to have a full workup to ensure that it is not a metastasis. In cases where there is a known primary malignant, it is mandatory to have a biopsy of the lesions in the mouth to exclude malignancy.

Majority of patients in this study were referred from the government or restructured institutions and polyclinics. A significant percentage was referred by private dental practitioners. One study stated that dental practitioners are more likely to refer asymptomatic, early-stage disease for biopsy after routine examination compared to medical practitioners.¹⁸ Due to the fact that treatment of premalignant conditions or early stage carcinoma improves survival significantly, this clearly indicates that dental surgeons play an important role in the detection of oral malignancy.

Conclusion

Although oral cancer is not a common pathology in Singapore, it remains as a disease of high mortality and morbidity. Armed with knowledge of risk factors that increases its incidence, efforts should be directed at the prevention of the disease by en masse education and behavioural modification. Medical and dental professionals would also be required to play an active role in identifying patients at risk and early referrals to institutions to achieve an optimum outcome.

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Diagnostic Value of Ultrasound-detected Calcification in Thyroid Nodules

Zhihong Wang, ¹MD, Hao Zhang, ¹MD, PhD, Ping Zhang, ¹MD, Liang He, ¹MD, Wenwu Dong, ¹MD

Abstract

Introduction: This study analyses the diagnostic value of ultrasonography (US) detection for calcification in thyroid nodules. **Materials and Methods:** We analysed the preoperative US findings and clinical characteristics of 577 malignant and 3434 benign thyroid patients who underwent surgery in our hospital. **Results:** The malignant rate in patients with microcalcification hyperechoic and tiny calcification foci ≤ 2 mm in diameter was significantly higher than the non-calcification and other calcification group ($P < 0.001$). The malignant rate in single calcification nodule was significantly higher than that in multiple nodule group ($P < 0.01$). Most of the patients (37/39) with lymph node calcification were malignant. The malignant rate of calcification and microcalcification was significantly higher in patients < 45 years old than in older patients ($P < 0.05$). **Conclusion:** Compared with other calcifications, microcalcification should be a better predictor of thyroid carcinoma. Malignancy should be highly suspected in patients with single calcification nodule, especially with lymph node calcification. Patients younger than 45 years of age with calcification or microcalcification have a greater risk for thyroid carcinoma.

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Key words: Age, Single nodule, Thyroid carcinoma, Ultrasonography

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Introduction

Thyroid nodules which can be palpated are present in between 4% and 7% of the population. Solid nodules as small as 3 mm and cysts of 2 mm can be detected with high-frequency ultrasonography (US).¹ US has been also commonly used to differentiate malignant nodules from all thyroid lesions using several sonographic features. Both benign and malignant thyroid nodules can present with calcifications in thyroid images but the incidence of calcification (especially microcalcification) is higher in malignant thyroid nodules.²⁻⁵ Thus, the purpose of this study is to further evaluate the association of different types of calcifications detected on the ultrasound to thyroid carcinoma, and to evaluate the diagnostic accuracy of different calcification types, in a large cohort of Chinese patients.

Materials and Methods

Patients

We retrospectively evaluated the records of 4011 consecutive patients who received preoperative high-resolution US examinations for thyroid nodule and underwent thyroid surgery between January 2005 and January 2010 at our Hospital.

US Examination Technique

The US examination was performed with a scanner of Apolio-80 (Toshiba, Tokyo, Japan) and a 7- to 12-MHz linear-array transducer. All of the calcification characteristics were recorded. The location, size, shape, boundary, envelope, echo feature of the nodules and the characteristic of the lymph nodes were examined. The

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final neck US examination was performed within 1 month before surgery.

Thyroid Nodule Calcification and Pathological Result

Microcalcifications were defined as hyperechoic and tiny calcification foci ≤ 2 mm in diameter, either with or without acoustic shadows (Fig. 1); macrocalcifications were indicated by the presence of irregular calcifications with maximum diameter > 2 mm (Fig. 2); rim calcifications were defined when a nodule had egg-shell or peripheral curvilinear calcification (Fig. 3).^{6,7}

The diagnoses were confirmed by postoperative histopathology. Microcarcinomas were ≤ 10 mm in maximum diameter and macrocarcinomas were > 10 mm in maximum diameter.

Gender and Age

Malignant rates with different types of calcification were calculated in male and female patients. According to a previous study, there is a significant difference in prognosis between patients < 45 years old and ≥ 45 years old.⁸ Vini et al found that patients aged ≥ 70 years old were significantly more likely to have advanced disease and higher incidence of recurrence and mortality than younger patients.^{9,10} The cut-off points of 45 and 70 years old were chosen for the analysis of malignancy rate with calcification according to age.

Statistical Analysis

The chi-square test with the 95% confidence interval (CI) of odds ratio (OR) was deployed. A value of $P < 0.05$ was considered statistically significant. The sensitivities, specificities, positive predictive value, negative predictive value and accuracy of calcification, microcalcifications, macrocalcifications and rim calcification in the diagnosis of thyroid malignancy were calculated. Statistical analysis was performed using SPSS statistical software, version 16.0 (SPSS Institute, Chicago, USA).

Results

A total of 4011 patients aged between 7 and 83 years old (mean 51.99 ± 10.92 years) who received thyroid surgery were analysed in our study. Among these, 873 were male and 3138 were female, with a male-to-female ratio of 1 : 3.59. Medical history, physical and imaging examinations and fine needle aspiration biopsy results were considered before deciding whether or not to have surgery. Operation types included excisional biopsy, hemi/total thyroidectomy and lymph node dissection. The postoperation histopathological diagnosis included thyroid carcinoma, nodular goitre,

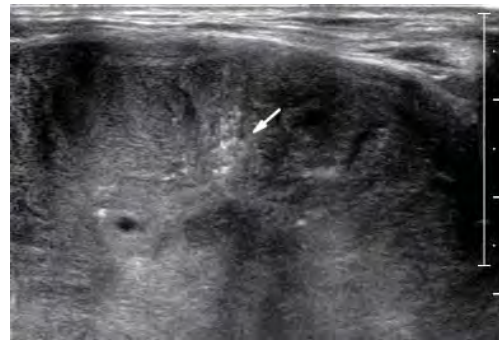


Fig. 1. A thyroid nodule (papillary thyroid cancer) with microcalcification (white arrow).



Fig. 2. A benign thyroid nodule with macrocalcification (white arrow).

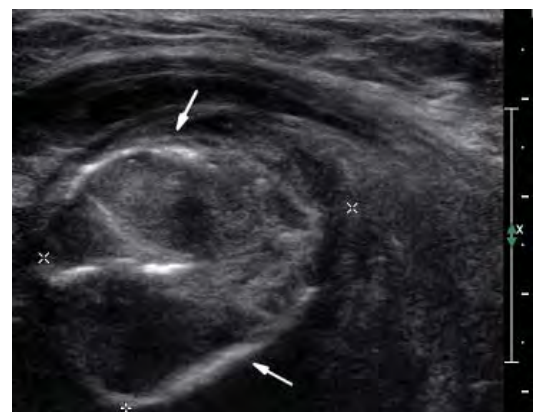


Fig. 3. A benign thyroid nodule with rim calcification (white arrow).

thyroid adenoma and Hashimoto's thyroiditis.

Calcifications were found in 39.37% (1579/4011) of all patients. Of the patients with calcifications, 33.69% (532/1579) had microcalcifications, 60.16% (950/1579) had macrocalcifications and 6.14% (97/1579) had rim calcifications. The incidence of malignancy was 56.02% (298/532) for patients with microcalcifications and 15.85% (166/1047) for patients with other calcifications including 16.11% (153/950) of macrocalcification and 13.40% (13/97) of rim calcification. The malignant rate in patients with calcifications were higher than that with non-calcification group ($P < 0.001$), with OR values of 8.54 (95% CI, 6.87 to 10.62). The malignant rate in patients with microcalcification was also significantly higher than that without microcalcification ($P < 0.001$), with OR value of 14.61 (95% CI, 11.84 to 18.03) (Table 1).

Of the 577 patients with malignant thyroid tumors, calcifications were detected in 80.42% (464/577): microcalcifications in 51.65% (298/577), macrocalcifications in 26.52% (153/577) and rim calcification in 2.25% (13/577). Ninety-six percent of the malignant thyroid tumours were papillary carcinomas in which 82.40% (440/534) had calcification, and 54.68% (292/577) had microcalcifications. The rate of calcification for other types of malignant tumours are summarised in Table 2.

In our group, the microcarcinomas were all micropapillary carcinomas histopathologically. The incidence of

microcalcification in microcarcinomas and macrocarcinomas were 52.23% (82/157) and 50.00% (210/420) respectively. There was no significant difference in the incidence of microcalcification in the 2 types of carcinomas (Table 2).

A total of 352 (22.29%) patients presented a single calcification nodule area in the 1579 patients with calcifications. Of them, 188 (53.41%) cases were malignant and 164 (46.59%) cases were benign. Of the 532 patients with microcalcifications, 174 (32.71%) showed a single calcification nodule area. Of these patients, 128 (73.56%) were malignant and 46 (26.44%) were benign. The incidence of malignancy was significantly higher in patients with single calcification nodule than in those with multiple calcification nodules ($P < 0.001$), with OR values of 3.95 (95% CI, 3.08 to 5.07). The malignant rate was also significantly higher in patients with single microcalcifications nodule ($P < 0.001$), with OR values of 3.08 (95% CI, 2.07 to 4.57) (Table 3).

Thirty-nine patients showed localised lymph node calcification. Thirty-seven of these patients were given a pathological diagnosis of metastatic papillary carcinoma while the other 2 were diagnosed with Hashimoto thyroiditis. Twenty-nine of these 37 patients had microcalcifications in the regional lymph nodes, whereas the others had macrocalcification in the lymph nodes.

There was no significant difference for the incidence of malignancy between male and female groups combined with calcification (31.61 % (98/310) vs 28.84 % (366/1269); P

Table 1. Calcification Types and Malignant Rates

Type of Calcification	Malignant cases	Benign cases	Malignant rate (%)	P value	Odds ratio	95% Confidence Interval
Microcalcification (n = 532)	298	234	56.02	<0.001	14.61	(11.84, 18.03)
Macrocalcification (n = 950)	153	797	16.11	0.084		
Rim calcification (n = 97)	13	84	13.40	0.78		
Calcification (n = 1579)	464	1115	29.39	<0.001	8.54	(6.87, 10.62)

Table 2. Pathological Diagnosis of 577 Malignant Thyroid Nodules with Different Calcifications

Pathological diagnosis	n	Microcalcification	Macrocalcification	P value
Papillary carcinoma	534	292	148	$P = 0.70$
Microcarcinoma (≤ 10 mm)	157	82	39	
Macrocarcinoma (> 10 mm)	377	210	109	
Medullary carcinoma	20	4	9	
Follicular carcinoma	2	0	0	
Anaplastic carcinoma	7	1	3	
Thyroid lymphoma	7	0	1	
Thyroid fibrosarcoma	3	0	2	
Others	4	1	3	
Total	577	298	166	

Table 3. Malignant Rates in Single and Multiple Nodule Combined with Calcifications

Single or multiple calcification nodule	n	Malignant Cases	Benign Cases	Malignant rate (%)	P value	Odds ratio	95% Confidence Interval
Single calcification	352	188	164	53.41	<0.001	3.95	(3.08, 5.07)
Multiple calcification	1227	276	951	21.67			
Single microcalcification	174	128	46	73.56	<0.001	3.08	(2.07, 4.57)
Multiple microcalcification	358	170	188	47.04			

Table 4. Malignant Rates in Different Age Status Combined with Calcifications

Age, years (n)	Age status combining with calcification (Malignant rate %)	P value	Age status combining with microcalcification (Malignant rate %)	P value
<45 years (1238)	49.20 (247/502)	< 0.001	74.90 (179/239)	< 0.001
45 to 70 years (2616)	20.18 (205/1016)	NA	40.43 (112/277)	NA
≥70 years (157)	19.67 (12/61)	NA	43.75 (7/16)	NA

Table 5. Diagnostic Value of Us Findings for Malignant Nodules According to Calcifications

Type of Calcification	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Accuracy (%)
Calcification (n = 1579)	80.42	67.53	29.39	95.35	69.38
Microcalcification (n = 532)	51.65	93.19	56.02	91.98	87.21
Macrocalcification (n = 950)	26.52	76.79	16.11	86.15	69.56
Rim calcification (n = 97)	2.25	97.55	13.40	85.59	83.84

>0.05), as well as with microcalcification (51.69 % (61/118) vs 57.25 % (237/414); $P > 0.05$).

The malignant rates with calcifications in different ages were shown in Table 4. The malignant rate of calcification and microcalcification was significantly higher in patients < 45-years old compared with older patients ($P < 0.05$). There was no significant difference between the 45 to 70 years and ≥ 70 years groups ($P > 0.05$).

The sensitivities of calcification, microcalcification, macrocalcification and rim calcification for predicting malignancy were 80.42%, 51.65%, 26.52% and 2.25%, respectively; and the corresponding specificity were 67.53%, 93.19%, 76.79% and 97.55%, respectively. In addition, calcification, microcalcification, macrocalcification and rim calcification had positive predictive values of 29.39%, 56.02%, 16.11% and 13.40%, respectively, and negative predictive values of 95.35%, 91.98%, 86.15% and 85.59%, respectively (Table 5).

Discussion

Several studies reported the association of calcification and thyroid carcinoma. Lu et al found that calcification was presented in 49.6% (128/258) of malignant nodules and 15.7% (292/1864) of benign nodules.¹¹ Similar findings by Wang et al reported calcification present in 68 (63.55%)

of the 107 patients with malignant thyroid tumours.¹² Our studies show that 80.42% (464/577) of malignant thyroid nodules exhibited calcifications. The result is consistent with the calcification rate in the previous study. The presence of calcification was associated with an OR value of 8.54 for malignancy, suggesting that there was 8.54 times more relative risk of carcinoma than without calcification. The possible reason for higher calcification rate in the malignant group was the difference in formation of calcification in benign and malignant diseases. The fast proliferation of cancer cells and the hyperplasia mixed with necrosis of cancer tissue promote calcium deposition and calcification formation. But in benign thyroid lesions, macrocalcifications present on the wall of nodules after the hematoma absorption in a few cases.¹²

A number of studies focus on the relationship between thyroid nodule microcalcification and malignancy. Lu et al studied 2122 thyroid nodules and found that the incidence of microcalcifications was significantly higher in malignant than benign thyroid nodules. They were 33.7% (87/258) and 6.4% (120/1864) respectively.¹¹ Moon et al reported a microcalcification rate of 44.2% in malignant nodules which was much higher than that of in benign nodules.¹³ Shi et al found that the malignant rate was significantly higher in patients with microcalcifications (96.5%) compared with macrocalcifications (41.1%).¹⁴ We also have the

similar findings. The presence of microcalcifications was associated with an OR value of 14.61 for malignancy, suggesting that thyroid nodules with calcification had 14.61 times more relative risk of carcinoma than those without microcalcification. High specificity (93.19%) and accuracy rate (87.21%) suggest that compared to other calcifications, microcalcification should be a better predictor of thyroid carcinoma.

In the present study, the incidence of malignancy is significantly higher in patients with single calcification nodule than in those with multiple calcification nodules. The malignant rate was also significantly higher in patients with single microcalcification nodule, which was similar to other studies.¹⁵ Our studies also suggest that the association between single calcification nodule and malignancy is closer than the association between multiple calcification nodules and malignancy. So malignancy should be highly suspected in patients with single calcification nodule, especially with single microcalcifications nodule.

Wang et al reported that the relative risk of presence of microcalcification with carcinoma was higher in patients <45 years old than those >45 years old.¹² In our group, the malignant rate of calcification and microcalcification was significantly higher in patients <45 years old compared with older patients ($P < 0.05$), which is consistent with a previous report.¹⁴

Choi et al¹⁶ evaluated the diagnostic accuracy of preoperative US and computed tomography (CT) examination of the neck in 589 patients. The specificity of both methods was higher than 90% for cervical lymph node metastasis. In our study, the malignant rate of patients with localised lymph node calcification was 94.87% (37/39). Moreover, 78.38% (29/37) of these lymph nodes presented microcalcification as in the primary cancer lesion. We consider that the patient should be recommended for surgery if the localised lymph node calcification, especially microcalcification, was found with the primary nodule calcification.

Conclusion

In conclusion, the association between calcification in ultrasonography and malignancy is confirmed in this study. Compared with other calcifications, microcalcification has a higher diagnosis value for thyroid carcinoma. Malignancy should be highly suspected in patients with single calcification or microcalcification nodule. It is necessary to recommend surgery for patients younger than 45 years old with calcifications, especially with lymph node calcification.

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Comparison of Adults with Insulin Resistance (IR) in Latent Autoimmune Diabetes Versus IR in Glutamic Acid Decarboxylase Antibody-negative Diabetes

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MBBS

Abstract

Introduction: Insulin resistance in latent autoimmune diabetes in adults (LADA) patients is controversial. The aim of this study was to evaluate insulin resistance and its related factors (metabolic syndrome parameters) among subjects with LADA and glutamic acid decarboxylase antibodies (GADA) negative diabetes, as well as the impact of these factors on insulin resistance. **Materials and Methods:** GADA levels were investigated in 1140 diabetic patients aged between 30 and 70 years. Insulin resistance and metabolic syndrome parameters were assessed in LADA and GAD-negative diabetic patients by general linear model. In addition, the impact of metabolic syndrome factors on insulin resistance was assessed in LADA and glutamic acid decarboxylase (GAD)-negative diabetic patients. **Results:** LADA was diagnosed in 33 subjects from 1140 Malaysian diabetic patients (prevalence = 2.9%). The results showed that LADA patients had higher insulin resistance and high density lipoprotein cholesterol (HDLc) ($P = 0.003$ and 0.00017 respectively) and lower body mass index (BMI) ($P = 0.007$) compared to GAD-negative diabetic patients. The HDLc was associated with decreased insulin resistance in LADA patients ($P = 0.041$), whereas HbA1c, triacylglycerides (TG) and waist were associated with increased insulin resistance in GAD-negative diabetic patients ($P = 3.6 \times 10^{-12}$, 1.01×10^{-5} and 0.004 respectively). HbA1c was highly associated with decreasing β -cell function in both LADA ($P = 0.009$) and GAD-negative diabetic subjects ($P = 2.2 \times 10^{-28}$). **Conclusion:** Insulin resistance is significantly higher in LADA than GAD-negative diabetic Malaysian subjects.

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Key words: GAD-negative diabetes, Insulin resistance, LADA

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Comparison of Adults with Insulin Resistance (IR) in Latent Autoimmune Diabetes Versus IR in Glutamic Acid Decarboxylase Antibody-negative Diabetes

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Introduction: Insulin resistance in latent autoimmune diabetes in adults (LADA) patients is controversial. The aim of this study was to evaluate insulin resistance and its related factors (metabolic syndrome parameters) among subjects with LADA and glutamic acid decarboxylase antibodies (GADA) negative diabetes, as well as the impact of these factors on insulin resistance. **Materials and Methods:** GADA levels were investigated in 1140 diabetic patients aged between 30 and 70 years. Insulin resistance and metabolic syndrome parameters were assessed in LADA and GAD-negative diabetic patients by general linear model. In addition, the impact of metabolic syndrome factors on insulin resistance was assessed in LADA and glutamic acid decarboxylase (GAD)-negative diabetic patients. **Results:** LADA was diagnosed in 33 subjects from 1140 Malaysian diabetic patients (prevalence = 2.9%). The results showed that LADA patients had higher insulin resistance and high density lipoprotein cholesterol (HDLc) ($P = 0.003$ and 0.00017 respectively) and lower body mass index (BMI) ($P = 0.007$) compared to GAD-negative diabetic patients. The HDLc was associated with decreased insulin resistance in LADA patients ($P = 0.041$), whereas HbA1c, triacylglycerides (TG) and waist were associated with increased insulin resistance in GAD-negative diabetic patients ($P = 3.6 \times 10^{-12}$, 1.01×10^{-5} and 0.004 respectively). HbA1c was highly associated with decreasing β -cell function in both LADA ($P = 0.009$) and GAD-negative diabetic subjects ($P = 2.2 \times 10^{-28}$). **Conclusion:** Insulin resistance is significantly higher in LADA than GAD-negative diabetic Malaysian subjects.

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Introduction

Latent autoimmune diabetes in adults (LADA) is a term used to describe the adult onset autoimmune diabetes, characterised by the presence of type 1 diabetes-associated antibodies but has a slower progression toward an absolute insulin requirement.¹ LADA is characterised by autoimmune destruction of pancreatic β cells with the presence of T-cell reactivity to circulating auto-antibodies, more frequently, glutamic acid decarboxylase antibodies (GADA).^{2,3} GADA is the most sensitive marker for LADA.^{4,6} Recent studies showed that GADA titers have a bimodal distribution in LADA and identify 2 subgroups of patients with distinct clinical, autoimmune and genetic features.⁷ Patients with high GADA titers tend to be younger, leaner and have a lower prevalence of metabolic syndrome and its components, with more prominent traits of insulin deficiency (lower C-peptide, higher HbA1c) than individuals with lower GADA titers.^{7,8} Epidemiological studies showed that

LADA prevalence among T2D varies from 2% to 12%⁹ and tends to be higher in Western countries compared to Asian countries.¹⁰⁻¹³ Prevalence of LADA has not been studied in Malaysia, which has a multiethnic population including Malays (50.4%), Chinese (23.7%) and Indian (7.1%). These 3 ethnic groups represent the major portion of the population residents in Asia.¹⁴

LADA patients do not require insulin at diagnosis. However, insulin dependency occurs within a short period (ranging from months to years). The final clinical features of these patients include weight loss, proneness to ketosis, unstable blood glucose levels and an extremely diminished C-peptide reserve.⁹ LADA patients may need treatment strategies different from that used for patients with T2D.¹⁵ An appropriate therapeutic approach would be one that offers a good metabolic control that maintains the mass and function of residual β -cell mass.⁷

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Insulin resistance is a clear feature of T2D and is sometimes affected by age, body mass index (BMI), ethnicity, physical activity, and medication.^{16,17} The role of insulin resistance and its contribution to the pathophysiology of LADA is controversial. Some studies showed that insulin resistance in LADA was lower than that in T2D and comparable to T1D.^{1,18–20} However, recent studies found no differences in insulin resistance between LADA and GAD-negative diabetes.^{17,21} The aim of this study was to evaluate insulin resistance and its related metabolic syndrome parameters, in LADA and GAD-negative diabetes, as well as study the impact of these factors on insulin resistance.

Materials and Methods

Ethics Statement

This study was approved by the Medical Ethics Committee of University Malaya Medical Centre (Reference No. 387.15) for data collection between 2009 and 2011.

Subjects and Data Collection

Malaysian diabetic patients aged between 30 and 70 years who attended the University Malaya Medical Centre (UMMC) Kuala Lumpur for treatment were invited to participate in this study. A total of 1140 diabetic subjects participated in this study. The subjects' height (in metre), weight (in kg) were measured using Seca scale (Seca gmbh & co. kg. Germany) and body mass index (BMI), defined as weight (kg)/height squared (m²), was calculated. Waist circumference was measured halfway between the lower rib margin and the anterior superior iliac spine. The antidiabetic and antilipid medications of the diabetic patients were obtained from the patient records at UMMC. Fasting venous blood (10 mL) was collected from each participant after obtaining written consent.

Biochemical Analysis

Fasting plasma glucose (FPG), high density lipoprotein cholesterol (HDLc), and triacylglycerides (TG) were measured on Dimension® RxL Max® Integrated Chemistry System (Siemens Healthcare Diagnostics Inc., Deerfield, IL USA). Fasting plasma insulin (FPI) was measured by ADVIA Centaur XP Immunoassay System (Siemens Healthcare Diagnostics Inc., Deerfield, IL USA) while variant haemoglobin A1c reorder pack (Catalogue number 270-0003) (Bio-Rad, USA) was used for the measurement of glycosylated haemoglobin (HbA1c). The β -cell function (HOMA- β) and insulin resistance (HOMA-IR) were assessed by the Homeostasis Model Assessment (HOMA2) Calculator v2.2, which is available online from Diabetes Trials Unit (University of Oxford www.dtu.ox.ac.uk) according to Matthews et al.²²

Immunological Analysis

GADA65 autoantibody was analysed by ELISA kit (RSR Limited, Cardiff, UK). The analysis was done manually according to the manufacturer's protocol. Plates were read at 450 nm using a microplate reader (BioRad, Calabasas, USA).

Statistical Analysis

Demographic and biochemical parameters were non-normally distributed, thus they were log-transformed before statistical analyses and then retransformed back and presented as geometric means. The statistical analyses were conducted using Social Package of Statistical Science (SPSS) 11.5 (LEAD Technologies; Inc. USA). Insulin resistance, β -cell secretion and metabolic syndrome parameters were assessed in LADA and GAD-negative diabetic patients by general linear model adjusted for age, gender, race, BMI, antidiabetes medications, and antilipid medications. The impact of metabolic syndrome factors on the insulin resistance, β -cell secretion among LADA patients and GAD-negative diabetes were evaluated by linear regression. The significance level was considered if $P < 0.05$.

Results

LADA was diagnosed in 33 subjects (2.9%) who had GADA titer above the cut-off point (5 u/ml) and did not require insulin at diagnosis of T2D for the previous 12 months. The demographic and biochemical parameters of the 2 groups are summarised in Table 1. Treatment data of diabetes revealed that 70% of LADA patients were insulin dependent while 43% of GAD-negative diabetic subjects were under insulin treatment ($P = 0.002$). Antilipid treatment data showed that 33% of LADA patients did not take any antilipid treatments versus 11% of GAD-negative diabetic patients ($P = 0.004$) (Table 2).

The general linear model (adjusted for age, gender, race, BMI, antidiabetes medications, and antilipid medications) showed that LADA patients had higher insulin resistance, fasting blood insulin and HDLc ($P = 0.003$; 0.015 ; 0.00017 , respectively) and lower BMI ($P = 0.007$) compared to GAD-negative diabetic patients (Table 3). There were no differences between the 2 groups with respect to β -cell secretion, FPG, HbA1c, waist circumference and TG. HDLc was associated with decreased insulin resistance in LADA patients ($P = 0.041$), whereas, HbA1c, TG and waist were associated with increased insulin resistance in GAD-negative diabetic patients ($P = 3.6 \times 10^{-12}$; 1.01×10^{-5} ; 0.004 , respectively) (Table 4). HbA1c was highly associated with decreasing β -cell function in both LADA ($B = -17.466$, $P = 0.009$) and GAD-negative diabetic subjects ($B = -15.342$, $P = 2.2 \times 10^{-28}$).

Table 1. Demographic Parameters Among LADA and GAD-negative Diabetes

Parameters	GAD-negative Diabetes (n = 1107) n (%)	LADA (n = 33) n (%)	P Value
Gender			
Male	748 (67.6)	21 (63.6)	
Female	359 (32.4)	12 (36.4)	
Race			
Malay	425 (38.4)	15 (45.5)	
Chinese	293 (26.5)	10 (30.3)	
Indian	389 (35.1)	8 (24.2)	
Age (years)	51.3 (50.8 – 51.9)	46.1 (42.8 – 49.6)	0.001
Weight (kg)	72.6 (71.5 – 73.7)	66.7 (59.2 – 75.3)	0.050
Height (m)	1.61 (1.60 – 1.61)	1.61 (1.57 – 1.65)	0.849
Insulin dependent	464 (43)	23 (70)	0.002
Antilipid medication	952 (89)	22 (67)	0.001

GAD: Glutamic acid decarboxylase; LADA: Latent autoimmune diabetes in adults

Note: The results presented represent geometric means (95% confidence interval of mean).

Table 2. Comparison of Antidiabetes and Antilipid Medications Among LADA and GAD-negative Diabetes

	GAD-negative Diabetes (n)%	LADA (n)%	P Value
Antidiabetes medications			
Non-treated	(42) 3.9%	0.00	-
Oral	(564) 52.7%	(10) 30.3%	0.038
Oral + insulin	(430) 40.2%	(18) 54.5%	0.173
Insulin	(34) 3.2%	(5) 15.2%	0.012
Antilipid medications			
Non-treated	(118) 11.0%	(11) 33.3%	0.004
Fibrates	(46) 4.3%	(1) 3%	0.704
Fibrates + statins	(165) 15.4%	(5) 15.2%	0.991
Statins	(741) 69.3%	(16) 48.5%	0.041

GAD: Glutamic acid decarboxylase; LADA: Latent autoimmune diabetes in adults

Table 3. Assessment of Insulin Resistance, Diabetic Parameters and Metabolic Syndrome Factors Among LADA and GAD-negative Diabetic Patients

Metabolic Syndrome Parameters	Univariate Model*			
	GAD-negative Diabetes	LADA	P Value	Eta Squared
HOMA-IR	2.44 (2.34 – 2.53)	3.47 (2.76 – 4.35)	0.003	0.8
HOMA-β	70.7 (67.4 – 74.2)	78.6 (59.3 – 104.1)	0.471	0.1
FPG (mmol/L)	7.88 (7.72 – 8.05)	8.63 (7.66 – 9.71)	0.145	0.2
FPI (pmol/L)	98 (94 – 102)	130 (104 – 164)	0.015	0.5
HbA1c (%)	7.98 (7.89 – 8.08)	8.41 (7.82 – 9.03)	0.167	0.2
BMI (kg/m ²)	27.4 (27.1 – 27.7)	25.2 (23.8 – 26.8)	0.007	0.6
Waist (cm)	96.1 (95.7 – 96.5)	95.5 (93.3 – 97.7)	0.592	0.01
TG (mmol/L)	1.56 (1.52 – 1.61)	1.39 (1.17 – 1.65)	0.183	0.2
HDLc (mmol/L)	1.09 (1.08 – 1.11)	1.27 (1.18 – 1.37)	0.00017	1.2

Note: The results presented represent geometric means and 95% confidence interval of the mean which evaluated by univariate (General Linear Model)

*adjusted for age, gender, race, BMI, antidiabetes and antilipid medications.

BMI: Body mass index; FPI: Fasting plasma insulin; FPG: Fasting plasma glucose; GAD: Glutamic acid decarboxylase; HOMA-β: β-cell function;

HOMA-IR: Insulin resistance, LADA: Latent autoimmune diabetes in adults; TG: Triacylglycerides

Table 4. Impact of Metabolic Syndrome Factors, on the HOMA- β and HOMA-IR

Parameters	GAD-negative Diabetes		LADA	
	HOMA- β B (P Value)	HOMA-IR B (P Value)	HOMA- β B (P Value)	HOMA-IR B (P Value)
Waist Circumference (cm)	1.313 (0.001)	0.041 (0.004)	– 5.126 (0.116)	– 0.012 (0.914)
BMI (kg/m ²)	0.416 (0.639)	0.059 (0.073)	12.876 (0.088)	0.077 (0.767)
Glycosylated HbA1c (%)	– 15.342 (2.2 $\times 10^{-28}$)	0.350 (3.6 $\times 10^{-12}$)	– 17.466 (0.009)	0.257 (0.242)
High Density Lipoprotein (mmol/L)	– 12.905 (0.204)	– 0.636 (0.091)	– 72.143 (0.152)	– 3.806 (0.041)
TG (mmol/L)	– 0.911 (0.686)	0.369 (1.01 $\times 10^{-5}$)	11.791 (0.641)	0.637 (0.484)

Note: The results are presented as unstandardised coefficients; B and (P value) the impact of each parameter on β -cell function and insulin resistance was assessed by linear regression controlled for the other parameters in addition to gender, race and age. The positive sign of the coefficient implies a direct relationship, and the negative sign implies an inverse relationship.

BMI: Body mass index; GAD: Glutamic acid decarboxylase; HOMA- β : β -cell function; HOMA-IR: Insulin resistance; LADA: Latent autoimmune diabetes in adults; TG: Triacylglycerides

Table 5. Assessment of Insulin Resistance, Diabetes Parameters and Metabolic Syndrome Factors Among LADA and GAD-negative Diabetic Patients

Metabolic Syndrome Parameters	1st Univariate Model*				2nd Univariate Model†			
	GAD-negative Diabetes	LADA	P Value	Eta Squared	GAD-negative Diabetes	LADA	P Value	Eta Squared
HOMA-IR	2.44 (2.35 – 2.54)	3.68 (2.93 – 4.63)	0.001	1.1	2.44 (2.35 – 2.54)	3.39 (2.71 – 4.25)	0.005	0.7
HOMA- β	70.5 (67.2 – 73.9)	76.0 (57.5 – 100.4)	0.602	0.02	70.8 (67.5 – 74.3)	76.4 (57.8 – 101.1)	0.596	0.3
FPG (mmol/L)	7.90 (7.74 – 8.06)	8.91 (7.91 – 10.04)	0.052	0.3	7.88 (7.72 – 8.05)	8.67 (7.70 – 9.76)	0.122	0.2
FPI (pmol/L)	97.8 (94.1 – 101.8)	136.4 (108.7 – 171.2)	0.005	0.7	97.9 (94.2 – 101.8)	127.7 (101.9 – 160.0)	0.023	0.4
HbA1c (%)	7.97 (7.87 – 8.07)	8.77 (8.13 – 9.46)	0.015	0.5	7.98 (7.89 – 8.08)	8.39 (7.81 – 9.01)	0.183	0.1
BMI (kg/m ²)	27.5 (27.2 – 27.7)	24.7 (23.3 – 26.3)	0.001	0.9	27.4 (27.1 – 27.7)	24.9 (23.5 – 26.5)	0.002	0.8
Waist (cm)	95.9 (95.6 – 96.3)	95.4 (93.2 – 97.6)	0.642	0.01	96.1 (95.7 – 96.5)	95.2 (93.1 – 97.4)	0.431	0.02
TG (mmol/L)	1.58 (1.53 – 1.63)	1.28 (1.07 – 1.52)	0.020	0.5	1.57 (1.52 – 1.62)	1.31 (1.10 – 1.56)	0.051	0.3
HDLc (mmol/L)	1.09 (1.08 – 1.10)	1.27 (1.17 – 1.37)	0.0001	1.2	1.09 (1.08 – 1.11)	1.28 (1.19 – 1.38)	7.03 $\times 10^{-5}$	1.3

Note: The results presented represent geometric means and 95% confidence interval of the mean which were evaluated by univariate (General Linear Model)

*adjusted for age, gender, race and BMI

†adjusted for age, gender, race, BMI and antidiabetes medications

BMI: Body mass index; FPI: Fasting plasma insulin; FPG: Fasting plasma glucose; GAD: Glutamic acid decarboxylase; HOMA- β : β -cell function; HOMA-IR: Insulin resistance; LADA: Latent autoimmune diabetes in adults; TG: Triacylglycerides

Discussion

The prevalence of LADA among Malaysian diabetic patients was 2.9%. This prevalence tends to be lower than that seen in Western countries and similar to that found in Asian countries. This finding was in agreement with previous reports^{11,12,23–25} in Japan and Korea.

The current study showed that LADA patients are younger and leaner than GAD-negative diabetes which is in agreement with Tuomi et al.²⁶ However, this is not in agreement with Zinman et al study.²⁰ LADA subjects showed poor glycaemic control, elucidated by the HbA1c levels, which is in agreement with Roh et al.¹² Furthermore, compared to GAD-negative diabetes, a large ratio of LADA patients were insulin-dependent. This finding which is in agreement with Lee et al¹¹ may be indicative of the progression of pancreatic β -cell decline and insulin

dependency in LADA patients. Currently there are no specific treatment guidelines for LADA patients, even though early insulin treatment may have beneficial effect. Early diagnosis of LADA and initiation of insulin treatment may maintain the function of residual β -cell mass.

The insulin resistance was higher in LADA patients compared to GAD-negative diabetes patients even when controlled for most confounders (age, gender, race, BMI, antidiabetes and antilipid medications). This finding is in contrast to the findings of Behme et al¹⁹ and Chiu et al.¹⁷ This variation might be due to differences in treatment profile between the current study subjects and subjects of Behme et al and Chiu et al which may affect insulin resistance. However the known metabolic syndrome parameters such as waist and TG that were associated with metabolic syndrome in T2D were not associated with increased insulin resistance

Table 6. Comparison of Fasting Plasma Glucose, Fasting Plasma Insulin and Insulin Resistance Between LADA Patients Subgroups According to the Diabetic Treatment

Parameters	LADA Subjects Under Oral Diabetic Treatment Only (n = 10)	LADA Subjects Under Oral and Insulin Treatment (n = 18)	LADA Subjects Under Insulin Treatment Only (n = 5)	P Value
Fasting Glucose (mmol/L)	9.77	9.71	7.49	0.59
Fasting Insulin (pmol/L)	132	119	122	0.96
HOMA-IR	3.47	3.48	3.20	0.99

Note: The results are presented as non-adjusted means.

HOMA-IR: Insulin resistance; LADA: Latent autoimmune diabetes in adults

in LADA patients. The HDLc was higher in LADA subjects than GAD-negative diabetes subjects and was unexplainably associated with insulin resistance among LADA patients. HbA1c was higher in LADA subjects compared to GAD-negative diabetes patients (Table 5), and was associated with decreased β -cell function in both LADA and GAD-negative diabetes. HbA1c reflect glycaemic control and on the other hand it indirectly reflects irreversible protein glycosylation inside the body. Abnormal and irreversible pancreatic β -cell damage caused by prolonged exposure of β -cell to supraphysiological glucose results in decreased insulin gene expression and is characterised by a decrease in insulin synthesis and secretion.²⁷ Once hyperglycaemia becomes apparent, β -cell function gradually deteriorates²⁸ and culturing of human islet with supraphysiological glucose induces β -cell apoptosis.²⁹ The mechanism responsible for the deleterious effect of chronic exposure to high glucose on islet cells is unclear. However, this effect may be mediated by increasing protein glycosylation (so-called advanced glycosylation end products or AGEs) and oxidative stress, which causes cell damage and ultimately cell death.³⁰ Hyperglycaemia increases production of reactive oxygen species (ROS) and ROS is thought to mediate glucose toxicity of β -cell and induce of β -cell apoptosis.³¹ It is still unclear whether LADA is a distinct entity and what the degree of its insulin resistance is.^{11,32} The limitations of the current study are that it is a hospital-based study and small sample size of T2D resulted in 33 subjects with LADA. Another potential weakness of this study using insulin test instead of C-peptide in comparison between LADA and GAD-negative diabetes in which the result may be affected by insulin treatment as 70% LADA patients were under insulin treatment. However, fasting insulin for LADA patients on insulin therapy was not higher than those on oral therapy (Table 6) suggesting that any spillover effect from the previous day's treatment is negligible. In conclusion, LADA patients were more insulin resistant than GAD-negative in diabetic Malaysian subjects. High HDLc is associated with decreased insulin resistance in

LADA patients. Early detection of LADA and initiation of insulin treatment may delay the decline of pancreatic β -cell function.

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Identification of Insulin Resistance in Subjects with Normal Glucose Tolerance

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Abstract

Introduction: Decreased insulin action (insulin resistance) is crucial in the pathogenesis of type 2 diabetes. Decreased insulin action can even be found in normoglycaemic patients, and they still bear increased risks for cardiovascular disease. In this study, we built models using data from metabolic syndrome (Mets) components and the oral glucose tolerance test (OGTT) to detect insulin resistance in subjects with normal glucose tolerance (NGT). **Materials and Methods:** In total, 292 participants with NGT were enrolled. Both an insulin suppression test (IST) and a 75-g OGTT were administered. The steady-state plasma glucose (SSPG) level derived from the IST was the measurement of insulin action. Participants in the highest tertile were defined as insulin-resistant. Five models were built: (i) Model 0: body mass index (BMI); (ii) Model 1: BMI, systolic and diastolic blood pressure, triglyceride; (iii) Model 2: Model 1 + fasting plasma insulin (FPI); (iv) Model 3: Model 2 + plasma glucose level at 120 minutes of the OGTT; and (v) Model 4: Model 3 + plasma insulin level at 120 min of the OGTT. **Results:** The area under the receiver operating characteristic curve (aROC curve) was observed to determine the predictive power of these models. BMI demonstrated the greatest aROC curve (71.6%) of Mets components. The aROC curves of Models 2, 3, and 4 were all substantially greater than that of BMI (77.1%, 80.1%, and 85.1%, respectively). **Conclusion:** A prediction equation using Mets components and FPI can be used to predict insulin resistance in a Chinese population with NGT. Further research is required to test the utility of the equation in other populations and its prediction of cardiovascular disease or diabetes mellitus.

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Key words: Area under the receiver operating characteristic curve, Body mass index, Insulin resistance, Metabolic syndrome, Oral glucose tolerance test

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Introduction

Currently, type 2 diabetes is a leading cause of death in Taiwan, as well as in many other countries.¹ It places a tremendous burden not only on patients themselves but also on patients' families and society. Although which process occurs first remains controversial, both insulin resistance and impaired insulin secretion are the 2 principal causes of type 2 diabetes.^{2,3} According to the Insulin Resistance Atherosclerosis Study, approximately 85.7% to 93.2%

of type 2 diabetes patients have insulin resistance.⁴ In addition, Reaven et al⁵ found that approximately 30% of normoglycaemic patients can be classified as insulin-resistant.⁶ Insulin-resistant patients were proven to have a higher cardiovascular risk than those without.⁷ Therefore, it is crucial to identify insulin resistance in normoglycaemic patients. Preventive interventions, such as lifestyle modification, can be suggested to this apparently healthy cohort.

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Insulin resistance and insulin sensitivity are conceptually reciprocal. In other words, a high level of insulin resistance corresponds to a low level of insulin sensitivity. Numerous methods have been developed for measuring insulin action (insulin-mediated glucose disposal). Among these methods, the glucose clamp technique is considered the “gold standard” for measuring insulin sensitivity, but because it is both time- and labour-consuming, it is not suitable for most hospitals and research institutes.⁸ Another sophisticated method, the insulin suppression test (IST), provides an accurate measurement of insulin resistance found to be closely correlated with the insulin sensitivity derived from the clamp technique.⁹ Because the oral glucose tolerance test (OGTT) is a simple method, it is a widely used test. Many surrogate tests used for measuring insulin action were derived from the OGTT, such as the Matsuda and Stumvoll Index.^{10,11} The homeostasis model assessment of insulin resistance (HOMA-IR) is the easiest method to conduct. However, it is less accurate than the mentioned tests, and can only be used for large cohorts.¹²

Metabolic syndrome (Mets), the clustering of glucose intolerance, hypertension, obesity, and dyslipidaemia, was found to be associated with insulin resistance and an increased risk of cardiovascular disease.¹³ Moreover, all Mets components have been found to be associated with insulin resistance.^{14–18} Combining these biological variables to identify insulin-resistant people has been assessed by several studies.^{19,20} For instance, Stern et al used routine clinical measurements to build a tree model that predicted insulin resistance with 78.7% sensitivity and 79.6% specificity in non-diabetic participants.¹⁹ In addition, McAuley et al developed an equation for the same purpose.²⁰

It is crucial to identify insulin-resistant normoglycaemic patients. Both Mets components and data from the OGTT were used in predictive models for this purpose. However, no known study has considered OGTT data, which could further increase the accuracy of predictive models.

In this study, we developed a simple but accurate multivariable risk score model to predict insulin resistance in patients with normal glucose tolerance (NGT) using both Mets components and data from the OGTT.

Materials and methods

Subjects

A total of 513 participants were enrolled and received the standard 75-g OGTT in Cardinal Tien Hospital. Participants were either self-referred or referred by health professionals who recommended screening for diabetes. The participants had no history of diabetes. After excluding frank diabetes, impaired fasting glucose, and impaired glucose tolerance (IGT), only 292 persons with NGT were included. They were defined to be “normal” according to the criteria published

by the American Diabetes Association in 2003.²¹ None of the participants had a notable medical or surgical history. Before the study, they were instructed by physicians and dietitians to avoid any medication known to affect glucose or lipid metabolism and to maintain a stable diet for at least 1 week before the study. On the day of the first study, a complete routine examination was administered to exclude the presence of cardiovascular, endocrine, renal, hepatic, and respiratory disorders. The study protocol had been approved by the hospital’s institutional review board and ethics committee, and all participants had provided written informed consent prior to participation.

Study Protocol

Both tests were performed in the Clinical Research Center. On the day of the OGTT, after a 10-hour overnight fast, a standard 75-g OGTT was performed at 8:00 am. Blood was drawn before the glucose load and at 30, 60, 90, and 120 minutes after the glucose load for the measurements of plasma glucose and insulin. The blood sample collected before the OGTT was also used to determine lipid profiles. At least 1 week from the first study visit, the IST for estimating insulin action (glucose-mediated glucose disposal) was performed.²² After a 10-hour overnight fast, an intravenous catheter was set in a forearm vein for administration of somatostatin (250 µg/h, preceded by a 125-µg bolus), insulin (25 mU/m²/min), and glucose (240 mg /m²/min) (m² refers to body surface area).²² A second intravenous catheter was placed in the contralateral forearm vein for blood collection. Blood was sampled at 30-minute intervals during the initial 150-minute period, and then at 10-minute intervals between 150 and 180 minutes of the infusion. The mean calculated from the last 4 measurements of glucose and insulin was used to determine the steady-state plasma glucose (SSPG) and the steady-state plasma insulin (SSPI) values. During this test, because the SSPI concentration was similar in all participants, the SSPG concentration was used as a direct measure of the ability of insulin to mediate glucose disposal; a high SSPG value indicated a high level of insulin resistance. According to the levels of SSPG, study participants were divided into tertiles. Participants in the highest tertile were defined as insulin-resistant. The remaining two-thirds of the participants were considered not insulin-resistant.²³ The HOMA-IR was determined by the formula of fasting plasma insulin (FPI): (mU/L) X (fasting plasma glucose, FPG)(mmol/L)/22.5.¹²

Laboratory Measurements

Plasma was separated within 1 hour of blood withdrawal and stored at –30°C until the time of analysis. Plasma glucose was measured using the oxidase method by employing a glucose analyzer (YSI Model 203, Scientific Division,

Yellow Spring Instrument Company, Inc, Yellow Spring, Ohio, USA). Plasma insulin was assayed using a commercial solid-phase radioimmunoassay technique (Coat-A-Count insulin kit, Diagnostic Products Corporation, Los Angeles, California, USA) with intra- and inter-assay coefficients of variance of 3.3% and 2.5%, respectively. Serum triglyceride (TG) was measured by employing a Fuji Dri-Chem 3000 analyzer (Fuji Photo Film Corporation, Minato-Ku, Tokyo, Japan) using the dry multilayer analytical slide method. Serum high-density lipoprotein cholesterol (HDL-C) concentration was determined with an enzymatic cholesterol assay method after dextran sulfate precipitation.

Statistical Analysis

Data were shown as mean $\pm$ standard deviation. One-way ANOVA was used to evaluate the demographic data, clinical characteristics, and parameters derived from the tests of the 3 groups. The Bonferroni test was used for post hoc examination.

All of the variables of interest were first evaluated for their predictive performance by logistic regression. The receiver operating characteristic (ROC) curve of each variable (or model) was then plotted as the sensitivity (true-positive rate, y axis) against the 1-specificity (false-positive rate, x-axis). The area under the ROC curve (aROC curve) was calculated by the trapezoidal rule, and used to determine the predictive accuracy of the models. In general, a large area corresponds to a high predictive accuracy of the variable (model).²⁴

Five binary logistic models were built. Insulin resistance was the dependent variable (i.e. 0 or 1 in statistical analysis), and all other baseline clinical and metabolic variables, including BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), TG, HDL-C, FPG, FPI, plasma glucose level at 120 minutes of OGTT (OGTT120g), and plasma insulin level at 120 minutes of OGTT (OGTT120i), were taken as the independent variables. Models were built to predict the occurrence of insulin resistance in the order of the simplest and most clinically available datum to the most complex datum that requires 2-hour OGTT results. The 5 models are as follows:

Model 0: BMI

Model 1: BMI + SBP + DBP + TG (Mets model)

Model 2: Model 1 + FPI

Model 3: Model 2 + OGTT120g

Model 4: Model 3 + OGTT120i (full model)

A model was selected using the enter method in binary logistic regression analysis. The Hosmer-Lemeshow test was used to assess the goodness of fit of these models. A comparison of the aROC curves of the models was performed using the method developed by Hanley et al.²⁴ From binary

logistic regression, equations were built for each model. After inputting all of the factors into the model, optimal cut-point values were derived, and the point with the highest sensitivity and specificity was selected. In other words, if the value derived from the equation is higher than the specified cutoff point, the chance of having insulin resistance is high.

For the sensitivity test (i.e. external validation), we divided the participants into 2 groups. To build the model and equation, three-fourths of the study participants ($n = 219$) were randomly selected. The remaining one-fourth ($n = 73$) were considered the external validation group. This procedure was repeated with Model 4 with these 219 participants only because it was the most accurate model. Because the study group was only three-fourth of the original Model 4 group, the equation derived from Model 4 to calculate the probability (P) of having insulin resistance was expected to contain the same factors but different coefficients. This equation was then used to calculate the P value of having insulin resistance in the external validation group. The sensitivity and specificity of this equation in the external validation group were evaluated.

To verify its accuracy, we not only compared our models with HOMA-IR but also the McAuley's equation. In order to do this, ROC curve was drawn by using McAuley's equation and HOMA-IR and their aROC curves were calculated in the study cohort.

All statistical analyses were performed using the SPSS software system, version 13.0 (SPSS Inc., Chicago, IL, USA). P values less than .05 were considered statistically significant.

Results

In total, 292 participants with NGT were enrolled and divided into 3 groups based on SSPG values. Group 1 represented the lowest tertile of insulin resistance, whereas Group 3 represented the highest. The demographic data of these 3 groups are shown in Table 1. Participants in Group 3 exhibited the highest BMI. After adjusting for age and BMI, Group 3 also had higher FPI, OGTT120g, OGTT120i, and HOMA-IR values than the other 2 groups. However, the TG level of Group 3 was only higher than that of Group 1. There were no statistically significant differences in age, sex, SBP, DBP, HDL-C, and FPG among these 3 groups.

The aROC curves of individual parameters and models, their Hosmer-Lemeshow goodness-of-fit tests, and tests of statistical difference between the models are shown in Table 2 and Figure 1, respectively. SBP, DBP, TG, and BMI had larger aROC curves than the diagonal reference line. This indicates that the prediction of insulin resistance can be improved by these parameters, but not by FPG or HDL-C. BMI demonstrated the greatest aROC curve (71.6%). Moreover, the aROC curves of FPI, OGTT120g,

Table 1. Baseline Demographic and Metabolic Characteristics of Study Participants Categorised by the Degree of Insulin Resistance

Variable	Group 1 (n = 97)	Group 2 (n = 98)	Group 3 (n = 97)
Age (y)	45.6 ± 10.3	44.9 ± 11.7	46.4 ± 11.4
Sex (F/M)	57/40	53/45	55/42
BMI (kg/m ²)	22.5 ± 2.1‡	22.8 ± 3.2‡	25.0 ± 3.3*†
SBP (mmHg)	114.7 ± 13.7	113.7 ± 14.0	119.1 ± 14.5
DBP (mmHg)	74.6 ± 7.9	74.3 ± 8.9	78.1 ± 9.5
TG (mmol/L)	1.0 ± 0.4‡	1.1 ± 0.6	1.1 ± 0.51
HDL-C (mmol/L)	1.2 ± 0.4	1.1 ± 0.3	1.1 ± 0.3
HOMA-IR	1.7 ± 0.9‡	1.9 ± 0.7‡	3.5 ± 3.2*†
FPG (mmol/L)	5.1 ± 0.3	5.1 ± 0.4	5.2 ± 0.3
OGTT120g (mmol/L)	6.1 ± 1.1‡	6.5 ± 1.2‡	7.4 ± 1.6*†
FPI (pmol/L)	55.6 ± 21.1‡	64.3 ± 28.2‡	125.4 ± 263.8*†
OGTT120i (pmol/L)	334.0 ± 198.4‡	443.5 ± 238.2‡	765.9 ± 408.0*†
SSPG (mmol/L)	4.6 ± 0.9†‡	7.9 ± 1.2*‡	12.9 ± 2.0*†
SSPI (pmol/L)	429.4 ± 77.3	453.4 ± 97.2	462.3 ± 104.2

BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; FPG: Fasting plasma glucose; FPI: Fasting plasma insulin; HOMA-IR: Homeostasis model assessment of insulin resistance; OGTT120g: Plasma glucose level at 120 min of the 75-g OGTT; OGTT120i: Plasma insulin level at 120 min of the 75-g OGTT; SSPG: Steady-state plasma glucose; SSPI: Steady-state plasma insulin. Data are expressed as (mean ± SD).

**P* < 0.05 against Group 1

†*P* < 0.05 against Group 2

‡*P* < 0.05 against Group 3

Table 2. Area Under the Receiver Operating Characteristic Curves of Metabolic Variables and Models Predicting Insulin Resistance

Models	aROC curve ± SE (95% CI)	* <i>P</i> value (Hosmer-Lemeshow)	† <i>P</i> value for omnibus test
BMI (Model 0)	0.716 ± 0.032 (0.653 – 0.778)	0.691	0.000
FPG	0.555 ± 0.036 (0.485 – 0.625)	0.340	Non-significant
SBP	0.614 ± 0.036 (0.534 – 0.675)	0.682	0.002
DBP	0.640 ± 0.036 (0.570 – 0.710)	0.027	0.000
logTG	0.618 ± 0.035 (0.549 – 0.686)	0.738	0.002
HDL-C	0.556 ± 0.035 (0.487 – 0.626)	0.830	Non-significant
FPI	0.710 ± 0.035 (0.641 – 0.780)	0.079	0.000
OGTT120g	0.714 ± 0.032 (0.651 – 0.777)	0.742	0.000
OGTT120i	0.828 ± 0.027 (0.776 – 0.881)	0.236	0.000
HOMA-IR	0.705 ± 0.034 (0.649 – 0.757)	0.100	0.000
McAuley' index	0.728 ± 0.032 (0.665 – 0.790)	0.086	0.000
Model 1	0.729 ± 0.031 (0.668 – 0.791)	0.665	0.000
Model 2	0.771 ± 0.030 (0.713 – 0.830)	0.500	0.000
Model 3	0.801 ± 0.029 (0.745 – 0.857)	0.297	0.000
Model 4	0.851 ± 0.025 (0.802 – 0.900)	0.201	0.000

aROC curve: Area under the receiver operating characteristic curve; Model 0: BMI; Model 1: BMI+ SBP+ DBP+ TG ; Model 2: Model 1+FPI; Model 3: Model 2+ OGTT120g; Model 4: Model 3+OGTT120i; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; FPG: Fasting plasma glucose; FPI: Fasting plasma insulin; OGTT120g: Plasma glucose level at 120 min of the 75-g OGTT; OGTT120i: Plasma insulin level at 120 min of the 75-g OGTT; HOMA-IR: Homeostasis model assessment of insulin resistance; McAuley' index, $\exp [2.63 - 0.28 \ln (\text{FPI}) - 0.31 \ln (\text{TG})]$

**P* values calculated using the Hosmer-Lemeshow goodness-of-fit test.

†*P* value calculated using an omnibus test.

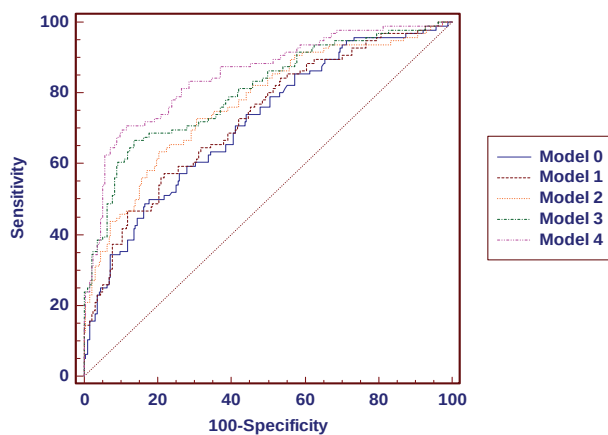


Fig. 1. Area under the receiver operating characteristic curves (aROC curves) of the models. The arrow indicates the arbitrarily selected risk score cut-off (0.29) of Model 4, which has a sensitivity and specificity of 76.0% and 77.1%, respectively.

Comparison of aROC curves	P values
Model 1 vs BMI	0.253
Model 2 vs BMI	0.013
Model 3 vs BMI	0.001
Model 4 vs BMI	<0.001
Model 2 vs Model 1	0.030
Model 3 vs Model 2	0.032
Model 4 vs Model 3	0.009
Model 4 vs Model 2	0.001

aROC curve: Area under the receiver operating characteristic curve; Model 0: body mass index (BMI); Model 1: BMI + systolic blood pressure + diastolic blood pressure + triglyceride; Model 2: Model 1+ fasting plasma insulin; Model 3: Model 2+ plasma glucose level at 120 min of the 75-g OGTT; Model 4: Model 3+ plasma insulin level at 120 min of the 75-g OGTT; P values, for testing the differences between 2 aROC curves (calculated using the method developed by Hanley et al).

and OGTT120i were similar to or greater than that of BMI (71.0%, 71.4%, and 82.8%, respectively).

Compared to Model 0, Models 2, 3, and 4 had significantly larger aROC curves and improved predictive accuracy (Table 2 and Fig. 1). Model 4 exhibited the largest aROC curve of the models and the optimal ability to predict insulin resistance. In addition to the observation of aROC curves, the probability of having insulin resistance was also be obtained from logistic regression. The basic equation for all models was the same: ($P = \text{ex}/(1+\text{ex})$). However, each model exhibited unique parameters for calculating probability. For instance, the equation of Model 4 for 293 participants is shown as follows:

$$x = -9.653 + 0.183(\text{BMI}) - 0.005(\text{SBP}) + 0.023(\text{DBP}) + 0.947(\log\text{TG}) + 0.007(\text{FPI}) + 0.159(\text{OGTT120g}) + 0.03(\text{OGTT120i}).$$

If the calculated P value is higher than the cut-off point (provided in the next paragraph), the value indicates a high probability of having insulin resistance. In the meanwhile, the aROC curves of the McAuley's index and HOMA-IR were also calculated and it could be noted that the aROC curves of Model 2, 3 and 4 were greater than those of McAuley's index and HOMA-IR.

Figure 1 shows the ROC curves of the 5 models. For Model 2, a cutoff of 0.30 provided the highest sum of sensitivity (70.1%) and specificity (70.3%). For Model 4, a cutoff of 0.29 provided the highest sum of sensitivity (76.0%) and specificity (77.1%).

Although the factors in the sensitivity test were the same, a reduction in sensitivity occurred compared to the equation

built from the whole study cohort. With this equation of Model 4, the P value was also calculated for the external validation group. Participants were classified as having or lacking insulin resistance according to the P value. These results were then compared to the classification of measured insulin resistance. The comparison yielded 68.0% sensitivity and 85.4% specificity.

Discussion

Several past studies have been conducted to identify non-diabetic patients with insulin resistance. Stern et al developed a tree model using routine clinical measurements in non-diabetic patients.¹⁹ The most accurate tree model for predicting insulin resistance demonstrated an 85.0% aROC curve, 78.7% sensitivity, and 70.6% specificity. In addition, McAuley et al used product-moment correlations to build an equation for predicting insulin sensitivity in patients.²⁰ They found that the variables with the most accurate predicting power were FPI and fasting TG. In the present study, we also built models to predict the occurrence of insulin resistance in non-diabetic patients. However, we added the results of the OGTT to the model rather than using only routine laboratory measurements. We intended to increase the aROC curve to enhance the sensitivity and specificity of the model.

Previous research has established that a high BMI corresponds to high insulin resistance.^{13,25} Our study results showed that BMI was the central component in predicting insulin resistance (Table 2). This is in agreement with Stern et al, who consistently used BMI to identify insulin

resistance in the first 2 layers of their 3 different tree models. However, the average BMI in the Stern et al study was much higher than that of our study. This is a common occurrence when similar studies are conducted between Caucasian and Chinese populations. In the McAuley et al study, only insulin and TG were important predictors. However, the BMI of the participants in the McAuley et al study was higher than that of our study ($27.5 \pm 5.3 \text{ kg/m}^2$ versus $23.3 \pm 3.1 \text{ kg/m}^2$). In addition, the strong correlation between BMI and TG might have also reduced the significance of BMI in their study. Nevertheless, BMI is a crucial factor in Chinese populations.

The association between insulin levels and insulin resistance in normoglycaemic patients has been widely explored, and it has been established that the insulin level is increased in patients with insulin resistance.^{26,27} In agreement with McAuley et al, McLaughlin et al reported that FPI was a valuable predictor of insulin resistance in normoglycemic patients.^{20,23} In the tree models proposed by Stern et al, the aROC curve increased from 85.1% to 90.0% after conducting HOMA-IR, which is a product of FPG and FPI. In the present study, we found that the aROC curve of FPI is similar to that of BMI (71.0% and 71.6%, respectively). After adding FPI to Model 1, the aROC curve of Model 2 significantly increased from 72.9% to 77.1%. This indicates that BMI and FPI contribute to insulin resistance using different pathways.

Postprandial hyperglycemia is one of the earliest glucose dysregulations detected in patients with type 2 diabetes and IGT.^{28,29} This metabolic abnormality initiates before the onset of clinical diabetes because of the loss of first-phase insulin secretion and increased insulin resistance.^{30,31} Therefore, measuring the postprandial plasma glucose and insulin levels can facilitate recognition of early metabolic derangements. Thus, the role of the OGTT is vital because it can provide changes in plasma glucose and insulin levels after glucose challenges. The OGTT is not only a standard method for diagnosing glucose intolerance but also a tool for assessing insulin resistance in population studies.^{21,32} In our study, Model 3 was built by adding OGTT120g to Model 2, forming a larger aROC curve (80.1%) that provided a significantly improved predictive performance ($P = 0.032$). This result indicates that OGTT120g exhibits an independent effect on insulin resistance, which is compatible with other studies on the topic.^{11,33,34}

OGTT120i demonstrated the largest aROC curve (82.8%) of all the individual factors. The inclusion of OGTT120i as one of the factors was the only difference between Model 3 and Model 4. Because of this inclusion, the aROC curve increased to 85.1%. The post-challenge insulin level may have contributed a certain percentage of insulin resistance that cannot be explained by the other factors. Accordingly,

Lakkso et al reported that the 2-hour post-load insulin level is associated with insulin resistance in patients with NGT.¹⁶ Our study extends the finding by Lakkso et al¹⁶ to clinical practice. In conclusion, although the OGTT is not routinely performed in clinical practice because of its inconvenience and high cost, it is considerably helpful in identifying insulin resistance in patients with NGT.

Compared to Model 4, Model 2 (the combination of Mets components and FPI) was simpler and more practical, but exhibited lower sensitivity and specificity. In contrast, Model 4 featured the greatest predictive power but requires OGTT data, which is impractical for use in routine clinical screening. Compared to the most widely used clinical equation, HOMA-IR (Table 2, aROC = 70.2%), Models 2, 3, and 4 all provided better predictive accuracy ($P = 0.006$, $P < 0.001$, and $P < 0.001$, respectively). Finally, since McAuley also built an equation for estimating IR, we also compared his equation with our models. It could be shown that the aROC was 72.8%. From this curve, the best specificity and sensitivity of the arbitrary cut-off point (0.34) were 67.0% and 66.7% respectively, which is less accurate than our proposed models. In conclusion, considering the economic aspects of health screening, Model 2 is more appropriate and practical than Model 4. Moreover, Model 2 also showed greater predictive accuracy than the available equations.

In our study, Model 4 was tested again for sensitivity with three-quarters of the participants. The results of the comparison showed high sensitivity and specificity. However, we are aware of the limitations of the study. First, this is a cross-sectional study. A longitudinal study may yield more conclusive results. Second, we did not consider the effects of exercise or smoking, which are all known to be related to insulin resistance. However, despite these limitations, our results can be used easily and extensively in clinical practice.

Conclusion

In conclusion, we demonstrated that FPI, OGTT120g, and OGTT120i are independently related to insulin resistance and can further improve the aROC curves of predictive models. In practice, insulin resistance can be accurately predicted in NGT patients with a sensitivity of 70.1% and a specificity of 70.3% using Mets components and FPI. In contrast, Model 4 provides the optimal prediction model for insulin resistance, but lacks practicality because OGTT data are required.

Acknowledgments

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Hepatic Adrenal Rest Tumour (HART): A Case Report

Dear Editor,

A 47-year-old Chinese woman was found to have mild transaminitis and a hepatic mass detected on ultrasound (US) at a routine medical check-up. She was subsequently referred to our institution for further investigations. She was asymptomatic and general physical examination was unremarkable. Hepatitis viral markers were negative and serum tumour markers, in particular alpha-fetoprotein (AFP), were normal. Magnetic resonance imaging (MRI), with primovist, revealed a slightly protuberant mass, measuring 3.4 cm in maximum dimension, arising from segment VII of the liver. It was hypointense on T1, isointense on T2 and demonstrated peripheral rim enhancement and minimal internal enhancement on the post-contrast phase. The radiologic features were suggestive of a fat-containing lesion with the differential diagnoses of hepatic adenoma, hepatocellular carcinoma (HCC) and angiomyolipoma. Given the concern for malignancy, a wedge resection was performed. At surgery, a well-defined subcapsular hepatic nodule was identified. There was no involvement of the adjacent organs or peritoneal disease. The right adrenal gland was distinctly demarcated from the liver. The specimen was sent for intraoperative frozen section examination. The surgery was uneventful and the patient was well 3 months postoperatively.

Pathologic Findings

The hepatic wedge resection specimen featured a well-demarcated subcapsular mass with a firm, yellow cut surface. No areas of haemorrhage or necrosis were present. The adjacent liver parenchyma did not appear cirrhotic. Both a hepatocellular adenoma and a well-differentiated HCC were considered on frozen section examination. However, in view of the limited sampling, the diagnosis was deferred to permanent sections.

The specimen was subsequently fixed in 10% formalin and routinely processed for light microscopy. Review of the paraffin embedded permanent sections showed a partially encapsulated nodular lesion, composed of polygonal clear cells, arranged in small clusters which occasionally merged into trabeculae. The cells demonstrated distinct cell membranes and moderate amounts of vacuolated cytoplasm.

The nuclei were uniform, small and round. Mitotic figures were extremely scarce.

The lesional cells showed strong cytoplasmic staining for α -inhibin and Melan-A as well as strong nuclear, cytoplasmic and membranous staining for calretinin. CD56 and synaptophysin showed patchy and weak membranous and cytoplasmic positivity; chromogranin was negative. No immunoreactivity was observed for HepPar-1, glypican-3, epithelial membrane antigen (EMA), CD10, renal cell carcinoma (RCC) marker and HMB-45. The Ki-67 proliferation index was less than 1%. Electron microscopic examination was not performed.

Based on the morphology, immunohistochemical profile and corroborative intraoperative and radiologic findings, the diagnosis of a hepatic adrenal rest tumour (HART) was rendered. In view of the tumour's relative small size, lack of cytological atypia, low proliferative activity and absence of invasion, it was postulated to behave in a benign fashion.

Discussion

Adrenal rest tumours are rare tumours derived from heterotopic adrenal cortical tissue. As the adrenocortical primordium develops in close association with the emerging gonad, some nests of adrenocortical cells may eventually be anchored alongside the gonad, or, when not sequestered by the adrenal capsule, be located in the adjacent retroperitoneal fat.¹ The liver is a rare site for accessory adrenal tissue, as is the pancreas, lung and brain. These aberrant nests may undergo marked hyperplasia in patients with increased adrenocorticotrophic hormone production, for instance in congenital adrenal cortical hyperplasia. Occasionally, these rests may give rise to ectopic adrenal cortical adenomas and even carcinomas.

HARTs are relatively rare compared to their gonadal and retroperitoneal counterparts. Only 6 cases were reported in the literature over the past 30 years (Table 1).²⁻⁷ The patients' ages ranged from 21 to 67 years with no gender predisposition. These tumours are frequently non-functional, presenting as an incidental finding. They are generally less than 5 cm in maximum dimension and demonstrate benign histological features. Our case reflects the aforementioned features. Two cases were described to be associated with

endocrine abnormalities.^{6,7} Both occurred in young female patients with relatively large lesions which demonstrated malignant histological features.

Though HART is typically diagnosed on histological grounds, correlating preoperative radiological findings are still imperative. MRI is the most specific imaging technique and the identification of fat, reflecting the intracytoplasmic lipid accumulation of the tumour cells, is the most suggestive feature (Fig. 1).⁸ However, fat-containing tumours of the liver are many, encompassing both benign and malignant lesions, which range from hepatocellular adenoma and angiomyolipoma to HCC with fatty change and metastases.

Hypervascularity is also characteristic of HART but is likewise non-specific.⁸ Likely due to its rarity, HART was not considered in the preoperative radiological assessment of all 6 reported cases.

The histological diagnosis of HART is still a challenging one owing to the wide range of lesions of similar appearance. Morphologically, they are reminiscent of the adrenal cortex, featuring trabeculae, cords and nests of large, polygonal cells with well-defined cell borders and containing clear cytoplasmic vacuoles (Fig. 2). They also exhibit the same immunohistochemical profile staining positively with low molecular weight cytokeratins, α -inhibin, calretinin

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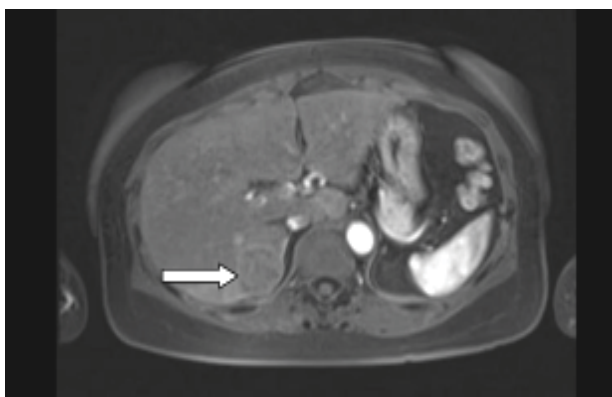


Fig. 1. An MRI image showing the lesion (white arrow) in segment VII of the liver with a distinct peripheral rim and internal enhancement (post-contrast phase, sagittal section).

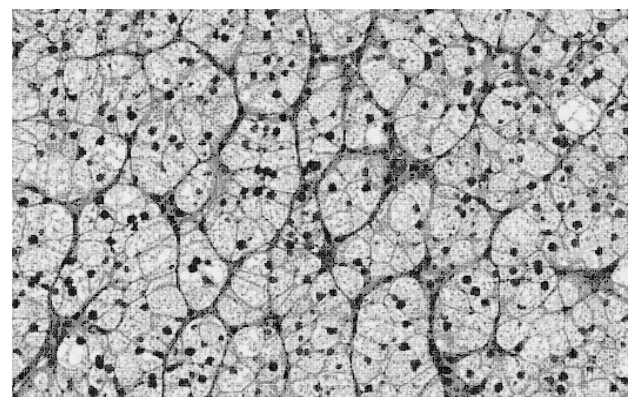


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The principle histological differential diagnoses are HCC, metastatic RCC and metastatic adrenocortical carcinoma. HCC can morphologically mimic a HART, especially when the hepatocytes accumulate cytoplasmic glycogen and/or fat, giving them a clear appearance. Histological features more in keeping with a HCC include Mallory-Denk bodies, bile production, cirrhosis in the adjacent liver parenchyma and a canalicular staining pattern demonstrated by CD10 and pCEA. HepPar1 positivity will further affirm the diagnosis. Among metastatic neoplasms with clear cells, RCC is the most likely to be confused with a HART. The presence of a network of small thin-walled blood vessels is a diagnostically helpful characteristic of this tumour. The lesional cells will show RCC antigen, CD10 and EMA positivity.

An intrahepatic malignant adrenocortical lesion can represent either malignant transformation of a HART or metastatic adrenocortical carcinoma. Radiological and intraoperative demonstration of the involvement of the adrenal gland is imperative in the determination of the primary site. Microscopic evidence of residual benign adrenocortical parenchyma can indicate an antecedent HART. Immunohistochemical studies are not discriminatory with regards to the site of origin. In our case, no malignant histological features were discerned. The right adrenal gland was clearly segregated from the liver on intraoperative examination, showing only mild fibrosis on its posterior aspect. The left adrenal gland was unremarkable.

Conclusion

In summary, we describe a case of an incidental HART, occurring in a 47-year-old woman, showing the classic histological and immunohistochemical features. The tumour's relative small size, lack of cytological atypia, low proliferative activity and absence of invasion, portends a benign nature. The lesion was completely excised and the patient was well postoperatively. The diagnosis of HART is both radiologically and histologically challenging with its wide range of differential diagnoses. This emphasises the value of clinical, radiological and histological correlation in guiding and ascertaining the correct diagnosis. On histological examination, it can potentially be misinterpreted as other primary or secondary neoplasms with clear cells if the key histological features are not appreciated and the appropriate immunohistochemical studies not performed. Despite its rarity, it is important to be aware of this entity and consider it in the differential diagnosis of clear cell neoplasms in the liver.

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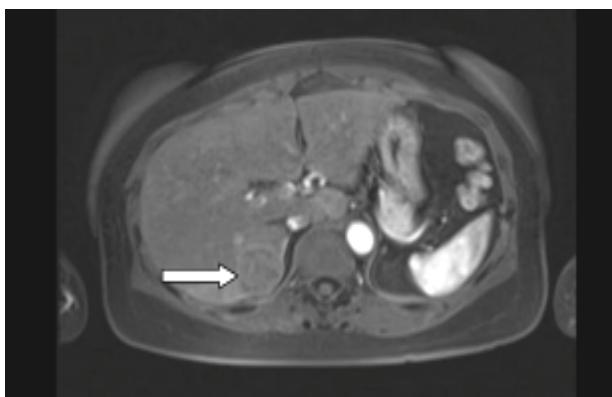


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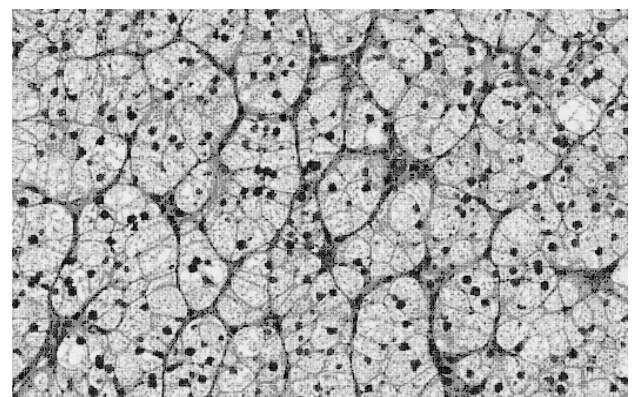


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Erythema Elevatum Diutinum (EED): A Distinctive Vasculitis with Acute-on-Chronic Features

Dear Editor,

A 53-year-old man presented at our institution in June 2012 for a painful rash on his hands and feet. He had been treated prior to hospitalisation with topical and oral antifungals and antibiotics without success. Microbiological stains and cultures done were also negative. His past medical history included hypertension, Type 2 diabetes mellitus and end-stage renal disease.

Physical Examination

There were multiple tender violaceous plaques and nodules on his feet, legs and hands with an extensor predilection (Fig. 1). Pustulation was noted on some lesions. Trunk and face were spared. There was no lymphadenopathy.

Progress

He was investigated thoroughly to exclude an underlying infective process. Repeated full blood counts were normal, C-reactive protein was not elevated and erythrocyte sedimentation rate was 27 mm/hr. Anti-nuclear antibody and human immunodeficiency virus (HIV) serology was negative. Tumour markers such as carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP) and prostate-specific antigen (PSA) were not elevated. A chest radiograph did not reveal any abnormalities. X-rays of the left foot showed chronic osteomyelitis of the second metatarsal (MT) head.

Histopathology

A skin punch biopsy of the left foot nodule was taken. Histopathology revealed dermal infiltrates of histiocytes and neutrophils (Fig. 2). There was epidermal acanthosis and parakeratosis. These features were considered to be in keeping with Sweet syndrome and he was started on oral prednisolone 30 mg per day.

Six weeks later, he reported minimal improvement. An alternative diagnosis of erythema elevatum diutinum (EED) was considered. On histopathological review, prominent endothelial cell swelling was noted and the neutrophilic infiltrates were confirmed to be perivascular rather than diffusely distributed, features less typical of Sweet syndrome and more consistent with EED (Fig. 2).

The patient was started on dapsone in August 2012. However shortly after, he was re-admitted with a severe pneumonia and eventually succumbed to sepsis. A review of the lesions while the patient was in the intensive care unit about 5 weeks after dapsone therapy showed a remarkable clearance of the lesions.

Discussion

EED is a rare, cutaneous form of leukocytoclastic vasculitis, characterised by nodules and plaques that may become indurated and tender over time.¹ Histopathologically, EED typically shows a perivascular



Fig 1. Photo showing the presence of reddish brown plaques and nodules involving the extensor surfaces of the small joints of the patient's hands.

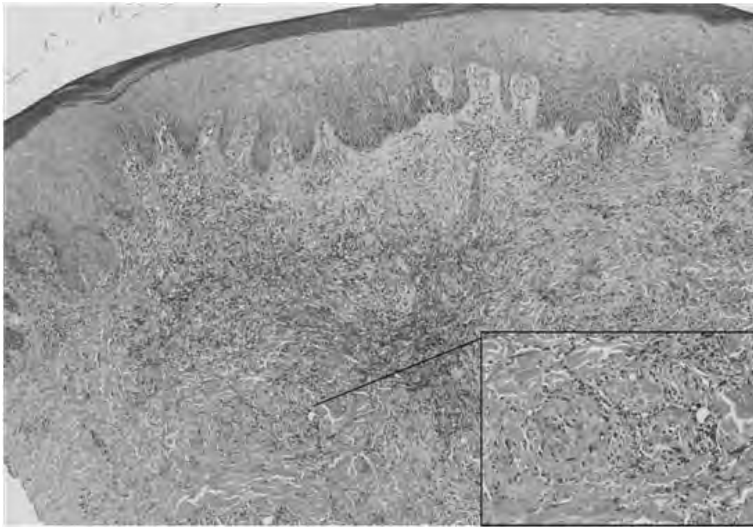


Fig. 2. A photomicrograph of the skin biopsy shows acanthotic epidermis with underlying perivascular and interstitial infiltrates of neutrophils, oedema and fibroplasia. Inset: Higher magnification view of the dermal blood vessels shows endothelial cell swelling with surrounding neutrophilic infiltrate (haematoxylin and eosin staining x40-main, x200-inset).

neutrophilic infiltrate that involves the superficial and mid dermis with fibrin deposition and endothelial swelling. Older lesions may demonstrate perivascular fibrosis and capillary proliferation.² The concomitant presence of the acute features of perivascular neutrophilic infiltrate and endothelial swelling and the more chronic features of dermal fibroplasia and epidermal acanthosis epitomises the histopathology of EED. Differential diagnoses of EED include urticarial vasculitis, neutrophilic dermatoses, Kaposi sarcoma, dermatitis herpetiformis, dermatofibroma, and granuloma annulare.³

Additionally, EED may occur in association with a variety of underlying disease entities such as malignancy, connective tissue and autoimmune disorders,⁴ immunocompromised states, and chronic infection.⁵ It was interesting to note that this patient had a concomitant chronic osteomyelitis of the second MT of his left foot. This patient had features consistent with EED but the diagnosis was unfortunately delayed due to a preoccupation to exclude infection and more common conditions like Sweet syndrome.

Although not usually fatal, EED can cause significant morbidity. Hence we hope to raise awareness of this rare condition so that prompt diagnosis and treatment can be instituted.

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Successful Management of Acute Mesenteric Ischaemia in a Pregnant Woman with Protein S Deficiency

Dear Editor,

Protein S is an endogenous anticoagulant that functions as a cofactor to activated protein C in the cleavage of clotting factors Va and VIIIa. Protein S deficiency, both hereditary and acquired forms, is a rare clinical condition, which usually manifests clinically as venous thromboembolism. Adverse pregnancy outcomes associated with protein S deficiency include recurrent fetal loss, intra-uterine growth retardation (IUGR) and intra-uterine death.¹ Acute mesenteric venous thrombosis is one of the rare manifestations of isolated protein S deficiency. We report a pregnant patient with hereditary protein S deficiency who presented with an acute abdomen, later diagnosed as acute mesenteric ischaemia secondary to acute mesenteric venous thrombosis.

Case Report

A 38-year-old primigravid Chinese woman presented at 7 weeks gestation of a pregnancy conceived via in vitro fertilisation (IVF), with a 2-day history of persistent, non-radiating epigastric pain associated with multiple episodes of vomiting. On examination, she was hypotensive with a blood pressure of 70/40 mmHg that responded to fluid challenge. Physical examination revealed a grossly-distended abdomen with prominent guarding and rebound tenderness in the epigastric and umbilical regions.

Investigations

Ultrasound of the abdomen and pelvis revealed free fluid around the liver and right kidney, a single in-utero viable fetus and moderate amount of free fluid in the pouch of Douglas. Laboratory studies demonstrated a haemoglobin level of 10.6 g/dL and white blood cell count of $35.7 \times 10^9/L$. The Prothrombin time was slightly prolonged—at 16.8 s, and the activated partial thromboplastin time was within normal range.

Treatment

Decision was made for emergency laparotomy.

Intraoperatively, moderate amount of hemoserous intra-peritoneal fluid was noted. A total length of 294 cm of ischaemic small bowel, extending from proximal jejunum to distal was resected, followed by primary anastomosis of the remaining ends, with a resultant viable bowel of approximately 60 cm. A thrombosis induced ischaemic event was suspected and confirmed with a duplex ultrasound performed immediately after the surgery, which revealed complete thrombosis of the superior mesenteric vein. The histological report showed haemorrhagic necrosis of the resected small bowel with moderate ischemic changes.

Postoperatively, the patient underwent a haematological work-up, results of which revealed that she had a significantly low functional protein S activity of 17% (first trimester: 55% to 130%) with mildly decreased levels of antithrombin activity—67% (range, 80% to 130%) and normal protein C activity—73% (range, 70% to 150%). Additionally, there was an absence of lupus anticoagulant, cardiolipin and B2 glycoprotein antibodies. It was thus surmised that protein S deficiency was the most likely cause of the venous thromboembolism in this patient, who was then started on 60 mg twice-daily dose of subcutaneous Clexane, titrated to a targeted anti-factor Xa assay of 0.5 to 1.0 IU/ML (therapeutic range). Total parental nutrition (TPN) via a peripherally inserted central catheter (PICC) was also instituted due to short gut syndrome following extensive small bowel resection.

Outcome and Follow-up

The patient successfully weaned off TPN at 20 weeks gestation to full enteral nutrition. Her pregnancy required luteal support with vaginal progesterone (Crinone) pessary and close antenatal surveillance by haematologists, surgeons and obstetricians. She remained antenatally well with satisfactory weight gain and normal fetal scans, subsequently delivering a baby girl weighing 2615 g. Subcutaneous Clexane was stopped on the morning of the delivery and was restarted on the second postoperative day. Recovery was uncomplicated without any thromboembolic events and subsequent 2-monthly follow-ups were uneventful.

Discussion

During pregnancy, levels of most procoagulant factors rise while that of some natural anticoagulants such as protein S fall to 40% to 60% of normal level² as part of the adaptive mechanism to ensure effective bleeding control during delivery and puerperium. With regard to this, the testing for protein S deficiency during early pregnancy using reference ranges derived from non-pregnant population is inappropriate and requires confirmation at least 3 months post-delivery. Subsequent blood tests done at 1 month and 3 months post-delivery showed persistently subnormal free protein S level of 55% and 60% (ref range, 65% to 140%) respectively, hence confirming an inherent protein S deficiency in the patient.

Obstetric complications in protein S deficiency such as recurrent early fetal loss, non-recurrent fetal loss and IUGR are postulated to be due to excessive thrombosis of the placental vessels, leading to placental infarction and secondary uteroplacental insufficiency. The most common manifestation of protein S deficiency is venous thrombosis of the lower extremities, accounting for up to 90% of all events. Our patient, in this case, presented with an atypical site of thrombosis—the superior mesenteric vein—which has an incidence of less than 5%. Such unusual sites, when observed, characteristically suggest an underlying inherited thrombophilia rather than acquired thrombophilia.³ This correlates to the significantly low free protein S levels in our patient (17%), which is likely hereditary.

The haematological workup for this patient included a screening panel for hereditary thrombophilia, consisting protein C, protein S and antithrombin III as recommended.⁴ Genetic testing for Factor V Leiden and prothrombin gene mutation were not done in view of the extremely low prevalence in local population as well as the lack of any significant family history or previous thrombotic events.

Even though the true cause of low protein S level in this patient had yet to be confirmed, the risk of another episode of catastrophic venous thrombosis and associated risks to the fetus was a strong indication to start her on low molecular weight heparin (Enoxaparin) administration. Furthermore, studies have concluded that starting Enoxaparin at either 40mg/day or 80 mg/day at 5 to 10 weeks of gestation, throughout pregnancy, and for 6 weeks postpartum to pregnant women with serious complications of thrombophilia was safe and effective for improving pregnancy outcome and reducing late pregnancy complications.⁵

Conclusion

In conclusion, maternal thrombophilic conditions such as protein S deficiency pose major risks for adverse maternal

and fetal outcomes. To our knowledge, this is the first locally reported case of successful management of a pregnancy complicated by mesenteric venous thrombosis secondary to protein S deficiency. Hence, the assessment of pregnant woman presenting with atypical venous thrombosis should include screening for underlying thrombophilias followed by appropriate treatment. Maternal treatment and careful fetal surveillance are mandatory in the management of these high-risk pregnancies.

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Vaginal Mass in a 13-year-old Girl

A 13-year-old girl, otherwise healthy, presented with complaints of a painless vaginal mass associated with intermittent abnormal vaginal bleeding and discharge over a few weeks. External examination of genitalia was unremarkable and pubertal development was appropriate for her age. No obvious abnormality was detected on recto-abdominal examination. No transvaginal examination was attempted in view of the patient's age and she was directly referred to a trans-perineal ultrasound scan that showed an ill-defined lobulated heterogeneous vaginal mass. On further evaluation with dynamic contrast magnetic resonance imaging (MRI) of the pelvis, a lobulated polypoid mass was noted protruding from the cervical orifice into the upper two thirds of the vagina. This mass demonstrated heterogeneous high T2 (Fig. 1a) and low T1 (Fig. 1b) signal intensity with mild central enhancement on delayed post contrast sequences (Fig. 1c). The rest of the pelvic organs were unremarkable.

Later, the patient underwent vaginal examination under anaesthesia followed by colposcopy and hysteroscopy that showed a fleshy polypoidal mass protruding from the endocervix into the vagina, which was subsequently resected. The vagina and uterine cavity were found to be unremarkable.

What is the diagnosis of the mass?

- A. Benign endocervical polyp
- B. Mullerian adenosarcoma
- C. Embryonal rhabdomyosarcoma
- D. Endometrial stromal sarcoma
- E. Haemangioma/venous malformation

The gross specimen appeared fleshy and gelatinous in consistency with dark haemorrhagic/ necrotic periphery and grey-white to tan central area, measuring 4.2 cm x 3.4 cm x 2.1 cm in size (Fig. 2). The final pathology revealed a Mullerian adenosarcoma (Figs. 3a and 3b) of endocervical origin.

Further investigations, including abdominal ultrasound as well as computed tomography (CT) thorax and abdomen were negative for nodal and distant metastasis. Clinically, there was no evidence of residual disease at the time of discharge. However, the patient was kept on close follow-up in view of high potential for recurrence of the primary lesion.

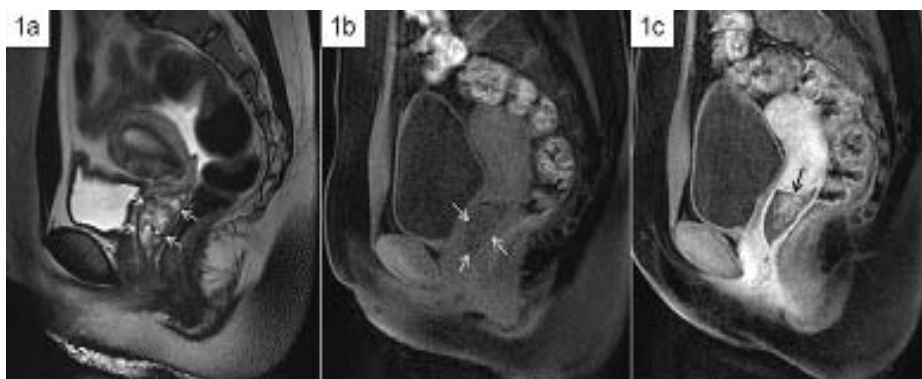


Fig. 1. MRI pelvis sagittal T2-weighted image (A) demonstrates a heterogeneously hyperintense lobulated mass in the upper two-thirds of the vagina (arrows) that is slightly low signal on T1-weighted images (B). Post contrast sagittal T1 fat saturation image (C) demonstrates delayed central enhancement of the lesion (arrow) close to the uterine cervix.

Answer: B

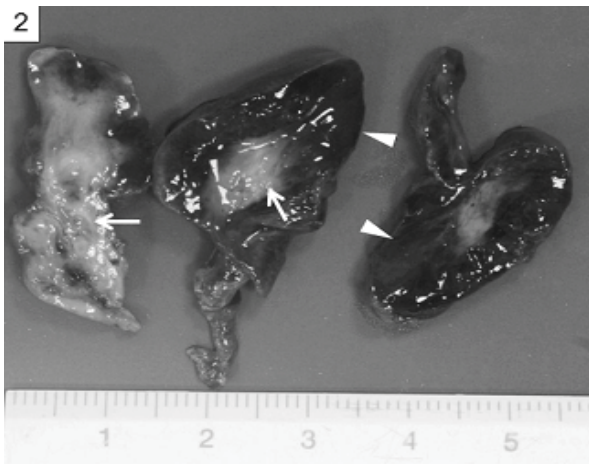


Fig. 2. Gross postsurgical specimen shows cut surfaces of the polypoid lesion demonstrating outer zone of haemorrhagic necrosis (arrowheads) and inner pale tan tissue (arrows).

Discussion

Adenosarcoma is an intermediate form of mixed Mullerian tumour with the benign adenofibroma at one end and aggressive carcinosarcoma at the other end of the spectrum.¹ Mullerian adenosarcoma is a biphasic tumour comprising benign glandular epithelium and low-grade malignant stromal component, usually involving the uterine corpus with rare occurrence in the uterine cervix, as in our case. Even rarer is occurrence in the extrauterine sites, such as in the vagina, ovary, fallopian tube and peritoneal surface. Cervical origin accounts for about 2% to 9% of all Mullerian adenosarcoma locations; and the younger age of presentation is more common with cervical lesions than other locations.

So far, only a few cases of cervical adenosarcomas have been reported in adolescents and paediatric patients in the literature, with the youngest patient reported being a 10-year-old girl.

Pelvic irradiation, hyperestrogenism, long-term oral contraceptive use and treatment with Tamoxifen have been found to be associated with Mullerian adenosarcoma of the uterus in elderly patients. However, these may be coincidental and no definite causal relationship or proven aetiological factors have been established. Unlike in cervical adenocarcinoma, human papilloma virus has not been implicated in the aetiology and prognosis of these mixed Mullerian tumours.

The patient may present with an abnormal vaginal discharge and/or bleeding with or without an introital mass. On MRI, typical low-grade adenosarcoma appears as a solitary polypoid mass containing a few tiny scattered cysts and rarely haemorrhagic foci. The solid component of the lesion is heterogeneously hyperintense on T2-weighted images and iso/hypointense on T1-weighted images relative to the myometrium.² The lesion is usually less vascular as demonstrated by the colour Doppler ultrasound and may show delayed heterogeneous enhancement on dynamic contrast MRI scan. Most of these MRI features were well demonstrated in our case.

Histologically, this mixed tumour is composed of benign glands distributed in a malignant stroma. The glands may be cystically dilated or show cleft like spaces, and are lined by bland epithelial cells usually of endometroid type. The stroma is cellular in general and characteristically shows cellular condensation around glands and beneath epithelium typical of Mullerian adenosarcoma. In most

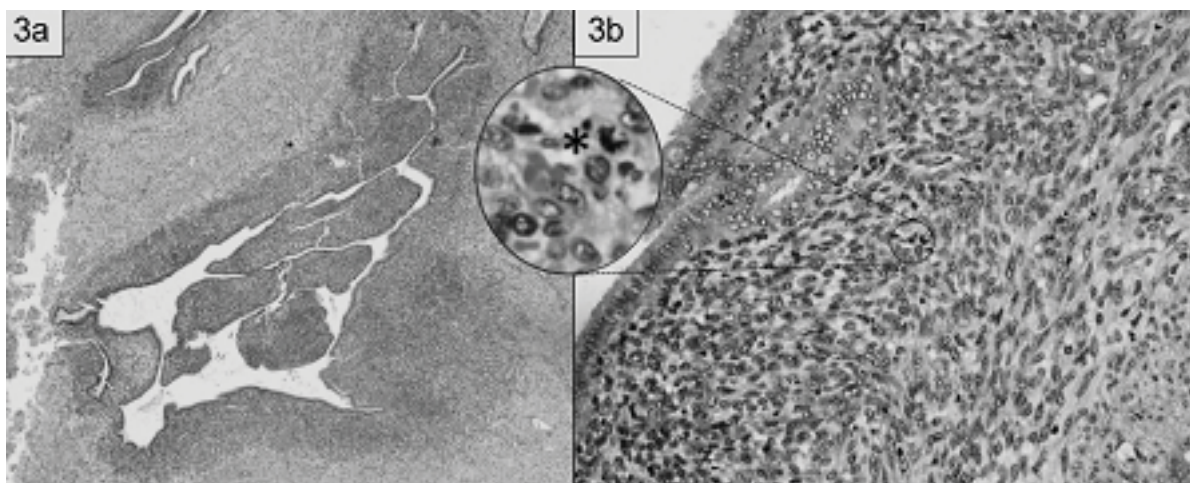


Fig. 3. Low power view of histopathology slides. (A) displays a dense periglandular condensation of stromal cells with polypoidal projections into the gland. (B) shows a high power view of mild nuclear atypia of the stromal cells and a mitotic figure (asterisk in the magnified window).

tumours, the stromal cells show mild to moderate nuclear atypia. Mitotic figures are readily identified in most cases, particularly in the hypercellular stromal cuffs around the glands. The stromal component of this neoplasm may be homologous, i.e. containing indigenous uterine elements or heterologous with differentiation into extrauterine stromal tissues, such as cartilage, osteoid and striated muscle.^{3,4} Heterologous elements are reported to be present in 20% to 25% of cases. A more aggressive variant of this entity is Mullerian adenosarcoma with sarcomatous overgrowth (MASO), which is associated with high postoperative recurrence and metastases even when diagnosed and treated at an early stage. Sarcomatous overgrowth occurs in approximately 10% of cases and is defined as pure high-grade sarcomatous component constituting 25% or more of the tumour showing increased cellularity, mitotic activity and nuclear atypia compared to the appearance of the background adenosarcoma.⁵

Differential diagnosis includes benign endometrial/endocervical polyps, adenofibroma, embryonal rhabdomyosarcoma and endometrial stromal sarcoma. In benign polyps and adenofibroma, both the epithelial and stromal components are morphologically benign, showing no stromal hypercellularity, periglandular cuffing, mitoses or nuclear atypia. In young patients, an embryonal rhabdomyosarcoma is an important consideration showing significant morphological overlap with adenosarcoma. In rhabdomyosarcomas, glands are not distributed throughout the tumour and sarcoma cells are more primitive and mitotically active than stromal cells of adenosarcoma. Immunohistochemical markers, such as myogenin and MyoD1, would show more diffuse staining in rhabdomyosarcoma. Whereas, the absence of periglandular cuffing, phyllodes-like architecture and even distribution of glands in the tumour differentiates the endometrial stromal sarcoma from a typical Mullerian adenosarcoma.

Although there is no current consensus with regard to the optimal therapy for cervical adenosarcomas, the aggressive lesions may warrant total hysterectomy and bilateral salpingo-oophorectomy in addition to adjuvant chemotherapy and radiation. Whereas the low-grade tumours, particularly in paediatric and younger patients, may be treated with conservative surgical methods such as wedge resection, cone biopsy or trachelectomy, to preserve the patient's fertility. However, review of the literature shows a high recurrence rate, often late, following conservative surgical management. High-grade malignant features in the stromal component, depth of invasion and sarcomatous overgrowth are some of the predictors for tumour recurrence and poor prognosis.^{4,5} In view of the known potential for delayed recurrence with conservative management, long-term close follow-up of these patients is warranted.

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Vesicles and Erosions in a Patient with Chronic Eczema: Is It Just Eczema?

An 80-year-old man was diagnosed with erythroderma secondary to seborrhoeic eczema 5 years before. His condition had been controlled with emollients, moderate potency topical steroids and antihistamines. He developed painful rashes over the genitals, suprapubic area and upper thighs of 1 week duration. There was associated low grade fever. The rashes were different from the appearance of his chronic eczema. On examination, there was a large area

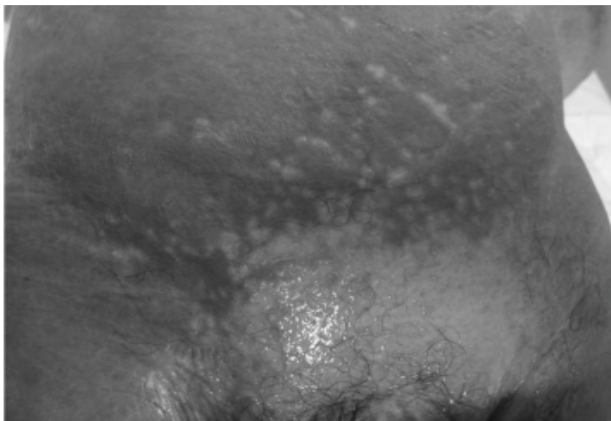


Fig. 1. Multiple punched out erosions surrounding a large denuded patch at the suprapubic area.

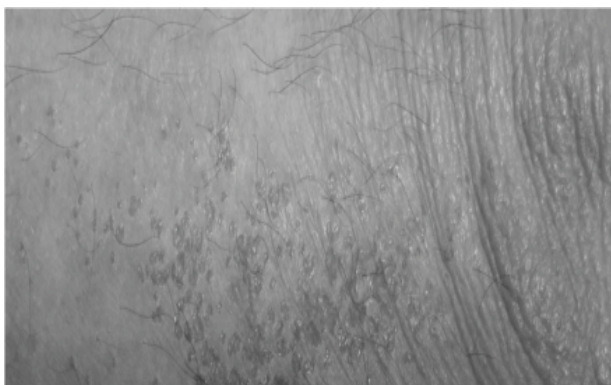


Fig. 2. Confluent monomorphic vesicles and erosions with erythema on the left thigh.

of erosion at the suprapubic area surrounded by satellite erosions (Fig. 1). Multiple smaller erosions were seen on the penile shaft, the glans and prepuce were spared. On the thighs, there were monomorphic grouped vesicles and erosions with erythema (Fig. 2). There were no oral ulcers.

What is the diagnosis for these cutaneous lesions?

- A. Acute eczema
- B. Candidiasis
- C. Eczema herpeticum
- D. Impetigo
- E. Herpes zoster

Discussion

The diagnosis of cutaneous herpes simplex infection including eczema herpeticum (EH) is primarily clinical. In this patient, Tzanck smear showed multinucleated giant cells. Polymerase chain reaction (PCR), viral culture and immunofluorescence were not performed. He was treated with oral acyclovir and cefuroxime, and all his lesions cleared within a week. EH is a recognised infective complication of eczema due to herpes simplex virus type 1 or type 2. Impetiginised eczema due to *Staphylococcus aureus* or *Streptococcus pyogenes* is a common bacterial super infection. EH occurs mainly in patients with severe atopic eczema,¹ it has also been described in patients with other chronic skin diseases. Patients with EH present with a rapid onset of vesicles in clusters. The vesicles break easily leaving small painful punched out erosions. Pustules may be observed. Frequently, there is associated fever, malaise and lymphadenopathy. Tzanck smear is a quick bedside test to support the clinical diagnosis of EH. Confirmatory investigations include immunofluorescence test of scraping from the vesicle base, PCR of the vesicle fluid and identification of the virus in the vesicle fluid by electron microscopy.¹ The management of EH includes systemic acyclovir 400 mg, administered 5 times per day for 7 days.² Cloxacillin or cephalexin with *S. aureus* coverage may be prescribed for concomitant bacterial superinfection. Continuous wet dressing with potassium permanganate is useful to dry up the lesions. Emollients like white soft

Answer: C

paraffin will facilitate the healing of dry crusted erosions. EH should be recognised and treated early to prevent morbidities due to viremia and multiorgan involvement.³

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