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CONTACT

Post:
Editor
UQU Medical Journal
Faculty of Medicine
Umm al Qura University
PO Box: 7607
MAKKAH
Kingdom of Saudi Arabia

Website:
www.uqumedicalju.com

E-mail:
mj@uqu.edu.sa

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UQU Medical Journal

Original Article

Rate of wound infection in surgical ward, King Faisal Hospital, Makkah, during hajj period of 2011 (1432 Hijri)

Professor Elbagir Ali A. Elfaki,. *Department of Surgery, Umm-Alqura faculty of Medicine,Consultant General surgeon, King Faisal Hospital,Makkah.KSA.*Dr. Mohummed Suliman Alrayes *Surgical resident, Department of surgery, King Faisal hospital.* Dr.Ahmed Salman Faisal MD. *Umm-Alqura,University Medical centre, Makkah,KSA.*

Correspondence :
Professor ; Elbagir Ali A. Elfaki :
Mobile: 0558803253 e.mail: albagirali@hotmail.com

معدل التهاب جروح ما بعد العمليات الجراحية في عنابر الجراحة- بمستشفى الملك فيصل- مكة المكرمة في موسم حج 1432

ا. د. الباقرعلي احمد الفكي استاذ , بقسم الجراحة كلية الطب – استشاري الجراحة- مستشفي الملك فيصل بمكة- د. محمد سليمان الريس- مقين – قسم الجراحة مستشفي الملك فيصل بمكة –د. احمد سلمان فيصل المركز الطبي - جامعة أم القري – مكة المكرمة- المملكة العربية السعودية .

الملخص العربي

.الاهداف: تهدث هذه الدراسة لتحديد معدل التهاب الجروح بعد العمليات الجراحية بمستشفى الملك فيصل بمكة المكرمة في موسم حج 1432 هجري (2011) ميلادي.

الطريقة: شملت الدراسة 131 مريض تم ادخالهم بقسم الجراحة بمستشفى الملك فيصل بمكة المكرمة في موسم حج 1432 هجري (2011) ميلادي . منهم 102 اجريت لهم نوع من العمليات الجراحية. قد تم تصنيف جروحهم الي: نظيفة ونظيفة ملوثة وملوثة وتم مناظرة علامات التهاب جروحهم بواسطة مقيم الجراحة وممرضة من قسم مكافحة العدوي واشراف ا سشاري الجراحة حسب الجدول المعد مسبقا لهذه الدراسة.قد تم استبعاد 29 مريض منهم لكون جروعهم سيئة الالتهاب ومفتوحة من البداية او تم علاجهم تحفظيا.

النتائج: 102 مريض تم علاجهم جراحيا منهم 8 تم تسجيل علامات التهاب الجروح عندهم بمعدل عام 7.8 % وعندما تم تصنيف جراحاتهم تقليديا وجدنا معدل التهاب الجروح النظيفة 0.98 % (مريض واحد استئصال زائدة) ونظيفة ملوثة 4 مرضى بمدل (3.94%) وملوثة 3 مرضى بمعدل (2.9%). جميع التهابات جروح العمليات سجلت لما بعد جراحة استكشاف البطن الحراحي الحاد. فى هذه الدراسة لم يسجل اى التهاب 0 % لاى مريض مكث 3 ايام او افل بالمستشفى وكل حالات الالتهابات سجلت لمرضى مكثوا ثلاثة ايام وما فوق.

الخاتمة : اكدت هذه الدراسة ان معدل التهابات جروح العمليات ذا علاقة وثيقة بمدة بقاء المرضى بالمستشفى مما تدعم وتشجع سياسات جراحة اليوم الواحد ووضع السياسات المستقبلية لترشيد التنويم ومكوث المرضى بعنابر الجراحة.

ABSTRACT

Objectives: The objective of this study to determine the rate of postoperative wound infection in surgical wards at King Faisal hospital (KFH), Makkah, during Hajj period of 1432Hij (2011).

Materials and Methods: From the total of 131 patients admitted to surgical wards at (KFH) during Hajj period of 1431Hij (2010), were studied for wound infection, 102 patients underwent surgical procedure (emergency or elective) and classified as clean, clean contaminated and contaminated. All post operative wounds were scrutinized for signs of infection included in this study by the team of surgical resident, trained infection control nurse and surgeon in charge. 29 patients were excluded either treated conservatively or badly infected open wounds at the time of presentation).

Results: Among the 102 operated patients, there were 8 post-operative wounds that became infected, yielding overall rate of 7.8%. When categorized operation by traditional wound classification, infection occurred in one patients (0.98%) of clean wound and 4 patients (3.94%) of clean contaminated and contaminated wound 3 patients (2.9%). One infection was seen after appendectomy and seven were reported in laparotomies for acute abdomen. In this study no wound infection was reported in any patient whom their stay in hospital less the 3 days revealed rate of wound infections (0%) while all 8 cases reported infected wound their stay more than 3 days and was over fifty years of age.

Conclusion: The incidence of infection was significantly related to period of hospital stay. However, the most obvious use of the present results is in long-term planning. This result may help decision-makers to enhance and support the policy of one day surgery and estimate the need for resources for these different patient groups in the future.

Keywords: *wound infection, Hajj, postoperative, rate.*

INTRODUCTION

Postoperative wound infection have been recognized to be the serious problem throughout the entire history of surgery. It causes significant postoperative morbidity and mortality in surgical wards. Despite of these evolution and elucidation of new techniques that led to better control of surgical infection, postoperative wound infection still remains the problem of major importance and source of illness and a less frequent cause of death in surgical patients[1].

There is considerable variation among infection reported from different studies varied from a 2.5% to 41% [2-6]. This discrepancy may be due to many factors such as patient and hospital characteristics, surgical subspecialty, type of surgery, prophylactic antibiotics and pre and postoperative hospital stay. Although the total elimination of wound infection is not possible, a reduction in the infection rate to a minimal level could have significant benefits in terms of both patient comfort and medical resources used [7].

OBJECTIVE

The objective of this study to determine the rate of postoperative wound infection in King Faisal hospital Makkah, during Hajj period of 1431Hij (2010).

MATERIAL AND METHODS

This cross sectional., study was conducted during the Hajj season of 1432 H., from 1st , Nov.2011 to 15th., Nov.2011. at King Faisal hospital (KFH),Makkah, KSA. It is one of the four Hospitals in Makkah serving pilgrims. It serves as first line hospital and nearest referral center for ritual site during Hajj season. The patients included in this study were all the patients admitted to general surgical wards for surgery, (elective and emergency) during the Hajj period.

One surgical resident and one certified infection control nurse visited daily each postoperative surgical patient and collected all the pertinent data. Surgical wound was inspected at the 2nd. post operative day and daily thereafter till discharge. Wound infection was diagnosed if any one of the criteria were fulfilled for diagnosing infection listed in Table 1. The charts of all discharged patients were reviewed to assure that no relevant data missing. The classical method, that classify surgical wound into one of the four categories according to degree of contamination has been generally used (clean, clean contaminated, contaminated and dirty wounds)[8]. Postoperative wound infection included incision surgical wound infection and deep surgical wound infection. No attempt was made to follow up the patients after discharge unless the patient was readmitted. Contaminated dirty wounds and severely infected diabetic septic cases were excluded from this study.

RESULTS

A total of 131 patients admitted to surgical wards during the 15 days period from 1st. Nov. 2011 to 15th., Nov.2011, through Haj period of 1432 H. were included and studied for postoperative rate of wound infection. Characteristics of enrolled patients are summarized in Table 2.

Out of 131 patient, 102(77.9), patients underwent surgical procedure and classified as clean ,clean contaminated and contaminated were included in this study. 29(22.1%) patients of the total were excluded right from the start (not operated or treated conservatively). Out of 29 patients excluded patients, 18 (62.1%) open infected and dirty wounds either badly infected diabetic wounds infection or unclassified wound infection (ano-rectal conditions), and 11 (37.9%) were treated conservatively Table 4.

Among all operated patients, there were 8 post-operative wounds that became infected, yielding an overall rate of 7.8%. When categorized operation by traditional wound classification infection applied. One patient (0.98%) reported of clean wound and 4 patients (3.9%) of clean-contaminated wound and three patients of contaminated wound (2.94%). Different surgeries performed no infection was seen after thyroidectomies, Laparoscopic cholecystectomies, hernia repairs, breast and testicular and scrotal surgery Table 3. In this study, predominance of wound infection is consistent with the prolonged hospital stay. Out of the 8 cases reported wound infection, none of the patients who were operated within the first three days of admission to the ward had wound infection identified (0%). While all 8 patients reported wound infection (100%) from the group whom stayed more than 3 days Table 2. The incidence of surgical site infection increased with increase in the duration of hospital stay.

When the 102 operations were classified by degree of contamination,52 (50.98%) patients classified clean wound , among them only one patient reported wound infection (12.5%), clean contaminated patients were 36 (35.29%) reported 4 wound infection with rate of (50%) and contaminated wound 14 (13.72%) reported three infected wound with rate of (37.5%).Table 4.

Table 1: Elements of wound infection

No	Clinical findings	Description	Rating and Record				
			1	2	3	4	5
1	Pain / Tenderness	Increased the level of pain, since operation					
2	Erythema	Bright red/ dark red skin immediately adjacent to the wound					
3	Edema	Shiny taut skin or pitting					
4	Heat	Detectable increase in temperature near by or up to 10 cm distance					
5	Serous/ sanguineous exudates	Thin watery or bloody fluids that present on a dry gauze dressing after 1 hr from cleaning					
6	Purulent exudates	Tan ,creamy, yellow or green thick fluids					
7	Discoloration	Pale , dusky or any obvious change in color.					
8	Foul odor	A putrid or distinctively unpleasant smell					
9	Wound breakdown	Small open area in a newly formed epithelial tissue					

Table 2. Characteristics of total admission of (131 patients)

Sex			
	Male 67 (51.1%)		
	Female 64 (48.9%)		
	Total 131 (100%)		
Age Groups	**Yrs Gps , *Total No. of Pts.&%***	***Number &% of operated Pts.***	***No. of infected Wound (%)***
	< 15 4 (3.8%)	1 (0.98%)	None
	16 - 25 16(12.2%)	8 (7.8%)	None
	26 - 35 14(10.7%)	21 (20.6%)	None
	36 - 45 20(15.3%)	14 (13.7%)	None
	46 - 55 28(21.4%)	20 (19.6%)	1(12.5%)
	56 - 65 32(24.4%)	26 (25.5%)	6 (75%)
	65 - 75 12(9.2%)	10 (9.8 %)	None
	76 - 85 5(3.8%)	2 (1.96 %)	1 (12.5%)
	Total 131 (100%)	**102 (100%)**	**8 (100%)**
Operated on	102 (55 Male & 47 Female)		
Not operated	29 (12 Male & 17 Female)		
Hospital stay			
< 3 days	87 (47 Male & 40 Female)	67 (69 %)	None (0 %)
> 3 days	44 (20 Male & 24 Female)	35 (72.7%)	8 (100%)

Table 3. types of surgery performed

Operations	Number of patients &%	No of infection & %
Appendicectomy	27 (26.5)	1 (1.1%)
Laparotomy (acute Abdomen)	19 (18.6)	7 (6.7%)
Perforated duodenal ulcer	8 (7.8)	-----
Hernial repair	10 (9.8)	-----
Laparoscopic cholecystectomy	7 (6.9)	-----
Chest tube insertion/lacerations	5 (4.9)	-----
Breast surgery	3 (2.9)	-----
Testicular and scrotal surgery	2 (1.96)	-----

Total	**102 (100%)**	**8 (7.8%)**

Table 4. Infection among total cases based on wound categories

Nature of admission	NO (%)	No of infected cases & (%)
Clean wound	52 (50.98%)	1 (12.5%)
Clean contaminated	36 (35.29%)	4 (50 %)
Contaminated	14 (13.72 %)	3 (37.5%)
Total	**102(100)**	**8 (100%)**
Dirty infected from the start	18 (71.8%)	Excluded
Non operated cases	11 (28.2%)	Exclude
Total	**29 (100%)**	

DISCUSSION

Rate of wound infection reports by many workers revealed considerable variation, ranging from 2.5 to 41.9 % [9-11]. This discrepancy may be due to many factors such as patient and hospital characteristics, surgical subspecialty and type of operation.

In the Hajj season, where this study performed we always operate upon the emergency, highly susceptible and most of them of unknown past history. So the infection rate suspected to be higher than in the previous hospital records, Despite of that reasons, the infection rate obtained from this study rather low. In this study, we found that the postoperative wound infection rate in patients underwent surgery is 8 patients (7.84%) which is acceptable when compares with other reported rates.

This accounts for 8 patients out of 102 patients treated surgically during the above mentioned period which compares favorably with other reported rates. In this study none of the patients whom their stay in hospital less the 3 days revealed wound infections while all 8 patients reported infected wounds their stay more than 3 days. This can be achieved by taking proper measures to improve and support the day case surgery.[12]

It has been observed that wound infection rate is influenced by duration of operation [13,14,15,16]. The findings of the present study are in agreement with the reported literature. With increase in duration of surgery, the rate of infection increased in direct proportion. Surgical site infection delays the recovery of the patient by about one week and in some cases significantly prolong the duration of hospital stay [17].

This finding is clear evidence that prolonged hospital pre/post-operative stay with exposure to hospital environment and its ubiquitous diagnostic procedures, therapies and micro flora have been shown to increase the rate of surgical site infection[17,18]. Study done at Fauji Foundation Hospital, Rawalpindi most cases of wound infection were noticed by the 6$^{th.,}$ postoperative day[19,20]. Prolonged hospital stay which is a major concern of most of the hospitals, has been evident in patients developing wound infection.

In our study correlation was seen between duration of hospital stay and the development of wound infection. Significant decrease in rate of surgical site infection was found in patients who had less than three days stay in hospital (0%) ,compared with 8 patients (7.8%) who had more than three days stay, which is match with other studies and findings.[21,22]

CONCLUSIONS

The incidence of infection was significantly related to period of hospital stay. In conclusion, modeling of hospital costs and prediction of length of stay is possible on the basis of preoperative risk scores. However, the most obvious use of the present results is in long-term planning. Risk stratification may help decision-makers to estimate the need for resources for these different patient groups in the future.

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Original Article

A novel tyrosine 516 stop mutation in the α subunit of the epithelial sodium channel causing Pseudohypoaldosteronism in the affected offspring and A subclinical, salt-losing phenotype in a heterozygous mother

Dr. Ahmad Zahrani , Pediatric Department , Alnoor Hospital,Makkah,KSA.

Correspondence:
Ahmad Zahrani,
Email: zz11ww@yahoo.com

طفرة غير مألوفة في وحيدة الفا من قنوات الصوديوم الطلائية مسببة لمرض الالدوستيرون الكاذب عند ام

د احمد الزهراني –استشاري الاطفال –مستشفي النور التخصصي- مكة المكرمة

الملخص العربي: :

المقدمة والخلفية : يعتبر النوع الأول من نقص الالدوستيرون الكاذب مرض نادر الحدوث يتميز بفقد شديد ومبكر للاملاح وارتفاع في بوتاسيوم الدم وحموضة دموية و مقاومة لعلاج المنيرالوكورتيكويد . يسبب هذا المرض تحول جيني متماثل او متنافر اللواقح في اي من الوحيدات الثلاث بقنوات الصوديوم الغشائية.

الحالات والمناقشة: هذه الدراسة عبارة عن تقرير لعائلة لديهم اربعة اطفال متأثرين بهذا المرض تم وصف حالتهم السريرية والكيميائية الحيوية والتحليل الجيني الذي اثبت وجود تحول جيني لم يتم كشفه من قبل في وحيدة الفا من قنوات الصوديوم الطلائية وذلك عند الحمض النووي 516 مستبدلا ترتيب ادت الى تحويل البروتين من 721 حمض نووي الى TAG بالاحماض النووية TAC الاحماض النووية . 516 حمض نووي مما يؤدي الى فقد وظيفة قنوات الصوديوم الطلائية وظهور المرض
ومع ان الام لاتعاني من المرض الا ان تحاليل الدم اثبتت ارتفاع الالدوستيرون وارتفاع نسبة الصوديوم في البول ربما فقد بسيط في الاملاح عند الام في طفولتها المبكرة لم يتم ملاحظته طبيا ، الاب حامل للمرض ولم يكن يعاني من أي اعراض وتحاليله كانت سليمة.

الخلاصة: طبقا لمعلوماتنا هذه الحاله الرابعة والعشرون المسجلة عالميا و الخامسة عربيا لمثل هذه الحالات مصابة بخلل في هذا الجين وتمثل تحولاجينيا حديثا ومكتشفا في هذه النقطة الجينية، واول تقرير عن ارتفاع الالدوستيرون وارتفاع الصوديوم اليومي في شخص ناقل للمرض.

الكلمات الدالة: الالدوستيرون الكاذب - المنيرالوكورتيكويد- التحول الجيني – اللواقح الحمض النووي

ABSTRACT

Rationale and Background:: Pseudohypoaldosteronism type I is a rare life-threatening condition characterized by Severe, early-onset salt wasting, hyperkalemia, metabolic acidosis and resistance to Mineralocorticoids. The condition is inherited in either an autosomal recessive or dominant manner. Autosomal recessive Pseudohypoaldosteronism type I is a multisystem, severe, life-long condition, caused by homozygous or compound heterozygous mutations in any of the three subunits, (α, β, or γ), of the Epithelial sodium channel.

The cases and discussion:

I reported a family with four affected siblings with autosomal recessive or multi-system Pseudohypoaldosteronism type I.,their clinical picture and biochemical abnormalities described. Genetic mutation analysis revealed a novel nonsense mutation in the alpha subunit of ENaC, at amino acid 516, substituting a **TAC [Y]** for a **TAG [STOP].** This truncated the protein, shortening it from 721 amino acids to 516 amino acids, leading to loss of channel activity and resulting in disease. Although asymptomatic, analysis of the laboratory values of the heterozygous mother revealed high serum aldosterone with high urinary sodium but normal clinical and biochemical analysis in the heterozygous father. However, a mild salt loss might have been missed in the infantile period.

Conclusion:This is the first report of a high serum aldosterone level with high urinary Na in a heterozygous carrier, and represented a novel mutation in this gene.

***Keywords*: Pseudohypoaldosteronism- mutation-DNA**

INTRODUCTION

Type I pseudohypoaldosteronism (PHA1) is a rare salt-wasting condition manifested soon after birth by vomiting, lethargy, severe dehydration, hyponatremia, hyperkalemia, metabolic acidosis with high plasma aldosterone and renin concentrations. [1,2,]

Two types of PHA1 have been described; the renal or autosomal dominant type due to aldosterone receptor defects, and the multisystem or autosomal recessive type, a severe, life long condition, caused by homozygous or compound heterozygous mutations in the subunits (α, β, or γ) of the Epithelial sodium channel (ENaC).[1,2,3]

The amiloride-sensitive epithelial sodium channel (ENaC) is a highly selective Na channel found at the apical membrane of salt-reabsorbing tight epithelia of tissues including the distal nephron, the distal colon, the salivary and sweat gland, and the lungs.[2] In these polarized epithelia, the ENaC mediated entry of sodium into the cell represents the rate-limiting step for vectorial movement of sodium from the mucosal to the serosal side. In the kidney, ENaC activity is controlled by aldosterone, serving to maintain salt homeostasis and blood pressure.[2, 3] and shares 35% identity in their amino acid sequences. [4,5] Each subunit has two transmembrane domains with short cytoplasmic N- and C- termini, and a large extra cellular

loop.[4,5,6,7] The human ENaC genes have been cloned, and several genetic mutations in the coding regions of the alpha, beta and gamma subunits of ENaC have been described recently.[8,9,10,14,16,18]

Herein, we describe the clinical and biochemical presentations, and a novel mutation, in the alpha subunit of ENaC, in a consanguineous family with four affected children.

Cases and Report

The subjects are four siblings who are the products of first-degree consanguineous Yamane parents. The mother, 29 years old and the father, 31 years old, are both asymptomatic and healthy.

Laboratory values showed serum aldosterone was normal in the father, 13ng/dl. All four siblings had the classic symptoms of PHAI with no respiratory manifestations. Three of the children died of complications from the disease. The third child is living at 3 years old. Table 1.

Case I

The first baby, a male, was brought to a hospital on the third day of life with vomiting and refusal to feed. He was admitted with severe dehydration, hyponatremia and hyperkalemia, and died two days later. No detailed information is available.

Case II

The second baby, a girl, born on 20/11/05, was brought to our hospital at 6 days old with vomiting, irritability and refusal to feed. She was found to be dehydrated with severe hyponatremia, hyperkalemia, and metabolic acidosis. System examination upon admission was unremarkable, with normal female external genitalia and no respiratory symptoms. Her serum aldosterone was elevated at 4200 ng/dl, (N <35.5ng/dl), PRA was 453 pg/ml/h, (N < 33 ng/ml/h), and was resistant to high dose fludrocortisone, (0.3mg /d). The patient was treated for PHAI with a large amount of sodium chloride, IV and PO, and Kayexalate. However, she developed severe hyperkalemic arrhythmia and died on 27/1/06 at 2 months old.

Case III

The third baby, a girl, was born on 23-6-2007 with a birth weight of 3 kg. She presented to the ER on the fourth day of life with vomiting, poor feeding, irritability and severe dehydration. Her blood pressure was 70/40; weight 2.7 kg with a normal system examination and normal female external genitalia.

Laboratory values showed persistent hyponatremia, severe hyperkalemia and metabolic acidosis, unresponsive to high dose fludrocortisone, 0.3mg /d(three tablets daily), and requiring large amounts of NaCl. Her serum aldosterone was elevated at 781 ng/dl N <35.5ng/dl. PRA of 381 pg/ml/h, (N<33 ng/ml/h), was also high, with normal serum cortisol at 25 ug/dl and 17-OH progesterone at 1.4 ug/ml. The patient was hospitalized for one year in our hospital due to frequent severe electrolytes disturbances needs frequent resuscitations. She had no respiratory symptoms during admission. She was discharged on 3% sodium chloride,

40cc PO every 4 hours, (about 7 gm/day), and Kayexalate, 7gm q 6 hourly. On follow up, she is thriving well, on these doses, with normal electrolytes.

Case IV

The fourth child, a baby boy, was born on 6-06-2009 with a birth weight of 3.5 kg. He was kept in our hospital as we were anticipating the condition. In the first few days, he was showing a picture of hyponatremia, hyperkalemia, metabolic acidosis, and resistance to high dose fludrocortisone 0.3mg /d. He was started on a 3% sodium chloride solution and Kayexalate rectal enema. His aldosterone was elevated at 137 ng/dl and PRA was high at 312 pg/ml/h. He had a stormy course in the hospital with frequent and severe episodes of hyperkalemia and hyponatremia, but no respiratory distress or chest infections. The last episode was at four months old when he developed severe hyperkalemia with serum potassium of 12 mmol/L. Cardiac arrhythmia and resistance to intensive measures complicated this episode. He arrested and died on 28-10-09 at almost four months old.

Mother

She was 29 years old healthy, asymptomatic lady, no history of neonatal admission, chest infection or significant illness, no history of polyurea or polydypsia or any other symptoms.
Her urinary sodium was elevated 185mmol/L (N <40 mmol/L) and her serum aldosterone of 93.5 ng/dl (Normal <35.5ng/dl).

Father

He was 31 years old with no clinical or biochemical abnormality including serum Na of 141 nmol/L ,K 4.1, serum aldosterone 13ng/dl , urinary Na 27 nmol/L.
No other family history of similar condition or early deaths

Genetic Mutation analysis

Blood samples for molecular genetic analyses were taken, after informed consent, from both parents and two siblings Cases III and IV and sent to the Genetic department at Yale University,USA, were Genomic DNA was extracted from peripheral blood leucocytes, and the subunits of the human amiloride-sensitive sodium channel, (ENaC), SCNN1A, SCNN1B and SCNN1G, were amplified using primers encompassing the coding regions and the flanking intronic sequences, as described previously. [8] The nucleotide sequences of both strands of the PCR products were directly determined using an automated fluorescent sequencer (ABI Prism 310 Genetic Analyzer, Perkin-Elmer Corp, Wellesley, MA). The PCR products were analyzed by agarose gel electrophoresis.

Table1: Patient's characteristics & lab results

Family	Presented	Age	Aldosteron	PRA	Serum Na	Serum	DNA	U Na
Case I	3ed day	Live5days	-	-	115	8.9	-	-
Case II	6th day	Live 2 mo	4200	453	117	9.5	-	-
Case III	4th day	A live	781	381	121	10.5	Y516 TER Homozygous	154
Case VI	4th day	Live 4 mo	137	312	124	12	Y516 TER Homozygous	195
father	31 yr	A live	13	-	141	4.1	Y516 TER Homozygous	27
mother	29 yr	A live	93.5	-	137	4.3	Y516 TER Homozygous	185

Aldosteron N <35.5ng/dl , PRA N < 33 pg/ml/h , U Na N <40

RESULTS

The clinical syndrome manifested in these siblings by salt wasting, hyperkalemia, and metabolic acidosis associated with elevated plasma renin activity and aldosterone levels is characteristic of PHAI. The phenotype arises from loss of function of the epithelial sodium channel. It is caused by mutations in any of the three subunits of ENaC, as an autosomal recessive disease or with mutations in the Mineralocorticoid Receptor (MR), as an autosomal dominant disease. [1, 2, 3]

We have demonstrated that the cause of PHA1 in our family is a nonsense mutation in the alpha subunit of the epithelial sodium channel, occurring at amino acid 516. This substitutes a TAC [Y]for a TAG [STOP], changing a Tyrosine to a Stop Codon. This truncates the SCNN1A protein at amino acid 516, shortening the protein from 721 amino acids to 516 amino acids. This truncation eliminates the second Transmembrane Domain and the PPXY domain of the protein. The second transmembrane domain is implicated in having a role in conferring ion selectivity to the protein, and in contributing to its conduction pore. The critical PPXY Domain has been shown to be the site of interaction with the E3 ubiquitin ligase, Nedd4-2. These are domains proven to be critical for functioning of the alpha ENaC subunit. The homozygous mutation confers affected status in the two children and the heterozygous mutation confers carrier status in both parents. We also observed that our patients did not have respiratory distress or get chest infections like most of the reported cases of PHA1. [11, 12, 13]

Surprisingly, the mother who is an asymptomatic heterozygous carrier demonstrated high serum aldosterone and high urinary sodium, a finding not reported before.

Table 2: ENaC subunit genes mutations responsible for PHA1 reported worldwide.

Phenotype	Ethnici	Subunit	Location	Mutation	Codon	change Reference
severe	Yaman	Alpha	Exon 11		Tyr516stop	This study
severe	Yaman	Alpha	Exon 11		Tyr516stop	This study
severe	Somali	Alpha	Exon 13	c.1684T>C	S562P	Felix et al14
Mild*	Polish	Alpha	Exon 5	1078G→	Gly 327 Cys*	Edelheit et al9
Mild *	Swedish	Alpha	Exon 13	1784C→	Ser 562 Leu*	Schaedel et al15
?	Northe	Alpha	Exon 2	256 C→	Arg 563 stop	Kerem et al11
Severe	Saudi	Alpha	Exon 2	302 del TC	Ile 68 fr	Chang et al8
Severe	Hispanic	Alpha	Exon 3	604 del AC	Thr169fr	Kerem et al 11
Severe	Swedish	Alpha	Exon 4	828 del A	Ser243fr	Schaedel et al15
Severe	Hispan	Alpha	Exon 8	140 del C	Phe435fr	Kerem et al11
Severe	Pakistan	Alpha	Exon 8	1439 ins T	Tyr447fr	Saxena et al10
Mild*	Polish	Alpha	Exon 8	1449 del C	His450fr*	Edelheit et al 9
Mild/sever	Swedish	Alpha	Exon 8	1449 del C	His450fr	Schaedel et al15
NLT	Turkish	Alpha	Exon 8	1455 del C	Ser 452 fr	Edelheit et al9
Severe	Dutch?	Alpha	Exon10	Arg492stop	Bonny et al16	
Severe	Indian	Alpha	Exon11	162 C →	Arg 508 stop	Saxena et al10
Severe	Jewish	Alpha	Exon11	1621 C →	Arg508stop	Chang et al8
?	Arab	Beta	Exon 2	236 G →	Gly 37 Ser	Chang et al8
?	Jewish	Beta	Exon 3	647 insA	Leul 74 fr	Kerem et al11
?	Jewish	Beta	Exon 5	915 del C	Ser 263 fr	Kerem et al11
Severe	Arab	Beta	Intron	1669+1G→	Abnormal	Edelheit et al9
Severe	Scottish	Beta	Intron	1669+1G→	Abnormal	Saxena el at 10
NLT	Indian	Gamma	Intron 2	318 - 1G→	Abnormal	Strautnieks
Severe	Japanese	Gamma	Intron	1570 - 1G→	Abnormal	Adachi et al18
Severe	Japanese	Gamma	Exon 13	1627delG→	Val 543fr	Adachi et al18

DISCUSSION

In this study, we reported a consanguineous family with autosomal-recessive, multisystem PHA1. Within the first few days of life their children manifested severe, early onset salt-wasting, dehydration, hyperkalemia, hyponatremia, metabolic acidosis, high urinary sodium, hyperaldosteronism, hyperreninemia with normal adrenal function, normal cortisol and 17-

OH progesterone, without respiratory distress. We have identified a novel nonsense mutation in the alpha subunit of the epithelial sodium channel, (SCNN1A), at amino acid 516 that substitutes a TAC (Y) for a TAG (Stop). This changes a tyrosine to a stop codon, truncating the protein at amino acid 516, shortening it from 721 to 516 amino acids. This truncation eliminates the second transmembrane domain and the critical **PPXY** domain, domains known to be significant for functioning of the epithelial sodium channel subunits.

The two affected children Cases III and IV were homozygous for the mutation and both parents were heterozygous carriers, indicative of autosomal recessive inheritance. To our knowledge, this finding brings to 24 the number of reported independent mutations, known worldwide, in the coding regions of the three subunits of ENaC (Table 2). The majority, (20 out of 24), of the multisystem PHA1 associated mutations leads to abnormal length mRNA or protein because of deletions, insertions or splice site mutations in the DNA. These structural changes within the proteins lead to loss of function of ENaC and a severe type of PHA1, resulting in the inability of the subject to regulate volume changes.
Interestingly, 16 out of 24 of the reported mutations[9], are in the alpha subunit of ENaC. (Tab 2)
The characteristic features of genetic mutations in the autosomal recessive multisystem PHA1 patients are as follows: [10]

1- All examined cases showed mutations in both alleles encoding one of the subunits of ENaC. The majority shows homozygous mutations, with both parents displaying heterozygous mutations. The others are compound heterozygote.

2- The mutations may be observed in any of the three subunits of ENaC.

3- The mutations observed include single nucleotide changes, deletions, insertions and splice site junction changes leading to the production of an inactive protein.

4- Most of the mutations appear in the alpha subunit, consistent with an important role of this subunit in ENaC function.

5- The mutations have helped define functional domains of the subunits.

6- In contrast to Liddle's syndrome, resulting from gain-of-function mutations in the ENaC subunits, none of the mutations in multisystem PHA1 appears in the carboxy-terminal region.[10]

The fact that none of the four affected siblings with systemic PHA1 exhibited chest symptoms, like many of the reported cases, [11, 12, 13] could be due to phenotypic heterogeneity of the condition.

The mother was an asymptomatic heterozygous carrier but she had an elevated serum aldosterone level of 93.5 ng/dl (N<35.5 ng/dl), and a high urinary sodium. Although, asymptomatic, a mild salt losing phenotype could have been missed during infancy. These findings, in a carrier, have not been reported in the literature. However, high sweat sodium and chloride levels in a heterozygous carrier have been reported by Felix G. Riepe et al.[14] This may be due to phenotypic heterogeneity in systemic PHA1 or a dominant-negative effect

of the mutant allele. This suggests that one copy of the mutant gene confers a mutant phenotypic effect that is subtle and not as severe as the two- copy mutation within the gene.

Conclusion: our sequence of systemic PHA1 patients, in a consanguineous kindred, revealed a novel homozygous, nonsense mutation at amino acid 516 in the alpha subunit of ENaC. This truncates the protein, shortening it from 721 AA to 516 AA, leading to a decrease in epithelial sodium channel activity.

The parents are heterozygous carriers, consistent with the autosomal recessive inheritance of the disease. The presence of a high serum Aldosterone and high urine sodium in the heterozygous mother cannot be fully explained. However, it may be due to the phenotypic heterogeneity of the disease, or the dominant effect of the mutated allele, (Dominant-Negative effect theory).

Identification of the molecular basis of PHA1 is helpful for early diagnosis, understanding of the path physiology of the condition, genetic counseling and possible pre-implantation selection in affected families.

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Original Article

Successful construction of functional bicistronic vector for transfer and concurrent expression of human oligodendrocyte transcription factor Olig2 and DsRed2 reporter gene sequence in human cells

Dr. Faisal A. Al-Allaf , *Department of Medical Genetics, Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia.*

Correspondence:
Dr. Faisal A. Al-Allaf
Department of Medical Genetics, Faculty of Medicine, Umm Al-Qura University, Al-Abdia Campus, P. O. Box 715, Makkah 21955, Saudi Arabia
Tel/Fax: +966 2 5270000 Ext: 4198
Email: fallaf@uqu.edu.sa

البناء الوظيفي الناجح لاوليق 2 والبروتين 2 الأحمر الفوسفوري في الخلايا البشرية

د فيصل العلاف – قسم المناعة كلية الطب –جامعة ام القري – مكة المكرمة

الملخص العربي:

الهدف: كثير من الأبحاث التى حاولت استخدام سلسلة دخول الريبوسوم الداخلية لبناء النواقل الوراثية المزدوجة واجهت صعوبة فى تحقيق تعبير جينى للبروتين الثانى فى السلسلة. فى هذه الدراسة قمنا ببناء ناقل وراثى مزدوج للتعبير الجينى المتزامن يحتوي على كاسيتكون من عامل الاستنساخ البشري اوليق 2 وسلسلة بين سيسترونية وسلسلة الريبوسوم الداخلية وسلسلة البروتين الأحمر الفوسفوري المعزز.

الطرائق: تم إعادة إستنساخ السلسلة المحتوية على الاستنساخ البشري اوليق 2 مع السلسلة بين سيسترونية وطولها 205 نيوكلتيد الى الناقل الوراثى للتعبير الجينى المسمى pIRES-2-DsRed2. الناقل الذي تم بناءه تم تحليله بواسطة التحليل التسلسلي للحمض النووي لتأكيد اندماج السلاسل بالشكل الصحيح. تم اختبار فعالية الناقل وقدرته على التعبير الجينى من سلسلة تحفيز السيتوميقالو فيرس بواسطة الويسترن بلوتينق و التحليل المجهري الفوسفوري.

النتائج: التحليل التسلسلي للحمض النووي للناقل الذي تم بناءه أكد اندماج سلسلة عامل الاستنساخ البشري اوليق 2 في الكاسيت الثنائى وبالتالى بناء الناقل المطلوب بالصورة الصحيحة. تحاليل الويسترن بلوتينق والتحليل المجهري الفوسفوري أكدت ايضا فعالية الناقل وقدرته على التعبير الجيني وإنتاج عامل الاستنساخ البشري اوليق 2 بمقاسه الصحيح والنشاط الحيوي للبروتين الأحمر الفسفوري في نموذج الخلايا المستزرعة.

الإستنتاج: سلسلة دخول الريبوسوم الداخلية تمثل اداة قيمة لإمكانية التعبير الجينى المتزامن والمتعدد السيسترون من نسخة الحمض النووي المراسل الناتج من النواقل الجينية والنواقل الوراثية العلاجية. الناقل المتعدد الذي تم إنتاجه سيكون مفيد للنقل الجيني ولدراسة تخصيص الخلايا الجذعية في المستقبل.

ABSTRACT

Objective: Internal ribosome entry site (IRES) sequences become a valuable tool for constructing gene transfer and therapeutic vectors for simultaneous expression of bicistrons from a single mRNA transcript. Many researchers using IRES elements for construction of bicistronic gene transfer vector reported difficulties to achieve significant expression of the second cistron. In the present study, we have constructed an expression vector containing a bicistronic cassette composed of the human oligodendrocyte transcription factor 2 cDNA, the IRES and enhanced red fluorescent protein coding sequences. Transcription of the bicistronic cassette is driven by a massage from a common upstream cytomegalovirus promoter. Translation of the two cistrons is uncoupled. Analyses of vector containing the cassette clearly confirm the functionality of the produced vector, correct size of the generated hOlig2 protein and the biological activity of the red fluorescent protein reporter in the cell culture model. The produced bicistronic hybrid vector in this configuration is intended to be used for gene transfer, gene based stem cell therapy and of great value for future stem cells transdifferentiation studies.

***Keywords*:** bicistronic vector, oligodendrocyte transcription factor, Olig2 and DsRed2

INTRODUCTION

Concurrent expression of more than one transgene from a single gene transfer vector is an important requirement in gene and cell therapy protocols {Ali, 2010 #1377;Chen, 2011 #1351;Ho, 2011 #1348;Licursi, 2011 #1368}. Concurrent expression can be achieved through the utilization of natural splicing signals of virally based vectors, in which multiple RNAs are produced from a single transcript {Kollen, 1999 #1197}. However, this is not a frequently used strategy because of the difficulties in controlling the splicing mechanism of the gene vector. Alternative approach involves in-frame fusion of chimeric sequences, ensuring concurrent expression of genes in one protein {Holt, 1999 #1188;Hoque, 2000 #1189;Kollen, 1999 #1197}. However, this strategy may not work for all combinations of proteins, some of which could result in protein misfolding or mistargeting. Instead, a vector with dual promoters expressing two separate transcriptional units can be constructed. The major disadvantage of constructing such a dual promoter vector is possible transcriptional interference and/or dissociated gene expression, with a fraction of the transfected cells expressing the selectable reporter but not the gene of interest and vice versa {Cullen, 1984 #1191;Emerman, 1984 #1195;Emerman, 1986 #1193}. To overcome the above limitations, heterologus chimeric gene sequences encoding the different cistrons can be separated by an element known as internal ribosome entry site (IRES) sequence. The IRES sequence is a *cis*-acting RNA element that has been found in many viral and human cellular RNAs {Fitzgerald, 2009 #1398}. Since its discovery, the IRES sequence has become a valuable tool for constructing gene therapeutic vectors for co-expressing multi-cistronic messenger RNA (mRNA) in tranfected cells and in transgenic animal models {Attal, 1999 #1203}. The function of these IRES sequences is to confer cap-independent translation initiation of internal cistrons by recruiting ribosomes directly to the mRNA without using the classical scanning mechanism suggested by Kozak {Kozak, 1995 #1201}. As a result, a single messenger unit including the bicistronic transcript of both genes spaced by IRES is produced. Translation initiation of the first cistron at the 5' of IRES is typically mediated by a cap-dependent translation initiation mechanism {Kozak, 1995 #1201} but the second cistron at the 3' of

IRES is translated via cap independent translation initiation, mediated by the IRES element which functions as ribosome-binding sites for internal initiation of translation.

Most of the described vectors utilize the IRES element from the Encephalomyocarditis virus (ECMV) for co-expression of a reporter gene together with therapeutic gene to permits titration of vector particles or to demonstrate gene transfer efficiency, and the level and longevity of gene expression {Borman, 1995 #1208;Gallardo, 1997 #1205;Licursi, 2011 #1368}. However, IRES efficiency is not absolute and it is a common occurrence for the subsequent gene in the expression cassette to fail translation or maybe translated at a lower level than the preceding gene {Attal, 2000 #1230;Mizuguchi, 2000 #1217}. Therefore, the optimal conditions for effective use of IRES element for constructing a functional expression vector are not precisely defined, but mounting evidence suggest that expression of the downstream genes can be improved by increasing or decreasing the sizes of the sequences flanking the 5' and/or 3' of IRES inserts {Attal, 1999 #1202;Romero-Lopez, 2011 #1300}.

In the present study, we have constructed an expression vector containing bicistronic cassette under control of the cytomegalovirus (*CMV)* promoter. This bicistronic cassette contains the human oligodendrocyte transcription factor 2 cDNA (hOlig2cDNA), 205bp inter cistronic sequence (ICS), the IRES element, and the DsRed2 reporter sequence. We then examined the efficiency of this vector with the aim to use it in future gene transfer studies for stem cells transdifferentiation and stem cells based gene therapy.

MATERIAL AND METHODS

1.1. Plasmid Construction and Cloning

The hOlig2cDNA insert was excised from pBluscript-Olig2 plasmid by digestion of 10µg with *SacI* (Promega) in a total volume of 200µl reaction mix topped with 3 drops of oil and incubated at 37°C for 14 hours. The tube was incubated at 65°C for 20 minutes to inactivate the *SacI* endonuclease. Digested DNA was then size fractionated on 1% agarose gel. The 1.2kb fragment containing hOlig2cDNA was cut out and DNA was purified using commercially available gel purification kit (Qiagen) and DNA was eluted in 50µl sterile Diethylpyrocarbonate (DPEC) treated H_2O. The eluted DNA was then blunt ended with Klenow enzyme (NEB), heat inactivated for 15 minutes at 65°C, column purified (Qiagen) and DNA was then eluted in 40µl sterile DPEC treated H_2O to ensure removal of enzymes and salts. Approximately, 10µg of the pIRES2-DsRed2 plasmid DNA was also digested with *BamHI/Bgl II* enzymes, heat inactivated at 65°C for 20 minutes, column purified again, blunt ended by Klenow (Roche) and alkaline phosphatase (NEB) treated for 8 hours at 37°C. Purified digested vector and insert DNA were mixed together in 1:10 ratio and ligated using T4 DNA ligase (Roche) for overnight at 16°C. Ligated mixture was purified again using the Qiagen column and eluted in 30µl molecular biology grade H_2O. Only 2µl of eluted ligation mixture was used to transform STBL2 competent bacterial cells (Invitrogen) {Sambrook, 1989 #1378}. Transformation was performed according to Invitrogen recommendations and 300µl of serially diluted SOC media containing transformed STBL2 competent cells was plated on *Kan* resistance plates. Colonies were allowed to grow for overnight and then subcultured on both *Kan* and *Amp* resistance plates. Only *Kan* resistance colonies were screened further together with ligation mixture by insert-vector PCR method {Al-Allaf, 2005 #1228} using the forward primer 5'-GCA CGG CCT ACT CAA GTC TC-3' and the reverse

primer 5'-GGA ACT GCT TCC TTC ACG AC-3' which anneals to the 5' and 3' restriction/ligation site. Colonies producing 527bp amplicon were inoculated for overnight culture and the integrity of the hOlig2-insert in the pOlig2cDNA-IRES-DsRed2 clones was examined by restriction digestion and DNA sequence analysis {Sambrook, 1989 #1378}.

1.2 Gene transfer into cultured cells

The human embryonic kidney epithelial (HEK 293), the cervix carcinoma Hela cells, and the human fetal mesenchymal stem cells (hfMSCs) were cultured with Dulbecco's modified Eagle's medium (Invitrogen or Sigma) supplemented with 10% fetal calf serum (FCS, Sigma). All cells were incubated at 37°C with 5% CO_2 in a humidified incubator. Cells were seeded into a 10cm^2 dishes at 60-70% confluence with a density of 5x10^6 cells and transfected the following day with 10µg of the expression vector DNA using FuGene6 (Roche) according to the manufacturer's instructions. Approximately 48 hours post-transfection, cells were analysed for cellular expression of DsRed2 using inverted fluorescence microscopy (Nikon). Mock transfected cells were used as controls. All transfection experiments were repeated at least three times.

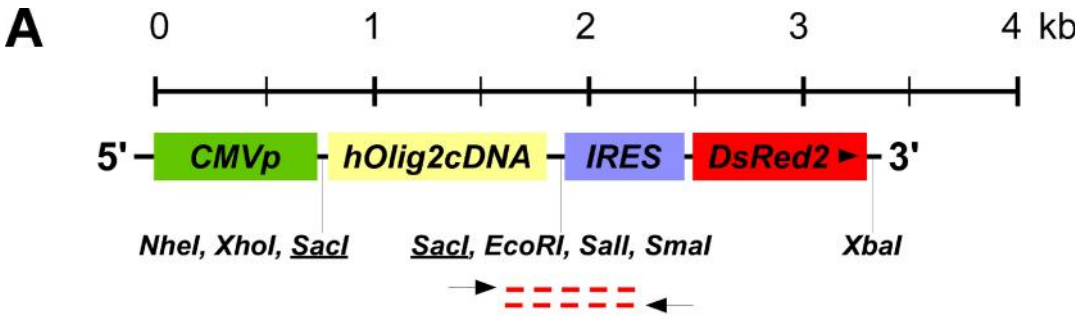
1.2. Western blot analysis for protein expression

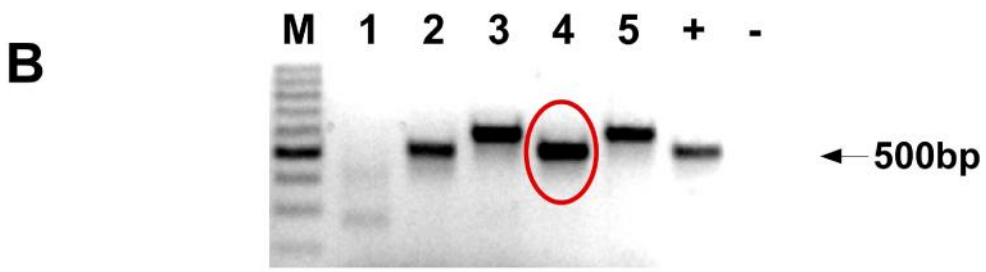
Forty eight hours after transfection, cells were harvested by scrubbing and re-suspended in 500µl/10^6 cells ice-cold lysis buffer (10mM Tris pH 7.5, 1mM EDTA, 1% NONIDET P-40 (Sigma), protease inhibitor cocktail at indicated dilutions (Roche)). Cell suspensions were then centrifuged at 13,000×*g* for 20 minutes at 4°C. The protein content of cell lysate supernatants was assayed by the bicinchoninic acid method using a commercial assay kit from Pierce with bovine serum albumin as a standard. Five milligrams protein of each lysate were combined with equal volumes of Laemmli sample buffer, boiled for 5 minutes and were size fractionated on a 13% or 11% SDS-PAGE gel for Olig2, and β-actin protein detection, respectively. Gels were then blotted onto PVDF membranes with a Hoefer apparatus (200 mA). After 1 hour incubation at room temperature in blocking solution (5% dried skimmed milk in PBS-Tween-20 0.1%), membranes were incubated overnight at 4°C with specific primary antibodies, diluted as specified below in blocking solution. After three 5-minutes washes in PBS-Tween-20, blots were incubated at room temperature for 1 hour with peroxides-conjugated anti-goat IgG horse radish peroxidase antibody (diluted 1:1000 in 5% milk solution). Following the final wash, detection on autoradiography hyper-films was performed after inducing a chemiluminescence reaction with supersignal detection kit (Amersham). Primary antibodies used in this study were polyclonal goat anti-hOlig2 antibody (diluted 1:500 in 5% milk solution in 1x TBS, Santa Cruz, USA) and anti-β-actin (1:2000; Sigma, UK) {Harlow, 1988 #1379}.

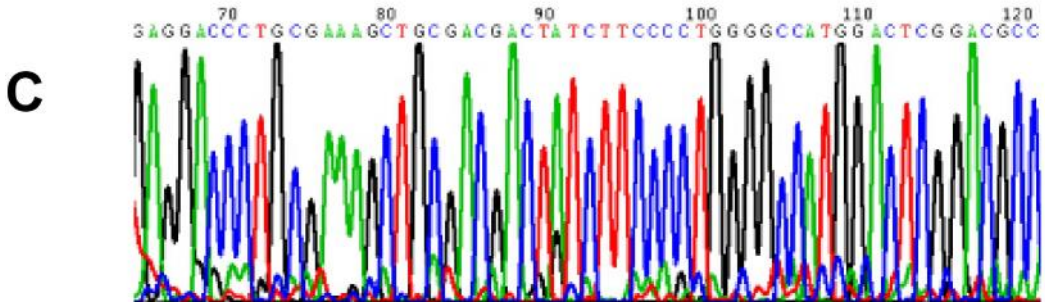
RESULTS

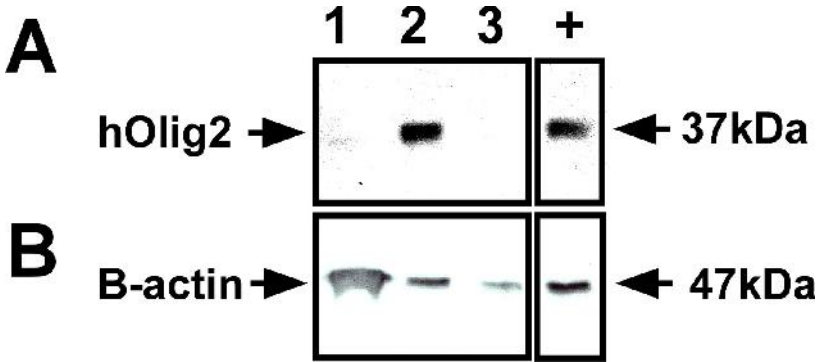
1.1 Structure of the bicistronic cassettes and proof of clonality

The pIRES2-DsRed2 expression vector was chosen as a backbone because it contains the IRES of the ECMV which has high translation efficiency compared to other IRES sequences {Borman, 1995 #1208;Borman, 1997 #1204;Gallardo, 1997 #1205;Ramesh, 1996 #1206;Saiz, 1999 #1207}. The multi-cloning site which located between the immediate early promoter of cytomegalovirus and the IRES sequence offers the possibility of convenience cloning. The hOlig2cDNA and the 205bp downstream ICS were sucloned from the pBluscript-Olig2 plasmid into pIRES2-DsRed2. The nasant pOlig2cDNA-IRES-DsRed2 vector in this configuration (Figure 1-A) permits high level of bicistronic expression. It also permits high scale of plasmid DNA production after propagation as it contains a pUC origin of replication and a bacterial promoter expresses kanamycin resistance in *E. Coli*.

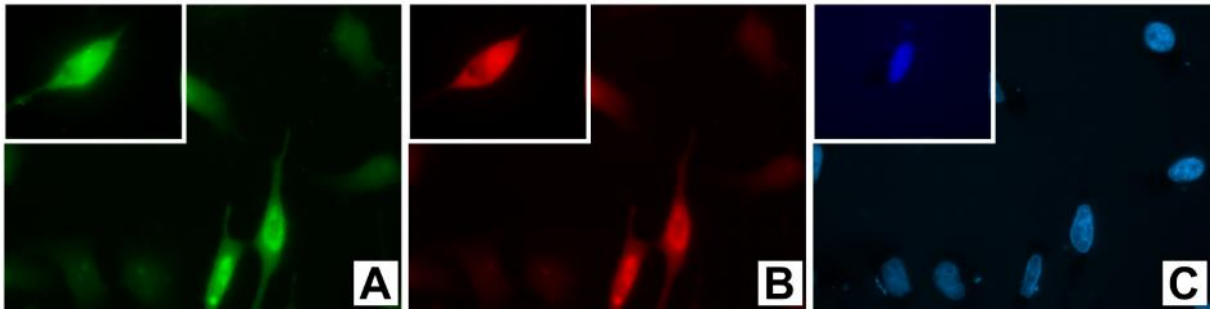








During cloning protocol, the ligation mixture was examined by insert-vector PCR {Al-Allaf, 2005 #1228} as positive control to confirm the physical presence of the expected recombinant plasmid containing the hOlig2cDNA in correct orientation. The correct PCR amplicon of 527bp in size was generated from 3 randomly screened colonies (Figure 1-B), indicating Olig2cDNA insertion in the desired orientation. The other three screened colonies showed no PCR product (lane 1) or non-specific amplification (lane 3 and 5) and therefore excluded from further screening. The ligation mixture was used as positive control. The PCR positive colonies were then grown individually in LB growth media under kanamycin selection. DNA sequence analysis was performed on nascent vector DNA which also confirmed the clonality and integrity of Olig2cDNA in the bicitronic cassette and the construction of the desired mammalian expression vector (Figure 1-C).



The vector backbone also contains an SV40 origin of replication expressing the SV40 T antigen. A neomycin-resistance cassette, consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the herpes simplex virus thymidine kinase gene, allows stably transfected eukaryotic cells to be selected using G418 {Nehlsen, 2010 #1399}.

The initiator AUG codon of the DsRed2 is located 12 bases downstream of the IRES element.

1.1. Analysis of hOlig2 expression by Western blotting

Analysis of gene expression was performed by Western blotting and fluorescence microscopy after cells transfected with bicistronic construct containing 5'-hOlig2-IRES-DsRed2-3' cassette. Western analysis ensures that the expressed proteins of a correct mature sizes and the fluorescence microscopy permits accurate assessment of gene transfer and expression at a single cell level. Immunoprobing with anti-hOlig2 antibody (Figure 2-A) shows the mature form of the hOlig2 protein migrating as one band of apparent molecular masses of 37kDa

from HEK 293 cell extracts transfected with the pOlig2cDNA-IRES-DsRed2 construct (lane 2) and from positive control lysate of MO3 cells (lane 4). No signals were detected from the mock transfected negative control (lane 1) or from extract of cell transfected with pIRES2-DsRed2 mother plasmid (lane 3). Immunoprobing of the membrane with anti-β-actin antibody in Figure 2-B shows the production of β-actin protein of the expected molecular mass of approximately 47kDa from cells. No other proteins were recognised from cell extract immunoprobed with these antibodies.

In summary, the data provided above show that the produced vector is functional and the generated proteins of correct sizes.

1.1 In vitro analysis of Olig2 and DsRed2 expression by immunofluorescence microscopy

In order to test the functionality of the constructed vectors and its biological activity at a single cell level, the pOlig2cDNA-IRES-DsRed2 vector DNA were transfected into Hela cells and expression was analysed using Immunofluorescence microscopy (Figure 3). Cellular co-expression of Olig2 and DsRed2 was detected approximately 48 hours post gene transfer in all transfected cells. This figure proof functionality of the produced vector and absence of transcriptional interference and/or dissociated gene expression between Olig2 and DsRed2. Transfected Hela cells cells were able to co-express both Olig2 and DsRed2 cistrons in approximately 60% of cell population.

DISCUSSION

Accurate assessment of gene transfer following transfection or transduction may require co-expression of a reporter gene in addition to the transgenic sequence in question. Methods used for co-expression of more than one gene have serious drawbacks {Cullen, 1984 #1191;Emerman, 1984 #1195;Emerman, 1986 #1193}. When an IRES element is used for the construction of a multi-cistronic cassette in gene transfer and expression vectors, it has been frequently observed that the DNA sequence subcloned at the 3' to the IRES is poorly expressed compared to the sequence subcloned at the 5' of the IRES {Attal, 1999 #1203;Attal, 1999 #1202;Brocard, 2007 #1303;Brown, 1994 #1299;Romero-Lopez, 2011 #1300}.

In the present work, we have overcome the previously reported problem of insignificant second cistron expression and have produced a functional 5'-hOlig2-IRES-DsRed2-3' expression cassette containing the following five components: the first component of the bicistronic cassette is a cDNA encoding hOlig2 which is a member of the basic helix-loop-helix nuclear transcription factor protein family {Wegner, 2001 #1380;Zhou, 2000 #1381}. Normally, the gene encoding hOlig2 is expressed in the spinal cord, brain and the optic nerve and has been shown to be involved in both oligodendrocyte differentiation and motor neuron specification {Fu, 2002 #1382;Lu, 2002 #1383;Novitch, 2001 #1384;Rowitch, 2002 #1385;Sun, 2001 #1386;Zhou, 2002 #1387;Zhou, 2001 #1388}. This dual function of Olig2 in neurogenesis and oligodendrogenesis can be explained by the possible interaction of Olig2 with other transcription factors {Lu, 2000 #1389;Novitch, 2001 #1384;Zhou, 2001 #1388;Zhou, 2000 #1381}. The second component of the cassette is a 205bp ICS located 5' of IRES element, which derived from the pBluscript-Olig2 plasmid. The third component of the

cassette is IRES of the ECMV. The fourth component is a 12bp ICS located 3' of IRES element. The fifth component in this vector represents the human codon-optimized DsRed2 {Matz, 1999 #1397} reporter coding sequence of *Discosoma* sp. red fluorescent protein {Cormack, 1996 #1213;Yang, 1996 #1214}. This cDNA is engineered for easy, rapid and less expensive detection and monitoring of expression. It also offers brighter fluorescence, faster maturation and lower non-specific aggregation with higher expression in mammalian cells {Cormack, 1996 #1213;Jakobs, 2000 #1395;Yang, 1996 #1214}. The DsRed2 coding sequence contains nine amino acid substitutions which improve solubility of the protein and reduce the time from transfection to detection of red fluorescence. In addition, these substitutions reduce the level of residual green emission {Haas, 1996 #1393}. Although DsRed2 most likely forms the same tetrameric structure as wild-type DsRed2, with reduced tendency to aggregate {Haas, 1996 #1393}. At the 3' downstream of DsRed2, the SV40 polyadenylation signal directs proper processing of the bicistronic mRNA transcript.

We analysed the expression levels of the respective transgenes systematically at the DNA and protein levels after gene delivery which showed 90% gene transfer in HEK 293 cells following transfection. Successful co-expression of hOlig2 and dsRed2 is attributed to the following two main factors: firstly, the length of the inter-cistronic sequence flanking the IRES element at both its 5' and 3' ends {Attal, 1999 #1203;Borman, 1995 #1208;Borman, 1997 #1204}. The effect of the length of these ICS on IRES mediated internal translation initiation is well documented in the literature {Attal, 1999 #1203;Borman, 1997 #1204}. Expression efficiency of the DsRed2 was obtained when the 5' ICS is 205bp in length. However, the expression levels of DsRed2 become completely undetectable at a spacer length of 530bp (data not shown). These results are in agreement with data published by Attal et al. {Attal, 1999 #1202} who found that IRES elements from ECMV as well as from poliovirus function optimally when about 100 nucleotides were added after the termination codon of the first cistron (luciferase). These authors also showed that IRES elements become completely inefficient when added after a 300-500 nucleotide spacer {Attal, 1999 #1202}. In a similar study using the IRES elements from poliovirus and from SV40, respectively in bicistronic cassettes containing the firefly luciferase gene as the first cistron and the *CAT* gene as the second cistron, Attal et al. {Attal, 1999 #1202} also showed that the expression of the second cistron was undetectable when the spacer fragment was 500 nucleotides. This phenomenon of low efficiency of IRES mediated translation initiation compared to cap dependent translation is possibly due to higher affinity of translation factors for the cap structure than for the IRES element, therefore, translation factors may become less available for the internal initiation of translation {Brown, 1994 #1299} but can be modulated by size. To exclude the effect of tissue tropism and the requirement of additional host *trans*-acting factors, which may modulate IRES function in a cell or tissue type specific manner {Dobrikova, 2006 #1283;Lourenco, 2008 #1280;Ray, 2002 #1287}, we have also transfected our construct into Hela and hfMSCs cells and obtained similar results. The second ICS located 3' of IRES is a short sequence contains a Kozak consensus translation initiation site {Kozak, 1995 #1201} to further increase the translation efficiency of second cistron in eukaryotic cells. Thus, the initiator AUG codon of the DsRed2 is located 12 bases downstream of the IRES element. Such a short distance is nevertheless sufficient to greatly affect the DsRed2 expression. The second factor attributed to the successful co-expression of hOlig2 and dsRed2 is the use of IRES of the ECMV which has high translation efficiency compared to other IRES sequences including those from human rhinovirus, poliovirus, foot-and-mouth disease virus and hepatitis A and C viruses {Borman, 1997 #1204;Gallardo, 1997 #1205;Ramesh, 1996 #1206;Saiz, 1999 #1207}. In addition to its high efficiency, ECMV IRES possess a broad tissue tropism

{Borman, 1995 #1208} which makes it the most widely used in gene transfer protocols {Azzouz, 2002 #1209;Morgan, 1992 #1210;Pizzato, 1998 #1211}.

The Olig2 gene has been shown to drive trans-differentiation of stem cells into oligodendrocyte {Copray, 2006 #1246;Zhang, 2005 #1240;Zhou, 2001 #1388}. In 2005, Zhang *et al.* {Zhang, 2005 #1240} used transfection methods and expression plasmids to deliver *Olig2* gene inside neural stem cells and accomplished a transient expression up to 12 days with a 60-80% efficiency of transfection. In the present study, we have achieved more than 90% tranfection in HEK 293 and approximately 20% transfection in hfMSCs and accomplished a transient expression up to 14 and 9 days respectively. We are also aware that transdifferentiation of stem cells by gene transfer may require long-lasting expression of the transgenic Olig2 in the relevant cell model, therefore, future work will focuses on subcloning the successfully constructed 5'-Olig2-IRES-DsRed2-3' into an integrated virally based gene transfer vector system to ensure permanent expression of the integrated transgenes.

In conclusion, we have overcome the previously reported problem of insignificant second cistron expression and have produced a functional 5'-hOlig2-IRES-DsRed2-3' expression cassette containing a 205bp spacer.

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Original Article

Assessment of Airway Remodeling in Bronchial Asthma by Multidetector CT and its Correlation to Pulmonary Function

Dr.Amani E A Garrad, Consultant Chest Disease King Faisal Hospital, Holly Mekkah. assistant. Prof of chest disease, Faculty of Medicine, Al Azhar University, Cairo, Egypt
Dr.Ahmed M Al Karoaby, *Consultant of Diagnostic Radiology, King Faisal Hospital, General Director of King Faisal Hospital. Dr.*Ahmed H Mohamed *Consultant of Radiology Al Noor Hospital, Ass. Prof Diagnostic Radiology, Mansoura University, Cairo, Egypt.*

Correspondence:
Dr.Amani E A Garrad.
Tel. 0562861574. Fax 966-2 5574350, Post Box 5592.
E-mail: amaniezz@hotmail.com.

تقييم التغيرات بالممرات الهوائية في مرضى الربو الشعبى بالأشعة المقطعية متعدّد الكاشف وعلاقته بالوظائفِ التنفسيةِ

د .اماني قراد
استشاري واستاذ مساعد امراض الصدر مستشفى الملك فيصل بمكة المكرمة وكلية طب الازهر – مصر.
د. أحمد محمد الخروبى
إستشاريى الأشعة التشخيصية ـ مستشفى الملك فيصل بمكة المكرمة. مدير مستشفى الملك فيصل بمكة المكرمة .
د.أحمد هارون محمد
إستشاريى الأشعة التشخيصية , مستشفى النور التخصصى بمكة المكرمة. أستاذ م الأشعة التشخيصية بكلية الطب جامعة المنصورة

الملخص العربي

الهدف من الدراسة:ان استخدام الأشعة المقطعية متعدّدة الكاشف تسْمحُ بمراقبة التأثيراتِ المُخْتَلِفةِ على الممرات الهوائية في مرضى الربو الشعبى. هدف دراستِنا هو تُقيّمَ التغيرات فى الممرات الهوائية بالأشعة المقطعية متعدّدة الكاشف و مقارنة قياسات الممرات الهوائية بين المرضى وغير المرضى و الربط بينها و بين وظائف التنفس.

الطرق: المرضى جُنّدوا مِنْ العيادة الخارجيةِ للأمراض الصدرية بمستشفى الملكَ فيصل بمكة المكرمة أثناء الفترةِ 2008-2009. تم قياس احداثيات الممرات الهوائية يدويا بالأشعة المقطعية متعدّدة الكاشف بمستشفى النور التخصصى. تم عمل مقَارنَّات بين سُمكَ و مساحة الممرات الهوائية وإيجاد علاقة بينهم و بين السمك و المساحة الكلية للممرات الهوائية لثمانى و عشرون شخصا مشاركين في الدراسةِ. أيضا قام جميع المشاركين في الدراسة بعمل وظائف التنفس

النَتائِج:وجد أن للمرضى الذين يعانون من ربو شعبى شديد زياده غير ملحوظة فى سُمكَ و مساحة الممرات الهوائية عند مقارنتهم بمرضى الربو متوسط الحده والمشاركين فى البحث من غير المرضى.وتبين عدم وجود علاقة بين سُمكَ و مساحة الممرات الهوائية عند مقارنتهم بوظائف التنفس.

الإستنتاج: نستنتج من الدراسة أن سُمكَ و مساحة الممرات الهوائية فى مرضى الربو الشعبى أكبر من الأشخاص الطبيعيين, و يزيد هذه القياسات كلما زادة شده الربو.و يعتبراستخدام الأشعة المقطعية متعدّدة الكاشف تقنية مفيدة لتَقييم الممرات الهوائية في مرضى الربو الشعبى.

ABSTRACT

Objective: Noninvasive measures of airway remodeling using MDCT scanning would allow monitoring the effects of various stimuli on remodeling in asthma patients. The objective of our study was to assess airway remodeling by MDCT and comparing airway wall measurements among patients with healthy subjects, and to correlate this with pulmonary function.

Methods: Patients were recruited from the respiratory outpatient clinic King Faisal Hospital; Mekkah during the period 2008-2009. Manual airway segment measurements using thin-section, MDCT scans done at Al Noor Specialized hospital, we compared airway wall thickness (WT) and wall area (WA) in 28 subjects participating in the study, the WT and WA measurements were corrected for total airway diameter and area: WT% and WA%, respectively. All subjects performed spirometric measurements within 1 to 2 days of the MDCT scan

Results: Subjects with severe asthma had a non-significantly greater WT% than patients with mild-to-moderate asthma and healthy subjects and a greater WA% compared to patients with mild-to-moderate asthma and healthy subjects. Both WT% and WA% were not correlated with FEV1 %. In the same individual, there is considerable regional heterogeneity in airway WT.

Conclusion: Asthma patients had greater airway wall thickening than the normal subjects, but patients with more severe asthma had greater airway wall thickening than mild asthma patients. Noninvasive measures of airway remodeling using MDCT scanning may be a useful technique for assessing airway remodeling in asthmatic patients.

Abbreviations: LA = luminal area; LA% =percent of luminal area; MDCT = multidetector-row CT; R = right upper lobe; L = left upper lobe; TA = total area; WA = wall area; WA% = wall area percent; WT = wall thickness; WT% = wall thickness percent; FEV1= forced expiratory volume in first second; FEF25-75 = forced expiratory flow between 25-75%.

INTRODUCTION

Asthma is the most prevalent of lung diseases and contributes an enormous burden of morbidity globally. Bronchial asthma is a heterogeneous disease characterized by reversible airway obstruction. It varies in severity from mild episodic attacks to life threatening ones.[1]

Asthma, characterized by reversible airflow obstruction and airway hyper-responsiveness, is described as a chronic inflammatory disease of the lungs. There are structural changes in airways of asthmatics, referred to collectively as airway remodeling, are most striking in chronic and fatal asthma.[2]

In cases of fatal asthma, airway wall thickness is increased 50–300% most marked in the small cartilaginous and large membranous airways, while in cases of nonfatal asthma the increase is 10–100% more marked in small membranous airways (diameter <0.6 mm) and mid-sized airways (diameter 1–3 mm), and is less marked in the central airways.[3] Postmortem studies of patients dying of asthmatic attacks have shown that the airway walls are thickened.[1] Although bronchial biopsy with a fiberoptic bronchoscope has been used to study changes of the asthmatic airway.[4,5] However, thickening of the total bronchial wall cannot be evaluated with this procedure.[6]

Computed tomography (CT) has been used to study airway dimensions, including airway wall thickening (WT), and airway wall area (WA) in asthmatic patients.[6] Kariyawasam and colleagues[7] and Orlandi and associates[8] have shown that the airways of asthmatic patients are thicker than those of normal controls, whereas Kosciuch and colleagues[9] have failed to show such a difference. The presence and significance of airway wall thickening as assessed by CT in asthmatic patients thus remains to be clarified.

Multidetector CT (MDCT) scan studies have recently been used to evaluate the extent of airway wall thickening as a noninvasive, highly reproducible method for studying individual airways[10]. Several studies[11,12,13] using MDCT scanning have demonstrated that the airway walls of asthmatic patients are thicker than those of healthy subjects, and that airway wall thickness (WT) is related to the severity of disease and airflow obstruction.

The objective of our study was to assess airway remodeling by Multidetector CT and comparing airway wall measurements among patients with severe and mild-to-moderate asthma and healthy subjects, and to correlate this with pulmonary function.

MATERIAL AND METHODS

Patients were recruited from the respiratory outpatient clinic King Faisal Hospital; Holly Mekkah during the period 2008-2009. All had a diagnosis of asthma, according to the American Thoracic Society criteria; In this study we assessed airway wall thickness (WT) and luminal area (LA) in 22 asthmatic patients and 6 healthy control subjects in a cross

sectional study. Airway wall dimensions were compared among groups of asthma patients. In addition, we investigated the correlation of airway wall thickness to the degree of airflow obstruction (FEV1, FEF25-75) of the asthmatic patient.
Excluded from the study patients with history of respiratory infection or asthma exacerbation within the previous 6 weeks, psychiatric illness, coronary heart disease, diabetes, or hypertension, and smokers. Patients were already receiving medication and were being followed at our clinic. Subjects included in the study were classified into 3 groups; mild-moderate (12 cases), severe (10 cases), and healthy control subjects (6). The healthy control subjects had no respiratory symptoms and no history of asthma or other respiratory diseases. All subjects were lifetime nonsmokers. The study was approved by the Human Research Ethical Committee.

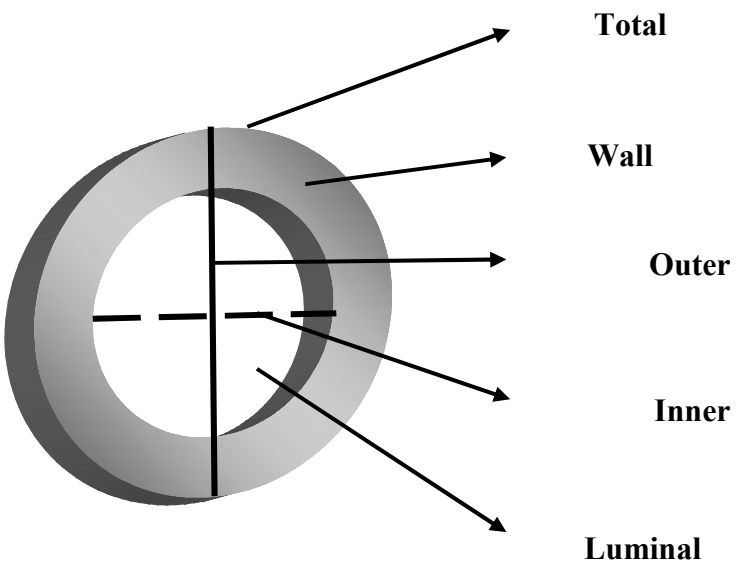
Spirometry measurements were performed within 1 to 2 days of the MDCT scan. Subjects were asked to abstain from the use of long-acting β2 agonists for 12 h and from the use of short-acting β 2 agonists for 4 h prior to assessment. Spirometry was performed before and after therapy with a short-acting β 2-agonist (2 puffs or 200 µg of albuterol) was delivered by metered-dose inhaler and spacer.

All subjects perform using thin-section (0.625 to 1.25 mm), MDCT scan of the chest with 24 detector rows (MOSA 1105514-T) at Al Noor Specialized Hospital. MDCT scans were analyzed by manual measurements. The specific MDCT scan measurements used included airway wall thickness (WT), percentage of WT (WT %), airway wall area (WA), percentage of WA (WA %), luminal area (LA) and percentage of LA (LA %). (Fig1)

The calculations are as follows:

- WT = average outer diameter - average inner diameter (Fig 1);
- WT% = (WT/average outer diameter) x 100;
- WA = total area (TA) – LA (Fig 3);
- WA% = (WA/TA) x 100; and
- LA% = (LA/TA) x 100

In the primary analysis, we averaged third-generation airway wall measurements for airways in each subject. An average of 8 third-generation airways per subject was measured (4 for each lung; right and left).



Wall Area %= $\frac{\text{Total Area – Luminal Area}}{\text{Total area}}$ x 100

Wall Thickness %= $\frac{\text{Wall Thickness}}{\text{Average Outer Diameter}}$ x 100

Wall Thickness = Average Outer Diameter- Average Inner Diameter

Figure 1. Airway measurements by MDCT scanning. WT is measured over the middle one third of each segment. The WT% for each segment is calculated by dividing the WT by the average outer diameter. TA and LA are similarly calculated and averaged over the middle one third of the segment. WA was calculated by subtracting the LA from the TA. WA% was calculated by dividing the WA by the TA.

STATISTICAL ANALYSIS

Unpaired t test was used to test the difference about mean values of wall thickness and area and LA among cases with moderate asthma, in comparison to severe asthma and normal subject's results were presented as mean and standard deviation. Pearson correlation coefficient, and correlation analysis: assessing the strength of association between FEV1 and LA and WT%, WA%, between FEF25-75 predicted and the same parameters [$P<0.05$ will be taken as a significant, the data were analyzed using SPSS (version 15)].

RESULTS

All subjects included in the study were male, their mean age was 37.4(9.6), all were non smoker, and was receiving long acting beta agonists and oral and inhaled corticosteroids. In subjects with severe asthma, conditions other than asthma were excluded, exacerbating

factors were treated, and the subject did not have a history of poor adherence to treatment.

Table 1, fig 2, shows MDCT Scan Airway Wall Measurements among Individual Airway Segments with Respect to Anatomic Location regarding WT%, WA%. Patients with severe asthma have slightly more thickened airway walls (WA %) compared to those with mild-to-moderate asthma and healthy subjects. There was no statistically significant difference between cases with severe asthma, mild-moderate asthma and control as regards the mean wall thickness. There was no statistical significant difference between the 3 groups although mean wall area in cases severe asthma was higher than those with mild-moderate asthma and control, Cases with severe asthma showed significant lower mean LAmm when compared to cases with mild-moderate asthma $P<0.05$ (table 2) From the results of this study we observed that there is no association was seen between wall thickness WT%, WA% and FEV_1 %, and FEF25-75%. (table 3).

Table 1: MDCT Scan Airway Wall Measurements among Individual Airway Segments With Respect to Anatomic Location regarding WT%, WA%

	Normal subjects Mean(SD) (n=6)		**Mild-moderate asthma** Mean(SD) (n=12)		**Severe asthma Mean(SD) (n=10)**	
WT% R1	17.58	(2.33) ¤¤c	17.80 b	(1.37)	19.28	(0.75)
WT% R2	19.00	(2.32)	18.91	(1.42)	18.85	(0.78)
WT% R3	18.45	(2.32)	17.88	(1.50)	17.29	(0.56)
WT% R4	19.97	(2.36)	18.83 ♦b	(1.46)	20.98	(0.88)
WT% L1	20.15	(2.41)	20.88	(2.45)	20.40	(1.86)
WT% L2	20.52	(2.51)	22.88 ♦b	(2.30)	19.16	(2.00)
WT% L3	18.60	(2.81)	18.68	(2.52)	17.83	(1.95)
WT% L4	18.22	(2.72)	20.78 *a♦b	(2.11)	18.59	(1.95)
WA% R1	59.82	(3.98)	61.00	(2.56)	61.42	(2.55)
WA% R2	59.95	(4.09)	58.95	(2.49)	59.26	(2.02)
WA% R3	58.47	(3.33)	57.94	(2.69)	58.73	(1.92)
WA% R4	61.35	(4.11)	59.39 ♦b	(2.71)	59.98	(1.97)
WA% L1	61.87	(2.76)	61.55	(2.11)	62.06	(1.50)
WA% L2	62.57	(2.60)	60.08 *a	(1.94)	60.82	(1.56)
WA% L3	55.90	(3.74)	55.37 ¤¤c	(9.27)	58.96	(1.47)
WA% L4	58.92	(3.13)	58.17 ♦b	(1.97)	59.62	(1.08)

a * normal vs mod, ♦b mod vs sever, c ¤¤ normal vs sever

Table 2: Comparison between luminal area (mm, %) among cases of Mild-moderate and severe asthma

	Mild-moderate asthma Mean(SD) (n=12)		Severe asthma Mean (SD) (n=10)		P	Sig.
LAmm	45.60	(1.02)	42.79	(2.02)	<0.0001	HS
LA%	42.74	(8.77)	43.99	(1.99)	0.66	NS

Cases with severe asthma showed significant lower mean LAmm when compared to cases with mild-moderate asthma P<0.05

Table 3: Correlation between MDCT Scan Airway Wall Measurements and pulmonary function parameters among cases with Mild-moderate and severe asthma

	Severe asthma (n=10)				Mild-moderate asthma (n=12)			
	FEV1 %		FEF25-75%		FEV1 %		FEF25-75%	
	R	p	R	p	r	p	r	p
WT% (right)	0.07	0.84	-0.06	0.86	0.58	0.05	0.75	0.005
WT% (left)	0.36	0.31	-0.17	0.64	0.59	0.04	-0.20	0.54
WA% (right)	0.58	0.08	0.06	0.87	-0.24	0.46	-0.35	0.26
WA% (left)	0.45	0.19	-0.41	0.24	0.55	0.06	-0.14	0.66

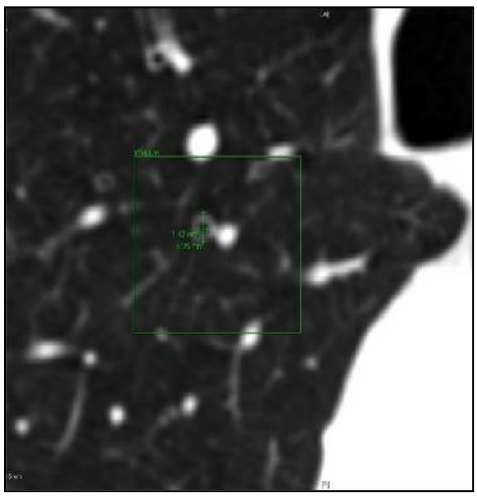


Figure: 2A

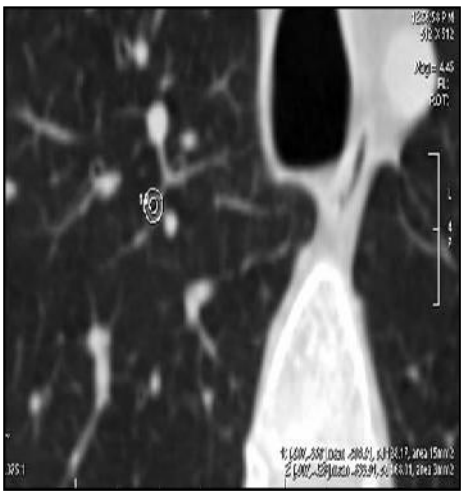


Figure: 2B

Figure 2: CT measurement for airways in asthmatic patient. 3rd generation bronchi are selected .
A- Wall Thickness = outer diameter – inner diameter.
B- Wall area = Total area – Luminal area
The average diameter is calculated.

DISCUSSION

Bronchial asthma has been characterized by chronic and allergic airway inflammation which induces cytological and histological changes in airway structure over time; these changed have been called airway remodeling. [14]

Studies on airway remodeling have been based on the use of histological examination, which require cross-sectional samples of the airways through surgical or autopsy techniques. Noninvasive methods are required to further investigation, follow up changes over time, and to allow the assessment of new therapeutic interventions designed to attenuate or control airway remodeling. Computed tomography (CT) allows an in vivo assessment of airway wall thickness. However, the information obtained from CT is essentially less detailed than that obtained on histological examination. Despite this limitation, the ability of MDCT to measure airways, noninvasively and frequently offers major potential advantages.[15]

In this study, we analyze and measure the differences in airway WT% between patients with severe asthma and those with milder disease manually. We found that when using MDCT scan indexes of patients with severe asthma have on average slightly more thickened airway walls compared to those with mild-to-moderate asthma and healthy subjects, although this differences in airway WT% was not statistically significant among the three groups.

Kosciuch and colleagues,[9] noticed that WT% did not differ significantly among the asthmatic groups due to airway secretions and bronchospasm as well as obliquity of section also affects airway measurements. It is therefore essential that asthma is optimally controlled before HRCT assessment.[14] In our study we use manual tracing of the air way which is extremely time consuming and is also prone to error.

On the other hand, Orlandi and colleagues[8] found that, bronchial wall thickness is probably overestimated on CT scans because of inaccuracies in boundary detection and inclusion of adjacent peribronchial interstitium. They found that all the asthmatic patients had greater airway wall thickening than the normal subjects by HRCT scanning, but patients with more severe asthma had greater airway wall thickening than those with mild asthma. He also found no difference in airway wall thickening, a marker of chronic airway inflammation between patients with a mild to moderate asthma.

In patients with fatal asthma thickening of the airway wall was observed in both large cartilaginous and small membranous bronchi. While in patients with non-fatal asthma the thickening occurred predominantly in the small airways. There was no difference in the thickness of the airway wall at the level of the bronchus intermedius between patients with asthma and normal controls.[12] In our study we assessed the bronchi at both segmental and subsegmental levels. Unlike Kosciuch and colleagues we did not measure airway wall thickness of the lobar or main bronchi.[9]

On the other hand Orlandi and colleagues, [8] Kariyawasam and colleagues[7] also found thickening of the airway wall in both large airways (L >2 mm) and small airways (L<2 mm) of asthma patients compared with normal controls.

In the present study, we noticed that patients with severe asthma have also slightly increased in WA% when compared to those with mild-to-moderate asthma and healthy subjects in most segmental airways. Also, Ravi, et al[10], found that there was no significant difference when comparing airway WT% or WA% in patients with mild-to-moderate asthma with those in healthy subjects. Kariyawasam and colleagues[7] measured WA and WA% in six asthmatic patients and four normal control subjects with airways of various size, and showed that smaller airways are thicker in asthmatic patients.

We also observed in our study, In cases with Mild-moderate asthma and severe asthma there was no correlation between FEV1 % predicted and WT% and WA%, also there was no correlation between FEF25-75% predicted and WT% and WA%. This is supported by Kosciuch and his colleague;[9] they measured airway dimensions (WT% and WA%) manually in asthmatic and normal subjects, they found that there was no correlation with the FEV_1.
On the other hand the study of Kasahara et al[11] found that the radiographic measurements of WT and WA were increased in asthma patients, and correlated inversely related to FEV1. Another study, Little, et al[12] found that no association was seen between wall thickness and FEV_1. Their results were towards an association with $FEF_{25–75}$ but not with FEV_1. Jeffery[16] found that there is a progressive increase in airway wall thickening with increasing duration as well as severity of asthma. Significant negative correlations were found between WA% and FEV_1, $FEF_{25-75\%}$. WA% may have been affected by bronchoconstriction, which is supposed to decrease WT and WA. In contrast, previous CT studies have shown that methacholine-induced bronchoconstriction does not affect WA in asthmatic patients or healthy control subjects.

CT scans of asthmatic patients have shown both decreased and increased bronchial lumen area, excessive airway narrowing in response to a variety of stimuli and airway wall thickening, in addition to mosaic perfusion and gas trapping on expiration.[17]
In this study Cases with severe asthma showed significant lower mean LA mm, but not LA%, when compared to cases with mild-moderate asthma. The same results were found in Ravi and his colleague;[10] they found that LA was not significantly different among the groups. Patients with severe asthma had a smaller LA compared to those with mild-to-moderate asthma and healthy subjects. There was no significant difference in LA% between patients with mild-to-moderate asthma and healthy subjects (difference was not significant).

Another study by Niimi and colleague[18] found that airway wall thickening, is related to airflow obstruction, and that luminal area did not decrease with increasing severity of asthma. Due to presence of bronchial dilatation or bronchiectasis reported in CT of airway in asthmatic patients which is more evidenced with increased severity of asthma. Also, Lung volumes increase with increasing severity in asthmatic patients, and airway diameter, or luminal area, becomes larger as lung volumes increase. [19]

A number of investigators have compared airway lumen area in normal and asthmatic subjects. Beigelman-Aubry et al, [20] demonstrated a lower baseline airway lumen area in asthmatics compared with controls pre-bronchodilator, but the difference was abolished after salbutamol. Conversely, Niimi et al.[17] found no decrease in lumen area in the right apical upper lobe bronchus in asthmatics, irrespective of disease severity, compared with a normal group.

CONCLUSIONS

Asthma patients had greater airway wall thickening than the normal subjects, but patients with more severe asthma had greater airway wall thickening than mild asthma patients. Noninvasive measures of airway remodeling using MDCT scanning may be a useful technique for assessing airway remodeling in asthmatic patients.

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UQU Medical Journal

Original Article

Demographic, clinical profile and survival outcome of relapsed pediatric acute myeloid leukemia

Wasil A. Jastaniah *Assistant Professor and Consultant Pediatric Hematology/Oncology/BMT Department of Pediatrics, Faculty of Medicine, Umm AlQura University, Makkah, and Princes Norah Oncology Center, King Abdulaziz Medical City, Jeddah, Saudi Arabia*

Correspondence:
Wasil Jastaniah
Email:wasilj@yahoo.com
jastaniahwa@ngha.med.sa
Tel: +966 2624 0000 ext 24082
Fax: +966 2624 0000 ext 2408

تحليل الخصائص الديموغرافية والسريرية مع دراسة متوسط نسبة الشفاء من مرض انتكاسة سرطان الدم النخاعي الحاد لدى الأطفال

واصل جستنية
استاذ مساعد , قسمم طب الاطفال
ص. ب. 9515- كلية الطب – جامعة أم القري – مكة المكرمة- المملكة العربية السعودية

الملخص العربي

الهدف:تحليل الخصائص الديموغرافية والخصائص السريرية و متوسط نسبة الشفاء من مرض انتكاسة سرطان الدم النخاعي الحاد لدى الأطفال الذين عولجوا في مركز الأميرة نورة للأورام بمدينة الملك عبدالعزيز الطبية، جدة، المملكة العربية السعودية.

الطرق:مراجعة استعادية على 86 طفلا تم تشخيصهم بسرطان الدم النخاعي الحاد حديثا وعلى التوالي من أغسطس 1986 إلى أبريل 2012، منهم 25 مريضا أصيب بانتكاسة وتأهل للدراسة الحالية.

النتائج:كان متوسط العمر عند التشخيص 3 سنوات (النطاق من 1 - 14) مع نسبة الذكور إلى الإناث (1.5:1) . وكانت الانتكاسة أكثر شيوعا في المرضى: الذين تتراوح أعمارهم بين 2 إلى 10 سنوات (58٪)، والذين تقل عدد كريات الدم البيضاء عندهم عن 100 (64%)، والذين يخلو سائل النخاع الشوكي عندهم من الخلايا السرطانية (84%)، وسرطان الدم النخلي ذو النمط الظاهري فصيلة M7 (24%). الانتكاسة النخاعية كانت الأكثر شيوعا بنسبة 80%, في حين كانت الانتكاسة في الجهاز العصبي المركزي بنسبة 4%. كان متوسط احتمالية البقاء على قيد الحياة لمدة 5 سنوات 12%. تباينت هذه النسبة اعتمادا على عمر المريض والشذوذ الكروموسومي في الخلايا السرطانية.

الخلاصه:المتغيرات الديموغرافية والسريرية تلعب دورا هاما في مرض انتكاسة سرطان الدم النخاعي الحاد لدى الأطفال. هذه الدراسة تثبت صعوبة شفاء انتكاسة مرض سرطان الدم النخاعي الحاد والحاجة الماسة لمزيد من الدراسات لاكتساب المزيد من المعرفة عن هذا المرض الصعب.

ABSTRACT

Objective: To analyze the demographic characteristics, clinical features and survival outcome of relapsed pediatric acute myeloid leukemia (AML) patients treated at the Princess Norah Oncology Center, King Abdulaziz Medical City, Jeddah, Saudi Arabia.

Methods: This is a retrospective review of 86 newly diagnosed children with AML treated consecutively from August 1986 to April 2012. A total of 25 relapsed patients qualified for the study.

Results: The median age at presentation was 3 years (range 1 - 14) with male to female ratio of 1.5:1. Relapse was more common in patients: aged 2 to 10 years (58%), with WBC less than $100x10^9$/L (64%), CSF negative (84%) and M7 phenotype (24%). Bone marrow was the most common site of relapse (80%) and isolated CNS relapse occurred in 4% of patients. The 5-year probability of post relapse overall survival (OS) was 12±6.5%. The OS varied significantly depending on patient's age (log-rank test, 7.072; *P = 0.029*) and cytogenetic risk group (*P = 0.019*).

Conclusion: Demographic and clinical variables play important role in relapsed pediatric AML. Our findings confirm that survival outcomes of relapsed pediatric AML were poor and there is a need for further studies to gain more insight on this challenging disease.

***Keywords*:** Relapsed AML, Pediatric, Demographic, Clinical Profile, Survival Outcome

INTRODUCTION

Over the past two decades, substantial improvements in the outcome for children diagnosed with acute myeloid leukemia (AML) have been made to reach as high as 70%[1]. The underlying reason for the success has been generally attributed to intensification in induction therapy and better supportive care [2-3]. Despite the current success, almost half of these children relapse and die from their disease [4]. Relapse remains the leading cause of treatment failure and accounts for about 40% of patients who achieved complete remission (CR) posing challenges to pediatric oncologists [5].

Even with the current risk assessment strategy, intensive frontline therapy and supportive care; treatment of relapsed pediatric AML patients remains unsatisfactory. Survival outcome following relapse is poor ranging from 10 to 33% [5-8]. Thus, there is a need to understand the underlying demographic factors, clinical profile and patterns of relapsed AML to gain further knowledge that may help improve outcome for these patients.

Studies addressing role of clinical and biological variables on survival outcome in relapsed pediatric AML are limited, and to our knowledge there are no published studies specifically addressing these issues in Saudi Arabia. The primary objective of the present study was to bridge this knowledge gap by describing the demographic, clinical profile and survival outcome of relapsed pediatric AML in a single institution, the Princess Norah Oncology Center, King Abdulaziz Medical City, Jeddah.

MATERIAL AND METHODS

Patients: This is a retrospective review of the records of 86 newly diagnosed children with AML treated consecutively between August 1986 and April 2012 at the Princess Norah Oncology Center, King Abdulaziz Medical City, Jeddah, Saudi Arabia (excluding AML-M3 patients). A total of 25 patients relapsed and qualified for the study. Data collected from hospital records include: age, gender, white blood cell count (WBC), cerebrospinal fluid (CSF), site of relapse, phenotype, cytogenetics, and the dates of diagnosis, relapse, last follow up, and death.

Chemotherapy Protocol

Patients with relapsed AML were treated with reinduction chemotherapy using two cylces of fludarabine, cytarabine and granulocyte colony-stimulating factor (G-CSF) followed by high dose cytarabine and l-asparaginase. Cycle 1 started with G-CSF (400 microgram/m^2) intravenously (IV) from day -1 until neutrophil count recovers to above 1 x 10^9/L, fludarabine (30 mg/m^2; maximum 50 mg) IV over 30 minutes daily for 5 days starting day 0 to day 4, and cytarabine (2000 mg/m^2) IV starting 4 hours after fludarabine and given as a 4 hour infusion daily for 5 days from day 0 to day 4. Cycle 2 started with G-CSF (400 microgram/m^2) IV from day 1 until neutrophil count recovers to above 1 x 10^9/L, fludarabine (30 mg/m^2; maximum 50 mg) IV over 30 minutes daily for 4 days starting day 0 to day 3, and cytarabine (2000 mg/m^2) IV starting 4 hours after fludarabine and given as a 4 hour infusion daily for 4 days from day 0 to day 3. Cycle 3 was given if the patient was not in remission or if hematopoietic stem cell transplant was delayed and consisted of cytarabine (3000 mg/m^2/dose) given IV over 3 hours every 12 hours on days 1, 2, 8, and 9 for a total of 8 doses, and l-asparaginase (6000 IU/m^2/dose) given intramuscularly 3 hours after the last cytarabine dose is completed on days 2 and 9.

Supportive care measures included pneumocysitis prophylaxis with spetrin, antifungal prophylaxias with fluconazole or itraconazole, irradiated blood products, antiemetic therapy using granisetrone, and steroid eye drops given every 6 hours beginning immediately before the first dose of cytarabine and continuing until 24 hours after the last dose. Patients who achieved remission following cycle 2 or 3 were referred for hematopoietic stem cell transplant if a match donor was available.

Definition of Risk Groups

Patients were stratified according to published cytogenetic risk profiles: low, intermediate and high risk groups[9]. High risk (HR) AML group was defined as patients with monosomy 7, monosomy 5, deletions of 5q, 3q rearrangements, complex cytogenetic abnormalities, and/or more than 5% blasts after the first course of chemotherapy, or patients with secondary AML. Low risk (LR) AML group was defined as patients with the favorable cytogenetic subtypes: inv(16) (p13q22) and t(8;21) (q22;q22). Intermediate risk (IR) AML group was defined as patients with all other aberrations not classified as low or high. Unknown risk (UR) AML group was defined to include patients with failed cytogenetics or were not done.

Statistical Methods

Relapse was defined as greater than 5% blasts in the bone marrow (BM) and/or the presence of 5% or more leukemic blasts per microliter in the CSF [10]. Time to treatment relapse (TTR) was calculated from the date of first remission until the date of relapse. Patients were categorized into three relapse groups: early, intermediate and late relapse depending on whether TTR was less than six months, 6 to 18 months or greater than 18 months. OS was measured from the time of diagnosis of first relapse to death from any cause or until the last follow-up date for surviving patients. OS was computed for the entire study population as well as for patients stratified by age group: less than 2 years, 2 to 10 years and greater than 10 years.

Descriptive statistics were used to describe demographic and clinical variables (gender, age, WBC, CSF, site of relapse and phenotype).The Fisher exact test was used to test for association between categorical variables. OS probabilities were calculated by the Kaplan-Meier method. The log-rank test was used to compare survival probabilities between age groups as well as between relapse categories. In all evaluations, *P*-values below 0.05 were considered significant. All statistical computations were performed using SPSS software package (SPSS Inc., Chicago Il. USA) and R software (version 2.13.2).

RESULTS

Demographic Characteristics

The diagnostic characteristics of relapsed patients are reported in Table 1. In total, 25 out of 86 (29%) pediatric AML patients experienced relapse between August 1986 and April 2012. The median age of these patients was 3 years (range 1 - 14) of which 15 (60%) were male and 10 (40%) female. Relapse by age group revealed that children aged 2 to 10 accounted for 56% of total relapse followed by children aged less than two years with 36% and children older than 10 years with 8%.

Of the 25 patients, 16 (64%) had WBC less than $100x10^9$/L and 6 (24%) greater or equal to$100x10^9$/L. The median leukocyte count was 36.6×10^9/L (range, 5.5 to 390×10^9/L). Patients with CSF negative status at diagnosis experienced more frequent relapse (84%) compared to patients with CSF positive results (16%).

Table 1. Characteristics of relapsed acute myeloid leukemia patients

Characteristic	No. (%)

Gender	
Male	15 (60.0)
Female	10 (40.0)
Age (years)	
<2	9 (36.0)
2-10	14 (56.0)
>10	2 (8.0)
WBC (x 10^9/L)	
< 100	16 (64.0)
≥ 100	6 (24.0)
Not available	3 (12.0)
CSF	
Positive	4 (16.0)
Negative	21 (84.0)
Site of Relapse	
Bone Marrow	20 (80.0)
CNS	1 (4.0)
Combined	3 (12.0)
Extramedullary	1 (4.0)
Phenotype	
M0	3 (12.0)
M1	2 (8.0)
M2	4 (16.0)
M4	3 (12.0)
M5	3 (12.0)
M7	6 (24.0)
Not available	4 (16.0)
Risk Groups	
Low	3 (12.0)
Intermediate	6 (24.0)
High	5 (20.0)
Unknown	11 (44.0)

White blood count (WBC), Cerebrospinal fluid (CSF)

Relapse by Risk Group

Risk based outcome revealed that 20% (5/25) of relapses occurred in HR: monosomy 7, abn(3q), and complex cytogenetics (one each); 24% (6/25) in IR: normal cytogenetics (n = 3) and other translocation (n = 3); and 12% (3/25) in LR: t(8;21) (q22;q22). None of the patients in the relapse group had inv(16) (p13q22). Cytogenetics were unknown in 11 patients.

Relapse by Phenotype

Relapse incidence grouped by French-American-British (FAB) classification for childhood AML showed 6 patients (24%) with subtype M7 experienced relapse followed by 4 patients (16%) with FAB subtype M2. Patients with FAB subtype M0, M4 and M5 each accounted for 12% (3/25) of relapse while FAB M1 had only 8% (2/25).

Site of Relapse

In the present study, BM relapse occurred in 20 children and accounted for 80% of relapse. Combined (BM+CNS) relapse occurred in 3/25 (12%), isolated CNS relapse in 1/25 (4%) and extramedullary in 1/25 (4%) patients. CNS relapse (isolated or combined) occurred in 4/25 (16%) children. The CSF status at diagnosis was negative in 3/4 (75%) patients and positive in one patient. The patient with positive CSF had simultaneously experienced a combined relapse.

Mortality

The overall mortality rate in the present study was 88% (22/25). Table 2 depicts factors associated with mortality. Mortality by risk group was statistically significant (P = 0.019). The mortality rate was 100%, 83%, and 33% for HR, IR, and LR group patients; respectively. The median TTR for the 25 patients was 7 months (range: 2 to 36 months). However, 11 of these patients had early relapse, 9 intermediate relapse and 5 late relapse. Early relapse patients had high incidence of death 91% (10/11) followed by intermediate 89% (8/9) and late relapse 80% (4/5). Similarly, all patients with phenotype M0, M5 and M7 had 100% mortality rate while M1, M2 and M4 had 50%, 75% and 67%; respectively (P = 0.314). Age, gender, WBC, CSF, and site of relapse, did not influence mortality with P values: 0.425, 0.250, 0.532, 1.00, and 0.257, respectively.

Table 2. Factors associated with mortality in relapsed acute myeloid leukemia

Factors	Alive	Dead

	n (%)	n (%)	*P-Values*
Age (median; range)	2.5 years; (1:10)	3.0 years; (1:14)	
Gender			
Male	3 (20%)	12 (80%)	0.250
Female	0 (0%)	10 (100%)	
WBC (median; range)	118 x 10^9/L (13.7:390)	32.6 x 10^9/L (5.5:360)	
CSF			
Positive	0 (0%)	4 (100%)	1.000
Negative	3 (14%)	18 (86%)	
Site of Relapse			
BM	2 (10%)	18 (90%)	0.257
CNS	1 (100%)	0 (0%)	
Combined	0 (0%)	3 (100%)	
Extramedullary	0 (0%)	1 (100%)	
TTR			
Early	1 (9%)	10 (91%)	1.000
Intermediate	1 (11%)	8 (89%)	
Late	1 (20%)	4 (80%)	
FAB:			
M0	0 (0%)	3 (100%)	0.301
M1	1 (50%)	1 (50%)	
M2	1 (25%)	3 (75%)	
M4	1 (33%)	2 (67%)	
M5	0 (0%)	3 (100%)	
M7	0 (0%)	6 (100%)	
Risk group			
HR	0 (0%)	5 (100%)	0.019
IR	1 (17%)	5 (83%)	
LR	2 (67%)	1 (33%)	
UR	0 (0%)	11 (100%)	

White blood count (WBC), cerebrospinal fluid (CSF), bone marrow (BM), central nervous system (CNS), time to relapse (TTR), French-American-British (FAB), high risk (HR), intermediate risk (IR), low risk (LR), unknown risk (UR).

Survival Outcome

The median post relapse survival was 3 months (range: 0 to 140 months). The 5-year probability of post relapse OS rate for all patients was 12%±6.5% as shown in (Figure 1). The OS by age-groups was statistically significant (log-rank test, 7.072; P = 0.029) as shown in (Figure 2). The OS for children less than 2 years was 11.1%±10.5% compared to 21.4%±11%

and 50%±35.4% for patients aged 2 to 10 years and for patients older than 10 years; respectively. The OS for early, intermediate and late relapsed patients were 9±9%, 11±10% and 20±18%; respectively (Figure 3). However, these OS rates were not statistically significant (log-rank test, 1.074; P = 0.584). Similarly, the 5 year OS by risk groups was 66.7%±27.2% for LR and 16.7%±15.2% for IR, however, the OS for HR and UR group patients was not achieved (log-rank test, 3.778; P = 0.286). Survival outcome by phenotype was not statistically different (log-rank test, 10.663; P = 0.099).

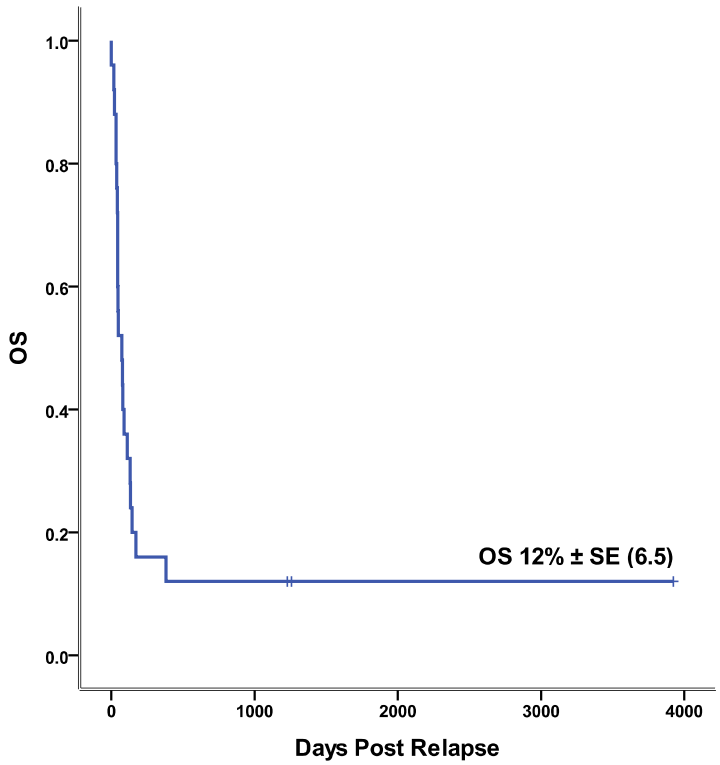

Figure 1: Five-year overall survival (OS) of relapsed pediatric acute myeloid leukemia.

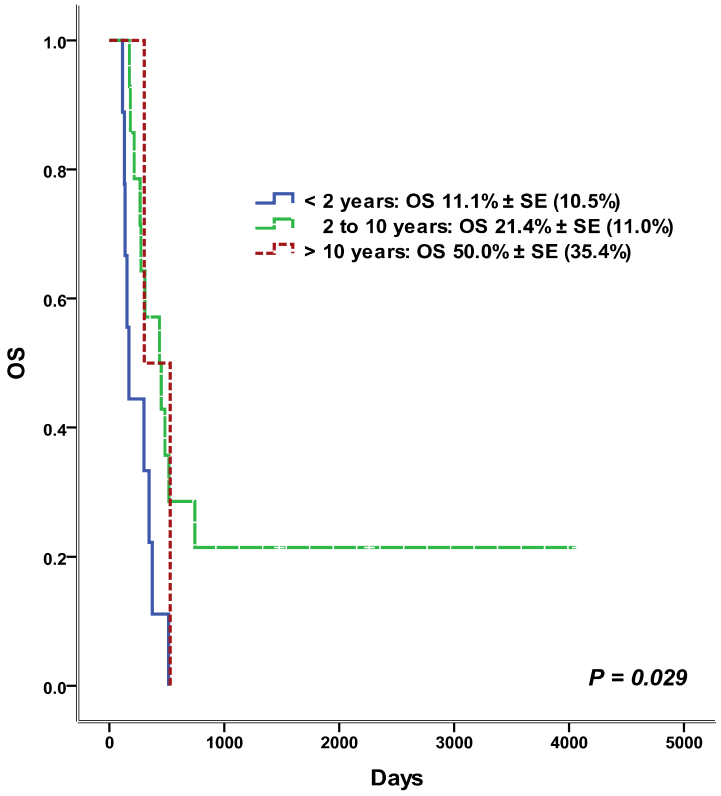

Figure 2: Overall Survival (OS) by age group.

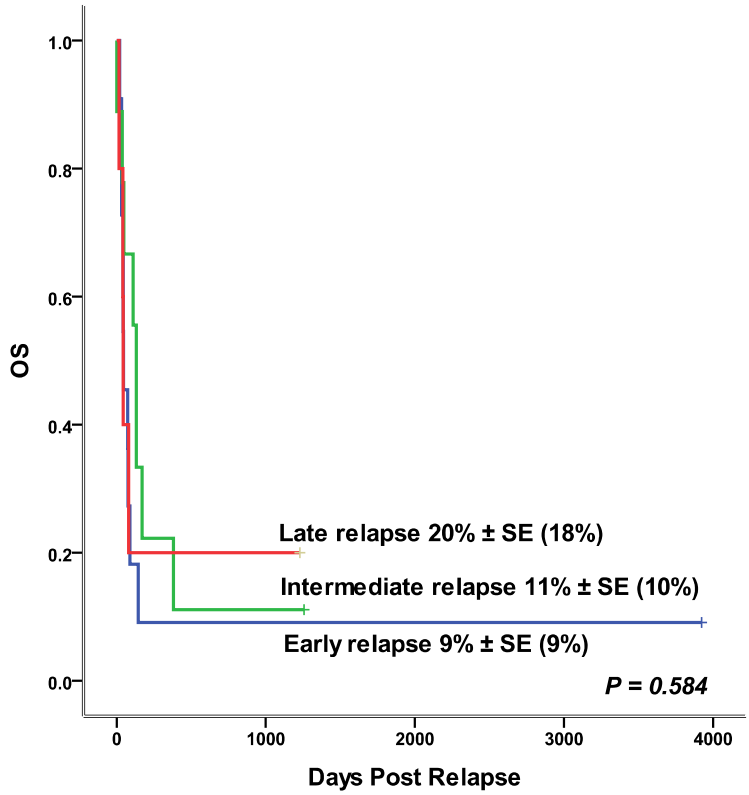

Figure 3: Overall Survival by time to relapse

DISCUSSION

Identifying factors that are related to poor outcome in relapsed pediatric AML patients is an effort to optimize treatment of these patients. The present study revealed demographic and clinical variability are associated with outcome of relapsed patients. The pattern of association that emerged from the analysis may help gain further understanding about the dynamics of relapse as well as recognizing patients who are at higher risk of treatment failure or may benefit from experimental therapies.

Demographic factors including gender and age have been associated with outcome in pediatric patients with AML[11]. The present study evaluated these variables in the context of relapsed AML patients and found that age at diagnosis did not influence risk of relapse but was found to be predictive of poor outcome. In particular, patients younger than 2 years of age had an inferior OS compared to other age groups.

In our study population, female patients were less likely to experience relapse compared to males and there was no statistically significant difference in mortality rates by gender. Despite this, the literature indicates that female patients have slight better outcome than males, however, this association was not strong enough to be included in therapeutic stratification[11]. The finding of the present study highlights the weakness of this association further.

Factors such as high WBC count (> 100,000 cells/ml), CNS disease at diagnosis and site of relapse have been associated with unfavorable outcome in pediatric AML patients [11-13]. The present study explored if these variables were associated with the incidence of relapse and did not find association between higher WBC at diagnosis and the risk of experiencing relapse.

Despite a high CSF positive rate at diagnosis, the most common site of relapse was the BM with an isolated CNS relapse rate of 4%. This finding is in line with the incidence of isolated CNS relapse range of 2% to 8.8% reported in the literature[14]. The incidence of any CNS relapse (isolated or combined) in our study was 16%. However, CSF positivity did not influence the risk of CNS relapse.

The present study revealed that Saudi Arabian pediatric AML patients with M7 phenotype were at higher risk of experiencing incidence of relapse. In comparison, patients with FAB subtype M1 had lower risk. Despite this, the mortality rates by phenotype were not significantly different.

Risk group using cytogenetics is considered as one of the most important significant prognostic factors in newly diagnosed AML[11]. Risk-based classification could have favorable and unfavorable outcomes in newly diagnosed AML. The present study evaluated the implication of risk-based classification with outcome in relapsed AML patients. It is worth noting that relapsed patients in LR group had a relatively low rate of relapse and a significantly lower mortality rate. This finding is indicative that risk-based stratification remains a significant prognostic factor in both relapsed and newly diagnosed AML patients.

In the present study, the OS of pediatric patients with relapsed AML remained poor. This finding is consistent with the outcomes reported in the literature[5-8]. However, post relapse OS rate was higher for late relapse patients followed by intermediate and early relapse. TTR has been well documented as a risk factor in pediatric acute lymphoblastic leukemia (ALL) and

its prognostic significance in influencing post relapse outcome is well established[2,10-12]. It is now possible to stratify relapsed ALL patients into different risk categories that would benefit from different treatment approaches. However, studies on the prognostic significance of TTR in pediatric AML are limited[2,6,10].

The present study was unable to demonstrate the prognostic significance of TTR in pediatric AML. However, given the retrospective design and the relatively small patient population of the study, the importance of TTR in determining the outcome of childhood AML cannot be ruled out. This highlights the need for further studies on prognostic variables that would help target improvement in therapeutic approach to this challenging disease.

CONCLUSIONS

Demographic, clinical and biological variables exist in relapsed pediatric AML and associated with important prognostic implications. The present study was able to demonstrate that the OS rate of relapsed pediatric AML treated in our institution is poor and within the range reported in the literature. In addition, age at diagnosis and risk group stratification was identified as factors significantly impacting survival in the present study. These findings are of potential relevance in individual treatment decisions as well as in improving the overall outcome of relapsed AML patients with poor-risk disease using risk-adapted treatment strategies.

The findings of the present single center study may not be generalized to all relapsed AML children in Saudi Arabia. However, conducting further multicenter studies that examine the significance of demographic factors, clinical characteristics and time to relapse may provide broader understanding of relapsed pediatric AML patients in the Kingdom. This in turn may improve the approach and management of this challenging disease.

ACNOWLEGMENT

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Case Report

Recurrent Giant Paratesticular Myxomatous Neoplasm

Anmar Nassir. MD, FRCS(C), *Assistant Prof. of Urology, Um Al-Qura University, Consultant urologist, King Abdullah Medical Center, Makkah, Saudi Arabia*

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Correspondence:
Dr. Anmar Nassir
E-mail: Amnassir@uqu.edu.sa
Fax: 966-2-6744321

الورم المخاطي العملاق ا لجارخصوى المتكرر

د.انمار محمد ناصر- استاذ مساعد المسالك البولية –كلية الطب جامعة ام القرى- مكة المكرمة-استشاري – مدينة الملك عبدالله الطبية بمكة المكرمة.

الملخص العربي:

ان المنطقة الجار خصوية من المناطق المعقدة تشريحيا وخلويا لذا نجد ان الاورام الناشئة منها قد تكون حميدة او عدوانية للانسجة المجاورة او حتى خبيثة . هنا نسجل نص سريري مفصل ومتابعة علاجية لحالة نادرة لورم مخاطي عملاق جارخصوى مع مناقشة التشريح المرضي النسيجي المثير للجد ل . لقد قدمنا حالة ورم جارخصوى مخاطي مع الوصف للتاريخ السريرية المرضي ، والنتائج على ضوء الفحص المجهري والمناعية، والمرضية المحتملة. على الرغم من ان الورم الجارخصوى المخاطي آفة نادرة، الا انه ينبغي النظر في ذلك في التشخيص التفريقي للأورام في هذه المنطقة .

ABSTRACT

The paratesticular region is complex in both anatomical and histogenetically. eoplasms arising from this region therefore might be benign, locally aggressive or even malignant. We present a 20 year old male case of giant paratesticular myxomatous tumor and describing clinical history, findings on light microscopy and immunohistochemistry, and possible pathogenesis and the controversial histopathological features of a rare giant paratesticular myxomatous neoplasm. Although primary paratesticular myxoma is a rare lesion, it should be considered in the differential diagnosis of intrascrotal mesenchymal tumors. The treatment of choice is radical inguinal orchidectomy and wide tumor excision with safe margins. Adjuvant therapy probably not necessary but long follow up is essential.

Keywords: Myxoid neoplasm, Myxomatous neoplasm, Myxolipoma, Spindle cell lipoma, Myxofibrosarcoma, Aggresive angiomyxoma, paratesticular tumor.

INTRODUCTION

The paratesticular region is complex anatomical area which includes the contents of the spermatic cord, testicular tunics, epididymis and vestigial remnants [1,2]. Histogenetically this area is composed of a variety of epithelial, mesothelial and mesenchymal elements. Neoplasms arising from this region therefore form a heterogeneous group of tumors with different behavioral patterns [3]. It may harbor rare varieties ranging from pure benign to locally aggressive lesions. Although malignant tumors are rare, their involvement in the differential diagnosis renders them clinically important [4]. Paratesticular myxomatous tumors are rare tumors that may arised in this area ,it occurs in middle aged men[5]. These tumors are prone to local recurrence following inadequate extirpation, even with adjuvant radiotherapy [6]. We report a rare entity of giant paratesticular myxomatous neoplasm which created some histopathological controversies. Initially mislabeled as an aggressive angiomyxoma. Then the possibility of myxofibrosarcoma was raised. Lastly, two soft tissue experts supported the view of angiomyofibroblastoma-like tumor, with final impression of myxomatous neoplasm with features of myxolipoma and spindle cell lipoma. The clinical scenario, work up, management, follow up and the differences in histopathological features are presented in this report.

CASE

A 20 year old male patient presented to us complaining of gradual and significant enlargement of his left scrotum associated with dragging dull ache pain. One and half year prior to presentation, he had undergone repeated partial excision of a left scrotal mass in a different hospital where he first presented as a case of hydrocele or/and hernia. According to the histopathological report from that hospital, diagnosis was “an aggressive angiomyxoma”. On physical examination he had a huge left inguino-scrotal soft mass measuring around 20 x 17 x 5 cm., not reducible or compressible. No lymph nodes were felt in inguinal or adjacent regions. MRI and CT scan (Fig. 1) showed no deeper lesions. The patient underwent left radical orchidectomy and wide inguinoscrotal mass excision (Fig. 2) with safe margins. Grossly, on slicing, the tumor contained mucoid and gelatinous material. Microscopically, general sections showed a hypocellularity with focal increase of cellularity and cytological atypia. The tumor appeared to be quite infiltrative of the soft tissue. The tumor cells showed mild pleomorphism although focally. Occasional large tumor cells were seen with irregular or multilobulated, hyperchromatic nuclei. Mitoses were not seen. (Fig. 3) The tumor cells were positive for vimentin, CD34 and for Bcl2. They were negative for SMA, MSA, desmin and for S100 protein. An impression of myxofibrosarcoma was raised as a possibility. The slides were reviewed at by two other international soft tissue experts who gave the impression of myxomatous neoplasm with features of myxolipoma / spindle cell lipoma. A possibility of angiomyofibroblastoma-like tumor was also considered. Due to the histopathological report of multiple projections and the adhesions from previous surgery, the patient was referred for radiation therapy to prevent possible recurrence. The patient refused this plan. Three years later, on follow up, no local recurrence was seen. This would favor the myxomatous neoplasm.

DISCUSSION

Benign and malignant tumors of the paratesticular tissues present an interesting spectrum of diagnostic entities often encountered in routine surgical pathology practice. Despite of the infrequent,it has been estimated that 70% of paratesticular tumours are benign and 30% are malignant. Although it is often difficult to determine with certainty the exact site of origin of paratesticular tumors, it is thought that the spermatic cord is the most common, accounting for 75- 90% of which 75-80% are primarily comprised of lipomas, with nearly all malignant counterparts being sarcomas [2,7]. There has been substantial research in the paratesticular soft tissue pathology with increasingly support by cytogenetics and molecular genetics, making it more logical, reproducible and substantially less confusing than it has ever been. As a consequence, the major beneficiaries are patients. Without question there will be continuing advances and conceptual shifts, as in any other area of pathology, and any future WHO classification will undoubtedly bring further improvement [8,9,10,11.]

In our case, at one stage, aggressive angiomyxoma was thought to be the diagnosis. This tumor is a rare but well described neoplasm. It was recognized as a separate histopathological entity in 1983. This is a distinctive tumour with a characteristic clinical course and specific gross and microscopic features. It has been reported in men, but mostly reported in women. In men it occurs over a wide variety of sites around the genital tract such as perineum, perianal region, scrotum, spermatic cord, inguinal region, and pelvic soft tissues.The final diagnosis is usually provided by the pathologist. Aggressive angiomyxoma occurs in ages ranging from 15 to 63 years. It is characteristically a slow growing, focally infiltrative tumour, lacking a capsule. Histologically, the tumour shows vascular channels of varying sizes distributed haphazardly in the myxoid stroma. Nuclear atypia and mitosis are not present. Although occasionally well circumscribed, they are usually not encapsulated and microscopically infiltrative. It is a locally aggressive but non-metastasising neoplasm. After excision, a significant rate of local recurrence of 20% is reported [11.]

On the other hand, myxofibrosarcoma is a myxoid subcategory of malignant fibrous histiocytoma, which is, in general, the most common soft tissue sarcoma of late adulthood (50-70 yrs) and second most common sarcoma of the retroperitoneum. Mostly a tumor of soft tissues, with 20% found in the abdomen and retroperitoneum. The Myxoid subtype represents 15% of this whole group. Composed of loose myxoid stroma which constitutes 50% of the tumor mass, interspersed with areas of higher cellularity, abundant plexiform vascular network, scattered giant cells. Metastases present at diagnosis in 25%. Five year survival is more than 50%.[12]. Angiomyofibroblastoma, shows a predilection for the vulvovaginal region during the reproductive years but may also occur in the inguino-scrotal region in men and is distinguished from aggressive angiomyxoma. Angiomyofibroblastoma is a recently described benign tumor in the scrotum and inguinal region of men. Microscopically it has high cellularity and vascularity. Unless there is a sarcomatous transformation, they behave benignly and are adequately treated by local excision [2,8,12.]

Myxolipoma is a very old term reported since 1857 in many different parts of the body but rarely reported in the scrotum [13]. Spindle cell lipoma is characteristically located at the upper trunk or rarely at the lower limbs. It shows microscopally a mixture of mature lipocytes and uniform, primitive, bland, S-100 negative spindle cells in a mucinous and fibrous background with frequent mast cells. It can be distinguished from myxoid liposarcoma by the thick collagen bundles, the absence of lipoblasts and a plexiform vascular pattern. No matter what are the underlining histological subtypes, the clinical presentation is mostly a mass or

swelling, which might be painless or painful and is occasionally accompanied by a hydrocele. These findings are by no means specific to a tumor type and it cannot distinguish a benign from a malignant tumor. High-resolution ultrasonography of the scrotum provides evidence of the location, extent, and relationship of intrascrotal masses with the testis, epididymis, or spermatic cord; the echogenicity and the internal echo patterns obtained allow cystic, fluid-filled lesions to be distinguished from solid ones. However, the technique cannot be used to discriminate between benign and malignant lesions. Unlike sonography, CT Scan can furnish densitometric evidence of tumor texture. MRI may locate the tumor better and define its relationship to various paratesticular structures in greater detail, which is not always possible with ultrasonography[14]. Careful histological assessment with the knowledge of the various pathological subtypes is crucial to formulating adequate treatment and predicting clinical outcome. All benign tumors of the paratesticular region are amenable to adequate surgical resection, but a close follow-up for some of these tumors has been recommended, because of occasional recurrences and rare malignant transformation, or even misdiagnosis for a rare unusual locally aggressive pathology.

CONCLUSION

Myxomatous neoplasm of the spermatic cord is a very rare tumor that can present during adulthood as a painless scrotal or inguinal mass that can be misdiagnosed as inguinal hernia, hydrocele or spermatocele. The new case that we present is an example of how similar tumor can be misleading to both surgeon as well as to general pathologist. Imaging can be useful, although definite diagnosis cannot be made without histological examination.Yet Careful histological assessment with the knowledge of the various pathological subtypes is crucial to formulating adequate treatment and predicting clinical outcome. The treatment of choice is radical inguinal orchidectomy and wide tumor excision with safe margins. However, conservative limited tumor excision has the risk of recurrence. Surgery seems also enough for local recurrences. Adjuvant therapy is probably not necessary since paratesticular myxomatous neoplasm have very good prognoses if completely excised and life-long follow up is accessible. The present case, which to date has had a follow-up of only medium duration, is an example of the above approach.

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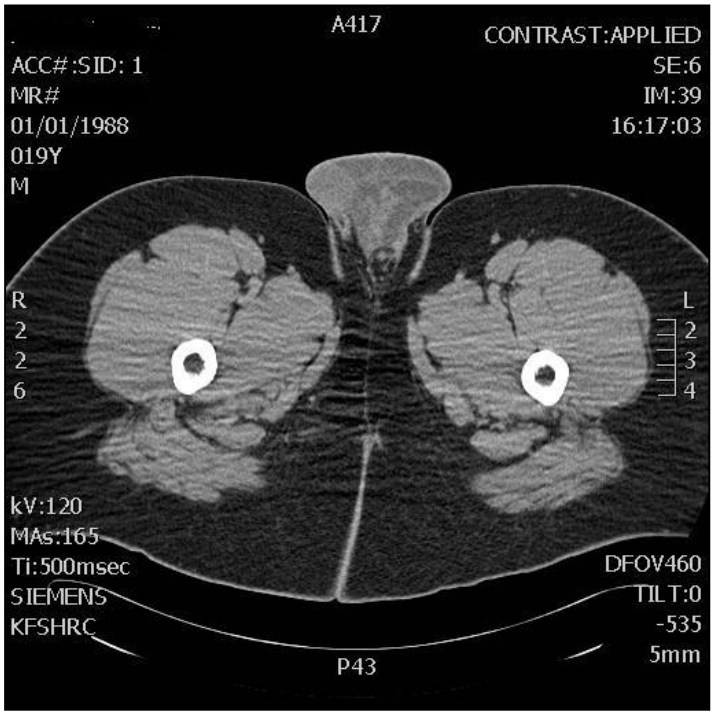
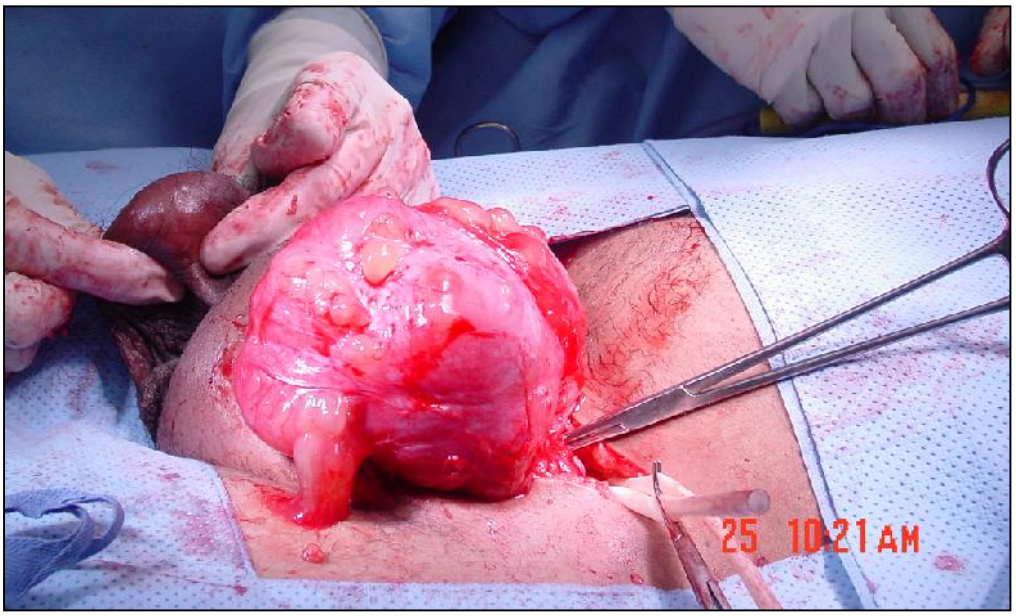
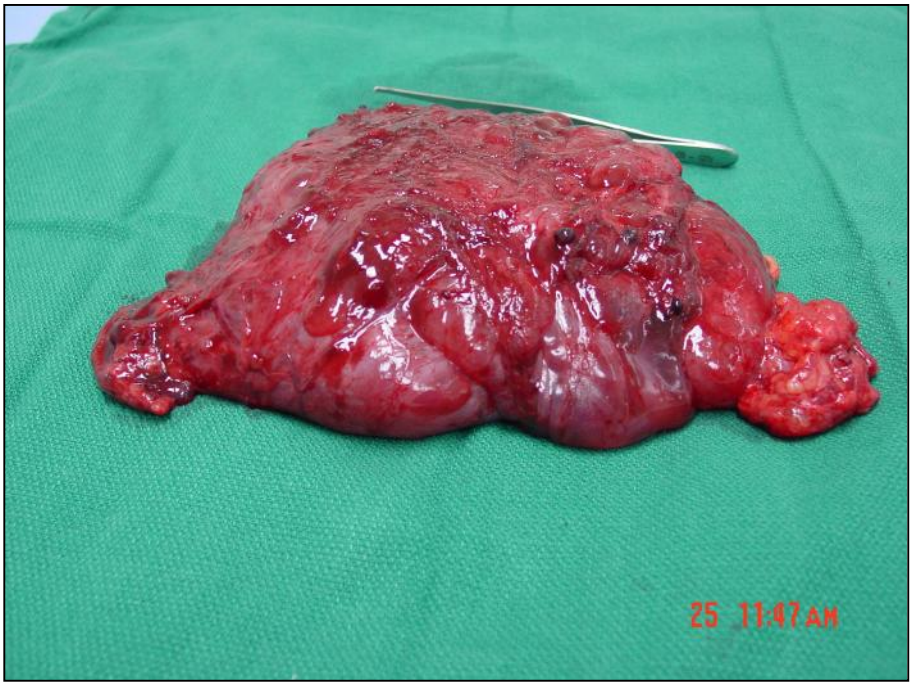
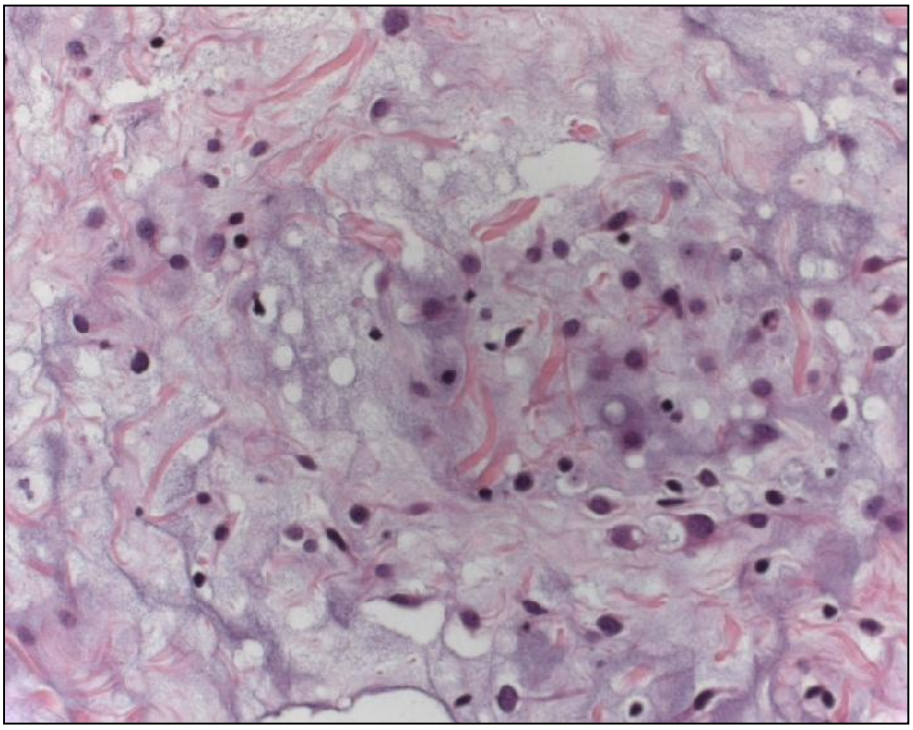


(Figure-1)
Figure 1. CT of the abdomen.



(Figure- 2)
Figure 2. gross pathology photograph of the tumor.





(Figure-3)
Figure 3. Microscopic photograph of the tumor

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