Middle East Journal of Internal Medicine

ISSN 1837 9052

July 2013 - Volume 6, Issue 4

From the Editor

Ahmad Husari *(Chief Editor)*
Email: editor@me-jim.com

This issue of the journal is rich with various papers dealing with metabolic syndrome. A paper from Turkey looked at Metabolic syndrome just after infancy. The authors studied consecutive children and adolescents between the ages of 2 and 15 years. Cases were divided into four groups according to their percentile for weight alone. The study included 299 cases. The authors concluded that metabolic syndrome is a reversible progression step between complete physical health and irreversible terminal diseases with a very high prevalence in adults. But these findings suggest that pathophysiological mechanisms of the syndrome are already going on even in childhood, and bases of the syndrome are starting to be built up just after the period of infancy, probably due to the eating habits of the families. Therefore, because of the irreversible nature of the terminal diseases of the syndrome, care to prevent should be started even in childhood.

A paper from Iraq looked at the Antibacterial potency of Some Medicinal Plant Extracts on Inhibiting Antibiotic Resistance property of Escherichia coli. The authors isolated Escherichia coli from different sources of human infections and 83 isolates of E. coli were obtained from 264 samples. The isolates were varied in their resistance to tested antimicrobials, and isolate E48 was resistant to all antimicrobials under study, while isolate E37 was resistant only to three antimicrobials. The results showed that the alcoholic extracts were more efficient for reducing antibiotic resistance in E. coli comparing with watery extracts. The authors concluded that all extracts have antibacterial action; alcoholic extracts were more efficient for reducing antibiotic resistance genes in E. coli comparing with watery extracts.

A paper from KSA reported a case of Chronic Lymphocytic thyroditis (Hashimoto's disease). A ten year old female child presented with anterior neck pain with neck swelling. Examination showed goiter with mild tenderness. Investigation showed high TSH and high thyroblobuin, thyroperoxidase antibodies. Patients diagnosed with chronic lymphocytic thyroiditis (Hashimoto's disease) and received Thyroxine which brought her thyroid function to normal. A follow up plan was designed and the patient is going well with management.

Another case report on a combination of Larsen and Adams - Oliver syndromes in a Jordanian newborn has been included. The author reports a full term female newborn product of cesarean section at Prince Hashem Ben Al- Hussein Military Hospital/ Zarqa, north of Jordan, with bilateral congenital knee dislocation, bilateral congenital hip dislocation, characteristic facies (prominent forehead, depressed nasal bridge, wide spaced eyes), congenital scalp defect, clubbed feet, bilateral hypoplastic toes and congenital heart defects presenting a previously not described in literature a combination of two very rare syndromes: - Larsen and Adams - Oliver syndromes.

A paper from Iraq looked at Cytotoxic and cytogenetic effects of Saliva officinalis on different tumor cell lines. The cytotoxicity of Salvia officinalis extracts were evaluated on two tumor cell lines Rabdomyosarcoma (RD) and Murine mammary adenocarcinoma (AMN3), and one normal cell line, Murine fibroblast (L20B). The results revealed that high significant cytotoxic effect was seen in highest concentrations on RD tumor cell line and AMN3 tumor cell with CC50 5400 µg/ml and 7810 µg/ml respectively. The cytogenetic effect of aqueous extracts of S. officinalis showed a significant decrease in mitotic index in all concentrations on both tumor cell lines. The authors concluded that 1-Aqueous extracts of S. officinalis has antiproliferative effects on both RD and AMN3 cell lines. 2-Their cytotoxic activity was more efficacious than Cisplatin. 3-The cytogenic effects of the plant extract were less than that of Cyclophosphamide.

A paper from Nigeria reports on a four months old baby who presented on account of bilateral congenital opacity .The patient could at least perceive light though the cornea of both eyes were opaque and there was no clear area at the peripheral portion of the cornea. Examination by Paediatricians revealed micrognatia, simian crease in the right palm and umbilical hernia. There was no similar occurrence in his family. There is need for early presentation of affected patients so as to prevent amblyopia. The Government should also support the affected patients so that they can be promptly treated in view of the challenges of the management of the condition.m

A prospective study for sample obtained from 45 male patients looked at fine needle aspiration versus open biopsy for testicular sperm recovery in infertile azoospermic patients. Categorical and continuous variables were compared using independent t test and -chi-square test. Logistic regression model was applied to develop a predictive model for SRR by TESA & PSA outcome. Sperm retrieval rate for TESA and PSA was 42.2% and 48.8%, respectively ($P = 0.03$). The authors concluded that serum FSH and testicular pathology were predictors of SRR by TESA. Patients with FSH < 23 IU/l and/or testicular pathology of hypospermatogenesis had comparable SRR by TESA versus PSA.

Fine needle aspiration versus open biopsy for testicular sperm recovery in infertile azoospermic patients

ABSTRACT

Background and objectives: This study aimed to develop a predictive model for sperm retrieval rate (SRR) sperm recovery by testicular fine-needle aspiration (TESA) and compare with (SRR) obtained by percutaneous open biopsy sperm aspiration (PSA) correlating with hormonal parameters.

Materials and Methods: This is a prospective study for a sample obtained from 45 male patients during the period from January 2011 - March 2012. Clinical, paraclinical, and histological information of patients were gathered. All patients underwent both TESA and PSA in a single operation. Predictors of SRR by TESA were identified comparing with predictive outcome of PSA.

Statistical Analysis Used: Categorical and continuous variables were compared using independent t test and Chi-square test. Logistic regression model was applied to develop a predictive model for SRR by TESA and PSA outcome.

Results: Sperm retrieval rate for TESA and PSA was 42.2% and 48.8%, respectively (P = 0.03). Regarding age group, more common in 4th (50.3%) while 3rd decade and 5th decades each of them (20%) the duration of infertility was variable ranging from 10-20 years, testis volume, luteinizing hormone, prolactin, and testosterone did not differ between patients.

Conclusions: Serum FSH and testicular pathology were predictors of SRR by TESA. Patients with FSH < 23 IU/l and/or testicular pathology of hypospermatogenesis had comparable SRR by TESA versus PSA.

Key words: Follicular-stimulating factor, sperm recovery rate, testicular fine-needle aspiration,

Salah A Ali (1)
Suhel Al-Najjar (2)

(1) Assist Professor, Dr Salah A Ali
Hawler Centre for Invitro Fertilization in Erbil, Iraq
(2) Senior lecturer, Dr Suhel Al-Najjar
Hawler Centre for Invitro Fertilization in Erbil, Iraq

Correspondence:
Assist Professor, Dr Salah A Ali
Hawler Centre for Invitro Fertilization in Erbil, Iraq
Email: Sala1955@yahoo.com

Introduction

In the normal male reproductive tract, sperm exiting the testis have minimal motility and limited egg fertilizing capacity. Sperm acquire the potential for improved motility and fertilizing ability during epididymal transit. So, in the unobstructed epididymis, sperm of optimal quality (as evaluated by percent motile cells) are found in the most distal epididymis(1). The obstructed epididymis shows the opposite pattern of sperm quality: optimal sperm quality in the proximal epididymis and very poor quality in the most distal segments. This finding of "inverted motility" is expected in the obstructed male reproductive tract since sperm production continues in the testis and reabsorption of those sperm is an active process in the most distal regions of the system. The most distal obstructed epididymis tends to contain dilated yellow tubules that are packed with macrophages reabsorbing old, degenerated sperm(2). Therefore, sperm retrieval should be performed from the proximal obstructed epididymis

Although excisional testicular biopsy is a less invasive surgical technique than microsurgical epididymal sperm aspiration, even less invasive procedures have been proposed for the recovery of either epididymal or testicular spermatozoa(3). Percutaneous sperm recovery procedures, in particular, are becoming more popular. However, so far the reports on this technique have been merely on a case by case basis and it has not been shown whether ICSI using percutaneous aspirated testicular spermatozoa offers success rates comparable to ICSI with spermatozoa retrieved by the open excisional approach. The present study therefore aimed at comparing the results of ICSI using testicular spermatozoa recovered by an open excisional technique and ICSI using testicular spermatozoa recovered by a percutaneous fine needle aspiration (FNA) technique.

Patients and Methods

In the current study the samples were taken by both methods TESA and PSA from 45 male patients who presented with azopsermia to perform invitro fertilization comparing the outcome from procedures.

Percutaneous sperm aspiration (PSA) procedures are performed by doing a small incision that is well tolerated under local anesthesia. The procedure is performed with the spermatic cord block and sedation. A tunica vaginalis space is entered via transverse 1-cm scrotal incision. A self-retaining eye-lid retractor is placed to create a window into the tunica vaginalis space. The posteriorly located epididymis is rotated into view by gentle traction or placement of 7-0 Prolene traction suture into the epididymal tunic. Under the operating microscope the epididymal tunic is incised and individual tubule isolated. After careful hemostasis with bipolar cautery, epididymal tubule is incised tangentially with microscissors. Fluid is aspirated with a 24-gauge angiocath sheath attached to a 1.0-ml syringe. About 10 microliters of aspirated fluid is examined under 400X light microscope. Aspiration sites then progress from cauda to caput in order to obtain best quality sperm. Epididymal tubule and tunic are closed with 10-0 and 9-0 nylon sutures respectively. Tunica vaginalis space is closed with 4-0 absorbable suture after irrigation with saline and local anesthetic without epinephrine. Skin edges are reapproximated with 4-0 absorbable suture(4).

The technique of Testicular Fine Needle Aspiration (TESA) of the testis was initially described as a diagnostic procedure in azoospermic men. Subsequently, testicular fine needle aspiration or biopsy for the recovery of spermatozoa has been described. Percutaneous puncture and aspiration of the testis can be performed using a 22 gauge needle connected to a 20 cc syringe in a Menghini syringe holder(5).

Results

A total of 45 cases of infertile males were included in this study; both TESA & PSA were performed for them to obtain sperm for purpose of in vitro fertilization.

The age distribution of different cases studied is shown in Table 1; the mean age (±SD) for cases of infertility was 45 years. Out of 45 cases of infertility, the majority of cases (53.3%) ranged between 31-40 years, and only 3 cases (6.7%) were above 50 years old.

Sperm retrieval rate for TESA and PSA was 42.2% and 48.8%, respectively (P = 0.03) which is significant. Regarding age group, it is more common in the 4th (50.3%)than 3rd and 5th decades, each of them 20%. The duration of infertility was variable ranging from 10-2) years. Testis volume, luteinizing hormone, prolactin, and testosterone did not differ between patients with and without mature sperm in TESA samples. Serum follicular-stimulating hormone (FSH) < 23 IU/l in 53.3% ranging from (6-28)IUI/l with P = 0.002) and histology of hypospermatogenesis was in 30 cases (66.6%) P < 0.001) (Figure 1) which is highly significant and were predictors of SRR by TESA. Patients with FSH < 23 IU/l (53.3%) versus less than 6 IU/l(46.7.%) and testicular histology of hypospermatogenesis accounted for 14 cases (33.4%) (Figure 2).

Discussion

In all azoospermic patients with normal spermatogenesis, i.e. patients with so-called obstructive azoospermia, testicular spermatozoa may be recovered by open excisional biopsy(6). The open excisional biopsy technique is an invasive procedure which may cause discomfort for the patient, even if only taken through a small incision and even after meticulous haemostasis. Percutaneous puncture of the testis using a fine 22-gauge needle is a less invasive procedure which has been used successfully to recover testicular samples for diagnostic purposes. Yet this sampling method allows only cytological investigation because it contains only a limited quantity of aspirated testicular cells which may, however, be sufficient in order to establish the diagnosis of normal spermatogenesis(7). A more invasive fine needle tissue aspiration technique has been reported to recover testicular spermatozoa for ICSI, and pregnancies have been obtained(8). However, many patients may suffer varying degrees of discomfort during these tissue aspiration procedures, in which a 24-gauge biopsy gun-type needle is used. Others have successfully used a thinner 22-gauge needle(3). In the present study, we used a fine 22-gauge needle for testicular aspiration. Although patient comfort was not assessed in the present retrospective study, in our experience patients undergoing percutaneous sperm aspiration tended to report less pain and discomfort once at home than those who had had an open biopsy Percutaneous sperm aspiration, however, risks recovering only a few spermatozoa but the testicular tissue is composed of seminiferouse tubules which are easily aspirated by special maneouver so sufficient amount of sperm can be aspirated. In the present study, we found that although the number of samples taken, i.e. aspirates or biopsies, was comparable, significantly fewer spermatozoa were harvested after FNA than after open biopsy. In 19 cases out of 45 FNAs (42.2%), a sufficient number of spermatozoa were recovered to allow ICSI, and in open biopsy 22 out of 45 cases (48.8%) a sufficient number of spermatozoa were recovered; the difference is not significant. Regarding the outcome in TESA 14 pregnancies took place while in PSA 15 pregnancies took place which is similar to a study done by Tournaye(9). The overall pregnancy rates were 27.5% for FNA-ICSI cycles (14 pregnancies) and 27.5% for excisional biopsy-ICSI cycles (14 pregnancies).

Conclusion

Testicular fine needle aspiration is a very good procedure for diagnosis and retrieving of sperm for infertile males with less side effects than open biopsy and producing the same number of pregnancies for infertile couples.

References

1- Schlegel P., Palermo G., Alkani M., Adler A., Reing A., Cohen J., Tpzenwaks Z. Micropuncture retrieval of epididymal sperm with in vitro fertilization: importance of in vitro micromanipulation techniques. Urology 1995;46:238

2- Craft I., Tsirigotis M., Bennett V., Taratnissi M., Khalifa Y et al. Percutaneous epididymal sperm aspiration and intracytoplasmic sperm injection in the management of infertility due to obstructive azoospermia. Fertil Steril 1995; 63:1038

Age groups(Years)	No of cases & %	Positive with PSA	Positive with TESA
21-30	9(20%)	2	1
31-40	24(53.3%)	9	7
41-50	9(20%)	4	4
Above 50	36.7%)	0	2
Total	45(100%)	15	14

Table 1: showing age distribution of patients with infertility and positive results in both procedures

Age groups (years)	FSH(IU/l)	LH(IU/l)	Testosterone(IU/l)
21-30	23	3-7	3-6
31-40	6 ,50% 23 50%	4-7	2-6
41-50	17	3.5-6	3-6
Above 50	28	3-5	3-4

Table 2: Hormonal levels related to age groups

SRR	TESA	PSA
No of cases	19	22
%	42.2	48.8

Table 3: Sperm retrieval rates(SRR) in TESA & PSA

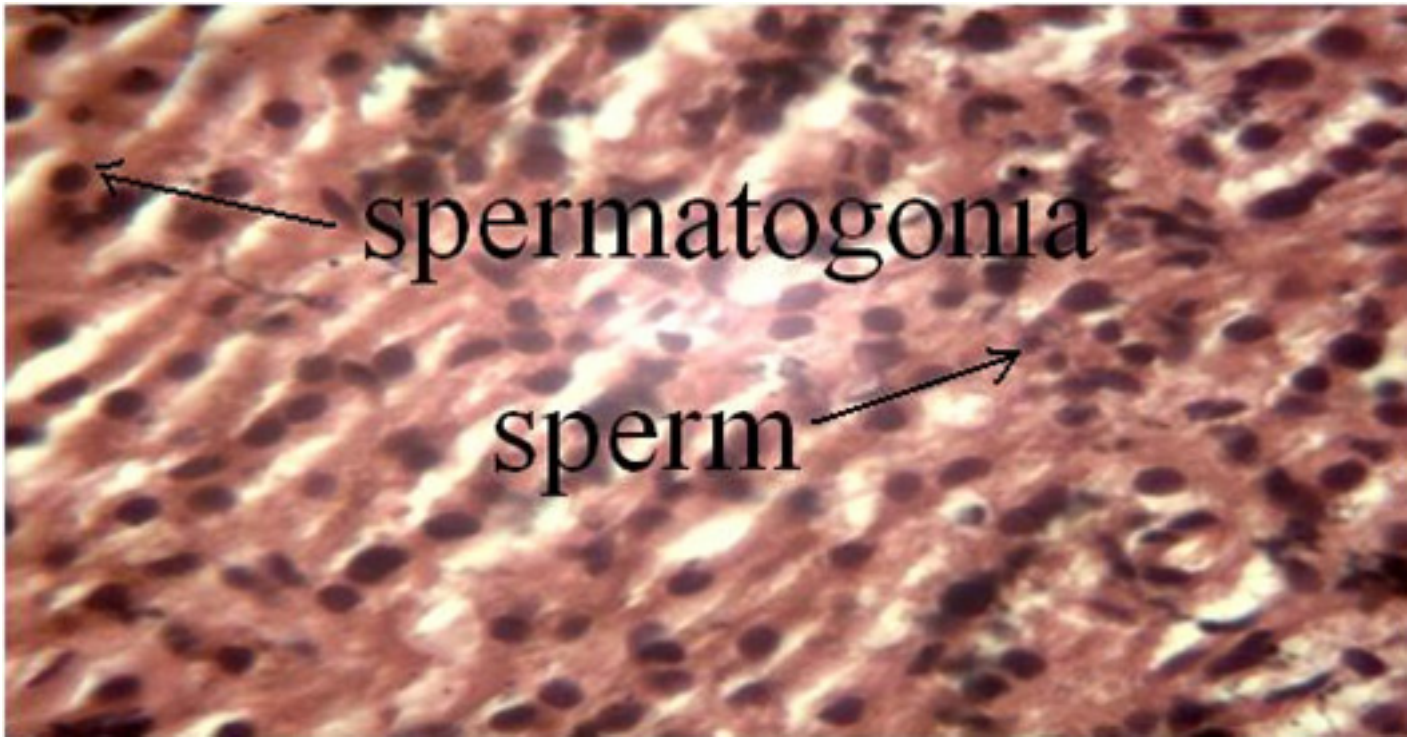


Figure 1: Open biopsy hypospermic testis

3- Lewin, A, Weiss, D.B., Friedler, S. et al. Delivery following intracytoplasmic sperm injection of mature sperm cells recovered by testicular fine needle aspiration in a case of hypergonadotrophic azoospermia due to maturation arrest. Hum. Reprod. (1996) 11, 769-773.

4- Nudell DM, Conaghan J., Pedersen RA., Givens CR., Schriock ED., Turek PJ. The mini-micro-epididymal sperm aspiration for sperm retrieval: a study of urological outcomes. Hum.Reprod. 1998;13:12601265

5- Friedler S., Raziel A., Strassburger D., Soffer Y., Komarovsky D., Ron-El R. Testicular sperm retrieval by percutaneous fine needle sperm aspiration compared with testicular sperm extraction by open biopsy in men with non-obstructive azoospermia. Hum Repord 1997;12:1488-1493

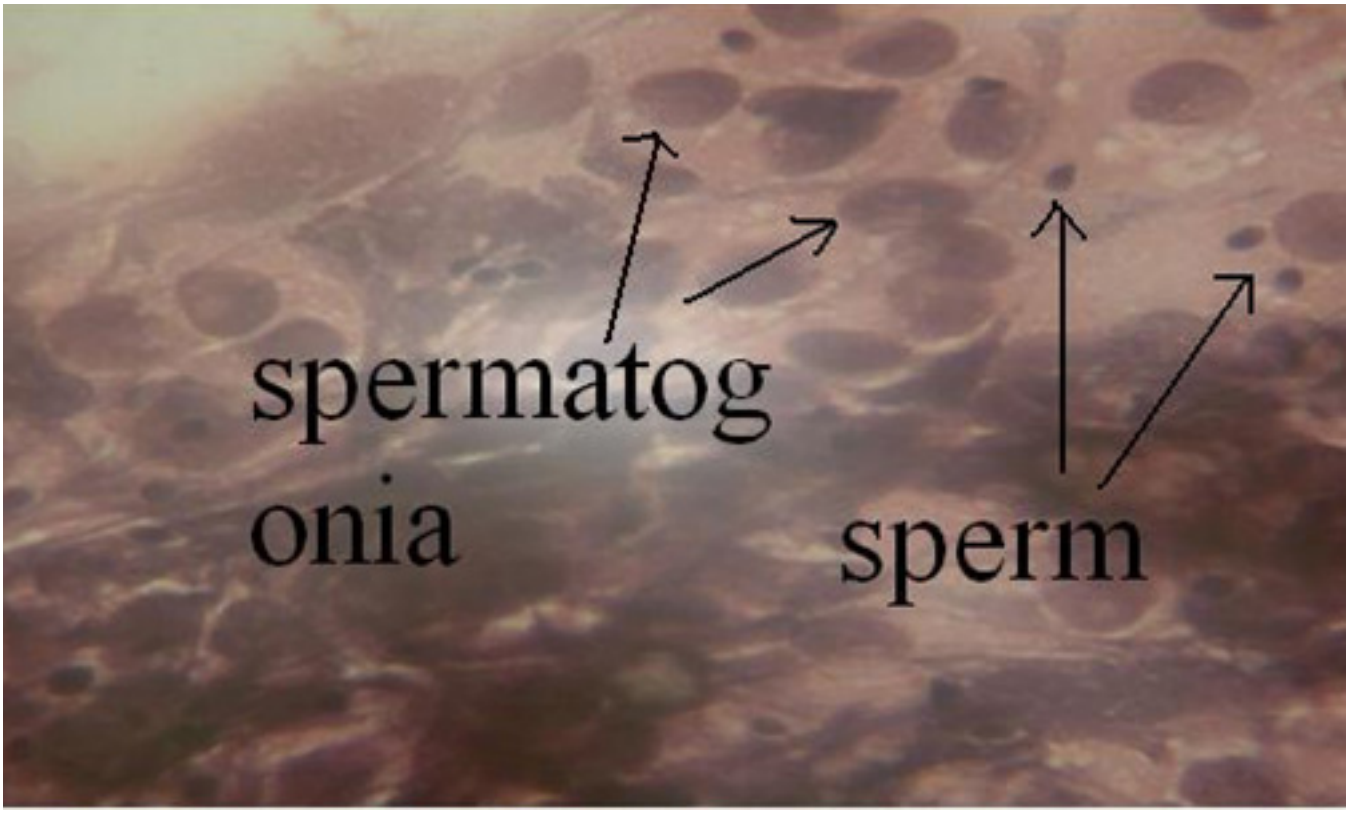


Figure 2: FNA of testis hypospermic testis

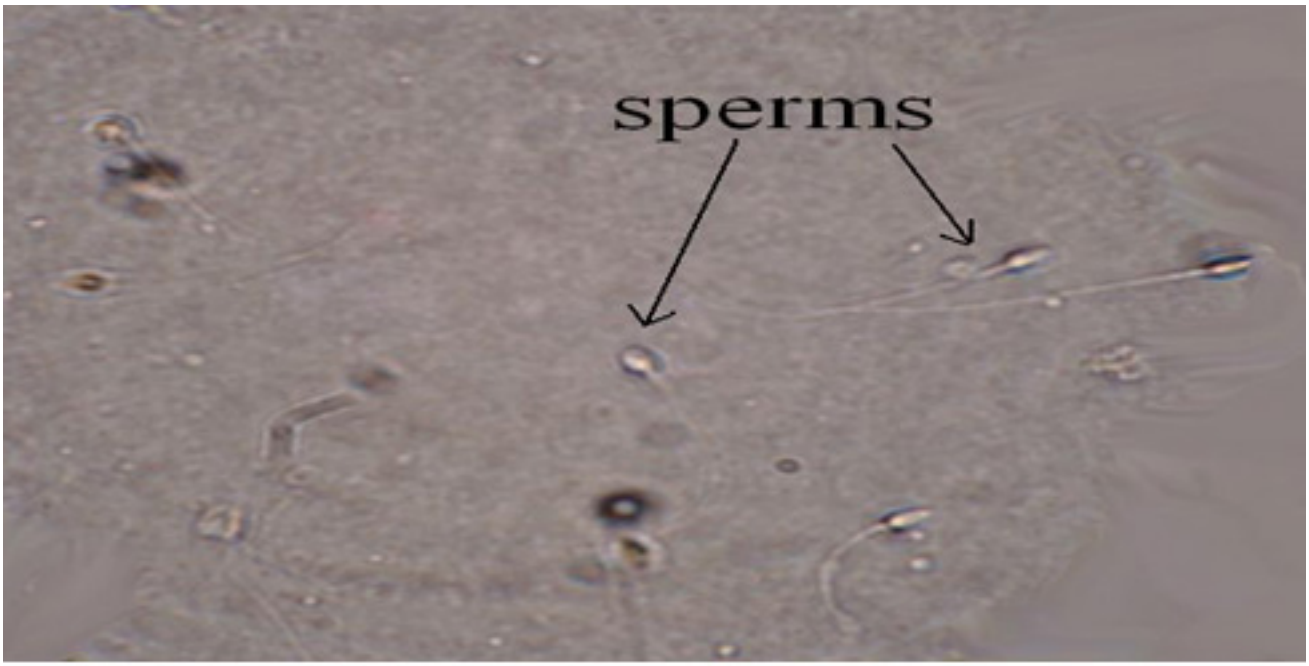


Figure 3: Sperm retrieval

6- Tournaye, H., Liu, J., Nagy, Z. et al. Correlation between testicular histology and outcome after intracytoplasmic sperm injection using testicular sperm. Hum. Reprod., (2006) 11, 127-132.

7- Foresta, C., Varotto, A. and Scandellari, C. Assessment of testicular cytology by fine needle aspiration as a diagnostic parameter in the evaluation of the azoospermic subject. Fertil. Steril(2002)., 57, 858-865.

8- Bourne, H., Watkins, W., Speirs, A. and Gordon Baker, H.W. Pregnancies after intracytoplasmic sperm injection of sperm collected by fine needle biopsy of the testis. Fertil. Steril. (2005), 64, 433-436.

9- Herman Tournaye, Koen Clasen, Ayse Aytoz, Zsolt Nagy, Andre´ Van Steirteghem and Paul Devroey Human Reproduction 2008 vol.13 no.4 pp.901-904,

Antibacterial potency of Some Medicinal Plant Extracts on Inhibiting Antibiotic Resistance property of Escherichia coli

ABSTRACT

Objectives: The present study was done to cure the antibiotic resistance gene by using medicinal plant extracts.

Methods: Isolation of Escherichia coli from different sources of human infections and 83 isolates of E. coli were obtained from 264 samples. The isolates were identified according to the cultural characteristics, morphological features and biochemical examination in addition to API 20E system. Antibiotic sensitivity for these isolates was tested. Curing antibiotic resistance genes was done by using alcoholic and watery extracts of three medicinal plants: Cinnamomum zeylanicum, Eugenia caryophyllata and Citrullus colocynthis and through determination of the minimum inhibitory concentration of these medicinal plants.

Results: The isolates were varied in their resistance to tested antimicrobials, and isolate E48 was resistant to all antimicrobials under study, while isolate E37 was resistant only to three antimicrobials. All isolates showed resistance 97.59% for Chm and less sensitivity for Amk 2.40%. The results of curing antibiotic resistance genes was done by using alcoholic and watery extracts of three medicinal plants: Cinnamomum zeylanicum, Eugenia caryophyllata and Citrullus colocynthis and through determination of the minimum inhibitory concentration of these medicinal plants which was 4000µg/ml for alcoholic extracts of Cinnamomum zeylanicum, Eugenia caryophyllata and Citrullus colocynthis and 2000 µg/ml, 3000 µg/ml and 4000 µg/ml for watery extracts of Cinnamomum zeylanicum, Eugenia caryophyllata and Citrullus colocynthis respectively and SMIC (Sub Minimum Inhibitory Concentration) of these plant extracts was used as curing agent. The results showed that the alcoholic extracts were more efficient for reducing antibiotic resistance in E. coli compared with watery extracts.

Conclusion: All extracts have antibacterial action; alcoholic extracts were more efficient for reducing antibiotic resistance genes in E. coli compared with watery extracts.

Keywords: Antibiotic resistance, Antibacterial, Medicinal plants, Curing agents, MIC, E. coli

Hêro Farhad Salah Akrayi
Adel Kamal Kheder

Biology Dept., College of Education, University of Salahaddin, Erbil, Iraq

Correspondence:
Hêro F. Salah Akrayi Instructor of Microbiology, College of Education,
Scientific Departments, University of Salahaddin, Kurdistan Region, Iraq
Email: hero_ferhad@yahoo.com

Introduction

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [1]. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotics, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient [2].

Plant-derived herbal remedies have remained a vital part of traditional medicine for thousands of years. It has been estimated by the World Health Organization that approximately 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care. Plant products also play an important role in the health care systems of the remaining 20% of the population, mainly residing in developed countries. The use of data on traditional medicine can provide a very valuable short cut by indicating plants with specific folk medicinal uses, which might be likely sources of biologically active compounds. Recent investigations on medicinal plants used in traditional medicine have led to the discovery of many new drugs and hundreds of pharmacologically active substances for synthetic modifications [3 and 4].

Spices and herbs have played a dramatic role in civilization and in the history of nations. The delightful flavour and pungency of spices make them indispensable in the preparation of palatable dishes. In addition, they are reputed to possess several medicinal and pharmacological properties and hence find position in the preparation of a number of medicines [5].

Antimicrobial activities of various species and their derivatives have been reported by many works. The use of alternative medical therapy has increased the interest of pharmacologists and herbalists over the past decade. Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made contributions to human health and well being. Many studies indicate that in some plants there are many substances such as peptides, unsaturated long chain aldehydes, alkaloidal constituents, some essential oils, phenols and water, ethanol, chloroform, methanol and butanol soluble compounds. These plants then emerged as compounds with potentially significant therapeutic application against human pathogens, including bacteria, fungi or viruses. The antimicrobial compound form plants may inhibit microbial growth by different mechanisms [4 and 6].

Materials and Methods

Specimens collection and identification

Two hundred and sixty four samples were collected from different sources of human infections (cerebrospinal fluid, urine, stool, wounds and burns), from Hawleri ferkari, Rezgary, Al-amal, Raparine hospitals and Khanzad lab in Erbil city, in addition to specimens taken from the sewage water as in Table 1. The collected specimens were identified by studying the cultural, morphological and some biochemical features such as IMViC, gelatenase, catalase oxidase, urease and SIM [7 and 8], in addition to performance of API 20E test.

The isolates were maintained and preserved on nutrient agar slants. For every experiment, freshly prepared sterile nutrient broth (10ml) was inoculated from the slants and incubated at 37^{o} for 24 hours.

Antibiotics Resistance test

To test the sensitivity of E. coli isolates to a variety of antimicrobials under study, Muller Hinton agar was used as growth medium, after sterilization and cooling at 45?, and final concentration of antibiotics (Amikacin (Amk), Cefixime (Cef), Cephalothin (Cph), Chloramphenicol (Chm), Ciprofloxacin (Cip), Clindamycin (Cln), Doxycillin (Dox), Gentamycin (Gen), Gulmentin (Gul), Kanamycin (Kan), Lincomycin (Lin), Nalidixic acid (Nal), Nitrofurantion (Nit), Pan-cloxacillin (Pac), Pipracillin (Pip), Rifampcin (Rif), Tetracyclin (Tet), Tobramycin (Tob) and Trimethoprim(Tri)) were added to medium and poured into sterile petri dishes. After solidification, the plates were inoculated by streaking method with E. coli then incubated at 37° for 24 hours. The results were recorded next day [9].

Extraction of selected plants

The medicinal plants used in this study included Cinnamomum zeylanicum (bark of cinnamon), Eugenia caryophyllata (fruits of clove), Citrullus colocynthis (fruits without seeds of colocynth), were obtained from the local market in Erbil city then washed with tap water. After drying at 37ºC for 24 hours the plants were ground in a grinding machine.

Preparation of Crude Extracts

Fifty gm of plant powder was weighed and soaked in 250ml of sterilized double distilled water and absolute ethanol, to obtain two different extracts according to the solvent used, and placed on a magnetic stirrer and left to mix by magnetic bar at room temperature for 72 hours, then filtrated by muslin cloth, then by filter paper. The above step was repeated 3-5 times to residue, until a clear colorless supernatant extraction liquid was obtained indicating that no more extraction from the plant material was possible. The prepared extract was also evaporated to dryness and stored in the refrigerator at 5°C till used [10].

Phytochemical Screening

The extracts of both aqueous and ethanolic plant extracts were screened for their phytochemical bases using the method described by Hasan [11] and Harborne [12].

For alkaloids, ten ml of plant extract acidified with HCl was taken, then tested with picric acid; formation of yellow participate indicated the presence of alkaloids.

For glycosides, two parts of Fahlang's reagent was mixed with plant extract, left in boiling water bath for 10 minutes; red color means presence of glycosides.

For flavonoides, ten ml of 50% ethanol was added to 10ml 50% KOH then this solution was mixed with an equal volume of plant extract. Yellow color referred to a positive result.

For tannins, ten ml from plant extract was divided into two equal parts, then drops of 1% CH3COOPb was added to the first part; the appearance of white pellet means a positive result. To the second part drops of 1% FeCl3 were added; formation of green bluish color means a positive result.

For saponin, five ml of plant extract was extremely shaken for half a minute, then left in a vertical case for 15 minutes; appearance of foam means presence of saponin.

For resins, ten ml of acidified D.W. with HCl was added to 10ml of plant extract; if turbidity appears indicates a positive reaction.

For phenols, three ml of plant extract was added to 2ml of potassium hexacyanofrrate and 2ml of FeCl3; the green bluish color indicates a positive result.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of medicinal plant extracts under study were determined by turbidity method (spectrophotometric method) at 600 nm, and the following dilutions were prepared for each extract (100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 4000, 5000, 6000) µg/ml [13]; in addition the SMIC of medicinal plant extracts was determined used as curing agents.

The MIC was determined for plant extract, which inhibited bacterial growth, contrast with control sample that consisted of 10 ml of nutrient broth and 0.1ml of overnight culture of bacterial suspension, then incubated at 37ºC for 24 hours.

Plasmid Curing

The curing of plasmid was performed by using medicinal plant extracts. SMIC of plant extract and 0.1 ml of overnight bacterial suspension were added to 10 ml nutrient broth then incubated at 37ºC for 24 hours. Next day 0.1 ml of it was spread on nutrient agar plate and incubated for 24 hours at 37 ºC; the following day 30 colonies were transferred to antibiotic agar plate. After incubation for 24 hours at 37 ºC the viable colonies were calculated, then percentage of curing colonies were calculated.

Results and Discussion

Two hundred and sixty four samples were collected from different sources of human infections (Table 1), from most hospitals in Erbil city, and also isolated from sewage water. The isolates were identified through studying their cultural, morphological and biochemical characteristics. Furthermore, API 20E was performed to support the obtained results, (Figure 1), [14 and 15], and according to results eighty three isolates of E. coli were obtained among two hundred and sixty four. In general, and according to analytical profile index (1997), the synonyms number obtained for tested samples ranged (1164552-7574552), which indicated that all isolates were E. coli.

Source of Isolation	Isolates number	No. of bacterial isolates
Body fluid	66	1
Burns	48	1
Cerebrospinal fluid	1	1
Sewage Water	4, 32	2
Urine	2, 6, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 23, 24, 27, 28, 29, 31, 33, 34, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 67, 68, 69, 70, 71, 72, 73, 74, 76, 77, 81, 82, 83	60
Stool	5, 8, 9, 10, 25, 26, 78,	9
	79, 80	
Wound	3, 7, 17, 30, 35, 36, 53, 65, 75	9
Total	**83**	**83**

Table 1: Distribution of E. coli isolates according to the source of isolations

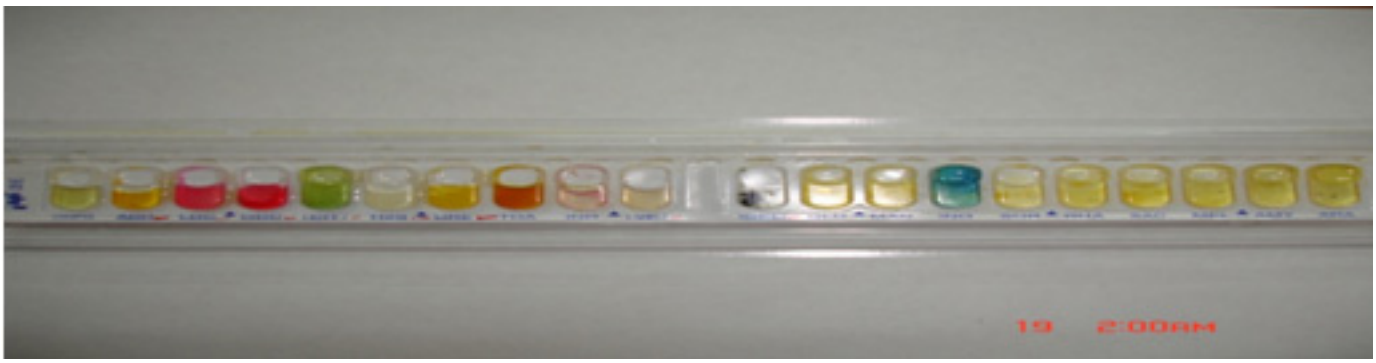


Figure 1: Result of API 20E test used for identification of E. coli.

The susceptibility of 83 isolates was tested against nineteen widely used antimicrobials (Amk, Cef, Cph, Chm, Cip, Cln, Dox, Gen, Gul, Kan, Lin, Nal, Nit, Pac, Pip, Rif, Tet, Tob and Tri). Table (2) illustrates that all isolates were varied in their response to use of antimicrobials, and the highest resistance percent (97.59%) was to Chloramphenicol, in contrast with the lowest percent (2.40%) was to Amikacin.

Antimicrobials	Symbol	No. of Resistant Isolate	Resistant %
Amikacin	Amk	2	2.40
Cifixime	Cef	30	36.14
Cephalothin	Cph	51	61.44
Chloramphenicol	Chm	81	97.59
Ciprofloxacin	Cip	22	26.50
Clindamycin	Cln	80	96.38
Doxicillin	Dox	37	44.57
Gentamycin	Gen	41	49.39
Glumentin	Gul	21	25.30
Kanamycin	Kan	27	32.53
Lincomycin	Lin	76	95.18
Nalidixic acid	Nal	41	49.39
Nitrofurantion	Nit	76	91.56
Pan-cloxacillin	Pac	24	28.91
Pipracillin	Pip	56	67.46
Rifampcin	Rif	3	3.61
Tetracyclin	Tet	57	68.67
Tobramycin	Tob	7	8.43
Trimethoprim	Tri	49	59.03

Table 2: The resistance of E. coli isolates to antimicrobials

The MIC of Cinnamomum zeylanicum, Eugenia caryophyllata, and Citrullus colocynthis, of alcoholic extracts were 4000 µg/ml, and for watery extracts were 2000µg/ml, 3000µg/ml, and 4000 µg/ml for cinnamon, clove and colocynth respectively, and the SMIC of these extracts was determined since the SMIC of alcoholic extracts of these plants was 3500 µg/ml while the SMIC of watery extracts of cinnamon, clove and colocynth was 1800 µg/ml, 2500 µg/ml and3500 µg/ml respectively and they were used as curing agents by transferring bacterial colonies for isolate E48.

The alcoholic and watery extracts of cinnamon exhibited as antibacterial activity against E. coli is shown in Table 3; cinnamon contains high amounts of cinnamaldehyde, linalool, eugenol and caryophllene. Aldehydes are known to possess powerful antimicrobial activity. It has been proposed that an aldehyde group conjugated to a carbon -to- carbon double bond is a highly electronegative arrangement. Such electronegative compounds may interfere in biological processes involving electron transfer and react with vital nitrogen compounds, e.g. protein and nucleic acid and therefore inhibit the growth of the microorganism [16].

*: Abbreviations are given in Table 2

Table 3: Curing percent of plasmid DNA of E. coli isolate (E48) by C. zeylanicum extracts at 3500 µg/ml for alcoholic extract & 1800 µg/ml for watery extract

Types of extract	Alcoholic Cinnamon 3500µg/ml	Watery Cinnamon 1800µg/ml	Spontaneous curing
Chemical groups	Flavonoids Tannins Glycosides Phenols	Flavonoids Tannins Glycosides Saponins	———
*Amk	80	100	0.00
Cef	73.33	3.33	0.00
Cph	83.33	20	0.00
Chm	76.66	16.66	0.00
Cip	3.33	0.00	0.00
Cln	90	3.33	0.00
Dox	13.33	0.00	0.00
Gen	80	10	0.00
Gul	3.33	0.00	0.00
Kan	43.33	33.33	0.00
Lin	3.33	23.33	0.00
Nal	100	76.66	0.00
Nit	90	100	0.00
Pac	33.33	36.66	0.00
Pip	46.66	20	0.00
Rif	0.00	3.33	0.00
Tet	0.00	0.00	0.00
Tob	100	0.00	0.00
Tri	0.00	0.00	0.00

Antimicrobial at final Concentrations

Types of extract	Chemical groups	Antimicrobial at final Concentrations		
Alcoholic Clove 3500µg/ml	Flavonoids Tannins Glycosides Phenols	Watery Clove 1800µg/ml	Flavonoids Tannins Glycosides Saponins Phenols Resins	Spontaneous curing
				———
100	*Amk	93.33		0.00
80	Cef	100		0.00
66.66	Cph	33.33		0.00
100	Chm	76.66		0.00
100	Cip	100		0.00
33.93	Cln	83.33		0.00
100	Dox	50		0.00
100	Gen	100		0.00
100	Gul	100		0.00
100	Kan	63.33		0.00
100	Lin	93.33		0.00
100	Nal	90		0.00
23.33	Nit	100		0.00
30	Pac	76.66		0.00
93.33	Pip	93.33		0.00
43.33	Rif	100		0.00
100	Tet	66.66		0.00
100	Tob	26.66		0.00
60	Tri	100		0.00

*: Abbreviations are given in Table 2

Table 4: Curing percent of plasmid DNA of E. coli isolate (E48) by E. caryophyllata extracts at 3500 µg/ml for alcoholic extract and 2500 µg/ml for watery extract

Types of extract	Alcoholic Colocynth 3500µg/ml	Watery Colocynth350 0µg/ml	Spontaneous curing
Chemical groups	Flavonoids Phenols	Tannins Saponins Resins	———
*Amk	56.66	0.00	0.00
Cef	86.66	0.00	0.00
Cph	23.33	0.00	0.00
Chm	100	0.00	0.00
Cip	90	6,66	0.00
Cln	66.66	0.00	0.00
Dox	53.33	0.00	0.00
Gen	33.33	23.33	0.00
Gul	70	0.00	0.00
Kan	73.33	0.00	0.00
Lin	56.66	0.00	0.00
Nal	100	100	0.00
Nit	90	0.00	0.00
Pac	56.66	0.00	0.00
Pip	100	0.00	0.00
Rif	56.66	0.00	0.00
Tet	76.66	0.00	0.00
Tob	100	43.33	0.00
Tri	76.66	0.00	0.00

Antimicrobial at final Concentrations

*: Abbreviations are given in Table 2

Table 5: Curing percent of plasmid DNA of E. coli isolate (48) by Citrullus colocynthis extracts at 3500µg/ml

Gill and Richard [17] mentioned that cinnamaldehyde has been inhibited the growth of Clostridium botulinum, Staphylococcus aureus, E.coli and Salmonella typhimurium. Yust and Fung [18] found that the combination of cinnamon with nisin accelerates decreasing bacterial number of E. coli O157:H7 and Salmonella typhimurium in apple juice. Klapthor [19] who mentioned that about a tea spoon (0.3%) of cinnamon killed 99.5% of a million E. coli O157: H7 cell when inoculated to apple juice in three days at room temperature 25°C. Niu and Gilbert [20] found that the cinnamaldehyde reduced the swimming motility of E. coli ATCC33456 by 60% ± 8%.

Table 4 shows the curing action of both extracts of clove to antibiotic resistance genes. Nascimento et al. [2], mentioned that the extract of clove inhibited 64.2% of the tested microorganisms, with higher activity against antibiotic-resistant bacteria 83%, and it is worth mentioning, the results were similar to our results of curing test; they associated clove extract with antibiotics, and they found that clove extract has a growth inhibitory effect on P. aeruginosa isolate which was resistant to 19 different antibiotics, but no effect was observed on E. coli.

The available experimental evidence for eugenol and cinnamaldehyde is contradictory, and there is evidence supporting both membrane interaction and inhibition of specific cellular processes or enzymes [21].

It is clear from Table 5 the extracts of colocynth affected on some resistance genes, while the other antibiotic resistant genes were not affected by watery extract, this may be due to the absence of phenols and flavonoids components present in alcoholic extract of colocynth, which has antimicrobial activity effect. The activity of colocynth may return to presence of glycosides including colocynthin and cucurbetacin; resins materials, pectins, saponins, in addition to alkaloids [22].

The effect of tested medicinal plant extracts and their actions as antimicrobial or curing effects for decreasing antibiotic resistant activity in E. coli isolates may be due to its containing active components such as flavonoids, phenols and tannins compounds for colocynth, cinnamon and clove which cleared in table 4, 5 and 6 respectively. The flavonoids have

been found to be effective in vitro and acting as the antimicrobial substances against a wide array of microorganisms [23], and their activity is maybe due to their ability to form a complex with extracellular and soluble proteins, and with bacterial walls, on the other hand the chemical structure of phenol, the site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity. In addition some authors have found that more highly oxidized phenols have more inhibitory effect. The mechanisms mentioned may be responsible for phenolic toxicity to microorganisms and include enzymes inhibition by the oxidized compounds possibly through reaction with sulfhydryl groups or through more non specific interactions with the proteins. While tannins have antimicrobial activity, one of their molecular actions is to form complex with proteins through so-called nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation, thus their mode of antimicrobial action may be related to their ability to inactivate microbial adhesions, enzymes, cell envelop transport proteins, etc [23]. The adhesion property which occurs on plasmid (consider a virulence factor), because they have the ability to inactivate bacterial adhesion maybe they have the ability to cure R- plasmid or some resistance genes carried on R-plasmid, as clear in the results of the present study. They also form complexes with polysaccharides. The medicinal extracts used contain glycosides; these have a protective role, which prevents growth of microbes during plant infection, through analysis of glycosides compound [23 and 24].

Conclusion

In general we found that alcoholic extracts of clove and cinnamon were more active for inhibition or decreasing the antimicrobial resistance on testing bacteria compared with watery extracts in spite of containing both extracts the same components except saponin compounds that present in all watery extracts for all tested plants.

References

1. Cohen ML Epidemiology of drug resistance: implications for a post antimicrobial era. Science, 1992; 257: 1050-1055.

2. Nascimento GGF, Locutelli J, Freitas PC, Silva GL. Antibacterial Activity of Plant Extracts and Phytochemicals on Antibiotic Resistant Bacteria. Brazilian Journal of Microbiology, 200; 31: 247-256.

3. Ibrahim T A, Opawale BO, Oyinloye JMA. Antibacterial Activity of Herbal Extracts against Multi Drug Resistant Strains of Bacteria from Clinical Origin. Life sciences Leaflets, 2011; 15:490 - 498.

4. Safary A, Motamedi H, Maleki S, Seyyednejad SM. A Preliminary Study on the Antibacterial Activity of Quercus brantii against Bacterial Pathogens, Particularly Enteric Pathogens. International Journal of Botany, 2009; 5: 176-180.

5. Parthasarathy VA, Chempakam B, Zachariah TJ. Chemistry of Spices. CAB International, 2008.

6. Seyyednejad SM, Maleki S, Mirzaei N, Motamedi H. Antibacterial Activity of Prunus mahaleb and Parsley (Petroselinum crispum) against Some Pathogen. Asian Journal of Biological Sciences, 2008; 1: 51-55.

7. Levinson W, Jawetz E. Medical Microbiology and Immunology. Hall International, 1995.

8. Atlas RM. Principles of microbiology. Mosby-Year Book, Inc., U.S.A, 1995.

9. Alnajjar AR. Studies on the Effect of Surfactants on the Antimicrobial Activity of Several Antibiotics. Ph.D. thesis. University of Manchester, UK, 1976.

10. Harborne JB, Mabray TJ, Mabray H. Physiology and Function of Flavonoids. Academic press, New York, 1975.

11. Hasan MKA. The Use of Some Plant Extracts for Inhibition of Genotoxic Effect for Some Anticancer Drugs in Mouse. Ph.D. thesis. College of Science. University of Babylon. Iraq, 2001.

12. Harborne JB. Phytochemical Methods (2nd ed.). Chapman and Hall, 1984.

13. Cruickshank R, Duguid JD, Marmion BP, Swain RHA. Medical Microbiology, the Practice of Medical Microbiology. (12th ed.). Churchill Livingstone, London and New York, 1975.

14. Johnson AG, Ziegler RJ, Lukasewycz OA, Hawley LB. Microbiology and Immunology (4thed.). Lippincott Williams and Wilkins, 2002.

15. Wistreich GA, Lechtman MD. Laboratory Exercises in Microbiology (4thed.). Glencoe publishing Co., Inc, 1980.

16. Ranasinghe LS, Jayawardena B, Abeywickrama K. Use of Waste Generated from Cinnamon Bark Oil (Cinnamonum zeylanicum Blume) Extraction as A Post Harvest Treatment for Embul Banana, 2003; 1: 340-344.

17. Gill AO, Holley RH. Mechanisms of Bactericidal Action of Cinnamaldehyde against Listeria monocytogenes and of Eugenol against L. monocytogenes and Lactobacillus sakei. Applied and Environmental Microbiology, 2004; 70: 5750-5755.

18. Yust J, Fung DY. In Activation of Salmonella typhimurium and Escherichia coli O157: H7 in Apple Juice by A Combination of Nisin and Cinnamon. Journal of Food Protection, 2004; 67: 371-7.

19. Klapthor J. Cinnamon is Lethal Weapon against E. coli O157: H7. The Society for Food science and Technology, 1999; 782-8424.

20. Niu C, Afre S, Gilbert ES. Chapter three "Interference with Quorum Sensing by Sub Inhibitory Concentrations of Cinnamaldehyde", 2006.

21. Filgueiras CT, Vanetti MC. Effect of Eugenol on Growth and Listeriolysin O Production by Listeria. Monocytogenes. An International Journal: Brazilian Archives of Biology and Technology, 2006; 49: 405-409.

22. Abdul-baqi IAA. The Inhibitory Effect of Some Medicinal Plants on Different Bacterial Isolated from Wounds Infections. M.Sc. thesis, College of Education. University of Mosul. Mosul, 2001.

23. Cowan MM. Plant Products as Antimicrobial Agents. American Society for Microbiology, 1999; 12: 564-582.

24. Hajawi Gh, Al-msaimi HH, Qasim MJ. Pharmacology and Medicinal Plants. Al-thakafa Library for Publishing. Amman. Jordan, 2004.

Cytotoxic and cytogenetic effects of Salvia officinalis on different tumor cell lines

ABSTRACT

Introduction and objectives: Reports indicated that extract of salvia officinalis has antioxidant and antihistaminic activities and could alleviate nephrotoxicity induced by cisplatin. This study was undertaken to investigate the possible cytotoxic and cytogenic effects of aqueous extract of Salvia officinalis on different tumor cell lines.

Methods: The cytotoxicity of Salvia officinalis extracts were evaluated on two tumor cell lines Rabdomyosarcoma (RD) and Murine mammary adenocarcinoma (AMN3), and one normal cell line, Murine fibroblast (L20B). The cytogenetic effects of the plant extract was studied after estimating the Cytotoxicity concentration 50% (CC50) value, on both tumor cell lines and human blood lymphocytes. Whereas 18 albino mice were used to study the in vivo cytogenic effects of Salvia officinalis after determining its Median Lethal dose (LD50).

Results: The aqueous extract of S. officinalis has dose dependant cytotoxic effects on tumor cell lines. The results revealed that high significant cytotoxic effect was seen in highest concentrations on RD tumor cell line and AMN3 tumor cell with CC50 5400 µg/ml and 7810 µg/ml respectively. Salvia officinalis extracts induced a significant increase in L20B cell line proliferation. AMN3 tumor cell line was more sensitive to Cisplatin than RD tumor cell line.

The cytogenetic effect of aqueous extracts of S. officinalis showed a significant decrease in mitotic index in all concentrations on both tumor cell lines. The plant extract caused a significant decrease in M.I of human blood lymphocyte at 48 hours. However their cytogenetic effect was less than that of Cyclophosphamide (CP).

The LD50 of aqueous extracts of S. officinalis leaves is estimated to be 4361 mg/kg. Cytogenetic studies showed significant decrease in mitotic index in all treated mice.

Conclusion:
1-Aqueous extracts of S. officinalis has antiproliferative effects on both RD and AMN3 cell lines. 2-Their cytotoxic activity was more efficacious than Cisplatin. 3-The cytogenic effects of the plant extract were less than that of Cyclophosphamide.

Key words: Salvia officinalis, antiproliferative effect. Cytogenic effect

Reshna K. Al-Barazanjy
Kawa Dizaye
Assad A. AL-Asadye

Department of Pharmacology, College of Medicine
Hawler Medical University, Iraq

Correspondence:
Dr. Kawa Dizaye
Professor of Pharmacology
Head of department of Pharmacology, College of Medicine
Hawler Medical University, Iraq
Cell phone: 009647504452392
Email: dr_kawadizaye@yahoo.com

Introduction

In recent years, medicinal plants have attracted a lot of attention globally. Since a long time evidence has accumulated to demonstrate promising potential of medicinal plants used in various traditional, complementary and alternative systems especially for cancer treatment (1).

Most people in developed and industrialized countries receive traditional health care (THC) for their everyday health care needs. The World Health Organization has consistently estimated that 70-80% of the population of these countries rely on THC for their basic health care needs, either on its own or in conjunction with modern medical care. (2). Many conventional drugs originate from plant sources: a century ago, most of the few effective drugs were plant based. Examples include aspirin (from willow bark), digoxin (from foxglove), morphine (from the opium poppy) and Vincristine from (Catharanthus roseus) (3).

Salvia is the largest genus of plants in the Lamiaceae family. The name Salvia officinalis derives from the Latin 'salveo', which means "to be saved". Salvia is a perennial herbaceous to shrubby herb growing up to 50cm in height (4).

Salvia officinalis has a very long history of effective medicinal use. The ancient Greeks used it to treat consumption, ulcers and snake bites, and it is an important domestic herbal remedy for disorders of the digestive system(5). Its antiseptic qualities make it an effective gargle for the mouth where it can heal sore throats, ulcers etc. The leaves applied to an aching tooth will often relieve the pain (6). Histopathological changes and in vitro studies revealed that the S. officinalis leaf extract possesses significant oxidative radical scavenging and antihistaminic activities (7).

Many studies have reported the effect of different crude and isolated compounds extracts of different species of salvia on different diseases especially cancer. Antimutagenic effect

of terpenoids from Salvia officinalis was tested by (Simic et al, (1997) (8). Slamenova et al, (2004) used three compounds isolated from the root of Salvia officinalis and they were tested for their cytotoxic activity in human colon carcinoma cells and human hepatoma cells cultured in vitro (9).

The search for novel anticancer drugs continues; agents that can eliminate the cancerous cells but do not affect the normal cells may have a therapeutic advantage for the elimination of cancer cells. The present study aims to determine 1- The Cytotoxic effect of aqueous leaves extracts of S. officinalis on the growth of (RD, AMN3 and L20B) cell lines. 2-Cytogenetic effect of aqueous leaves extracts of S. officinalis on tumor cell lines, human blood lymphocyte and bone marrow of albino mice by evaluation of the Mitotic Index.

Materials and Methods

Preparation of aqueous extracts

Aqueous extract of Salvia leaves was prepared as follows:

Amount of 50 gm of the powdered plant was suspended in 200 ml of distilled water and stirred by magnetic stirrer overnight at 45?C, then was filtered once through gauze and once through filter paper. Filtrate extract was gently poured into pre-weighed glass Petri dishes and left at 37°C for 48-72 hours. The crude dried extract then was placed in labeled, tightly sealed plastic tubes and stored at -20°C until used.

Cell lines

1. Rhabdomyosarcoma (RD) Cell Line: This is a human cell line derived from a biopsy specimen obtained from a pelvic rhabdomyosarcoma of a 7-year-old Caucasian girl (10). Passages 258-263 of RD cell line were used throughout this study and RPMI-1640 was used for maintaining the cells.

2. Ahmed-Mohammed-Nahi-2003 (AMN3) Cell Line: This cell line is a murine mammary adenocarcinoma cell line derived from a spontaneous mammary adenocarcinoma of female BALB/c mice (11). The cells were maintained on RPMI-1640 medium. Passages 128-135 of AMN3 cell line was used throughout this study.

3. L20B Cell Line: This cell line is a murine cell line derived from mouse L cells (fibroblasts) expressing the human poliovirus receptor (12). Passage numbers (14-18) of L20B cell line was used in this study and it was maintained in RPMI-1640 medium containig 10% Bovine Calf Serum.

Cytogenetic studies for the effect of aqueous and methanolic leaves extract of S. officinalis on both AMN3 and RD cell lines were carried out to determine the mitotic index (M.I).

The M.I % was determined as a ratio of the mitotic cells (cells in interphase) to the number of dividing cells plus number of non-dividing cells (total calculated cells) (3).

Cytotoxicity assay

The cytotoxicity protocol was depending on Flick and Gifford, 1984. Cells were plated in a 96-well flat-bottomed plate, after adhesion, Serial dilutions of aqueous extract three replicate for each concentration was placed in three columns for each type (200 µl from each extract) to the appropriate wells and incubated for 24, 48 or 72 hours at 37°C, 5-10% CO2 in a humidified environment. Untreated cells were used as controls. Then the supernatants were removed from the wells, and 50 µl of 0.01% neutral red dye was added to each well, and re-incubated for 2 hours; at the end of incubation, excess dye was removed by washing the wells twice with 150 µl PBS, then 125 µl of extraction dye solution was added (14). The optical density (O.D) of each well was read using Enzyme Linked Immunosorbent Assay (ELISA) Reader at a transmitting wavelength on 492 nm.

Determination of Median lethal dose (LD50) of aqueous leaves extracts of S. officinalis

Adult male Swiss albino Balb/C mice 20-25 gm body weight (B.W) were used to determine the intraperitoneal median lethal dose (LD50) of aqueous leaves extracts of S. officinalis. The animals were kept in well air-conditioned rooms at the animal house of the Collage of medicine in Hawler medical University, and were given pellets animal feed and water.

Median Lethal Dose (LD50) was determined in mice using the up and down method (15).

Eighteen adult male Swiss albino mice aged 9-10 weeks, weighing 20-25 g were used to study the in vivo cytogenetic effect of the plant extract and cyclophosphamide

Statistical analysis

Significance level was ascertained by one way analysis of variance, followed by student Newman Keul's multiple tests. Results were expressed as the mean ±SEM. A p-value of < 0.05 was considered significant. All statistical procedures were performed with SPSS software version 16.

Results

In vitro studies

Cytotoxic effects of Aqueous of S. officinalis and Cisplatin on different tumor Cell Lines

Cytotoxic effects of Aqueous extract of S. officinalis leaves on RD tumor cell line

Table 1 shows the cytotoxic effect of aqueous leaves extract of S. officinalis on RD tumor cell line at 24, 48 and 72 hours. The extract showed a time dependent effect. The viability of RD cells was decreased with time reaching its lowest level after 72 hours of treatment with high concentrations 5000 and 10000 µg/ml compared with the controls.

Only an increase in the cell viability was detected at 24 hours with high concentration of S. officinalis aqueous leaves extract. However, the cell viability was remarkably decreased when they were treated for longer periods (48 and 72) hours by the extract. When the tumor cells were exposed to aqueous extracts for 72 hours a significant decrease was detected at concentrations particularly at the concentrations 5000 µg/ml and10000 µg/ml in which the optical density was (0.0823±0.0130) and (0.0837±0.0069).

The CC50 value was found to be >10000 µg/ml at 48 hrs and 5400 µg/ml at 72hours of exposure to aqueous extract.

Table 1: Cytotoxic effect of aqueous extract of S. officinalis leaves on RD tumor cell line

Concentrations (µg/ml)	Exposure period		
	24 hrs	48 hrs	72 hrs
Control	0.3670±0.0012 bc	0.4483±0.0301 b	0.1147±0.0030 a
78.125	0.2997±0.0049 a	0.303±0.0215 a	0.0903±0.0802 a
156.25	0.3073±0.0027 a	0.29±0.0270 a	0.0897±0.002 a
312.5	0.3143±0.0046 a	0.2643±0.0269 a	0.0960±0.0042 a
625	0.3527±0.0019 b	0.2747±0.0263 a	0.0977±0.0009 a
1250	0.3440±0.0006 b	0.271±0.0318 a	0.0993±0.0032 a
2500	0.3877±0.0041 c	0.273±0.033 a	.0.1087±0.0023 a
5000	0.4733±0.0104 d	0.4747±0.0563 b	0.0823±0.0130 b
10000	0.5093±0.0203 e	0.5193±0.0275 b	0.0837±0.0069 b

*Similar letters indicate no significant differences.

*Different letters indicate significant differences at $p<0.05$.

Cytotoxic effects of Cisplatin on RD tumor cell line

Non-significant differences were detected in proliferation of RD tumor cells at three periods of exposure (24, 48 and 72 hours), when compared with control group for each period (Table 2 - next page).

Cytotoxic effects of aqueous extract of S. officinalis leaves on AMN3 tumor cell line

The effect of treating AMN3 cells with S. officinalis aqueous leaves extracts is shown in Table (3). The results showed that the incubation of AMN3 cells with aqueous extract have affected the viability of those cells when compared with the untreated cells (controls).

All concentrations (from 78.125 µg/ml and up) and after 48 hours have showed significant reduction in cell proliferation, except 5000 µg/ml shows non-significant differences with optical density (0.1897±0.00633) recorded when compared with that of the control group with its recorded optical density of (0.2260±0.0057), while a non significant decrease was recorded at both 24 and 72 hours.

The aqueous of S. officinalis extract exhibited CC50 value of 7810 µg/ml after 48 hours of exposure.

Cytotoxic effects of Cisplatin on AMN3 tumor cell line

As shown in Table 4 the different concentrations of cisplatin started their significant decreasing effects after 48 hours of treatment from lowest concentration (78.125 µg/ml) to highest concentration (312.5 µg/ml) with optical density (0.1843 ±0.0146), (0.1913±0.00524) and (0.1937± 0.00536), respectively. Cisplatin suppressed the proliferation of AMN3 cells in low concentrations. This effect also exhibited at 72 hours of treatment with low concentration 78.125 µg/ml and its optical density was (0.1777± 0.02924) when compared with that of control group (0.2430± 0.0005).

Cytotoxic effects of aqueous extract of S. officinalis and Cisplatin on L20B cell lines in vitro

Cytotoxic effects of aqueous extract of S. officinalis leaves on L20B cell line

The cytotoxic effect of aqueous leaves extracts of S. officinalis on L20B cells were as seen in Table 5.

Table 2: Cytotoxic effect of cisplatin on the growth of RD tumor cell Line

Concentrations (µg/ml)	Exposure period		
	24 hrs	48 hrs	72 hrs
Control	0.0787±0.0044 a	0.0643±0.0007 ab	0.0560±0.0025 ab
78.125	0.0897±0.0027 a	0.0663±0.0009 ab	0.0587±0.00133 ab
156.25	0.0867±0.0032 a	0.0653±0.0027 ab	0.0587±0.0022 ab
312.5	0.0903±0.0038 a	0.0693±0.0013 b	0.0563±0.0013 ab
625	0.0887±0.0019 a	0.0683±0.0009 ab	0.0620±0.0025 b
1250	0.0873±0.00203 a	0.0623±0.0009 a	0.0637±0.0038 b
2500	0.0870±0.0015 a	0.0653±0.0009 ab	0.0560±0.001 ab
5000	0.0847±0.0017 a	0.0647±0.0009 ab	0.0543±0.0015 ab
10000	0.0843±0.0033 a	0.0643±0.0019 ab	0.0510±0.0006 a

*Similar letters indicate no significant differences.

*Different letters indicate significant differences at $p<0.05$.

Significant increases were detected in proliferation of L20B cells at all three period of exposure, particularly at high concentrations (5000 µg/ml and 10000 µg/ml).

Cytotoxic effects of Cisplatin on L20B cell line

Incubation of L20B cells for all three periods with cisplatin caused non significant differences in proliferation of the cells at all three periods 24, 48 and 72 hours as compared with controls Table 6.

Cytogenetic effect of S. officinalis extract on cell lines

Cytogenetic effect of aqueous extracts of S. officinalis leaves on RD tumor cell line.

The results showed the presence of significant reduction in M.I Index in RD tumor cells exposed to 5800µg/ml, 2900µg/ml and 1450µg/ml of methanolic leaves extract of S. officinalis for 48 hours when compared to its specific control groups. The percentage of M.I was 0.2109±0.00639, 0.2283±0.0060 and 0.2504±0.00496 respectively. The same results were obtained after exposure of RD cells to 5400µg/ml, 2700µg/ml and 1350µg /ml of aqueous leaves extract of S. officinalis for 72 hours, and all show a significant decreases in mitotic activity which was revealed as mitotic activity in Table 7.

Cytogenetic effect of aqueous extract of S. officinalis leaves on AMN3 tumor cell line

Table 8 shows that different concentrations of aqueous leaves extract of S. officinalis has a significant reduction effect on mitotic index of AMN3 cells in all concentrations 7810 µg/ml, 3905 µg/ml and 1952 µg/ml, respectively and the lowest value was seen in the highest concentration which was 0.1568±0.01255 when compared with control group 0.2902± 0.01683.

Cytogenetic effect of aqueous extract of S. officinalis leaves on chromosomes of human lymphocyte

The effect of aqueous leaves extract of S. officinalis on human lymphocytes

Table 3: Cytotoxic effect of aqueous extract of S. officinalis leaves on the growth of AMN3 tumor cell line

Concentrations (µg/ml)	Exposure period		
	24 hrs	48 hrs	72 hrs
Control	0.0649±0.0620 ab	0.2260±0.0057 b	0.2267±0.0034 a
78.125	0.0613±0.0009 ab	0.1150 ± 0.002 a	0.1127±0.005 a
156.25	0.0697±0.0012 b	0.1037±0.0036 a	0.1097±0.0012 a
312.5	0.0687±0.0027 b	0.0907±0.0027 a	0.3780±0.2810 a
625	0.0623±0.0029 ab	0.1183±0.0052 a	0.1223±0.0015 a
1250	0.0617±0.00133 ab	0.1087±0.00713 a	0.1227±0.0015 a
2500	0.0620±0.0035 ab	0.1090±0.0045 a	0.1740±0.0145 a
5000	0.0580±0.0025 a	0.1897±0.00633 b	0.2263±0.0024 a
10000	0.0570±0.001 a	0.2407±0.01936 a	0.2370±0.0035 a

*Similar letters indicate no significant differences.

*Different letters indicate significant differences at $p<0.05$.

in peripheral blood were studied by using doses of aqueous extract 7810 µg/ml, 3905µg/ml and 195µg/ml at 48 hours and 5400µg/ml, 2700µg/ml and 1350µg/ml at 72 hours exposure period. Cell division was induced by mitogen (PHA). Cyclophosphamide (CP) 50µg/ml was used as a positive control group. Table 9 shows in 48 hours, significant decreases in the M.I in CP group when compared with negative control group. However there was a significant reduction in M.I of all groups when compared with negative control group, but when the treated groups with plant extract were compared with CP group a significant increase in M.I was recorded. While the effect of these three concentrations of aqueous extract of S. officinalis at 72 hours showed also a significant reduction in mitotic index found in positive control group when compared with the negative control one. But another three groups treated with S. officinalis for 72 hours exhibited non significant differences when compared with the negative control group, Table 10.

In vivo studies

The effect of aqueous extracts of S. officinalis leaves on mitotic index in male albino mice.

The oral median lethal dose (LD50) of the plant extract was determined to be 4361 mg/kg.

Table 11 shows the effect of CP and 4361 mg/kg of aqueous leaves extract of S. officinalis on M.I in bone marrow of albino mice.

Discussion

The present study showed that the growth inhibition was significantly progressed as the concentrations of aqueous extracts increased, as well as the time of exposure. The effect of extract type was variable according to the type of tumor cell line, extract type and the time of exposure. However in normal cell line L20B there was a significant increase in proliferation of cell growth. The growth was time and concentration dependant in which the growth was highly increased in higher concentrations (5000& 10000 µg/ml) with time exposure (72hours). This result of L20B cells is an agreement with that obtained by Lima et al, (2007) who found that S. officinalis contains some phenolic compound and the most abundant one is Rosmarinic acid and Luteolin-7-glucoside, which have shown a significant protection potential against cell death in the HepG2 cell line which is a normal cell line. The results of RD tumor cell line showed a significant decrease of its growth in the aqueous extracts after 72hours exposure, which may be due to the effect of different phytochemical compounds found in S. officinalis, especially triterpinoids.

Table 4: Cytotoxic effect of Cisplatin on the growth of AMN3 tumor cell line.

Concentrations (µg/ml)	Exposure period		
	24 hrs	48 hrs	72 hrs
Control	0.1117±0.0049 a	0.2260±0.0056 a	0.2430±0.0005 a
78.125	0.0933±0.0117 a	0.1843 ±0.0146 b	0.1777±0.0292 b
156.25	0.0950±0.0031 a	0.1913±0.0052 b	0.2026±0.0066 ab
312.5	0.0623±0.0312 a	0.1937±0.0053 b	0.2147±0.0107 ab
625	0.0987±0.0059 a	0.1957±0.0034 ab	0.2427±0.0228 ac
1250	0.0990±0.0127 a	0.1990±0.0005 ab	0.2383±0.0107 ac
2500	0.1013±0.0012 a	0.1997±0.0021 ab	0.2350±0.0071 ac
5000	0.1093±0.0116 a	0.2013±0.0008 ab	0.2377±0.0034 ac
10000	0.1140±0.0330 a	0.2023±0.0096 ab	0.2377±0.0006 ac

*Similar letters indicate no significant differences.

*Different letters indicate significant differences at $p<0.05$.

This effect of triterpinoids on tumor cell lines was also indicated by Slamenova et al, (2004) and Amirghofran et al, (2010) who found that different species of Salvia show inhibitory effects on tumor cell lines (16, 17). Ibrahim and Aqel, (2010) found that aqueous and methanolic extracts of Salvia triloba has a time dependent effect on RD tumor cell line (18). The cytotoxic effect of plant extract could be attributed to the presence of triterpinoids compounds in the extracts of S. officinalis.

This study showed significant growth inhibition in AMN3 after treatment with the aqueous extracts of S. officinalis, especially in the highest concentration; the results showed time and dose dependent inhibitory effects. The effect of S. officinalis extract was also investigated by Keshavarz et al, (2010) who found that the ethanolic extract of S. officinalis showed a dose-dependent inhibition activity on the migration of human umbilical vein endothelial cells (HUVEC) (19).

(AMN3) cells were more sensitive to the aqueous extract with CC50 and was 7810 µg/ml in 48 hours hrs of exposure. This result was supported by Li et al, (2002) who found that the tumor cell varies in its response to different drugs or crude extracts during chemotherapy treatment according to the types of cell membrane receptors (20). Moreover Shoieb et al, (2003) observed that the different plant crude extracts have revealed different activities against the proliferation of tumor cells, according to the properties of their compounds (21).

The effects of S. officinalis leaves extracts was effectible on RD tumor cell line with CC50 (5800 µg/ml) for aqueous extract, and it has a significant anti proliferative effect on AMN3 tumor cell line with CC50 (7810 µg/ml) for aqueous extract.

The aqueous extract of S. officinalis could cause 50% inhibition to L20B cells. This could be an indication of the relative safety of aqueous extracts of S. officinalis towards non-malignant cells. Compared with malignant cell lines, normal cell line (L20B) showed induction in viability after exposure to the extracts especially high concentrations. This could be indicative of the safety of S. officinalis extracts to normal cells by selectively affecting malignant cells. Lima et al, (2007) estimated that S. officinalis contains some phenolic compound that shows a significant protection potential against cell death in normal cell line (22).

Beljanski (2000) reported that plant-derived alkaloid flavopereirine, utilized in the form of a purified plant extract, selectively destroyed cancer cells; yet it

Table 5: Cytotoxic effect of aqueous extract of S. officinalis leaves on the growth of L20B cell line

Concentrations (µg/ml)	Exposure period		
	24 hrs	48 hrs	72 hrs
Control	0.1007±0.0009 ab	0.1030±0.0035 a	0.5720±0.0235 a
78.125	0.0730±0.003 a	0.091±0.0015 a	0.6373±0.0026 b
156.25	0.0947±0.0038 ab	0.0933±0.0012 a	0.6250±0.0136 ab
312.5	0.0743±0.0007 a	0.0940±0.0021 a	0.6227±0.0126 ab
625	0.0740±0.001 a	0.0947±0.0024 a	0.6123±0.0103 ab
1250	0.0813±0.0007 a	0.1177±0.0069 a	0.6113±0.0148 ab
2500	0.1120±0.007 b	0.1373±0.0133 a	0.6033±0.0041 ab
5000	0.2963±0.0174 d	0.2103±0.0289 b	0.7089±0.0101 c
10000	0.1970±0.0100 c	0.2100±0.0463 b	0.7117±0.0124 c

*Similar letters indicate no significant differences.

*Different letters indicate significant differences at $p<0.05$.

did not inhibit normal (non-malignant) cell multiplication (23). The selectivity of this anticancer agent could be due to the difference in membrane properties between cancer and normal cells. Nair et al, (2004) also found that the natural flavonoid, (quercetin) significantly inhibited the growth of the prostate cancer cell lines, whereas it did not affect colony formation by the normal fibroblast cell line BG-9. This effect is due to the fact that quercetin significantly inhibited the expression of specific oncogenes and genes controlling G1, S, G2 and M periods of the cell cycle.

Keshavarz et al, (2010) found that S. officinalis extract could inhibit proliferation of different cells at the concentrations 200 µg/ml without toxic effect on the cells in doses that ranged from 0-500 µg/ml (24).

Cisplatin, one of the most widely used cytotoxic drugs in the chemotherapy of human cancers, is a potent DNA-damaging anticancer agent, and its cytotoxic action is exerted by the induction of apoptosis. We evaluated the cytotoxicity of cisplatin on cell lines (RD, AMN3 and L20B), comparing it with the cytotoxic effect of the plant extracts, and in the present study the results revealed that no significant effect was detected on proliferation of RD tumor cell line for three periods(24, 48 and 72hours) as compared with controls. The anti proliferation effect of cisplatin is in agreement with the study of Leuschner et al, (2002) who suggested (RD) tumor cell line is one of the resistant types of tumor cell lines and this resistance against chemotherapy drugs especially cisplatin results from mutations (25).

The results of the present study on AMN3 cell line showed that there was significant growth inhibition of AMN3 cells due to treatment with cisplatin in both 48 and 72 hours and this result agrees with Ott and Gust, (2007) and Silver et al, (2010) who concluded that platinum (cisplatin) has antiproliferative/cytotoxic effects in human breast cancer cell lines and also in patients with breast cancer (26, 27).

The results showed the presence of a significant decrease in M.I in RD cells exposed to aqueous leaves extract of S. officinalis for 72 hours. Whereas the M.I of AMN3 tumor cells was significantly decreased after exposure to aqueous leaves extract of S. officinalis for 48 hours. Chang et al, (2004) found that (salvinal) isolated compound from aqueous extracts of Salvia miltiorrhizae, showed inhibitory activity against tumor cell growth and induced apoptosis in human cancer cells. They

Table 6: Cytotoxic effect of Cisplatin on the growth of L20B cell line

Concentrations (µg/ml)	Exposure period		
	24 hrs	48 hrs	72 hrs
Control	0.1117±0.0049 a	0.2260±0.005 a	0.2430±0.0005 a
78.125	0.0877±0.0960 a	0.1844 ±0.0148 a	0.2510±0.0455 a
156.25	0.0893±0.0054 a	0.2210±0.0146 a	0.2700±0.0288 a
312.5	0.0910±0.0036 a	0.2233±0.0133 a	0.2973±0.0578 a
625	0.0987±0.0059 a	0.2307 ± 0.2034 a	0.3187±0.0242 a
1250	0.0990±0.0127 a	0.2497±0.0267 a	0.3230±0.0412 a
2500	0.1050±0.0025 a	0.2693±0.0311 a	0.3330±0.03727 a
5000	0.1210±0.0055 a	0.2833±0.0375 a	0.3370±0.0199 a
10000	0.1243 ± 0.0219 a	0.3167±0.00491 a	0.3710±0.03051 a

*Similar letters indicate no significant differences.
*Different letters indicate significant differences at $p<0.05$.

Table 7: Mean ±SE for mitotic index (M.I) of RD tumor cells after treatment with aqueous extracts of S. officinalis leaves separately in vitro

Treatment	Concentration µg/ml	M.I%
S. officinalis aqueous extract (72hrs)	0	0.3840±0.00624 a
	5400	0.2182±0.01173 c
	2700	0.2727±0.01125 b
	1350	0.2938±0.00415 b

*SE=standard error; *Similar letters indicate no significant differences.*Different letters indicate significant differences at $p<0.05$.

	Concentration µg/ml	M.I%
Control	0	0.2902±0.01683 a
S. officinalis aqueous extract (48hrs)	7810	0.1568±0.01255 c
	3905	0.1740±0.01617 c
	1952	0.2209±0.00697 b

Table 8: Mean ±SE for mitotic index of AMNS tumor cells after 48 hours treatment with aqueous extract of S. officinalis leaves in vitro
*SE=standard error; *Similar letters indicate no significant differences.*Different letters indicate significant differences at p<0.05.

Table 9: Mean±SE for mitotic index of human blood lymphocytes after 48 hours treatment with aqueous extract of S. officinalis leaves in vitro

	Concentration µg/ml	M.I%
Negative control (PBS)	0	0.4893±0.006445 a
Positive control (Cyclophosphamide)	50	0.1038±0.00185 b
Aqueous leaves extract (48 hours)	7810	0.23420±0.01264 c
	3905	0.3206±0.02327 c
	1952	0.3333±0.01469 c

*Similar letters indicate no significant differences. *Different letters indicate significant differences at p<0.05.

Table 10: Mean±SE for mitotic index of human blood lymphocytes after 72 hours treatment with aqueous extract of S. officinalis leaves in vitro

	Concentration µg/ml	M.I%
Negative control (PBS)	0	0.4906±0.00561 a
Positive control (Cyclophosphamide)	50	0.1962±0.29093 b
Aqueous leaves extract (72 hrs)	5400	0.2737±0.01208 a
	2700	0.3084±0.00609 a
	1350	0.3269±0.00660 a

*Similar letters indicate no significant differences. *Different letters indicate significant differences at p<0.05.

Table 11: Effect of aqueous extracts of S. officinalis leaves and Cyclophosphamide on mitotic index in bone marrow cells of albino mice

	Concentration (mg/kg)	M.I%
Negative Control (PBS)	0	0.4657±0.01205 a
Positive control (Cyclophosphamide)	20	0.1857±0.01597 b
Aqueous leaves extract	4361	0.3652±0.00931 c

*Similar letters indicate no significant differences.*Different letters indicate significant differences at $p<0.05$.

suggest that the effect of Salvinal is due to inhibition of cell growth (IC50 range, 4-17 ?M) in a variety of human cancer cell lines using flow cytometry analysis which showed that salvinal treatment resulted in a concentration-dependent accumulation of cells in the G2/M phase by inhibited tubulin polymerization in a concentration-dependent manner (28).

The results of the present study revealed that the inhibitory effect of S. officinalis on both tumor cell lines may be due to the presence of one or more of phytochemical compounds such as flavanoid that have been identified in the extracts.

These results agree with that obtained by Regenbrecht et al, (2008) who found that a special type of isoflavonoid exhibits anti-carcinogenic activity. Cancer cells treated with this isoflavonoid undergo cell-cycle arrest at different checkpoints; this arrest was associated with a decrease in the mRNA levels of core regulatory genes (29).

In this study CP has the most mutagenic effect and caused a significant reduction in M.I. This result was the same results as that of Porter and Singh, (1988) who mentioned that rats treated with increasing doses of CP, caused high mutagenic and toxic effects on embryos during 48 hours in vitro cultures. (30).

Cyclophosphamide is one of the widely used anticancer drugs. Acrolein and phosphoramide are the active compounds of CP. These active compounds of the CP slow down the growth of cancerous cells by interfering with the actions of DNA within those cells. The mutagenicity of CP in particular is related to formation of the ultimate cytotoxic metabolite phosphoramide mustard through the intermediate agents' hydroxycyclo-phosphamide and deschloroethylcyclophosphamide, which is capable of inducing DNA cross links and strand lesions. (31).

This effect also appears in the present study of treatment of lymphocyte cells with CP. Salvia officinalis extracts have the ability to reduce M.I but with lower affect than CP. That means S. officinalis extracts have less mutagenic effect than that of CP. This result is in agreement with Vujosevic and Blagojevic, (2004) who found that the percentage of chromosomal aberrations decreased with increasing concentrations of S. officinalis (32). Also this effect may be due to antioxidant activity and suppression of metabolic activation, which could be mechanisms through which sage or some of its components act as desmutagen. The antioxidant effect of S. officinalis was also recorded by Patenkovic et al, (2009) and Dizaye, (2010) (33, 34).

The results of the present study showed significant decreases in M.I value in all tested doses of aqueous extract of S. officinalis leaves. The ability of S. officinalis extracts to reduce the M.I value can be traced to its chemical constituents such as taninnes that have the ability to block cell cycle progression. The study of Patenkovic et al, (2009) and Grzegorczyk and Wysokinska, (2008) explained the effect of different extracts of S. officinalis on M.I attributed to the antioxidant effects of terpenoids which enhanced the cell death (33, 35). The reduction in the M.I revealed a mitodepressive effect of the extract on dividing bone marrow cells of mice (36).

References

1- Zhou L, Chan WK, Xu N, Xiao K, Luo H, Luo KQ and Chang DC. Tanshinone. IIA, an isolated compound from Salvia miltiorrhiza Bunge, induces apoptosis in HeLa cells through mitotic arrest. Life Sci; 2008, 83 (11-12): 394-03.

2- World Health Organization (WHO). Fact sheet N°134. December 2008.

3- Weiss R, Fintelmann V: Herbal medicine. (2000); 2nd edition Thieme Stuttgart. New York.

4- Bartram T: Encyclopedia of Herbal Medicine, (1995); 1st edn., Grace Publishers, Bournemouth.

5- Betsy C: Book of Salvias: Sages for Every Garden by, (1997); Timber Press.

6- Bown D: Encyclopaedia of Herbs and their Uses. (1995); Dorling Kindersley, London.

7- Dizaye KF. The pharmacological and antimicrobial studies of Salvia officinalis infusion. ZJMS; (2005) .9(2): 55-63.

8- Simic D, Vukovic-Gacic B, Knezevic-Vukcevic J, Trninic S and Jankov RM. Antimutagenic effect of terpenoids from sage (Salvia officinalis L.). J. Environ Pathol Toxicol Oncol; 16(4): 1997, 293-01.

9- Slamenova D, Masterova I, Labaj J, Horvathova E, Kubala P, Jakubikova J and Wsolova L. Cytotoxic and DNA-damaging effects of diterpenoid quinones from the roots of Salvia officinalis L. on colonic and hepatic human cells cultured in vitro. Basic ClinPharmacol Toxicol; 2008, 94(6): 282-90.

10- McAllister R. M., Melnyk J., Finklestein J.Z., Adams E.C. and Gardner M.B.Cultivation in Vitro of Cells Derived from a Human Rhabdomyosarcoma. Cancer. 1969; 24(3) 520-526.

11- Al-Shamery, A. M. H.. The Study of Newcastle Disease Virus Effect in the Treatment of Transplanted Tumors in Mice. M.V.M. Thesis, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.2003.

12- Duizer E., Schwab K. J., Neill F. H., Atmar R. L., Koopmans M. P. G. and Estes M. K. Laboratory efforts to cultivate noroviruses. J Gen Virol. 2004; 85: 79-87.

13- Bhatta P and Sakya SR. Study of Mitotic Activity and Chromosomal Behaviour in Root Meristem of Allium cepa L. Treated with Magnesium Sulphate. ECOPRINT; 2008, 15: 83-88.

14- Hayslett, H. T. and Patrick, M. Statistics. Heinemann. London 1981; p:246.

15- Dixon WJ (1980). Efficient analysis of experimental observations. Ann. Rev. Pharmacol. Toxicol.1980, ; 20: 441-62.

16- Slamenova D, Masterova I, Labaj J, Horvathova E, Kubala P, Jakubikova J and Wsolova L. Cytotoxic and DNA-damaging effects of diterpenoid quinones from the roots of Salvia officinalis L. on colonic and hepatic human cells cultured in vitro. Basic Clin Pharmacol Toxicol; 2004, 94(6): 282-90.

17- Amigorena S . Cancer immunotherapy using dendritic cell derived exosomes. MEDICINA (Buenos Aires); 2000, 60 (2): 51-4.

18- Ibrahim A and Aqel AA . Effect of Salvia triloba L. f. extracts on neoplastic cell lines. Jordan Journal of Biological Sciences; 2010, 3(2): 69-76.

19- Keshavarz M, Mostafaie A, Mansouri K, Bidmeshkipour A, Motlagh HR and Parvaneh S.(2010). In vitro and ex vivo antiangiogenic activity of Salvia officinalis. Phytother Res; 24(10):1526-31.

20- Li J, Gyo WJ and Yang QY. Effects of ursolic acid and oleanolic acid on human colon carcinoma cell line HCT15. World J Gastoenterol; 2002, 15(3): 493-95.

21- Shibata Sh. Chemistry and cancer preventing activities. J. Korean Med Sci; 2001, 16(1): 28-37.

22- Lima CF, Valentao PCR, Andrade PB, Seabra RM, Fernandes-Ferreira M and Pereira Wilson C. Water and methanolic extracts of Salvia officinalis protect HepG2 cells from t-BHP induced oxidative damage. Chem Biol Interact; 2007, 167 (2): 107-15.

23- Beljanski M. The anticancer agent PB-100, selectively active on malignant cells, inhibits multiplication of sixteen malignant cell lines, even multidrug resistant. Genet Mol Biol.; 2000, 23(1): 29-33.

24- Keshavarz M, Mostafaie A, Mansouri K, Bidmeshkipour A, Motlagh HR and Parvaneh S.. In vitro and ex vivo antiangiogenic activity of Salvia officinalis. Phytother Res; 2010, 24(10):1526-31.

25- Leuschner I, Heuer Th and Harms D. Induction of drug resistance in human rhabdomyosarcoma cell lines is associated with increased maturation: Possible explanation for differentiation in recurrences. Pediatric and Developmental Pathology.; 2002, 5(30): 276-82.

26- Ott I and Gust R. Preclinical and clinical studies on the use of platinum complexes for breast cancer treatment. Anti-Cancer agents Med Chem; 2007, 7: 95-10.

27- Silver DP, Richardson AL, Eklund AC, Wang ZC, Szallasi Z, Li O, Juul N, et al. Efficacy of neoadjuvant cisplatin in triple-negative breast cancer. J Clin Oncol.; 28(7): 2010, 1145-53.

28- Chang J-Y, Chang CY, Kuo C-C, Chen L-T, Wein YS and Kuo Y-H. Salvinal, a novel microtubule inhibitor isolated from Salvia miltiorrhizae bunge (Danshen), with antimitotic activity in multidrug-sensitive and -resistant human tumor cells. Mol Pharmacol.; 2004, 65(1): 77-84.

29- Regenbrecht ChR, Jung M, Lehrach H and Adjaye J. The molecular basis of genistein-induced mitotic arrest and exit of self-renewal in embryonal carcinoma and primary cancer cell lines. BMC Medical Genomics; 2008, 1: 49.

30- Porter AJ and Singh SM. Toxicity and sister chromatid exchange in culture preimplantation mouse embryos exposed to serum from cyclophosphamide-treated rats: possible implications for testing maternal serum genotoxicity. Mutagenesis; 1988, 3: 45 9.

31- Sowjanya L, Devi KR and Madhavi D. Modulatory effects of garlic extract against the cyclophosphamide induced genotoxicity in human lymphocytes in vitro. Journal of Environmental Biology; 2009, 30(5): 663-66.

32- Vujosevic M and Blagojevic J. Antimutagenic effects of extracts from Sage (Salvia officinalis) in mammalian system in vivo. Acta Veterinaria Hungarica; 2004, 52 (4): 439-43.

33- Patenkovic A, Stamenkovic-Radak M, Banjanac T and Andjelkovic M. Antimutagenic effect of sage tea in the wing spot test of Drosophila melanogaster. Food Chem Toxicol.; 47(1): 2009, 180-83.

34- Dizaye K. Allevation of Cisplatin-induced nephrotoxicity in albino rats by aqueous extract of Salvia officinalis. Proceeding of the CB medical treatment symposium, spiez laboratory, Switzerland, 2010 (CBMTS VIII).

35- Grzegorczyk I and Wysokinska H. Liquid shoot culture of Salvia officinalis L. for micropropagation and production of antioxidant compounds; Effect of triacontanol. Acta Societatis Botanicorum. 2008, 77(2): 99-104.

36- Modallal N, Abderrahman S and Papini A (2008). Cytogenetic effect of Arum maculatum extract on the bone marrow. CaryologiaA.; 2008, 61(4): 383-87.

Metabolic deterioration just after infancy

ABSTRACT

Background: We tried to understand whether or not there are some relationships between body weight alone and systolic and diastolic blood pressures (BP) and other metabolic parameters even in childhood.

Methods: Consecutive children and adolescents between the ages of 2 and 15 years were studied. Patients with devastating illnesses were excluded to avoid their possible effects on weight. Cases were divided into the four groups according to their percentile for weight alone, including cases below the 3rd percentile in the first, below the 50th percentile in the second, at and above the 50th percentile in the third, and above the 97th percentile in the fourth group.

Results: The study included 299 cases. Although mean values of the systolic and diastolic BPs, plasma glucose, total cholesterol, low density lipoprotein cholesterol, triglyceride, and alanine aminotransferase increased, mean value of the high density lipoprotein cholesterol decreased significantly from the first towards the fourth group with a gradual manner nearly in all steps.

Conclusion: Metabolic syndrome is a reversible progression step between complete physical health and irreversible terminal diseases with a very high prevalence in adults. But these findings suggest that pathophysiological mechanisms of the syndrome are already going on even in childhood, and bases of the syndrome are started to be built up just after the period of infancy, probably due to the eating habits of the families. Therefore, because of the irreversible natures of the terminal diseases of the syndrome, the care to prevent should be started even in childhood.

Key words: Body weight, blood pressure, metabolic syndrome, infancy

Mehmet Rami Helvaci (1)
Murat Tutanc (2)
Vefik Arica (3)

(1) Medical Faculty of the Mustafa Kemal University, Antakya, Associated Professor of Internal Medicine, M.D.
(2) Medical Faculty of the Mustafa Kemal University, Antakya, Assistant Professor of Pediatrics, M.D.

Correspondence:
Mehmet Rami Helvaci, M.D.
Medical Faculty of the Mustafa Kemal University,
31100, Serinyol, Antakya, Hatay, Turkey
Phone: +903262291000 (3333) Fax: +903262455654 Mobile phone: +905362894692
Email: mramihelvaci@hotmail.com

Introduction

An association between certain metabolic parameters and hypertension (HT), type 2 diabetes mellitus (DM), coronary heart disease (CHD), stroke, and eventually an increased all-cause mortality has been known for many years, and defined as the metabolic syndrome.(1,2) It has become increasingly common in developed countries, for example, it is estimated that 50 million Americans have it.(3) The metabolic syndrome is characterized by a group of metabolic risk factors, including overweight, impaired glucose tolerance (IGT), impaired fasting glucose (IFG), hyperbetalipoproteinemia, hypertriglyceridemia, dyslipidemia, white coat hypertension (WCH), and a prothrombotic and proinflammatory state(4,5) instead of being a certain disease because it can be reversed completely with appropriate nonpharmaceutical approaches, including lifestyle changes, diet, and exercise.(6) So the syndrome actually contains the risk factors for the development of irreversible terminal diseases which decrease duration or quality of life, such as HT, DM, CHD, peripheric artery disease, renal failure, and stroke. Although it is a well known life threatening role and there is a high prevalence of the syndrome in adults, its significance is not so clear in children. We tried to understand whether or not there are some relationships between body mass, as the major component of the syndrome, and systolic and diastolic blood pressures (BP) and other metabolic parameters even in children, but we preferred to use body weight alone instead of the body mass index (BMI) as an indicator of excess body fat in the study.

Materials and Methods

The study was performed in the Polyclinic for the Pediatrics of the Mustafa Kemal University between May 2009 and February 2010, prospectively. We studied consecutive children and adolescents applying for any complaint between the ages of 2 and 15 years. Infants were excluded due probably to some protective effects of breastfeeding. A detailed medical and family history was obtained from all patients, and a physical examination was performed. Body weight was measured with a digital scale to the nearest 0.1 kg, and percentile for weight alone for each case and it was calculated by the measurements of the physician instead of verbal expressions. A routine check up procedure including plasma glucose, total cholesterol, low density lipoprotein cholesterol (LDL-C), triglyceride (TG), high density lipoprotein cholesterol (HDL-C), and alanine aminotransferase (ALT) values was initially performed without a fasting state because of the difficulty of fasting in children. A fasting plasma glucose (FPG) was obtained at the second procedure just in cases with a plasma glucose level of 126 mg/dL or higher on the random sample, not to overlook diabetic cases. Eventually, patients with devastating illnesses including type 1 DM, malignancies, acute or chronic renal failure, chronic liver diseases, celiac disease, and hyper- or hypothyroidism were excluded to avoid their possible effects on weight. BP was checked after a 5-minute silent state of the children with a mercury sphygmomanometer (ERKA, Germany). All readings were taken from the right arm. Appropriate sized cuffs were used with cuff-width 40% of mid-arm circumference, and cuff bladders covering 80-100% of the arm circumference and approximately two thirds of the length of the upper arm without overlapping. The first measured BP was not used alone, and a second was obtained just after. The average of the two measurements was recorded and included in the analysis. Eventually, all cases were divided into the four groups according to their percentile for weight alone, including cases below the 3rd percentile for his or her age in the first, cases below the 50th percentile in the second, cases at and above the 50th percentile in the third, and cases above the 97th percentile in the fourth group. Finally, the mean age, female ratio, and mean values of the systolic and diastolic BPs, plasma glucose, total cholesterol, LDL-C, HDL-C, TG, and ALT were detected in each group and compared. Mann-Whitney U Test, Independent-Samples T Test, and comparison of proportions were used as the methods of statistical analyses.

Results

The study included 299 children and adolescents (173 females and 126 males), totally. Anthropometric and metabolic data are shown in Table 1 (next page). Mean ages of the groups were 8.6 ± 4.3, 8.0 ± 3.6, 8.0 ± 3.8, and 8.0 ± 3.5 years from the first towards the fourth group, respectively, without any statistical significance between groups. Similarly, female ratios of the groups were similar, too (59.5%, 58.3%, 58.5%, and 55.9%, respectively, $p>0.05$ in all steps). When we compared the four groups according to the mean values of the systolic and diastolic BPs, plasma glucose, total cholesterol, LDL-C, HDL-C, TG, and ALT, except one, all of the parameters increased significantly from the first towards the fourth group with a gradual manner ($p<0.05$ nearly in all steps). The only parameter showing no significant increase was the HDL-C, and as an opposite feature, its mean value decreased significantly in the same direction with a gradual manner again ($p<0.05$ nearly in all steps).

Discussion

The metabolic syndrome is a collection of metabolic risk factors for many terminal diseases. Although there is not any universally accepted definition for the syndrome, it basically includes five features: obesity (high body weight, BMI, or waist circumference), high glucose and insulin levels, low HDL-C, high TG, and high BP.(7) But the already used definitions as a BP of 135/85 or 140/90 mmHg or above and a FPG of 100 or 110 mg/dL or above also include patients with DM and HT. But actually the syndrome is a collection of risk factors instead of the final diseases, and it is a reversible condition with appropriate nonpharmaceutical approaches, whereas the diseases, obesity, HT, DM, and symptomatic atherosclerosis, are irreversible and final states which almost always require drug therapy to delay complications. For example, in a previous study by us(5), prevalences of hyperbetalipoproteinemia, hypertriglyceridemia, dyslipidemia, IGT, and WCH showed a parallel fashion to excess weight by increasing until the seventh decade of life and decreasing afterwards, significantly ($p<0.05$ nearly in all steps). On the other hand, prevalences of HT, DM, and CHD always continued to increase by aging without any decrease ($p<0.05$ nearly in all steps), indicating their irreversible properties.(5) So metabolic syndrome alone is a disadvantageous but reversible status but not a final disease, and after the development of one of the final metabolic diseases, the term of metabolic syndrome probably loses most of its significance, since from now on, the nonpharmaceutical approaches will provide little benefit to prevent development of the others, probably due to cumulative effects of the risk factors on systems for a long period of time. So the definition of metabolic syndrome should include reversible metabolic risk factors such as overweight, hyperbetalipoproteinemia, hypertriglyceridemia, dyslipidemia, IGT, IFG, and WCH but not obesity, HT, DM, CHD, and stroke like terminal diseases.

HT has been recognized as a cardiovascular risk factor for several decades, and it is a prevalent pathology in adults. For example, it has been reported that more than 85% of cases with the metabolic syndrome have elevated BP levels.(6) Because BP tends to tract along the same percentile throughout life, children with higher BPs are more likely to become adults with HT in the near future. So it is now widely accepted that cardiovascular health originates in childhood. Similarly, we observed very high prevalences of WCH even in early decades in a previous study(8), 23.2% in the third and 24.2% in the fourth decades of life. The high prevalences of WCH in society were shown in some other studies, too.(9-11) When we compared the sustained normotension (NT), WCH, and HT groups in another study in adults(12), prevalences of nearly all of the health problems including obesity, IGT, DM, and CHD showed significant progressions from the sustained NT towards the WCH and

Variables	Cases below the 3rd percentile for weight	*p*-value	Cases below the 50th percentile for weight	*p*-value	Cases at and above the 50th percentile for weight	*p*-value	Cases above the 97th percentile for weight
Prevalence	15.7% (47)	ns*	16.0% (48)	<***0.001***	37.1% (111)	ns	31.1% (93)
Mean age (years)	8.6 ± 4.3 (2-15)	ns	8.0 ± 3.6 (2-15)	ns	8.0 ± 3.8 (2-15)	ns	8.0 ± 3.5 (2-15)
Female ratio	59.5% (28)	ns	58.3% (28)	ns	58.5% (65)	ns	55.9% (52)
Mean value of systolic BP† (mmHg)	102.0 ± 13.3 (79-136)	ns	104.4 ± 10.9 (78-124)	***0.000***	112.1 ± 12.5 (84-145)	***0.009***	116.4 ± 10.2 (83-136)
Mean value of diastolic BP (mmHg)	59.2 ± 11.7 (34-86)	ns	62.2 ± 11.0 (39-81)	***0.000***	69.9 ± 11.9 (41-92)	***0.001***	75.0 ± 10.4 (40-93)
Mean value of blood glucose (mg/dL)	85.2 ± 20.9 (45-131)	***0.014***	94.5 ± 14.6 (65-128)	ns	98.4 ± 17.0 (71-146)	***0.000***	132.5 ± 30.3 (71-225)
Mean value of total cholesterol (mg/dL)	122.5 ± 46.0 (40-245)	ns	137.2 ± 46.3 (68-266)	ns	141.6 ± 51.6 (52-274)	***0.000***	218.1 ± 71.3 (74-352)
Mean value of LDL-C‡ (mg/dL)	75.0 ± 33.0 (32-162)	ns	84.8 ± 33.6 (33-152)	ns	91.0 ± 41.8 (25-205)	***0.000***	141.7 ± 52.0 (32-257)
Mean value of HDL-C§ (mg/dL)	76.2 ± 26.8 (27-142)	ns	69.2 ± 22.3 (22-118)	***0.000***	52.8 ± 18.0 (15-96)	***0.000***	41.2 ± 19.0 (8-81)
Mean value of TG‖ (mg/dL)	72.8 ± 45.4 (20-184)	***0.000***	106.6 ± 38.0 (26-186)	ns	116.1 ± 44.7 (33-225)	***0.000***	233.0 ± 50.3 (42-312)
Mean value of ALT¶ (U/L)	29.8 ± 16.9 (10-66)	ns	34.1 ± 16.9 (8-66)	ns	36.5 ± 14.1 (12-74)	***0.000***	50.7 ± 20.9 (10-91)

*Nonsignificant ($p>0.05$) †Blood pressure ‡Low density lipoprotein cholesterol §High density lipoprotein cholesterol ‖Triglyceride ¶Alanine aminotransferase

Table 1: Characteristics of the study cases

HT groups, and the WCH group was found as a progression step in between. So the detected high prevalences of WCH even in early decades, despite the low prevalences of excess weight in these age groups, may show a trend of gaining weight and several terminal diseases. As an interesting finding of the present study, although the increased systolic BP values alone parallel to the increased BMI in some previous studies(13), the mean diastolic BP values were also increased parallel to the increased body weight alone, significantly.

It is already known that excess weight leads to both structural and functional abnormalities in many systems of body, and risk of death from all causes, including cardiovascular diseases and cancers, increases parallel to the range of moderate to severe weight excess in all age groups.(14,15) The effects of body mass on BP were also shown previously by us(16) that the prevalence of sustained NT was significantly higher in the underweight (80.3%) than the normal weight (64.0%) and overweight cases (31.5%) ($p<0.05$ for both), and 55.1% of cases with HT had obesity against 26.6% of cases with NT ($p<0.001$) in another study.(17) So the dominant underlying risk factor of the metabolic syndrome appears as an already existing excess weight(6), or a trend towards excess weight, which is probably the main cause of insulin resistance, dyslipidemia, IGT, IFG, and WCH. Even prevention of the accelerating trend of body weight with diet or exercise, even in the absence of a prominent weight loss, will probably result with resolution of many parameters of the metabolic syndrome.(18-20) Since obesity tends to tract along the same percentile throughout life, children with higher body weights are more likely to become adults with obesity in the future. So it is now widely accepted again that obesity

originates in childhood. But according to our opinion, limitation of excess weight as an excessive fat tissue in and around the abdomen under the heading of abdominal obesity is meaningless; instead it should be defined as excess weight including overweight and obesity via body weight alone or BMI, since adipocytes function as an endocrine organ that produces a variety of cytokines and hormones anywhere in the body.(6) The resulting hyperactivity of sympathetic nervous system and renin-angiotensin-aldosterone system is probably associated with insulin resistance, endothelial dysfunction, and elevated systolic and diastolic BPs.

Body weight alone may also be a sensitive method to detect excess fat in the body.(21) The Adult Treatment Panel III(22) reportedthat although some people with a large muscular mass are classified as overweight according to the BMI, most of them also have excess fat tissue, so they are actually obese according to body weight alone. So BMI should not be accepted as the final progression step for the detection of excess body fat, and research should be continued to find some more appropriate methods. For example, the detected significant positive correlations of body weight with systolic and diastolic BPs, plasma glucose, total cholesterol, LDL-C, and TG and the negative correlation with HDL-C in the present study may also support the sensitivity of body weight alone for detection of the metabolic syndrome. As a similar result to ours, increasing weight showed significant increases in prevalence of HT in a linear relationship in the some other studies in adults.(21,23)

Nonalcoholic fatty liver disease (NAFLD) is another consequence of excess weight, and probably is the hepatic component of the metabolic syndrome. NAFLD is a term used to define a spectrum of disorders characterized by macrovesicular steatosis which occur in the absence of consumption of alcohol in amounts considered to be harmful to the liver. Since the possibility of having NAFLD is directly proportional to body weight, and there is an increasing prevalence of excess weight in society, NAFLD is becoming an important health problem, nowadays. According to the literature, sustained liver injury will lead to progressive fibrosis and cirrhosis in 10% to 25% of affected individuals.(24) There are two histologic patterns of NAFLD, including fatty liver alone and nonalcoholic steatohepatitis (NASH). NASH represents a shift from simple steatosis to an inflammatory component. Obesity and insulin resistance are main factors in exacerbating hepatic inflammation and fibrogenesis in NASH. NAFLD markers such as ALT may independently predict the metabolic syndrome in adults. But as a similar result to ours, a strong association of the metabolic syndrome with elevated ALT levels was detected even in children and adolescents, and this association existed in a graded fashion across the number of metabolic components.(25) Additionally, a correlation between degree of obesity and severity of the hepatic steatosis has also been reported, ultrasonographically.(26,27)

As a conclusion, the metabolic syndrome is a reversible progression step between physical health and irreversible terminal diseases with a very high prevalence in adults. But these findings suggest that pathophysiological mechanisms related to the syndrome are already going on even in childhood, and the bases of the syndrome are starting to be built up just after the period of infancy, probably due to the eating habits of the families. Therefore, because of the irreversible natures of the terminal points of the syndrome, the care to prevent should be started even in childhood.

References

1. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet 2005; 365: 1415-1428.

2. Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation 2004; 109: 433-438.

3. Clark LT, El-Atat F. Metabolic Syndrome in African Americans: implications for preventing coronary heart disease. Clin Cardiol 2007; 30: 161-164.

4. Tonkin AM. The metabolic syndrome(s)? Curr Atheroscler Rep 2004; 6: 165-166.

5. Helvaci MR, Kaya H, Seyhanli M, Yalcin A. White coat hypertension in definition of metabolic syndrome. Int Heart J 2008; 49: 449-457.

6. Franklin SS, Barboza MG, Pio JR, Wong ND. Blood pressure categories, hypertensive subtypes, and the metabolic syndrome. J Hypertens 2006; 24: 2009-2016.

7. Hunt KJ, Resendez RG, Williams K, Haffner SM, Stern MP. National Cholesterol Education Program versus World Health Organization metabolic syndrome in relation to all-cause and cardiovascular mortality in the San Antonio Heart Study. Circulation 2004; 110: 1251-1257.

8. Helvaci MR, Seyhanli M. What a high prevalence of white coat hypertension in society! Intern Med 2006; 45: 671-674.

9. Hozawa A, Ohkubo T, Kikuya M, Yamaguchi J, Ohmori K, Fujiwara T, et al. Blood pressure control assessed by home, ambulatory and conventional blood pressure measurements in the Japanese general population: the Ohasama study. Hypertens Res 2002; 25: 57-63.

10. Celis H, Fagard RH. White-coat hypertension: a clinical review. Eur J Intern Med 2004; 15: 348-357.

11. Helvaci MR, Kaya H, Seyhanli M, Cosar E. White Coat Hypertension Is Associated with a Greater All-cause Mortality. J Health Sci 2007; 53: 156-160.

12. Helvaci MR, Kaya H, Duru M, Yalcin A. What is the relationship between white coat hypertension and dyslipidemia? Int Heart J 2008; 49: 87-93.

13. Kelishadi R, Cook SR, Adibi A, Faghihimani Z, Ghatrehsamani S, Beihaghi A, et al. Association of the components of the metabolic syndrome with non-alcoholic fatty liver disease among normal-weight, overweight and obese children and adolescents. Diabetol Metab Syndr 2009; 1: 29.

14. Calle EE, Thun MJ, Petrelli JM, Rodriguez C, Heath CW Jr. Body-mass index and mortality in a prospective cohort of U.S. adults. N Engl J Med 1999; 341: 1097-1105.

15. Helvaci MR, Kaya H, Sevinc A, Camci C. Body weight and white coat hypertension. Pak J Med Sci 2009; 25: 6: 916-921.

16. Helvaci MR, Ozcura F, Kaya H, Yalcin A. Funduscopic examination has limited benefit for management of hypertension. Int Heart J 2007; 48: 187-194.

17. Helvaci MR, Kaya H, Yalcin A, Kuvandik G. Prevalence of white coat hypertension in underweight and overweight subjects. Int Heart J 2007; 48: 605-613.

18. Azadbakht L, Mirmiran P, Esmaillzadeh A, Azizi T, Azizi F. Beneficial effects of a Dietary Approaches to Stop Hypertension eating plan on features of the metabolic syndrome. Diabetes Care 2005; 28: 2823-2831.

19. Volek JS, Feinman RD. Carbohydrate restriction improves the features of Metabolic Syndrome. Metabolic Syndrome may be defined by the response to carbohydrate restriction. Nutr Metab (Lond) 2005; 2: 31.

20. Helvaci MR, Kaya H, Borazan A, Ozer C, Seyhanli M, Yalcin A. Metformin and parameters of physical health. Intern Med 2008; 47: 697-703.

21. Helvaci MR, Ozer C, Kaya H, Yalcin A. Practical cut off values to determine body mass index. Med J Malaysia 2008; 63: 122-124.

22. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002; 17:106: 3143-3421.

23. Al-Nozha MM, Abdullah M, Arafah MR, Khalil MZ, Khan NB, Al-Mazrou YY, et al. Hypertension in Saudi Arabia. Saudi Med J 2007; 28: 77-84.

24. Sanyal AJ; American Gastroenterological Association. AGA technical review on nonalcoholic fatty liver disease. Gastroenterology 2002; 123: 1705-1725.

25. Oliveira AC, Oliveira AM, Almeida MS, Silva AM, Adan L, Ladeia AM. Alanine aminotransferase and high sensitivity C-reactive protein: correlates of cardiovascular risk factors in youth. J Pediatr 2008; 152: 337-342.

26. Chan DF, Li AM, Chu WC, Chan MH, Wong EM, Liu EK, et al. Hepatic steatosis in obese Chinese children. Int J Obes Relat Metab Disord 2004; 28: 1257-1263.

27. Franzese A, Vajro P, Argenziano A, Puzziello A, Iannucci MP, Saviano MC, et al. Liver involvement in obese children. Ultrasonography and liver enzyme levels a

Case report: Chronic Lymphocytic thyroiditis (Hashimoto's disease)

Ibrahim Mohamed ELSaeed (1)
Almoutaz Alkhier Ahmed (2)
Ali Alsharief (3)
Khalid Abdullah Khalid (4)
Mahmoud Khalif Ismael (5)

1) Dr. Ibrahim Mohamed Elsaeed, Internist
.MSc internal medicine. Medical Specialist Center/National Guard Health Affairs, Jeddah - KSA
2) Dr. Almoutaz Alkhier Ahmed, Diabetologist, MSc in diabetes, [MRCGP[intr]
Medical Specialist Center, National Guard Health Affairs, Jeddah - KSA
3) Dr. Ali Alsharief, consultant family medicine SBFM,ABFM. Medical Specialist Center/National Guard Health Affairs, Jeddah - KSA
4) Dr. Khalid Abdullah Khalid ,MRCPH. Pediatrician. King Abdulaziz Medical City. Jeddah - KSA
5) Dr. Mahmoud Khalifa Ismael,Radiologist.MSc in Radiology. Medical Specialist Center/National Guard Health Affairs, Jeddah - KSA

Correspondence:
Dr. Almoutaz Alkhier Ahmed , Diabetologist , MSc in diabetes, [MRCGP[intr]
Medical Specialist Center/National Guard Health Affairs, Jeddah, Kingdom of Saudi Arabia
Email: khier2@yahoo.com

ABSTRACT

A ten year old female child presented with anterior neck pain with neck swelling. Examination showed goiter with mild tenderness. Investigation showed high TSH and high thyroglobulin, thyroperoxidase antibodies. Patient was diagnosed with chronic lymphocytic thyroiditis (Hashimoto's disease) and received Thyroxine which brought her thyroid function to normal. Follow up plan was designed and patient going well with management.

Introduction

Hashimoto's thyroiditis is a condition caused by inflammation of the thyroid gland. It is an autoimmune disease. The underlying cause of the autoimmune process still is unknown. Hashimoto's thyroiditis tends to occur in families, and is associated with a clustering of other autoimmune conditions. In diagnosing Hashimoto's thyroiditis, assess symptoms and complaints commonly seen in hypothyroidism, examine the neck, and take a detailed history of family members. Blood tests are extremely useful in diagnosing Hashimoto's thyroiditis. The blood tests look at the thyroid function in general. With hypothyroidism, a high thyroid stimulating hormone and low thyroid hormone would be expected. Thyroid hormone medication can replace the hormones the thyroid made before the inflammation started.

Case Report

"S" is a female child aged 10 years. She attended with her parent complaining of pain with swallowing plus pain in the front of the neck since one month.

"S" informed that neck pain started gradually on the front of her neck .The pain is not sharp, dull in character and radiates to the jaw. There are no associated symptoms and she did not notice by herself or her parent any factors increasing or decreasing the pain. There were no symptoms suggestive of thyroid dysfunction.

"S" did not have any history of a similar condition.

There was no family history of a similar condition. She did not suffer from any illness in the near future or undergo any surgical interventions.

Her immunization was up to date.

On examination, she looks well. She did not have fever, her temperature was 36.6 oC . There was no pallor or jaundice. Her weight was 39kg and her height was 145cm (BMI 18.5 which is normal for her age). She was correlated well to her developmental standards.

Chest, cardiac and abdominal examinations were normal.

Neck examination showed swelling at the site of the thyroid gland. Her thyroid examination showed slight enlargement of both lobes which was mildly tender on palpation. Cervical lymph nodes were normal .There was no thyroid bruit.

Investigations were requested and showed:

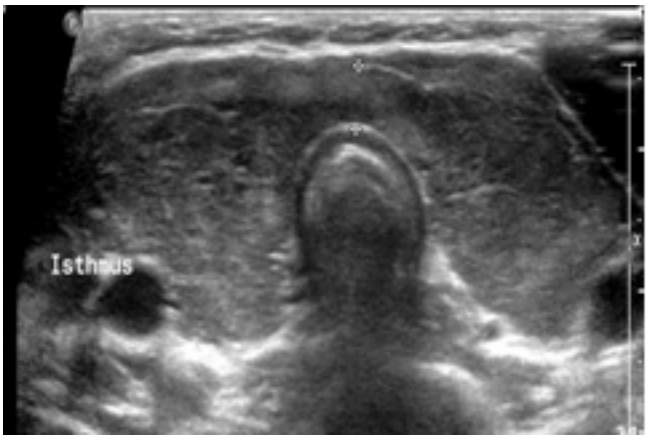
A) Routine Hematology

Test	Result	Normal value
WBC	5.9	4 – 12 (10^9/L)
RBC	5.04	3.9 – 5 (10^12/L)
Hemoglobin	13.0	11.3 – 15g/dl
Hematocrit	37.9	34-45%
MCV	75.3	77-90fl
MCH	25.8	25-30pg
MCHC	34.3	31-36g/dl
RDW	12.4	11.5-14.5%
PLT	432	150-450 10^9/L
Absolute LYPHS	3.29	1.3-7.2 10^9/L
Absolute MONOS	0.38	0.2-0.8 10^9/L
Absolute EOS	1.91	1.1-7.2 10^9/L
Absolute BASO	0.29	0.1-0.7 10^9/L
	0.06	0.0-0.1 10^9/L

B) Chemistry

Test	Result	Normal value
NA	136	136-145 mmol/L
K	4.4	3.1-5.1 mmol/L
Chloride	103	98-107 mmol/L
Bicarbonate	24	20-28 mmol/L
Urea	2.7	2.5-6 mmol/L
Creatinine	52	27-62 mmol/L
C-Reactive Protein	1.2	<5 mg/L

Thyroid ultrasound was done with the following report:



Ultrasound report

Endocrinology

Date	Test	Before Thyroxin therapy	After Thyroxin therapy
17 Nov 2012	TSH	93.09 mIU/L (0.35-4.94 mIU/L)	
	Free T4	9.1 pmol/L (9-19pmol/l)	
	Free T3	4.9 pmol/l (2.6-5.7pmol/L)	
	Vit D3	20.9 nmol/L (<25nmolLl deficiency)	
23/11/2012	TPO Ab	213.7	
	Thyroglob Ab	230.2	
22/12/2012	TSH		9.4 mIU/L
	Free T4		13.5 pmol/l
20/1/2013	TSH		6.73 mIU/L
	Free T4		13.5 pmol/L

Findings

Both thyroid lobes are enlarged with heterogeneous echotexture and increased vascularity with no focal nodules seen.

The right thyroid lobe measures 1.9 x 2.7 x 5 cm. The left thyroid lobe measures 1.6 x 2.2 x 5 cm.

The isthmus measures 0.4 cm.

Benign looking small bilateral cervical lymph node.

Both submandibular and parotid gland appear unremarkable.

Conclusion

Bilateral enlarged hypervascular & heterogeneous thyroid lobes likely in keeping with thyroiditis, for clinical correlation.

At this stage we discussed with the parent the possibility of thyroid inflammation. The clinical picture showed mixed presentation between subacute thyroiditis and chronic thyroiditis especially as the patient did not attend at the time of initial presentation.

Table 1 (top of next page) shows symptoms and signs suggestive of either subacute or chronic thyroiditis.

The most likely diagnosis is chronic lymphocytic thyroiditis (Hashimoto's disease)

Replacement therapy was started in the form of Eltroxin 50mcg.

As chronic thyroiditis is an autoimmune disease we explained to parent the necessity of long run follow up to screen the patient from other autoimmune diseases such as diabetes type 1, Celiac disease and pernicious anemia. Patient will be reviewed every three weeks to adjust the dose of Eltroxine to reach the normal TSH value. Vit D is also vit D deficiency and attention should be directed to correct this deficiency as there are some studies that have shown that progression of thyroiditis may correlate with severity of vit D deficiency.

Discussion

Chronic lymphocytic thyroiditis (Hashimoto's disease) is the most common form of thyroiditis in childhood (1) . Weetman AP (2) reported clinical HT prevalence rate at 1 in 182 or 0.55% in the US. In the UK, Tunbridge et al (3) reported an overall HT prevalence of 0.8%. However, diagnosis based fine needle aspiration biopsy study; the cytology of HT seems to be much more prevalent, at 13.4% (4). This difference may be partially explained by the fact that for diagnosing clinical HT, abnormally elevated TSH, low thyroid hormones (2-3) and the confirmatory presence of thyroid autoantibodies are usually accounted for.

Every year 5% of Hashimoto's disease develops into overt hypothyroidism (5). At the time of diagnosis, children and adolescents with chronic thyroiditis may be asymptomatic and the main reasons for referral are goiter, hypothyroid symptoms and findings which occur while working on unrelated problems or for high risk groups (6). Thyroid function at presentation

	Subacute thyroiditis	Chronic thyroiditis
Symptom(s)	• Neck pain • Pain with swallowing • Age : 10 years	• Neck swelling (goiter) • No fever
Sign(s)	• Tenderness on examination	• Firm swelling on palpation
Investigation(s)		• CRP normal • TSH high (93 mIU/L) • Low free T4 (9.1Pmol/l) • Low free T3 (4.9Pmol/l) • High TPO (213.7) • High Throglopulin (230.2) • U/S thyroid : enlarged, hypervascular and heterogenous thyroid lobes

Table 1: Subacute vs chronic lymphocyctic thryroiditis

may significantly vary in different pediatric reports (7-10) , ranging from euthyroid to overt hypothyroidism or occasionally, hyperthyroidism. Also the thyroid function may present with subclinical hypothyroidism or more rarely subclinical hyperthyroidism.

There are few studies investigating the factors which may affect different biochemical presentations of chronic lymphocytic thyroiditis (Hashimoto's disease) and these are frequently based on limited pediatric populations. An interesting recent study (11) tried to answer the question if there are factors affecting the incidence of thyroid dysfunction in chronic lymphocytic thyroiditis. The researchers found that at presentation, test results showed euthyroidism in 52.1% of patients (subgroup A), overt or subclinical hypothyroidism in 41.4%, and overt or subclinical hyperthyroidism in 6.5%. The mean age of patients with thyroid dysfunctions (subgroup B) was significantly lower than that of subgroup A, and the rate of children below 10 years of age was significantly greater in subgroup B. Other variables related to thyroid function patterns were prepubertal status; association with either Down or Turner syndromes, which correlated with increased risk of thyroid dysfunctions, and association with other autoimmune diseases, which correlated with decreased risk of thyroid dysfunctions. None of the remaining factors analyzed were associated with increased risk of thyroid dysfunctions. The transient hyperthyroid phase of hashitoxicosis may be due to unregulated release of stored thyroid hormones from destructive cells due to the inflammatory mediated process (12). Presentation of hashitoxicosis could be very similar to Grave's disease in childhood (13). So, differential diagnosis of hashitoxicosis from Grave's disease can be a real challenge when the diagnosis is based only on clinical and biochemical features (14). The presence of goiter and elevated thyroglobulin (Tg) autoantibodies at presentation, together with progressive increase in both thyroid peroxidase antibodies and TSH may be considered as predictive factors for the future development of hypothyroidism in euthyroid patients (15). The presence of additional risk factors such as celiac disease or elevated TSH and thyroid peroxidase antibodies at presentation seems to significantly increase the risk of developing overt hypothyroidism after 3 years in the chronic thyroiditis children (16-17).

An association between severity of vitamin D deficiency and chronic autoimmune thyroiditis has long been investigated. Bozkurt NC et al (18) studied this relation and they concluded that level of vit D(3) in patients with chronic lymphocytic thyroiditis was significantly lower than in controls and the severity of vit D deficiency was correlated with duration of thyroiditis, and thyroid volume antibody levels.

In conclusion chronic lymphocytic thyroiditis is the commonest thyroid dysfunction in childhood. High clinical sense can be of benefit in suspecting diagnosis of Hashimoto's disease. Full assessment was needed to diagnose the condition.

References

1) Wasniewska M, Vigone MC, Cappa M, Aversa T, Rubino M, De Luca F: Study Group for Thyroid diseases of Italian Society for Pediatric Endocrinology: Acute suppurative thyroiditis in childhood: relative frequency among thyroid inflammatory diseases. J Endocrinol Invest 2007, 30:346-347.

2) Weetman AP: Thyroid disease. In: the Autoimmune Disease. Rose NR, Mackay IR (eds). 2006; 467-482.

3) Tunbridge WM, Evered DC, Hall R, Appleton D, Brewis M, Clark F, Evans JG, Young E, Bird T, Smith PA. The spectrum of thyroid disease in a community: The Whickham survey. Clin Endocrinol (Oxf) 1977; 7: 481-493.

Key message

Criteria for diagnosing chronic lymphocytic thyroiditis (Hashimoto's disease)

- Seropositivity for Tg autoantibodies and/or TPO autoantibodies plus one at least of the following:

✔ Abnormal thyroid function
✔ Enlarged thyroid gland
✔ Morphological changes on thyroid ultrasound

Key message

When to start Thyroxin therapy (19)?

✔ If TSH values >10 IU/mL
✔ If TSH values >5 IU/mL in combination with goiter or thyroid autoantibodies

What is the target of treatment?

Normalization of TSH level

When to recheck thyroid function after initiation of Thyroxin?

Usually after 6 - 8 weeks

At whitch interval should the thyroid function be repeated?

Once biochemical euthyroidism has been achieved, TSH can be monitored every 4-6 months in the growing child and yearly up to the attainment of final height

How to take medication?

Treatment should be administered at least 20 min, before eating or ingestion of any medication known to impair its absorption, such as calcium and iron supplements, sucralfate, potassium-binding resins, antacids containing aluminium, and bile-acids binding resins.

4) Staii A, Mirocha S, Todorova-Koteva K, Glinberg S, Jaume JC. Hashimoto thyroiditis is more frequent than expected when diagnosed by cytology which uncovers a preclinical state. Thyroid Research 2010, 3: 11.

5) Nagataki S. The concept of Hashimoto disease. In: Nagataki S, Mori T, Torizuka K, eds. Eighty years of Hashimoto disease. Amsterdam: Elsevier Science, 1993:539-45.

6) de Vries L, Bulvik S, Phillip M: Chronic autoimmune thyroiditis in children and adolescents: at presentation and during long-term follow-up. Arch Dis Child 2009 , 94:33-37.

7) Zak T, Noczyñska A, Wasikowa R, Zaleska-Dorobisz U, Golenko A: Chronic autoimmune thyroid disease in children and adolescents in the years 1999-2004 in Lower Silesia. Poland. Hormones. 2005, 4:45-48.

8) Gopalakrishnan S, Chugh PK, Chhillar M, Ambardar VK, Sahoo M, Sankar R: Goitrous autoimmune thyroiditis in a pediatric population: a longitudinal study. Pediatrics 2008, 122: e670-e674.

9) Demirbilek H, Kandemir N, Gonc EN, Ozon A, Alikasifoglu A: Assessment of thyroid function during the long course of Hashimoto's thyroiditis in children and adolescents. Clin Endocrinol 2009, 71:451-454.

10) Özen S, Berk Ö, ªimºek DG, Darcan S: Clinical course of Hashimoto's thyroiditis and effects of levothyroxine therapy on the clinical course of the disease in children and adolescents. J Clin Res Pediatr Endocrinol 2011, 3:192-197.

11) Wasniewska M, Corrias A, Salerno M, Mussa A, Capalbo D, Messina MF, Aversa T, Bombaci S, De Luca F, Valenzise M: Thyroid Function Patterns at Hashimoto's Thyroiditis Presentation in Childhood and Adolescence Are Mainly Conditioned by Patients' Age. Horm Res Paediatr 2012, 78:232-236.

12) Rallison ML, Dobyns BM, Keating FR, Rall JE, Tyler FH: Occurrence and natural history of chronic lymphocytic thyroiditis in childhood. J Pediatr 1975, 86:675-82.

13) Nabhan ZM, Kreher NC, Eugster EA: Hashitoxicosis in children: clinical features and natural history. J Pediatr 2005, 146:533-536.

14) Reinwein D, Benker G, König MP, Pinchera A, Schatz H, Schleusener A: The different types of hyperthyroidism in Europe. Results of a prospective survey of 924 patients. J Endocrinol Invest 1988, 11:193-200.

15) Radetti G, Gottardi E, Bona G, Corrias A, Salardi S, Loche S: Study group for thyroid diseases of the Italian society for pediatric endocrinology and diabetes (SIEDP/ISPED): the natural history of euthyroid Hashimoto's thyroiditis in children. J Pediatr 2006, 149:827-832.

16) Wasniewska M, Corrias A, Aversa T, Valenzise M, Mussa A, De Martino L, Lombardo F, De Luca F, Salerno M: Comparative evaluation of therapy with L-thyroxine versus no treatment in children with idiopathic and mild subclinical hypothyroidism. Horm Res Paediatr 2012, 77:376-381

17) Díez JJ, Iglesias P, Burman KD: Spontaneous normalization of thyrotropin concentrations in patients with subclinical hypothyroidism. J Clin Endocrinol Metab 2005, 90:4124-4127.

18) Bozkurt NC , Karbek B ,Ucan B et al. The association between severity of vitamin D deficiency and Hashimoto's thyroiditis. Endocr Pract 2013,21:1-14

19) Mandel SJ, Brent GA, Larsen PR. Levothyroxine therapy in patients with thyroid disease. Ann Intern Med 1993; 119: 492-502.

A Combination of Larsen and Adams - Oliver syndromes in a Jordanian newborn. A case report

Ghassan S.A. Salama (1)
Mohammed Al-Raqad (2)
Kurdi Nael (3)
Manar M. Abu-Karaki (4)

(1) Ghassan S.A.Salama, MD
Neonatologist. Department of Pediatrics / Jordanian Royal Medical Services.
(2) Mohammed Al-Raqad,MD
PhD clinical genetics. Department of Pediatrics / Jordanian Royal Medical Services.
(3) Kurdi Nael, MD
Physical and medical rehabilitation center/ Jordanian Royal Medical Services.
(4) Manar M. Abu-Karaki, MD
Gyn. & Obs. Department / Jordanian Royal Medical Services

Correspondence:
Dr Ghassan Saleh Salama.
Neonatologist. Department of Pediatrics / Jordanian Royal Medical Services.
Tel No: 00962799376016.
Email: gh_surra@yahoo.com

ABSTRACT

We are reporting a full term female newborn, product of cesarean section at Prince Hashem Ben Al- Hussein Military Hospital/ Zarqa, north of Jordan, with bilateral congenital knee dislocation , bilateral congenital hip dislocation, characteristic facies (prominent forehead, depressed nasal bridge, wide spaced eyes), congenital scalp defect, clubbed feet, bilateral hypoplastic toes and congenital heart defects presenting with a previously not described in literature, combination of two very rare syndromes: - Larsen and Adams - Oliver syndromes.

Key words: scalp defect, knee dislocation, and toes hypoplasia

Introduction

Larsen syndrome: - A rare osteochondrodysplastic genetic disorder that has been associated with a wide variety of different clinical findings(1,2,3). The cardinal features of this condition are dislocations of the large joints, skeletal malformations, and distinctive facial features (1,4).

Adams-Oliver syndrome: - Is a very rare congenital anomaly complex characterized by terminal transverse limb defects and aplasia cutis congenital (5,6,7,8,9).

Here we are reporting a previously not described in literature, combination of these two very rare syndromes in a Jordanian full term female newborn.

Case Report

A 2850 grams female Jordanian newborn of 40 weeks gestation, product of smooth caesarian section due to breech presentation after an uneventful pregnancy, for non-consanguineous parents. The Mother was 22 years old, primigravida, non-smoker, with no medical illnesses or history of drug ingestion, and with no family history of congenital deformity.

Apgar scores at birth, first minute and five minutes were 8/10 and needed no intervention.

On examination the following abnormalities were detected: - two separate but one near to the other congenital scalp defects of the parietal area (aplasia cutis congenita) measuring 3x3 cm. And 3x1 cm., depressed nasal bridge , small, low set and posteriorlly rotated ears, wide spaced eyes, abnormal position of both knees in the form of gene recurvatum, bilateral club feet and bilateral toes hypoplasia. Heart examination revealed ejection systolic murmur grade II/VI.

Complete blood count, kidney and liver function tests all were within the normal range for the age.

AP and lateral radiograph of both knees at birth -showed anterior dislocation of both knees.

An AP radiograph of hips at birth, showed bilateral acetabular dysplasia.

Bilateral foot radiograph, bilateral hypoplasia of toes.

Chest X-ray - no lungs or chest wall abnormalities.

D -ECHO- revealed patent ductus arteriosus and atrial septal defect secondum = 0.3 cm. Abdominal and renal ultrasonography - showed normal intra-abdominal structures.

Figure 1: - **(A)** The characteristic appearance of bilateral congenital dislocation of the knees and club foot at birth. **(B)** characteristic facial features (prominent forehead, depressed nasal bridge, wide spaced eyes). **(C)** Low set, posteriorly rotated ear. **(D)** Bilateral hypoplasia of the toes. **(E)** Congenital scalp defect (aplasia cutis congenita).

Abdominal and renal ultrasonography - showed normal intra-abdominal structures.

Brain CT scan- showed no abnormal brain structures.

Hearing assessment revealed normal hearing pattern.

Discussion

Larsen syndrome (LS, OMIM number: 150250) is a rare genetic disorder, with an incidence of about one in 100,000(1,2,3,10,11,12). Originally described by Larsen et al, in 1950(4) when they reported a syndrome of multiple congenital dislocations (bilateral dislocation of elbows, hips and knees), and characteristic facies (prominent forehead, depressed nasal bridge, wide spaced eyes). Clubfoot and short metacarpals with cylindrical fingers, cleft palate hydrocephalus and abnormalities of spinal segmentation. In 1988 a mixed hearing loss in a child with Larsen syndrome was described by Stanley et al(13).

Le Marec et al. (1994)(14) described a male infant who had laryngomalacia with apnea, kyphosis and atlantoaxial dislocation in addition to the typical manifestations of Larsen syndrome. Both autosomal dominant and autosomal recessive pattern of inheritance are described in Larsen syndrome. The autosomal dominant Larsen syndrome is caused by heterozygous mutation in the gene encoding Fleming B (FLNB) on chromosome 3p14.3(10,12). While the autosomal recessive syndrome has been found to be caused by mutation in the B3GAT3 gene on chromosome 11q12.3(10,11). Meanwhile, Fints et al. (2000)(15) and Dabeer et al. (2003)(16) described a case of asymmetric Larsen syndrome which was explained by unilateral somatic mosaicism.

Adams - Oliver syndrome (AOS, OMIM number: 100300) first came to light back in 1945 and fortunately it is an extremely rare genetic condition with an incidence of about 1in 200,000(5,6,7,8,9), and only 125 cases were reported. Most reported cases follow an autosomal dominant pattern of inheritance but sporadic and autosomal recessive cases, were reported(7). Autosomal dominant form of Adams-Oliver syndrome is caused by heterozygous mutation in the ARHGAP31 gene on chromosome 3q13(7).

Figure 2: - (A) Lateral radiograph of the knee at birth shows anterior dislocation of the knee. (B) An AP radiograph of hips at birth showing bilateral acetabular dysplasia. (C) Radiograph before reduction demonstrating anterior subluxation in the bilateral knees. (D) Radiograph demonstrating anterior dislocation of the left knee.
(E) Bilateral foot radiograph- bilateral hypoplasia of toes.

Adams - Oliver syndrome is characterized by a congenital scalp defect (aplasia cutis congenital) and variable degrees of terminal transverse limb defects. Hand deformities include postaxial polydactyly, short fingers or absent hand, phalanges, or digits and syndactyly. Foot deformities include short toes, absent foot, distal phalanges, toes or feet, widely spaced toes, clubfoot. Other associated anomalies include: - congenital heart defects and cutis marmorata of the skin(5,6,7,8,9). Recently, Adams-Oliver syndrome has been hypothesized to occur early in embryo formation as a result of a genetic defect causing abnormalities in small vessel structures which in the presence of specific forces could be the cause of the described anomalies(5).

After a prolonged search in literature and discussions with orthopedics and genetics we all agreed that our female newborn presented with complex congenital abnormalities not described together before but a combination of two different very rare syndromes namely Larsen syndrome and Adams-Oliver syndrome.

The orthopedic department started early reduction of the dislocated knees with flexion > 90°, and the extremity was immobilized with a splint. Splinting was changed every 2 weeks. Concomitant treatment using a Pavlik harness for associated DDH was initiated at the age of 6 weeks and to be maintained for at least 4 months.

The newborn is still under regular monitoring by both neonatologist and orthopedic for any unexpected sequelae or complications and planned to be examined every 2 months until walking, then annually.

References

1- Chun-Chien Cheng, MD; Jih-Yang Ko, MD. Early Reduction for Congenital Dislocation of the Knee within Twenty-four Hours of Birth. Chang Gung Med J 2010, 33(3) :266-272.

2- Mohammad Arshad Ikram, Ali Saleh Abdullah, Eman Al Khalfan. Congenital Genu Recurvatum with Dislocation of the Knees: A Case Report. Kuwait Medical Journal 2004, 36 (3):203-205.

3- Diclehan Orhan, Sevim Balc, Özgür Deren, Eda Gülen Ütine Ahmet Ba?aran, Gülsev Kale. Prenatally diagnosed lethal type Larsen-like syndrome associated with bifid tongue. The Turkish Journal of Pediatrics 2008; 50: 395-399.

4- Larsen, L. J., Schottstaedt, E. R., Bost, F. C. Multiple congenital dislocations associated with characteristic facial abnormality.J. Pediat. 37: 574-581, 1950.

5- Baskar.S, Kulkarni L, Kulkarni A.M et al. Adams-Oliver syndrome; Additions to the clinical features and possible role of BMP pathway.AJMG 2009;149A(8):1678-1684.

6- Rajabian M H, Aghaei S. Adams-Oliver syndrome and isolated cutis congenital in two sibs. Dermatology

online Journal 2006;12(6):7.

7- Tentany S A, Aglan M S, Ashour A Met al. Adams-Oliver syndrome:further evidence of an autosomal recessive variant. Clin. Dysmorphol.2007;16(3);141-149.

8- Whitty C B, Gorlin R J. Adams-Oliver syndrome revisited. Am. J Med Genrt.1991;40(3):319-326.

9- Amor D J, Leventer R J, Hayllar S et al. Polymicrogyria associated with scalp and limb defects; variant of Adams-oliver syndrome.Am J Med Genet.2000;93(4):328-334.

10- Knoblauch H, Urban M, Tinschert S. Autosomal recessive versus autosomal dominant inheritance in Larsen syndrome: report of two affected sisters. Genet Couns 1999; 10: 315-320.

11- Bicknell, L. S., Farrington-Rock, C., Shafeghati, Y., Rump, P., Alanay, Y., Alembik, Y., Al-Madani, N., Firth, H., Karimi-Nejad, M. H., Kim, C. A., Leask, K., Maisenbacher, M., and 14 others. A molecular and clinical study of Larsen syndrome caused by mutations in FLNB.J. Med. Genet. 44: 89-98, 2007.

12- Vujic, M., Hallstensson, K., Wahlstrom, J., Lundberg, A., Langmaack, C., Martinsson, T. Localization of a gene for autosomal dominant Larsen syndrome to chromosome region 3p21.1-14.1 in the proximity of, but distinct from, the COL7A1 locus. Am. J. Hum. Genet. 57: 1104-1113, 1995.

13- Stanley, C. S., Thelin, J. W., Miles, J. H.Mixed hearing loss in Larsen syndrome. Clin. Genet. 33: 395-398, 1988.

14- Le Marec B, Chapuis M, Treguier C, Odent S, Bracq H. A case of Larsen syndrome with severe cervical malformations. Genet Couns 1994; 5: 179-181.

15- Frints, S. G. M., De Smet, L., Fabry, G., Fryns, J. P.A young female with asymmetric manifestations of Larsen syndrome: another example of unilateral somatic cell-line mosaicism.Clin. Dysmorph. 9: 273-276, 2000.

16- Debeer, P. H., De Borre, L., De Smet, L., Fryns, J. P. Asymmetrical Larsen syndrome in a young girl: a second example of somatic mosaicism in this syndrome. Genet. Counsel. 14: 95-100, 2003.

Congenital bilateral cornea opacities in a Nigerian child: a case report

C.O. Omolase (1)
A.O. Alonge (2)
B.O.Omolase (3)
O.A.Sotiloye (1)

(1) Department of Ophthalmology, Federal Medical Centre, Owo, Ondo State, Nigeria.
2) Department of Paediatrics, Federal Medical Centre, Owo, Ondo State, Nigeria.
3) Department of Radiology, Federal Medical Centre, Owo, Ondo State,Nigeria

Correspondence:
Dr Omolase Charles Oluwole
Federal Medical Centre,
PMB 1053,Owo,Ondo State,
Nigeria
Email: omolash2000@yahoo.com

ABSTRACT

This report is of a four months old baby who presented to the Eye Clinic of Federal Medical Centre, Owo, Ondo State, Nigeria in April,2011 on account of bilateral congenital opacity. The patient could at least perceive light though the cornea of both eyes which were opaque and there was no clear area at the peripheral portion of the cornea. Examination by Paediatricians revealed micrognatia, simian crease in the right palm and umbilical hernia. There was no similar occurrence in his family. In a bid to restore vision in the patient in spite of the challenges involved in the Management of Peters' anomaly as well as for cosmetic reasons, the patient was referred for keratoplasty as we felt the patient would not benefit from optical iridectomy. There is need for early presentation of affected patients so as to prevent amblyopia. The Government should also support the affected patients so that they can be promptly treated in view of the challenges of the management of the condition.

Keywords: **Cornea opacity, amblyopia, keratoplasty, Nigeria.**

Introduction

The extent and location of cornea opacities vary greatly. Congenital corneal opacities present in approximately 3 in 100,000 new born.[1] It can be classified to primary or secondary categories.[2] Primary congenital cornea opacity encompasses choriostomas and cornea dystrophies. Secondary cornea opacity includes cases of kerato-irido-lenticular dygenesis, infection, iatrogenic like forceps delivery injury, developmental anomalies of the irido trabecular system / lens and developmental anomalies of the adnexa like Peters' anomaly as well as sclerocornea.[2]

Peters' anomaly was described by Peters about one hundred years ago.[3] It is a mesenchymal dysgenesis of the cornea.[4] Peters' anomaly is likely to arise from faulty migration of the first wave of neural crest cells.[5] It is a rare form of anterior segment dysgenesis in which abnormal cleavage of the anterior chamber occurs.[6] The affected patients have congenital central cornea opacity with corresponding defects in the posterior stroma, Descemet's membrane and endothelium.[4] The cornea is usually clear at the periphery and vascularisation is not common however there may be sclerization of the limbus. There are two types of Peters'anomaly, namely type 1 and type 2. Type 1 is unilateral characterized by a central or paracentral cornea opacity with iris strands that arise from the iris collarete and attach to the cornea. Type 2 is bilateral in 60% of cases and shows lens adherence to the posterior cornea due to lack of separation between the cornea and the lens. This latter type is associated with cataract.

Case Report

A four month old infant was brought into our clinic in April, 2011 with a complaint of whitish appearance of the normal black of the eyes since birth. A few hours after birth, the mother noticed that the normal black of the eye were white in the child. Mother claimed she applied breast milk to baby's eyes before he could open them on the third day of life. The child is a product of term pregnancy, which was uneventful. Mother denied any history of febrile illness during pregnancy. No history of rashes in the mother in the course of the pregnancy and no history of use of any drugs apart from routine ante-natal haematinics. There was no history of ingestion of native concoctions during pregnancy. She received routine antenatal T.T. vaccination. She neither smoked cigarettes nor drank alcohol. She did not chew kolanut as well. The child was a product of spontaneous vertex delivery. He cried immediately after birth and the umbilical cord was cut using sterile cord scissors. He had been receiving immunization according to National Programme of Immunization schedule. He had also been achieving all the normal developmental milestones. No history of any febrile illness since birth.

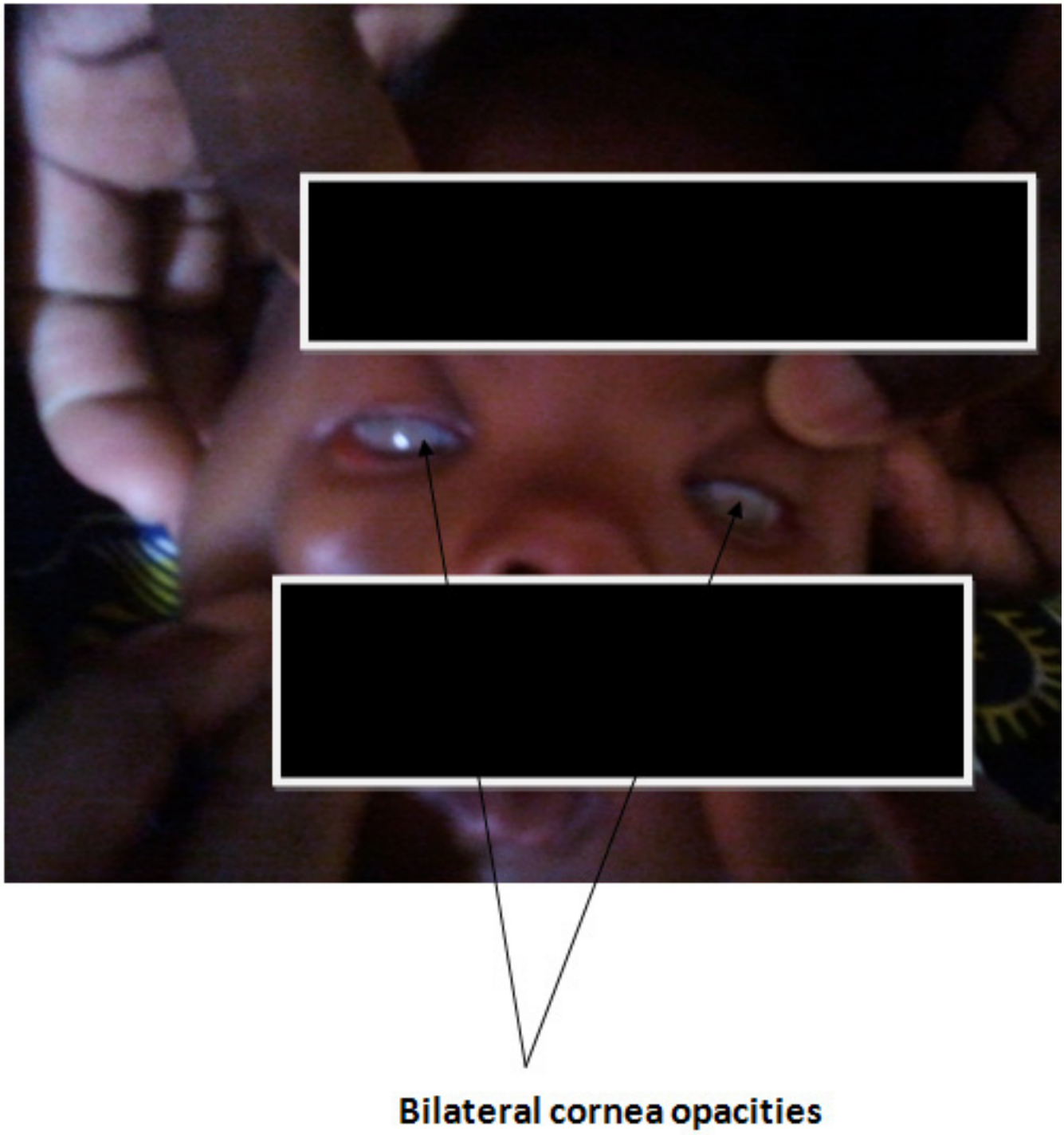


Figure 1: Bilateral congenital cornea opacities

The father is a 40-year-old automobile panel beater. He drank alcohol sparingly, chewed kolanut occasionally but did not smoke. The mother is a 30-year-old trader. The patient is the last of the four surviving children from five children they previously had. There is no similar history in both the paternal and maternal families or in other siblings.

Further evaluation by our Paediatricians revealed micrognathia, simian crease on right palm and umbilical hernia. Ocular examination revealed bilateral corneal opacity with no further view into both eyes.

An assessment of Peters' anomaly was made. The nature of this condition was explained to patient's parents with counselling on how to raise the patient. The patient was referred to a corneal surgeon in one of the Teaching Hospitals in Nigeria for keratoplasy with guarded prognosis. However the parents were yet to take the child to the referral centre at the time of this report due to financial constraint.

Discussion

Most cases of Peters' anomaly are sporadic but there are autosomal recessive and dominant modes of inheritance. It is associated with abnormalities of chromosome 4.[7] We could not establish a family history of a similar presentation in the family of this patient. Thus we had the impression that this presentation may actually be a sporadic one. Peters' anomaly is often an isolated ocular defect but may be associated with other ocular and systemic anomalies.[4] The associated anterior segment anomalies with

Peters' anomaly include glaucoma, anterior polar cataract, cornea plana, sclerocornea, microphthalmos, colobomata as well as mesodermal dysgenesis of the anterior chamber angle and iris.[8] Peters' anomaly may be associated with systemic anomalies like limb deformities, craniofacial anomalies, cleft lip and palate as well as genitourinary defects.[9-11]

In view of the associated systemic anomalies, this patient was sent to the Paediatricians for further evaluation. None of the detected associated anomalies in this patient was life threatening. The early presentation of the patient is commendable in view of the fact that the parents were not enlightened. The need for prompt presentation and early surgical intervention at the referral centre cannot be overemphasized. Penetrating keratoplasty is the most common surgical procedure for Peters' anomaly but it is associated with low graft survival rates due to difficulties in surgical technique and high rate of immune rejection.[12] Optical iridectomy is an alternative to penetrating keratoplasty in cases of Peters' anomaly with peripheral clear cornea.[13] A good result was achieved in a previous report of Peters' anomaly with central cornea opacity and clear cornea supero temporally.[12] We could not consider doing optical iridectomy in this particular case in view of the fact that the whole extent of the cornea of both eyes were opaque. The Management of Peters' anomaly is challenging in view of the attendant poor visual outcome arising from the high incidence of post operative complications. Poor visual outcome may also be due to anterior and posterior segment pathology as well as amblyopia.[14] The difficulty in correction of refractive error and enforcement of refractive correction may also contribute to poor visual outcome.

We conclude that prompt treatment of patients with Peters' anomaly is advised. In view of the capital intensive nature of the treatment, the Government and Non-Governmental Organization should come to the aid of affected patients. There is also the need to create more awareness among the populace about this rare condition so that affected children can present early.

Acknowledgement

The contribution of other health workers involved in the management of this patient is hereby acknowledged. We also appreciate the parents of this baby for their cooperation.

References

1) Ciralsky J, Colby K. Congenital cornea opacities: a review with a focus on genetics. Semin Ophthalmol 2007;22(4):241-6.

2) Nischal KK. Congenital cornea opacities - a surgical approach to nomenclature and classification. Eye 2007;21:1326-37.

3) Peters A.Ueber angeborne Defektbildung der Descemetschen Membran. Klin Mbl Augenheilk 1906;44:27-40,105-119.

4) Bleyen I, Bartels MC, Wolfs RCW. Newborn with bilateral hazy corneas. Bull Soc.belge Ophtalmol 2007;303:29-32.

5) Bahn CF, Falls HF, Varley GA, Meyer RF, Edelhauser HF, Bourne WM. Classification of corneal endothelial disorders based on neural crest origin. Ophthalmology 1984;91:558-563.

6) Park SJ, Byun JL, Lees ST, Shin KC, Kim MH. A case of Peters' anomaly. Korean J Perinatol 2007;18(3):298-302.

7) Hittner HM, Ferrel RE, Antoszyk JH, Kretzler FL. Autosomal dominant anterior dysgenesis with variable expressivity-probable linkage to MNS blood group on chromosome 4. Pediatr Res 1981;15:56.

8) Kenyon KR. Mesenchymal dysgenesis in Peters' anomaly, sclerocornea and congenital endothelial dystrophy Ex Eye Res 1975;21:125-142.

9) Bhat YR, Sanoj KM. Sclerocornea. Indian Pediatr 2005;42:277.

10) Rezende RA, Uchoa UB, Uchoa R, Rapuano CJ, Laibson PR, Cohen EJ. Congenital cornea opacities in a cornea referral practice. Cornea 2004;23:565-70.

11) Kivlin JD, Fineman RM, Crandall AS, Olson RJ. Peters' anomaly as a cause of genetic and non genetic syndromes. Arch Ophthalmol 1986;104:61-4.

12) Altan-Yaycioglu R, Akpek EK, Stark WJ. Improvement of cornea opacity in Peters' anomaly. The Internet Journal of Ophthalmology and Visual Science 2004;3(1).

13) Junemann A, Gusek GC, Naumann GO. Optical sector iridectomy : an alternative to perforating keratoplasty in Peters' anomaly. Klin Monatsbl Augenheikd 1996;209:117-124.

14) Yang LL, Lambert SR, Lynn MJ, Stulting RD. Surgical Management of glaucoma in infants and children with Peters' anomaly : Long-term structural and functional outcome. Ophthalmology 2004;111:112-17.

Middle East Journal of Internal Medicine
medi+WORLD International 2013