nonsense mutation. The mutation was predicted to result in an E-cadherin peptide lacking both the transmembrane and cytoplasmic domains. Same authors [35] described a family in which multiple members with gastric cancer were heterozygous for the insertion of an additional C residue in a run of 5 cytosines at positions 2382 to 2386. The resulting frameshift led to an E-cadherin molecule lacking about half of its cytoplasmic domain. In another diffuse gastric cancer family, a heterozygous G-to-T transversion at nucleotide 70 in exon 2 of the CDH1 gene, was also identified. The mutation converted a glutamic acid (glu24) to a TAG stop codon in the signal peptide of the E-cadherin precursor protein.

Richards *et al*. [41] identified a splice acceptor site mutation, an A-to-G transition at position -2 from nucleotide 49 at the start of exon 2 of the CDH1 gene and also a germline G-to-A transition at nucleotide 59 in exon 2. The mutation, a trp20-to-ter substitution was predicted to truncate the E-cadherin gene product in the signal peptide domain, which is cleaved from the N terminus of the mature protein.

In a family segregating diffuse gastric cancer, Gayther *et al*. [42] found a 2095C-T transition in the CDH1 gene, resulting in a truncating mutation, arg598 to ter.

Same authors [42] found a 1-bp insertion in the CDH1 gene in the proband of a family with familial diffuse gastric cancer. Insertion of a G after nucleotide 1711 created a frameshift that would truncate the protein at codon 587. Another 1-bp insertion (C) at nucleotide 1588 in exon 11 was also identified in a family segregating diffuse gastric cancer.

Mutations were also found in other types of carcinoma. In an endometrial carcinoma, Risinger *et al*. [44] identified a GCA-to-ACA transition in codon 617 predicting a substitution of thr for ala in the E-cadherin molecule. Somatic loss of heterozygosity was identified in the tumor tissue. They also identified a CTG-to-GTG transversion resulting in a leu711-to-val amino acid substitution in E-cadherin. The wild type allele was not lost.

In an ovarian carcinoma, same authors [44] identified an AGC-to-GGC transition in codon 838 resulting in substitution of glycine for serine. The tumor tissue also showed somatic loss of heterozygosity. In an infiltrative lobular breast carcinoma, Berx et al. [1] found a GAA (glu)-to-TAA (stop) nonsense mutation in the CDH1 gene. Tumor-specific loss of heterozygosity of chromosomal region 16q22.1 was demonstrated in this case. Here only selected examples of CDH1 mutations in carcinoma tissue have been assessed. The number of mutations is growing each day .

Pećina-Šlaus *et al*. [45] indicated yet another type of genomic instability of CDH1 gene encountered in tumor tissue. When searching for allelic loss i. e. Loss of Heterozygosity (LOH) of the CDH1 gene in clear cell renal cell carcinoma, they came across Replication ERror (RER) positive samples, an instability problem [46] that characterizes tumor development and progression. Replication/repair machinery seems to be targeted in 10% of clear cell renal cell carcinoma sample.

Conclusions

Reduced expression of E-cadherin is regarded as one of the main molecular events involved in dysfunction of the cell-cell adhesion system, triggering cancer invasion and metastasis. Therefore, E-cadherin is an important tumor suppressor gene. Research on E-cadherin has elucidated insights into both embryogenesis and oncogenesis. One of the most crucial exhibit of E-cadherin's function in development is the controlled epithelial - mesenchymal conversion.

The involvement of E-cadherin in wnt signaling, indicates that same molecule may have different functions and that E-cadherin can regulate cellular response generated by external signals the cell receives. In this way it can regulate migration, proliferation, apoptosis and cell differentiation.

The method of blocking E-cadherin downregulation in tumors is one of the important future approaches in gene therapy. To target this molecule is the logical path to prevent metastazing potential of almost any epithelial tumor. Nevertheless, it will not be an easy enterprise since its downregulation is caused by many different mechanisms, ranging from mutations and gross deletions to repression of gene transcription, as well as signal transduction stimulation of E-cadherin adhesion complex formation.

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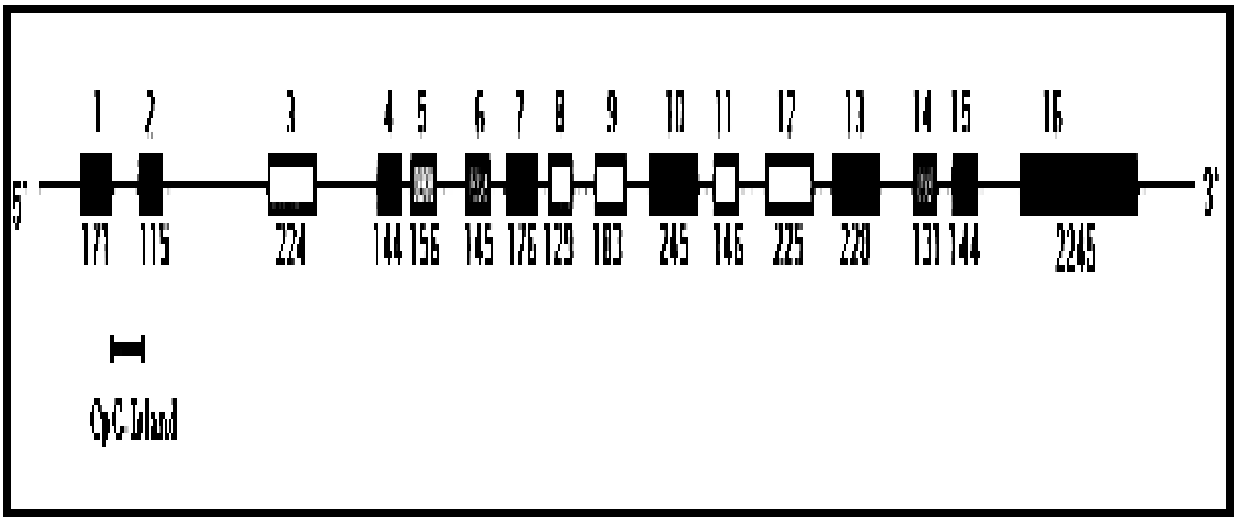

Fig1. Genomic organization of the human E-cadherin gene.

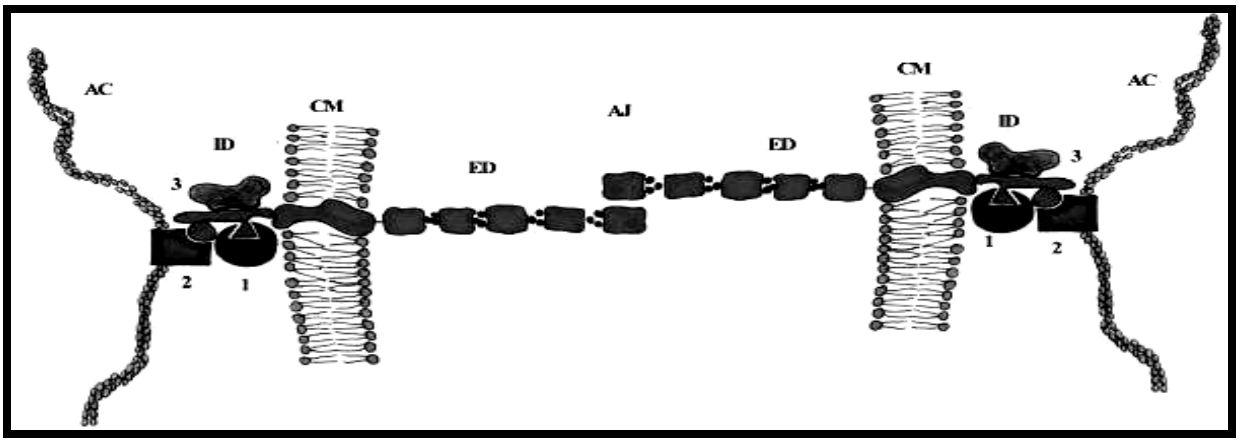

Fig 2 Shematic illustration of E-cadherin in adherens junction

Surgical Treatment of Penile Fracture

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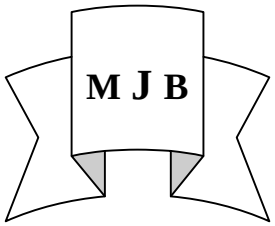

Abstract

This study was done in order to evaluate surgical correction of penile fracture, between 1996-2003, twenty patients [25-54] years old were studied after blunt trauma to erect penis the time between injury and presentation [2-24] hours, mechanism of injury were vaginal intercourse, masturbation and rolling on bed at early morning on erected penis.

All patients underwent surgical correction as emergency condition by degloving of skin of the penis with suturing of injured corpus.

All injury were unilateral corporeal,no urethral injury follow up of the patients show adequate erection in all cases with no curvature.

Early surgical correction of rupture corporeal injury give good result with nearly no complications.

الخلاصة

تمت دراسة كسر القضيب في المرضى الذين يتعرضون لشدة خارجية على القضيب تضمنت الدراسة عشرين مريضا تتراوح اعمارهم من 25 سنة الى 54 سنة بعد المواقعة الجنسية او بعد عملية الاستمناء او النوم على القضيب في حالة انتصاب.و قد اجريت لجميعهم عملية خياطة جرح الاعصاب الانتفاضية كحالة طارئة بعد تعرضهم للحادث مباشرة خلال فترة تتراوح بين ساعتين و 24 ساعة من الحادث كانت جميع النتائج ايجابية و لم تسجل مضاعفات كبيرة و مهمة.

Introduction

Penile Fracture is rupture of the tumescent corpora cavernosa due to non physiological bending of the penile shaft[1].The most common etiology is vaginal intercourse but it may occur after manipulation rolling over onto erect penis[1,3].Patients present with pain at shaft of penis with swelling and ecchymosis[2,3].

Available treatment were splinting,compresses and combined anti-inflammatory antifibrinolytic and analgesic treatment[2,4,5-9],but long term follow up of conservative treatment show significant complications such as curved or painful erection,fibrotic plaque affect erection arteriovenous fistula,infection and impotence[1,6-7].

Most recent studies advocate the immediate surgical repair with excellent initial and long term result,including a decrease in the complications associated with conservative treatment as well as decrease in hospital stay[8-15].

Patients and Methods

Between 1996-2003 , 20 patients their age range betwwen 24-54 years old at presentation were evaluated after history of suspected

rupture penis following blunt trauma to erected penis.

Interval between injury to time of presentation were 2-24 hours.Mechanism of injury were vaginal intercourse in 10 patients,masturbation in 5 patients,and rolling on erected penis in another 5 patients.The first 10 patients were married while others were not.

All patients were complained of severe pain at the penis with swelling and ecchymosis depend on severity of injury, on examination the penis swollen,,tender on examination.

All patients were voided normally.

All patients admitted to hospital as emergency condition and the condition were explained to the patient with possible complications.

Under general anasthesia ,the penile skin degloved completely by circumferential incision below coronal sulcus,the site of injury identified ,clot evacuated irrigated with saline to identify extent of injury the suture with absorpable suture usually chromic catgut [2-0].

All these were done after insertion of Foleys catheter and remained for 5 days post operatively.

Post operative care by antibiotic "broad spectrum",analgesia,dressing and catheter removed after 5 days ,patient usually discharged from hospital within 48hours.

Follow up of the patients between 6 months and one year by questioneir and phisical examination.

Results

Surgical exploration showed corporeal injury in all cases of ours patients, 12 patients had left corpus injury and 8 patients had right corpus , 15 patients had injury in ventral side and 5 patients had lateral side, 14 patients had injury at midshaft, 5 patients had distal shaft and only one patient had at base of the shaft.

The causes of injury were vaginal intercourse in 10 cases,masturbation in 5 cases and rolling on erected penis in other 5 cases.

Table 1 Causes of Fracture Penis

Cause	No. of Patients
Vaginal intercourse	10
Masturbation	5
Rolling on erected Penis	5

Table 2 Site of Injury

Site	No. of Patients
Ventral	15
Lateral	5

Table 3 Side of Injury

Side	No. of Patients
Left	12
Right	8

Discussion

The diagnosis of penile fracture is usually based on typical clinical feature and history with corporeal disruption only confirmed at exploration[3,8,10].This role was adopted by us we depend on history and clinical examination and diagnosis confirmed by surgical exploration which was true in all cases explored.

The classical presentation of penile fracture lend to its case of diagnosis.

Cavernosography,Sonography and Magnetic Resonance Imaging for evaluating these patients contribute little to over all assessment[16-18].

There have been isolated reports in the literature of dorsal vein and artery rupture mimicking penile fracture at presentation.

In our patients no case of dorsal vein or artery rupture were reported ,but all cases corporeal injury were reported[17,18,20].

Reports of penile fracture on non operative management,the reported complication rate after conservative treatment is 10 % to 53%[2,9,21].

In our patient the follow up time between 6 months and one year show no complications were reported this explained that all patients were identified early ands submitted to rapid surgical exploration and suturing the injured area.

The most common etiological factor that cause fracture penis were vaginal intercourse[1-3] this is also true in our patient, 10 patients had history of vaginal intercourse which account of 50% of our patients.

Conclusion

Early identification and rapid surgical correction of corporeal injury as a cause of penile fracture give good result with few or no complicatio

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Cervical Lymphadenopathy in Children . A Pathological Study in Mosul

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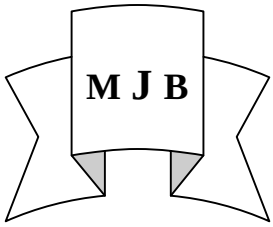

Abstract

Cervical lymph node are the most frequently enlarged and biopsies of all peripheral lymph node affecting all age groups including children.

The aim of the study was to find the main cause of cervical lymphodenopathy in children and to compare the result with other studies.

This study was conducted in Alzahrawi Teaching Hospital Ands three private laboratories in Mosul over 10 years period 1993-2002 which included two hundred fourteen cases. Clinical data including the age and sex in addition to the histological study of the slides were reviewed .

The cervical lymphadenopathy showed distribution of [p46, 72 and 96] within the three childhood quinquennia respectively. The male : female ratio was 1.3:1. The most common histological findings were reactive hyperplasia [51.4] followed by tuberculosis [28.5%] and lymphoma [17.3%] One out of each five excised lymph node show malignant changes..

In developing countries about ½ of the cervical lymph nodes examined show significant and clinically important pathologies, there by justifying an active management of all pediatric patients with persistent CLA.

الخلاصة

إن العقد اللمفاوية العنقية هي أكثر احتمالية لإصابتها بالتضخم وخضوعها للخزع النسيجية من باقي العقد اللمفاوية المنتشرة في أنحاء الجسم في جميع المراحل العمرية وخاصة عند الأطفال.

وكان الهدف من هذه الدراسة الرجعية لمعرفة الأسباب الرئيسية لتضخم العقد اللمفاوية العنقية عند الأطفال ومقارنة النتـــائج بمثيلاتها في الدراسات الأخرى.

شملت الدراسة جميع حالات تضخم العقد اللمفاوية عند الأطفال والمشخصة في مستشفى ألزهراوي وثلاث مختبرات أهلية في مدينة الموصل خلال عشر سنوات للفترة بين 1993-2002 0 وضحت الدراسة المعلومات السريرية كالعمر والجـــنس إضـــافة إلـــى التشخيص النسيجي.

شملت الدراسة مائتان وأربعة عشر حالة موزعة على خماسيات العمر الثلاثة في الطفولة [46، 72، 96] على الترتيب، وكانت نـــسبة الذكور إلى الإناث: 1.3:1وأكثر الحالات المشخصة هي الالتهابات الغير محددة للغدد اللمفاوية [51.4] يعقبها التدرن [28.5] واللمفوما [17.3%].

في الأقطار النامية حوالي نصف حالات تضخم العقد اللمفاوية العنقية أظهرت تشخيصاً نسيجياً مهما يستدعي أخذ خزع نسيجية من العقد اللمفاوية. وأن أكثر الحالات شيوعاً هي الالتهاب الغير محدد للعقد اللمفاوية يعقبه التدرن وثم اللمفوما.

مفتاح ألكلمات:تضخم ألعقدألللمفاوية ،ألاطفال، ألتكرار،تدرن ألعقد أللمفاوية .

Introduction

Cervical lymphadenopathy [CLA] is a common clinical presentation due to a variety of pathological conditions in response to autoimmune disease and immunization [1,2]. Most lymph nodes are not palpable in the newborns, with subsequent antigenic exposure, lymphoid tissue continue to enlarge through puberty[3]. As a result most normal children have axillary and inguinal lymph nodes enlargements[2]. In general lymph nodes are considered to be enlarged if their diameter exceed 1 cm for cervical and axillary one and 1.5cm for inguinal ones[4,5,6,7]. The exact incidence of CLA at any given time may vary in children and has been reported as high as 55% in cohort of 222 children under 6 years of age, being high in sick children than in those attending a well baby clinic[8]. The indications for lymph node biopsy include; failure of response to antibiotics, rapid increase in size without abscess formation, hard or matte lymph nodes especially in the posterior triangle , development of new signs or symptoms e.g. [fever, night sweating, weight loss], supraclavicular lymphadenopathy and inconclusive or difficulty in the diagnosis of fine needle aspiration[9,10].

Children are not miniadults, the cause of their lymphadenopathy are not replica of their adults counterpart. In fact CLA in children is mainly due to benign conditions [70-75%][11-12], whereas in patients over the age of 50 benign conditions account for [30-40%][12,13]. The aims of the study are to identify different types of diseases in children with this problem and to compare the results with other studies .

including reaction to local or systemic inflammatory conditions, lymphoreticular malignancies ,metastatic process,

Material and Methods

The files of pathology reports from Al-Zahrawi teaching hospital and from three private laboratories in Mosul [Ninavah , Al-Ahali and Al-Kindey private laboratories] were reviewed for the period between 1993-2002 included .

Haematoxylen and Eosin slides Of all cases with clinical diagnosis of CLA of patients aging 14 years and younger were reviewed .Any files with missed data were excluded , and the cases were analyzed according to the type of the lesion , age and sex .

Results

Two hundred and fourteen cases of CLA were collected during 10 years period 1993-2002. Their ages ranged between 1-14 years with male: female ratio of 1.3:1. The cases were divided into 3 groups covering the three quinquennia of childhood including [46, 72,96] cases respectively [Table 1].

The most common histological diagnosis was reactive hyperplasia which was seen in 110 cases [Table 2]. Tuberculosis accounted for 61 cases, about 62% of them were seen in 41 over the age of 9 years with female predominance.

Malignant lesions were seen in 41 cases in which Hodgkin,s disease was the commonest diagnosis [53.6%] followed by Non Hodgkin lymphoma (36.6%]. Only in 4 cases [9.8%] secondary

deposits were seen [2 retinoblastoma, l neuroblastoma and 1 papillary carcinoma of the thyroid].

Table 1 Age and sex distribution in patients with CLA

Age group (Years]	Male	Female	Total	%
0-5	24	22	46	21.5
5-12	54	18	72	33.6
10-15	42	54	96	44.9

Table 2 Age distribution in different Pathologies of CLA in children

.

Types of lesions	0-5	5-10	10-15	Total
Reactive hyperplasia	22	47	41	110
Tuberculosis	13	10	38	61
Hodgkin disease	4	10	8	22
Non Hodgkin lymphoma	6	3	6	15
Secondaries	1	1	2	4
Others*	-	1	1	2

* include eosinophlic granulama .

Table 3 The most common diagnosis of CLA in (208]cases.

Type of the lesions	No.	M:F	%
Reactive hyperplasia	110	2.4:1	51.4
Tuberculosis	61	1.0:2	28.5
Hodgkin disease	22	2.0:1	10.3
Non-Hodgkin lymphoma	15	1.8:1	7.0

Discussion

Cervical lymph nodes are the most frequently enlarged and biopsied of all peripheral lymph nodes affecting all age groups including children[14,15,16,17]. Once the physician decides that the child has an abnormal peripheral lymph node, an attempt must be made to determine the cause of this abnormalities to avoid delay in the diagnosis of serious conditions.

The diagnostic yields of lymph node biopsies vary among different series and different anatomical sites. Cervical

lymph node biopsy gives the highest diagnostic yield [35-70%] among peripheral lymphadenopathy[9,12,18]. In this study the positive diagnostic yield rate is 48%. These differences in positivity may be a reflection of the combined effects of the lack of available facilities and the readiness of the surgeon to perform biopsy, as it is the most encouraging and easily available procedure.

The majority of enlarged lymph nodes in children occur as a result of infective agents. However, in this study 51.4% of cases lymph nodes show reactive hyperplasia without specific causes being identified. This is similar to others[19,20,21]. This may be due to number of factors; lymphoid tissue in children is more labile and more responsive than that in later life, to the empiric use of antibiotics, and the difficulty in identifying the causative viruses.

Tuberculous lymphadenitis is the commonest form of extrapulmonary tuberculosis[15,21] and despite the declining incidence of tuberculosis in many parts of the world it still forms a common cause of peripheral lymph node enlargement[15,17,22] especially in the cervical region which is often due to direct extension from the primary site in the lung through the mediastinal lymph nodes . It is found that 4-9% of children with pulmonary tuberculosis have cervical lymph node involvement[23,24].

Although a definitive diagnosis of tuberculous adenitis can't be made without the demonstration of mycobacterium in culture and/or tissue section by special stain, yet the presence of epitheloid granulomatous inflammation and necrosis with or without giant cells in children make tuberculosis the most likely diagnosis in our locality[23,25]. In our series tuberculous lymphadenitis account for 28.5% of the cases forming the most common specific cause.

Malignant lymph nodes were identified in 41 cases [19.2%] accounting approximately 1 in every 5 excised lymph node. This figure is higher than that in other studies from different parts of the world[9,26,27]. However, Al-Talib et al in 2004 conducted a survey on childhood cancer in Ninevah province covering the same period and found that there is 22.8% increase in the incidence of childhood cancer including lymphoma which is almost double the rate compared to other studies[28,29,30].

Although excisional biopsy remains the gold standard in diagnosis, fine needle aspiration changed the practice in adult surgery in the modern era and may provide material to establish an early diagnosis in children[31]. However, it is difficult to perform without sedation in younger children and should be regarded as a diagnostic triage tool only as far as positive findings are concerned, also it yields a smaller sample, gives no information on lymph node architecture and care should therefore be expressed interpreting negative results to exclude false one for security.

Conclusions

1. Although there are many causes of lymph node enlargement, the most likely diagnosis are reactive hyperplasia, tuberculosis, lymphoma and less commonly metastatic cancer [Table 3].
2. In developing countries about ½ of the cervical lymph nodes examined show significant and clinically important pathologies, there by justifying an active

management of all pediatric patients with persistent CLA.

3. CLA in children has a high incidence of infective causes including a significant number of mycobacterium tuberculosis.

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Effect of Different Caffeine Concentrations on Sperm Parameters of Asthenospermic Patients

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Abstract

The study is an attempt to improve the sperm parameters of asthenospermic patients by using centrifugation-washing technique, with Earle's balanced salt and glucosaline medium and addition three different concentrations of caffeine (0.043, 0.074 and 0.097 molar separately]. Three incubation periods (30, 45 and 60 minute) were used for all sperm activation specimens.

A 0.043 molar of caffeine with Earle's or glucosaline media caused significant increase ($P<0.001$) in sperm motility percent and grade activity compared to the control group (Earle's or glucosaline alone) in 30 minute of incubation period also a significant differences were noticed between this concentration and other two concentrations (0.074 and 0.097 molar) when added to glucosaline in different incubation period. The results showed insignificant difference in sperm motility index between Earle's medium with caffeine and glucosaline medium with caffeine in different incubation periods and concentrations.

الخلاصة

اجريت الدراسة الحالية لتحسين قيم معالم النطف لمرضى العقم المصابين بوهن النطف وذلك بتنشيط النطف خارج الجسم، باستخدام تقنية الغسل والنبذ وباضافة ثلاث تراكيز مختلفة (0.043 و 0.074 و 0.097 مولاري] من الكافائين الى وسط ايرل الملحي مرة والى المحلول الفسيولوجي السكري مرة اخرى. ثم حضنت العينات لـ 30 و 45 و 60 دقيقة ودرست معالم النطف في فترات الحضن المذكورة وقورنت مع قيمها قبل التنشيط.

سبب اضافة الكافئين بتركيز 0.043 مولاري الى وسط ايرل والمحلول الفسيولوجي السكري زيادة معنوية [$p<0.001$] في النسبة المئوية للنطف المتحركة ودرجة نشاط النطف مقارنة بمجموعة السيطرة (وسط ايرل لوحده او المحلول الفسيولوجي السكري لوحده] عند التحضين لفترة 30 دقيقة، كذلك لوحظ فرق معنوي بين هذا التركيز والتركيزين الاخرين[0.074 و 0.097 مولاري] عندما اضيف الى المحلول الفسيولوجي السكري في فترات الحضن المختلفة.

اشارت النتائج الى عدم حدوث فرق معنوي في منسب حركة النطف بين قيمها عند استخدام الكافائين مع وسط ايرل مقابل استخدامه مع المحلول الفسيولوجي السكري ولفترات الحضن والتراكيز جميعها.

Introduction

Spermatozoa must be vigorously motile to penetrate the cervical mucus, migrate to the fertilization site and then penetrate zona pellucida after capacitation. There is a strong relationship between sperm motility and fertility [1]. Caffeine or other methylxanthines when added to semen could help in improve the fertility of

patient's semen with low sperm motility [2].

A similar response to caffeine has been demonstrated to occur in fertile semen and it improved the fertilizing capacity [3], this due to the positive effect of caffeine on spermatozoal penetration [4]. These observations may have clinical importance for the improvement the motility of asthenospermic samples used for artificial insemination and for the possible improvement of pregnancy [5].

According to those finding, it was deemed necessary to evaluate the effect of caffeine on human sperm motility and other parameters. In addition to study the optimum caffeine concentration which can be used for *in vitro* sperm activation, especially caffeine was considered as metabolically active compound can passing blood-testicular barrier and affecting on sperm motility in vivo.

This study was aimed to evaluate the efficiency of caffeine in sperm activation in vitro. Another aim of the study is finding a suitable media instead of expansive media like Earle's salt media.

Materials and Methods

Semen Collection and processing

Twenty semen samples were obtained by masturbation from twenty patients after 3 days of sexual abstinence. The specimens were allowed to liquefy at 37C for 20-30 minutes. The mean data of the semen parameters were estimated, including sperm concentration, sperm motility percent, grade activity, sperm motility index leukocytes and phagocytes concentration, and percentage of spermatozoa with normal morphology. The values of these parameters considered as sperm parameters before activation.

In Vitro Sperm activation technique

Two ml of each semen sample was divided into four equal splits, putting separately in four centrifuged tube. Each 0.5ml of semen was mixed with 1ml of Earle's media or glucosaline supplemented with 20% inactive maternal serum. The mixture was centrifuged, the supernatant was discarded and the final pellet in four tubes were overlayered with Earle's alone, Earls +0.043 molar of caffeine, Earles + 0.074 molar of caffeine and Earles +0.097 molar of caffeine. Also other samples which were activated by glucosaline alone or with the same above concentrations of caffeine were treated with same technique of Earls media. The tubes contain samples kept in incubator at 37°C.

After 30, 45 and 60 minutes of incubation, a drop of top part of media (Earle's alone, Earle's with caffeine, glucosaline alone and glucosaline with caffeine) was aspirated by pipette and examined to evaluate sperm parameters after activation.

The results were analyzed by using analysis of variance (ANOVA) and LSD to indicate the significancy [6].

Results

The result of in vitro sperm activation showed a significant improvement ($p<0.001$) in all sperm parameters compared to those values before activation by using Earle's media, or glucosaline alone or with different concentrations of caffeine in three incubation period used.

Sperm activation by using 0.043. molar of caffeine added to Earle's or glucosaline caused significant increase

($p<0.001$) in sperm motility percent and grade activity compared to sperm activation by using Earle's or glucosa line alone, in 30 minutes of incubation period (Table 1 and 4). The same Tables revealed insignificant differences in other values of activated sperm parameters between media alone and media with caffeine.

Table 5 showed a significant increase ($p<0.001$) by using 0.043 molar of caffeine with glucosaline compared to those values by using other concentrations of caffeine (0.074 and 0.097 molar) or glucosaline alone in 45 minutes of incubation period.

In 60 minutes of incubation period, sperm motility percent and grade activity showed significant decreased ($p<0.001$) by using 0.074 and 0.097 molar of caffeine compared to those values by using glucosaline alone or with 0.043 molar of caffeine (Table 6).

The comparative study of sperm motility index of three concentrations of caffeine between its using with Earle's media contrast glucosaline, revealed insignificant differences in all concentrations and incubation periods (Figures1, 2 and 3).

Table 1 Sperm Function parameters before and after activation of asthenospermic patients by using Earle's medium with different concentration of caffeine for 30 minutes of incubation period

Sperm parameters	Before activation (mean ± SD)	After activation (mean ± SD)			
		Control	Caffeine concentration (molar)]		
			0.043	0.074	0.097
Sperm concentration ($x10^6$)	49.00^a ± 23.66	8.40^b ±4.81	8.50^b ±5.57	8.20^b ±5.51	8.90^b ±5.78
Sperm motility percent	29.00^a ±11.25	57.00^b ±26.37	79.00^c ±28.16	64.00^{cb} ±23.30	58.50^{cb} ±23.81
Grade activity	1.70^a ±0.67	2.70^b ±1.18	3.95^c ±1.36	3.17^{cb} ±1.00	3.05^{cb} ±0.99
Abnormal sperm morphology percentage	46.50^a ±11.06	19.56^b ±5.99	17.86^b ±14.36	18.56^b ±13.51	18.35^{cb} ±9.45
Leukocytes & phagocytes concentration ($x\ 10^6$)	4.80^a ±2.20	0.30^b ±0.48	0.30^b ±0.48	0.30^b ±0.48	0.40^b ±0.51

Patients number=10
P<0.001 significant difference
Different letter indicate for significant

Table 2 Sperm function parameters before and after activation of asthenospermic patients by using Earle's medium with different concentration of caffeine for 45 minutes of incubation period

Sperm parameters	Before activation (mean ± SD)	After activation (mean ± SD)			
		Control	Caffeine concentration (molar)		
			0.043	0.074	0.097
Sperm concentration ($x10^6$)	49.00[a] ± 23.66	8.80[b] ±5.45	9.30[b] ±6.07	8.70[b] ±6.16	8.80[b] ±4.80
Sperm motility percent	29.00[a] ±11.25	63.50[b] ±26.14	73.00[b] ±19.15	63.50[cb] ±21.99	57.00[b] ±14.56
Grade activity	1.70[a] ±0.67	3.05[b] ±1.12	3.52[b] ±0.72	3.12[b] ±0.84	2.90[b] ±0.55
Abnormal sperm morphology percentage	46.50[a] ±11.06	18.48[b] ±12.83	18.66[b] ±10.52	19.85[b] ±10.98	20.32[b] ±6.98
Leukocytes & phagocytes concentration (x 10^6)	4.80[a] ±2.20	0.40[b] ±0.51	0.40[b] ±0.51	0.20[b] ±0.42	0.30[b] ±0.48

Patients number=10
P<0.001 significant difference
Different letter indicate for significant

Table 3 Sperm function parameters before and after activation of asthenospermic patients by using Earle's medium with different concentration of caffeine for 60 minutes of incubation period

Sperm parameters	Before activation (mean ± SD)	After activation (mean ± SD)			
		Control	Caffeine concentration (molar)		
			0.043	0.074	0.097
Sperm concentration ($x10^6$)	49.00[a] ± 23.66	8.80[b] ±6.16	9.20[b] ±5.57	10.00[b] ±5.45	9.00[b] ±6.03
Sperm motility percent	29.00[a] ±11.25	53.50[b] ±23.81	66.50[b] ±17.00	55.50[cb] ±18.32	52.00[b] ±15.49
Grade activity	1.70[a] ±0.67	2.72*[b] ±1.21	3.52*[b] ±0.76	3.25*[b] ±0.76	2.70*[b] ±0.67
Abnormal sperm morphology percentage	46.50[a] ±11.06	19.41[b] ±13.83	20.64[b] ±5.47	18.46[b] ±8.94	19.26[b] ±9.84
Leukocytes & phagocytes concentration (x 10^6)	4.80[a] ±2.20	0.40[b] ±0.51	0.50[b] ±0.52	0.40[b] ±0.51	0.20[b] ±0.42

Patients number=10
P<0.001 significant difference
Different letter indicate for significant

Table 4 Sperm function parameters before and after activation of asthenospermic patients by using glucosaline medium with different concentration of caffeine for 30 minutes of incubation period

Sperm parameters	Before activation (mean ± SD)	After activation (mean ± SD)			
		Control	Caffeine concentration (molar)		
			0.043	0.074	0.097
Sperm concentration ($x10^6$)	55.60[a] ± 2.7.65	10.00[b] ±5.23	10.40[b] ±5.94	9.90[b] ±5.78	10.10[b] ±6.87
Sperm motility percent	33.50[a] ±8.51	51.00[b] ±9.94	72.00[c] ±12.51	59.50[b] ±14.42	58.50[b] ±16.50
Grade activity	2.02[a] ±0.39	3.00[b] ±0.53	3.74[b] ±0.53	3.30[b] ±0.42	3.21[b] ±0.43
Abnormal sperm morphology percentage	51.45[a]	20.68[b]	21.06[b]	19.57[b]	22.40[b]

Patients number=10
P<0.001 significant difference
Different letter indicate for significant

Table 5 Sperm function parameters before and after activation of asthenospermic patients by using glucosaline medium with different concentration of caffeine for 45 minutes of incubation period

Sperm parameters	Before activation (mean ± SD)	After activation (mean ± SD)			
		Control	Caffeine concentration (molar)		
			0.043	0.074	0.097
Sperm concentration ($x10^6$)	55.60[a] ± 27.65	10.70[b] ±6.09	12.30[b] ±5.94	11.30[b] ±5.96	11.60[b] ±6.52
Sperm motility percent	33.50[a] ±8.51	60.00[b] ±14.52	67.50[b] ±9.20	58.00[b] ±8.23	56.00[b] ±8.09
Grade activity	2.02[a] ±0.39	3.30[b] ±0.32	3.52[b] ±0.24	3.22[b] ±0.24	3.15[b] ±0.21
Abnormal sperm morphology percentage	51.45[a] ±13.42	22.40[b] ±6.80	20.91[b] ±4.59	20.24[b] ±4.50	21.53[b] ±5.66
Leukocytes & phagocytes concentration (x 10^6)	4.20[a] ±2.39	0.40[b] ±0.69	0.30[b] ±0.48	0.30[b] ±0.48	0.40[b] ±0.51

Patients number=10
P<0.001 significant difference
Different letter indicate for significant

Table 6 Sperm function parameters before and after activation of asthenospermic patients by using glucosaline medium with different concentration of caffeine for 60 minutes of incubation period

Sperm parameters	Before activation (mean ± SD)	After activation (mean ± SD)			
		Control	Caffeine concentration (molar)		
			0.043	0.074	0.097
Sperm concentration ($x10^6$)	55.60[a] ± 27.65	11.20[b] ±7.19	14.00[b] ±8.16	11.20[b] ±6.76	11.00[b] ±7.00
Sperm motility percent	33.50[a] ±8.51	61.50[b] ±9.73	62.00[b] ±11.59	52.50[c] ±9.20	48.50[c] ±7.83
Grade activity	2.02[a] ±0.39	3.26[b] ±0.47	3.18[b] ±0.39	2.82[c] ±0.23	2.58[c] ±0.28
Abnormal sperm morphology percentage	51.45[a] ±13.42	22.77[b] ±12.73	20.62[b] ±9.32	21.92[b] ±9.72	21.18[b] ±8.64
Leukocytes & phagocytes concentration (x 10^6)	4.20[a] ±2.39	0.30[b] ±0.48	0.30[b] ±0.48	0.40[b] ±0.51	0.20[b] ±0.42

Patients number=10
P<0.001 significant difference
Different letter indicate for significant

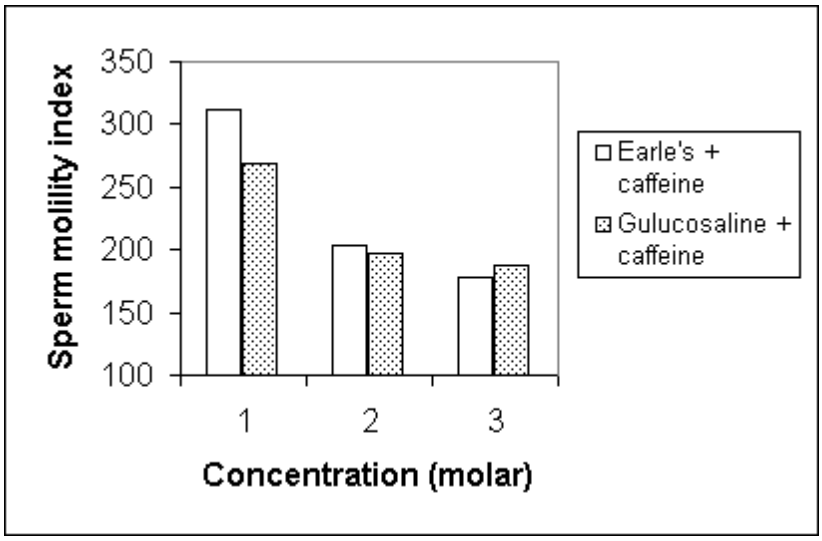

Figure 1 Sperm motility index for different caffeine concentrations with Earle's and glucosaline at 30 minutes of incubation period.
Where the concentration 1,2 and 3 was 0.043, 0.074 and 0.097 molal respectively

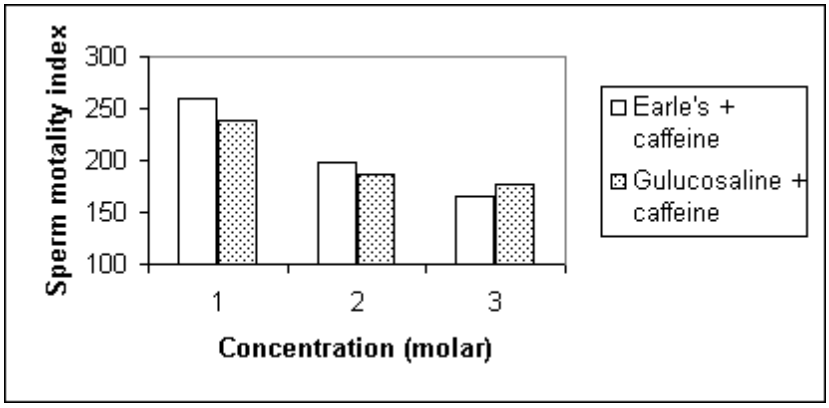

Figure 2 Sperm motility index for different caffeine concentrations with Earle's and glucosaline at 45 minutes of incubation period.
Where the concentration 1,2 and 3 was 0.043, 0.074 and 0.097 molal respectively

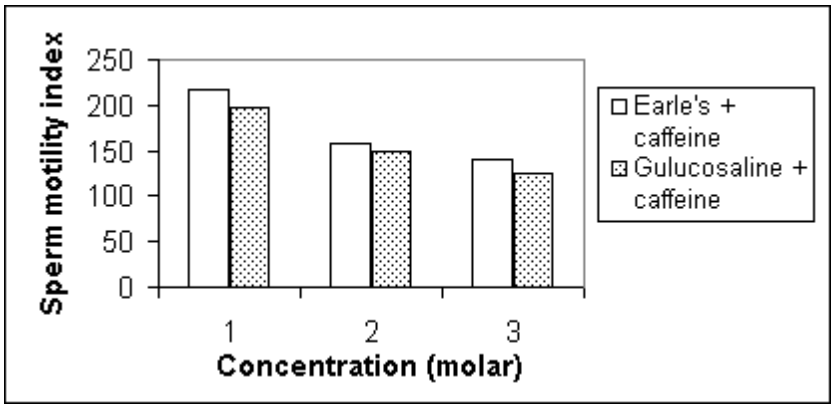

Figure 2 Sperm motlity index for different caffeine concentrations with Earle's and glucosaline at 60 minutes of incubation period.
Where the concentration 1,2 and 3 was 0.043, 0.074 and 0.097 molal respectively

Discussion

The in our experiment results present demonstrate that caffeine effect was dose dependent, this agree with another's study that showed the concentration of 2.5 mM caffeine and 2.5 mM 2-deoxyadenosine are optimum to improve and maintain motility to increase the number of sperm reached the upper reproductive tract[7]. Also it was seen that adding 0.043 molar of caffeine to Earle's media or glucosaline media caused significant increase ($p<0.001$) in sperm motility percent and sperm grade activity compared to Earle's or glucosaline alone, this result is synrigist with several studies that showed the adding 6mM of caffeine to frozen thawed semen specimens or to fresh, asthenospermatic human sperm [8-10], caused a significant increase in sperm motility percent and sperm activity.

The addition of 7.2 µm of caffeine to the spermatozoa before freezing cause significant increase in motility percentage from 40% to 80%

[11,12]. Another studies showed that nonmotile live spermatozoa were activated by 10 mM of caffeine without any effect on the percentage of dead sperm by using the combined multiple exposure photography and supravital staining techniques [13]. This due to the elevated level of cAMP in caffeine-stimulated human spermatozoa which may be increase energy production by accelerating glycolysis and tricarboxylic acid cycle [14]. This energy was used to convert ADP to ATP for sperm motion [15].

Our results showed that the increase in sperm motility percent and sperm grad activity at first 30 minute of incubation only. This result was agreed with another study that demonstrated an immediate response to caffeine was occur by a marked increase in mean velocity at 30 minute only when sperms were incubated with tyrode's solution that supplied with 6 mM of caffeine. In this technique showed a sharp decline in mean velocity after 60-90 minutes [16]. The incubation of spermatozoa of asthenospermic specimen with 2 mg/ml of pentoxifylline caused significant improvement in motility and progressive motility at first 30 minute [17]. In other study noticed that pentoxifylline enhanced several motion parameters as well as bovin cervical mucus penetration at 30 minute only in asthenospermic samples when incubated with 3.6 mM of pentoxifylline [18].

The addition of 0.074 and 0.097 molar of caffeine to Earle's media or glucosaline media at 30 and 45 minutes were not appear any significant differences compared to sperm parameters by using Earle's or Glucosaline alone (Table1,2,4&5). While in 60 minute incubation period noticed a significant decrease in sperm motility percent and grad activity when these concentrations were added to glucosaline media compared to glucosaline media alone, this agreement with another authors which demonstrated a highly significant inhibitory effect of caffeine in a dose of 60 mM/ml on all parameters of sperm motility [19].

The present study revealed that insignificant difference in sperm motility index when different caffeine concentrations were used with Earle's or glucosaline for all different incubation periods (30, 45 and 60 minutes), (Figure 1, 2, 3). This results discussed due to the role that methylxanthine act as inhibiting phosphodiestrase and improving counts of total motile and total progressively motile spermatozoa in case of oligospermia/asthenospermia [20,21], and the percentage of acrosome-reacted sperm in fertile men under capacitating conditions and these effects occur despite decreasing calcium inside human sperm cell[22]. When sperm was incubated in medium without calcium, calcium restoration caused calcium influx[23], and the release of calcium from internal calcium stores was sufficient to initiate hyper activation[24].

The results showed that insignificant differences in recovery sperm concentration, abnormal sperm morphology percentage, white blood cells concentration with phagocytes in different treatments incomparing with control, this may be due to that centrifugation technique only was used in our study for sperm activation in vitro and only different concentrations of caffeine have to effect on sperm motility parameters.

In conclusion, it was found that 0.043 molar of caffeine was the optimum

concentration for *in vitro* sperm activation, this may have clinical importance of the motility of asthenospermic samples at the time of artificial insemination.

Moreover the most important result of our study was revealed that it can be used glucosaline medium instead of an expensive media (Earle's media) after providing it with caffeine.

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