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From the Editor

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This is the last issue this year with good papers from the region that deal with various topics. A paper from Jordan looked at newly diagnosed cases of Vitiligo. The aim of this study is to show the efficacy and safety of Tacrolimus ointment in children with localized Vitiligo, particularly when located on the head and neck. Patients are asked to apply topical Tacrolimus twice daily for at least 6 months with regular visits each month to estimate the area of reduction and record the site effect. A total of 210 patients were included in the study. At least partial response to Tacrolimus ointment was noted on the head and neck in 182 (86.6%), and on the trunk and extremities 63.3%. Facial Vitiligo of the localized type showed the best response rate. The author concluded that topical Tacrolimus is an effective and safe therapy in children with localized Vitiligo particularly involving the head and neck with fewer side effects.

Management of Neck Masses Causing Difficulty in Diagnosis and Treatment: A retrospective study from Turkey in which 11 patients who underwent surgery due to a neck mass between 2006 and 2013 were evaluated . The aim of this study was to analyze surgical interventions performed for neck masses causing difficulty during diagnosis and treatment process as well as to discuss recommendations of solutions.

The authors concluded that during the diagnostic process, the most challenging issue had been failure of radiological data in the terms of establishing paravertebral muscles or major vascular structures. During the treatment process, the control of unexpected ruptures in internal carotid and internal jugular veins had been rather difficult.

A retrospective- descriptive study from Iraq attempts to verify the prevalence of TORCH infections in women with Bad Obstetric History (BOH) in Kirkuk Governorate. The information about the pregnant women was gathered from data available in the laboratory of Kirkuk General Hospital records. Of 2566 women with BOH, 27 (1.05%) were anti- T.gondii IgM positive and 505 (19.68%) anti-T. gondii IgG positive. Anti-rubella IgM was detected in 238 out of 2566 (9.28%) women with BOH. The authors concluded that the present study has confirmed the significant association of TORCH and BOH. The study being retrospective and without controls has its limitation, still the observations obtained cannot be ignored. We recommend that all antenatal cases with BOH be routinely screened for TORCH complex.

Problem based learning implementation outcomes from students perspectives

Were explored in a cross sectional study which was conducted on 40% of Tikrit University College of Medicine students. Data collected from 215 students by using a questionnaire by simple quota sampling. The aim of the study was to assess Iraqi medical student's perceptions for implementation of problem based learning as an educational approach to improve medical education quality. The study indicated that 150 students (69.71%) chose problem based learning curriculum as a favored curriculum, while 65 students (30.2%) chose classical curriculum as a favored curriculum. The authors concluded that the quality of learning and teaching by Problem based learning curriculum is better than that of the classical curriculum. The major limitation of this study is the lack of control group.

Management of Neck Masses Causing Difficulty in Diagnosis and Treatment

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ABSTRACT

Objective: **The aim of this study is to analyze surgical interventions performed for neck masses causing difficulty during diagnosis and treatment process as well as to discuss recommendations of solution.**

Study design: **This is a retrospective study in which 11 patients who underwent surgery due to a neck mass between 2006 and 2013 were evaluated.**

Results: **Mean follow-up was 25.70±28.36 (min .6 – max .84) months. The mean age was 47.00±15.74 (min.16 - max.63) years in 11 patients (7 women, 4 men) included in the study. By the histopathological evaluation, it was found that there was a metastatic squamous cell carcinoma of unknown primary in 3 patients, a benign paraganglioma in 4 patients, a schwannoma in 2 patients, Burkitt lymphoma in one patient and tuberculosis lymphadenitis in one patient. Carotid rupture occurred in 2 of 3 patients who underwent surgery for glomus caroticum. Of these, graft repair was performed in one patient while hemiplegia developed in the other. The patient who underwent surgery with an initial diagnosis of giant vagal paraganglioma died on the postoperative hour 24 due to pulmonary thromboembolism. Internal jugular vein rupture developed in the patient with Burkitt lymphoma during neck biopsy, leading neck exploration. Mean blood loss was estimated as 695.45±739.41 (min.100 - max.2200) cc during surgery.**

Conclusion: **During the diagnostic process, the most challenging issue had been failure of radiological data in terms of establishing paravertebral muscles or major vascular structures. During treatment process, the control of unexpected ruptures in internal carotid and internal jugular veins had been rather difficult.**

Keywords: **Squamous cell carcinoma, glomus caroticum, glomus vagale, schwannoma, tuberculosis, radiological findings.**

Introduction

Metastatic squamous cell carcinomas (SCCs) of unknown primary are being encountered in the neck. There are challenges about how to act in the treatment of these tumors where radiological evaluations and needle biopsies are inconclusive during the process of making diagnosis.

Paragangliomas are the tumors of paraganglionic tissues originating from the neural crest [1]. Paragangliomas of head and neck region are associated with four distinct regions including jugular bulb, middle ear cavity, vagal nerve and carotid body. The carotid body (CB) tumors are the most commonly seen paragangliomas. CB tumor, also known as glomus caroticum, is a pulsatile mass with rubber-like consistency localized at deep plane at upper part of the neck. These tumors are typically mobile at horizontal plane but fixed at vertical plane. These tumors can cause hypertension and arrhythmia due to their hormone-active characteristics and the most common findings include mass at neck, pain, balance disorders, dizziness, hearing loss, dyspnea and transient ischemic attacks.

Vagal paragangliomas (VPs) are rarer than glomus jugulare and CB tumors, accounting for approximately 5% of all paragangliomas at head and neck region [2, 3]. VPs can be managed by one of the following options: surgery, radiotherapy and observation in selected cases [4].

Schwannoma is a solid and insidiously growing mass with benign nature. It can arise from any peripheral, cranial or autonomic nerve that has Schwann cell sheath. It generally has a medial or lateral localization at neck. Those with lateral localization frequently originate from muscular or cutaneous branches of cervical plexus or brachial plexus [5].

Chemotherapy is the choice of treatment in the lymphomas of head and neck region; however, biopsy is performed to reach a histopathological diagnosis in these cases. Several complications are being encountered during excisional biopsies.

Again, the surgery is performed for excisional biopsy in tuberculosis (Tbc) lymphadenitis localized at neck.

In the assessment of head and neck masses, the importance of radiological imaging techniques cannot be denied. In particular, the diagnostic value is higher when high-resolution computed tomography (CT) and magnetic resonance imaging (MRI) are used in combination in the assessment of lymphadenopathy and tumors [6]. For head-neck surgeons, the most important advantage of imaging modalities is not only providing an initial diagnosis but also providing information about invasion of major vascular structures and paravertebral muscles, as this information is extremely important for surgical indications and selection of surgical technique.

The knowledge about whether these masses have malignant character by fine needle aspiration cytology (FNAC) at preoperative period will guide to planning of the surgical technique used. FNAC is a safe, sensitive, specific and cost-effective technique for preoperative evaluation of head and neck masses.

The major complications of neck surgery can be discussed in 3 category including wound site, neural and vascular complications. Among these, vascular complications are the immediate and life-threatening complications including the ruptures of carotid artery (CA) or internal jugular vein (IJV).

In the present study, it was aimed to discuss the importance of radiological and cytopathological data in the initial diagnosis of neck masses, diagnostic and therapeutic approaches in challenging neck masses, major complications and their treatments.

Patients and Methods

Among the patients who presented with a neck mass and underwent surgical intervention between 2006 and 2013, eleven cases in which difficulties were experienced in the diagnosis and treatment processes were retrospectively evaluated.

Exclusion criteria: The patients without a surgical complication or difficulty in diagnosis or management and/or those with a histopathological diagnosis at preoperative period, were excluded.

After physical examination, the patients underwent sonography, CT scan, MRI or a combination of these evaluations according to their initial diagnoses (Table 1). FNAC was performed in case of suspicion. Endoscopic evaluation was performed by larynx and nasopharynx in all patients. Biopsies were taken from nasopharynx and root of tongue in cases with metastatic neck masses of unknown primary. In patients with a suspicion of malignant tumor, systemic metastasis screening was performed with abdominal sonography, thorax CT and brain CT scans at preoperative period. Patients were directed to one of the following according to results of pathological evaluation: radiotherapy, chemotherapy or follow-up.

Results

Of the 11 patients included, 7 were women and 4 were men with a mean age of 47.00±15.74 (min.16 - max.63) years (Table 2 - page 7). The smallest amount of bleeding observed was 100 cc in Ancient Sch excision (Figure 1 - page 8), whereas the greatest amount of bleeding observed was 2200 cc in the rupture of internal carotid (ICA) and common carotid arteries (CCA) during CB surgery. Mean bleeding volume was estimated as 695±739.41 cc in surgical interventions.

Based on the physical examination, imaging and biopsy results, a malignant focus out of neck or distant metastasis wasn't detected in any of the patients. Preoperative FNAC was performed in 7 patients. Atypical cells were observed in FNAC in 2 patients who underwent surgery with SCC of unknown primary, and in another patient with multiple

Table 1 Part A:

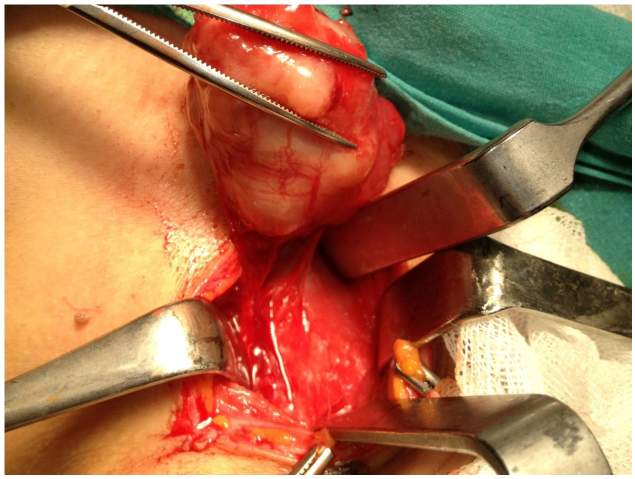
#	Diagnosis	Radiological Findings	Pathological Results
1	SCC unknown of primary	**Neck CT:** A solid mass lesion (4 x 5 cm in size) with lobulated contours and heterogeneous contrast enhancement is observed at right parapharyngeal region. **Boyun MRI:** Prevertebral fascia and muscles were normal. A mass lesion (7.5 x 5.5 cm in size) with lobulated contours, heterogeneous appearance, contrast enhancement and necrotic areas was observed at right cervical region which extended to parapharyngeal area at inferior to parotid gland (paraganglioma?)	**FNAC:** Malign cells were observed. **Histopathology:** SCC with moderate differentiation. Abundant perineural invasion.
2	SCC unknown of primary	**Neck USG:** A solid, heterogeneous, well-defined mass lesion (4 x 3 cm in size) with cystic areas at periphery is observed at left upper cervical region. **Neck CT:** A mass lesion (3.5 x 4 cm in size) with peripheral contrast enhancement which exhibits slight lobulation and hypo-dense appearance is observed at left parapharyngeal area (paraganglioma?). **Neck MRI:** A mass lesion (3.5 x 4 cm in size) with contrast enhancement which involves hyper-intense cystic areas as well as occasional areas of necrosis is observed at left parapharyngeal area (it is striking to observe that mass lesion compresses left IVJ at medial, while intermediate plane with left SCM is vague at lateral (paraganglioma?).	**FNAC:** Malign cells were observed. **Frozen:** Malign epithelial tumor. **Histopathology:** SCC with moderate differentiation. Diffuse necrosis areas and occasional areas of abscess are observed in the tumor.
3	SCC unknown of primary	**Postoperative control MRI:** A voluminous, heterogeneous image at the level of left palatine tonsil. Compared to previous MRI, no marked difference is observed. However, it is striking not to observe a conglomerate lymphadenopathy observed in previous MRI.	**Histopathology:** No malignity is detected in the biopsy of left palatine tonsil. **Frozen section:** Malign SCC. **Dissection Histopathology:** SCC with moderate differentiation.
4	Glomus Caroticum	**Neck MRI:** A mass lesion (5 x 3 cm in size) with marked contrast enhancement which displaces left CA and its branches is observed, favoring CB tumor. **Postoperative Cervical Doppler USG:** It has continuity as left ICA at left CA bulb, and blood flow and signal pattern is normal. No ECA is observed at the left.	**Histopathology:** Paraganglioma.
5	Glomus Caroticum	**Neck MRI:** A mass lesion (7 x 3.5 cm in size) with marked contrast enhancement which displaces CA and its branches is observed at the level of bifurcation at right, favoring CB tumor. No lymphadenopathy is detected at both sides.	**Histopathology:** Paraganglioma.
6	Glomus Caroticum	**Neck CT:** A mass lesion (6 x 4 cm in size) with marked contrast enhancement which displaces ICA and its branches is observed at left CA bifurcation, favoring CB tumor.	**Histopathology:** Paraganglioma. No malign cells are observed.
7	Giant Vagal Paraganglioma (17 x 13 cm in size)	**Neck CT:** A well-defined mass lesion (16.5 x 14 cm in size) with lobulated contours and central necrosis which extends from right parapharyngeal region up to supraclavicular level, displaces surrounding soft tissues and CA and its branches to anteriomedial deviates midline structures to left is observed. **Neck MRI:** A well-defined mass lesion (16.5 x 14 cm in size) with lobulated contours, central necrosis and heterogeneous contrast enhancement which extends from right cervical region to right parapharyngeal area and displaces CA and its branched to anteriomedial is observed.	**FNAC:** Benign cytology. **Histopathology:** Paraganglioma. No malign cells are observed.

8	Ancient Schwannoma	**Neck USG:** A well-defined, heterogeneous, hypoechoic solid lesion (approximately 4 x 2.5 cm in size) which involves patchy cystic areas one-third distal and posterior to left SCM is observed. This lesion is considered to be independent from thyroid parenchyma and has vascular signals on Doppler sonography.	**FNAC:** Benign cells were observed. **Histopathology:** Ancient schwannoma.
9	Parapharyngeal Schwannoma (10 x 8 cm in size)	**Neck CT:** A hypo-dense, mass lesion (6 x 4 cm in size) is observed which extends from mandibular angle up to level of skull base at right parapharyngeal region. The lesion is adjacent to pterygoid muscles at anterior, parotid gland at lateral and nasopharynx at medial. It displaces wall of nasopharynx to posteromedial and has marked margins which can be identified across surrounding soft tissue. **Neck MRI:** A mass lesion with prominent contrast enhancement which has close proximity to CA at posteroinferior region and signal void areas is observed (paraganglioma?).	**FNAC:** Benign cytology. **Histopathology:** Findings compatible with Schwannoma are observed. No malign cells are observed.
10	Parapharyngeal Burkitt Lymphoma	**Neck USG:** A well-defined, hypoechoic lymphadenopathy (5 x 3 cm in size) is observed at right cervical region. Bilateral cervical vascular structures appear normal. **Neck CT:** Multiple lymphadenopathies (largest one being 5 x 4 cm in size) with spherical shape are observed at right submandibular region. Main vascular structures are at normal localization and course.	**FNAC:** The prominent nuclei in some cells rise suspicion of malignity. Excisional biopsy is recommended. **Histopathology:** It is compatible with high-grade Non-Hodgkin B cell Lymphoma (Preferably Atypical Burkitt Lymphoma).
11	Tbc lymphadenitis at neck	**Neck USG:** Multiple, well-defined lymphadenopathies (largest one being 34 x 13 mm in size at posterior to SCM) which involve calcified areas (millimeters in size) at the right side of neck. Central echogenicity is lost. **Neck CT:** Multiple, necrotic and conglomerate lymphadenopathies (largest one being 29 mm in short axis) are observed adjacent to right parotid gland.	**FNAC:** Benign cells are observed. **Histopathology:** Caseified granulomatous inflammation (Tbc?).

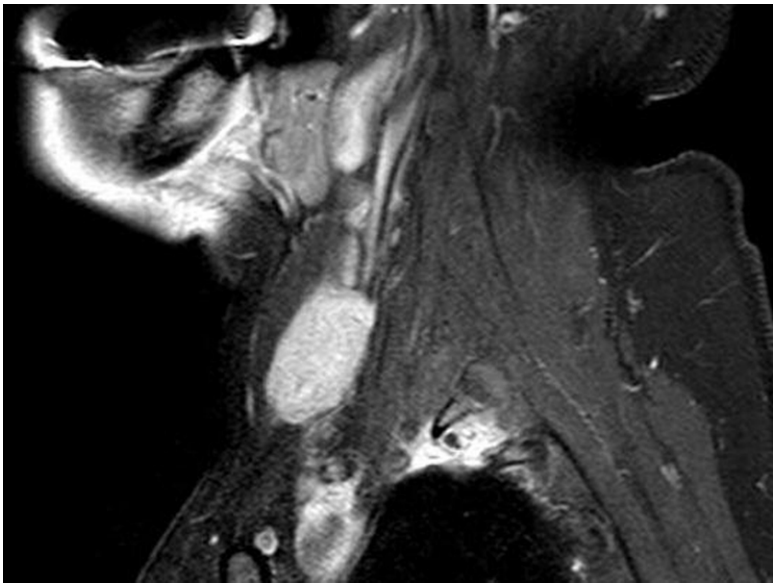
◂ Table 1 Part B

#	Age	Gender	Localisation	Diagnosis	Treatment	Difficulty	Bleeding volume	Outcome	Follow-up period (months)
1	63	M	Right	SCC of unknown primary	Surgical excision and adjuvant radiotherapy	Paravertebral invasion which didn't detected in radiological evaluations	200 cc	Ongoing radiotherapy	12
2	51	M	Left	SCC of unknown primary	Radical neck dissection and adjuvant radiotherapy	ECA ligation due to ECA invasion	250 cc	No recurrence at month 6	11
3	62	F	Left	SCC of unknown primary	Modified radical neck dissection and adjuvant radiotherapy	Challenging diagnosis	250 cc	No recurrence at month 12	24
4	29	F	Left	Glomus caroticum	Surgical excision and ICA + CCA grafting	Perioperative ICA + CCA rupture and grafting	2000 cc	No complication	6
5	60	M	Right	Glomus caroticum	Surgical excision and ICA + CCA ligation	ICA + CCA rupture	2200 cc	Hemiplegic	72
6	57	F	Left	Glomus caroticum	Surgical excision	Difficult dissection due to bleeding	1000 cc	No complication	84
7	62	F	Right	Giant Vagal Paraganglioma (17x13 cm in size)	Surgical excision	Difficult dissection and postoperative mortality due to thromboembolism	500 cc	Died on postoperative hour 24	-
8	41	F	Left	Ancient Schwannoma	Surgical excision	Difficulty in making distinction between malign and benign lesion	100 cc	No complication	12
9	37	F	Right	Parapharyngeal Schwannoma (10 x 8 cm in size)	Surgical excision	Difficult dissection due to exposure and proximity to major vascular structures	500 cc	No complication	22
10	39	M	Right	Parapharyngeal Burkitt Lymphoma	Chemotherapy following incisional biopsy	IJV rupture	500 cc	IVJ ligation	6
11	16	F	Right	Tbc Lymphadenitis at neck	Excisional biopsy and anti-tuberculosis treatment	Difficulty in dissection while preserving capsule integrity	150 cc	No complication	8

Table 2

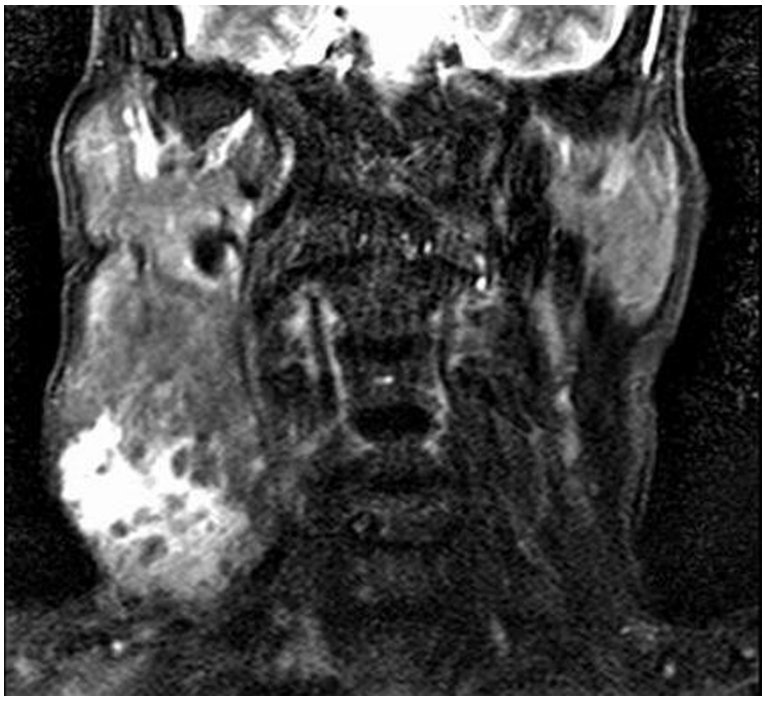


1 (a)

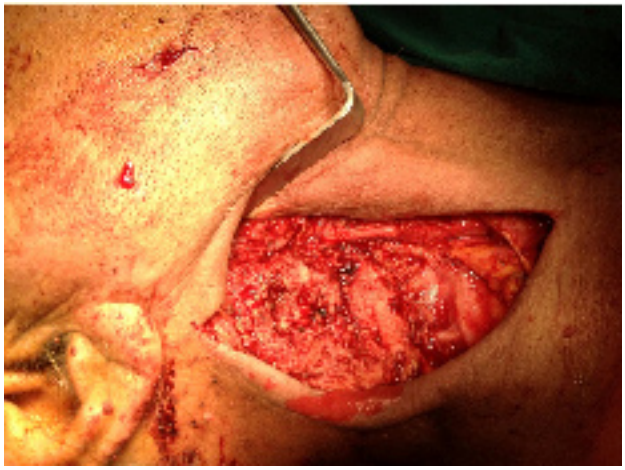


1 (b)

Figure 1: Dissection **(a)** was readily achieved in the case of ancient schwannoma in which no definitive diagnosis was reached by clinical examination and preoperative MRI **(b)**.



2 (a)



2 (b)

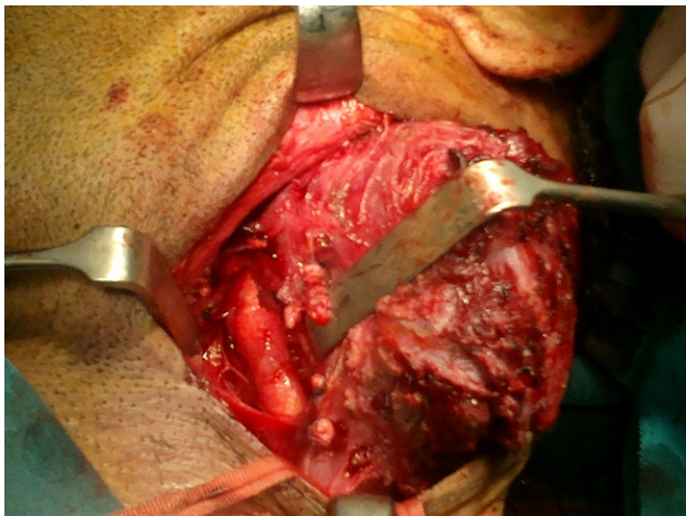
Figure 2: The invasion of paravertebral muscles **(b)** was observed during surgery in the case which was initially diagnosed as paraganglioma on preoperative MRI. Paravertebral muscles were reported as normal on preoperative MRI **(a)**. This case was considered as inoperable.

lymphadenopathies. In the histopathological evaluation, it was found that there was a metastatic SCC of unknown primary in 3 patients, a benign paraganglioma (CB tumors in 3 and VP in 1) in 4 patients, a Sch in 2 patients, Burkitt lymphoma in one and Tbc lymphadenitis in another patient.

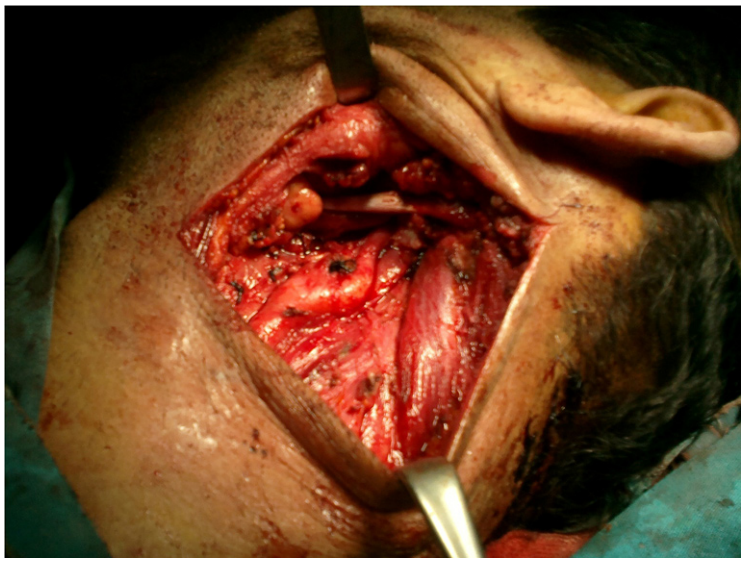
Although there was no involvement in major vascular structures and paravertebral muscles or fascias on the radiological evaluations (Figure 2a) before surgery, paravertebral muscle invasion was detected in one patient (Figure 2b) whereas external carotid artery (ECA) invasion was seen in another patient (Figure 3).

No recurrence was detected during follow-up in 2 of 3 cases who underwent surgery and adjuvant radiotherapy for SCC of unknown primary (Figure 4), while it failed to achieve cure in the remaining case in which resection was insufficient because of paravertebral muscle invasion.

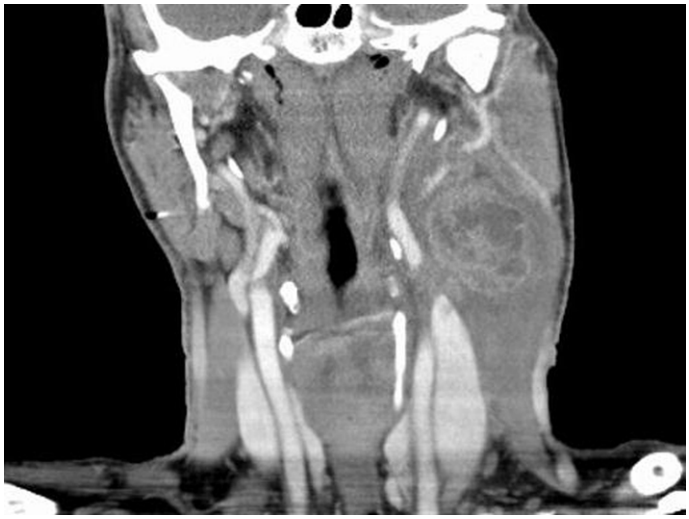
No CA rupture was observed in excisions of CB tumor performed by sparing adventitia of carotid artery (Figure 5), while ICA and CCA rupture was observed in 2 cases in which dissection was performed at subadventitial plane. In the former case, grafting was performed by using synthetic material (Figure 6) and the patient recovered without a complication. In the latter case, CA was ligated and hemiplegia developed. The patient who underwent surgery with an initial diagnosis of giant vagal paraganglioma died on the postoperative hour 24 due to pulmonary thromboembolism (Figure 7).



3(a)

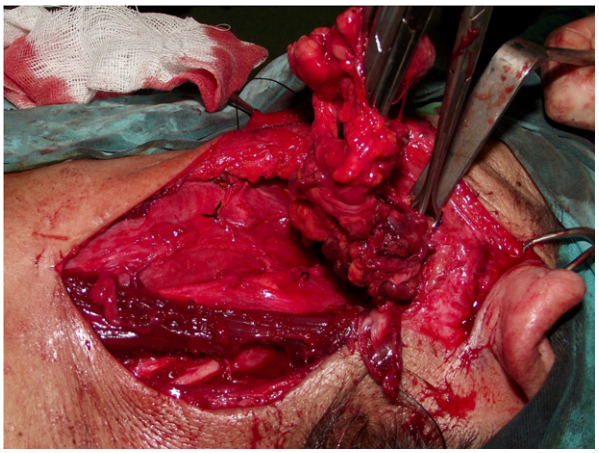


3 (b)



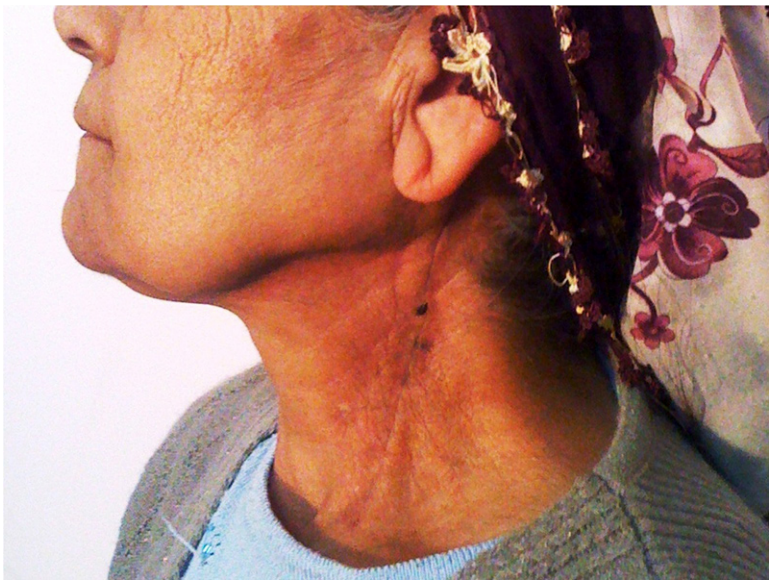
3 (c)

Figure 3: In the patient who underwent surgery due to cervical SCC of unknown primary, ECA invasion was observed **(a)** and the artery was ligated with radial neck dissection **(b)**. There was no CA invasion on preoperative CT scan in this patient **(c).**



4 (a)

Figure 4: The appearance of the patient with cervical SCC of unknown primary after dissection **(a)** and adjuvant radiotherapy **(b)**.



4 (b)

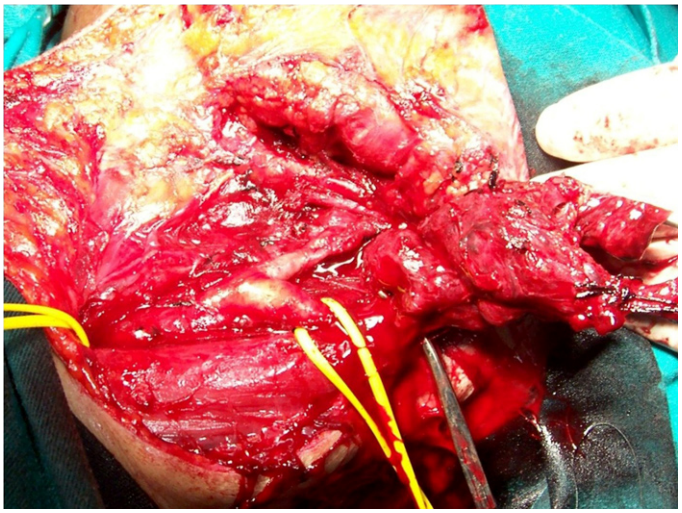


Figure 5: The likelihood of rupture reduces in glomus tumor surgery by sparing adventitia of CA.

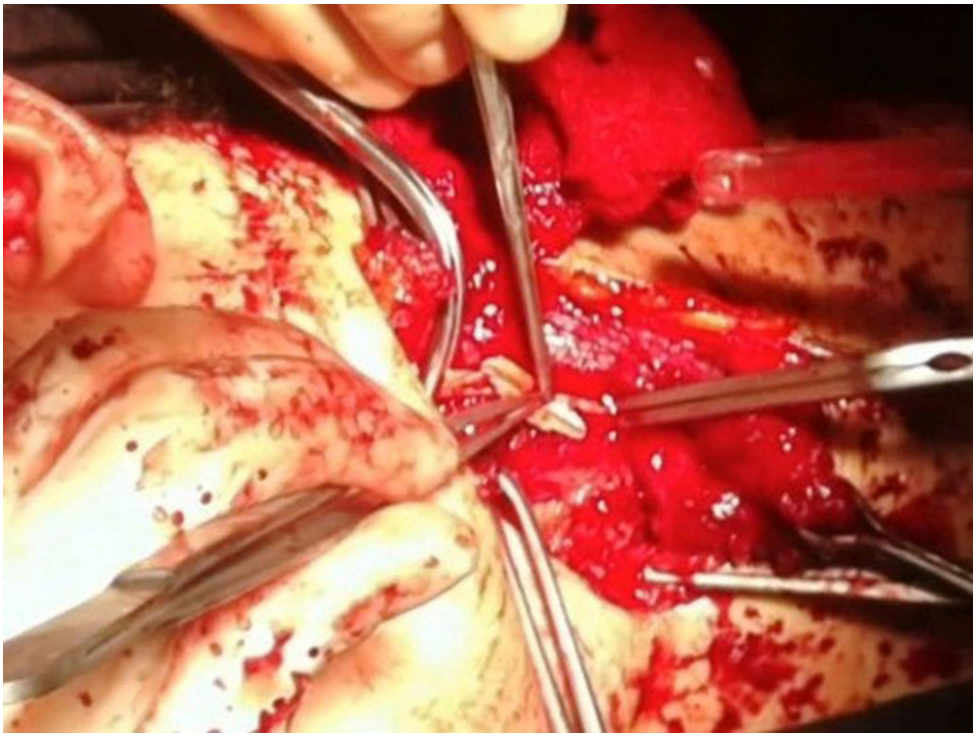
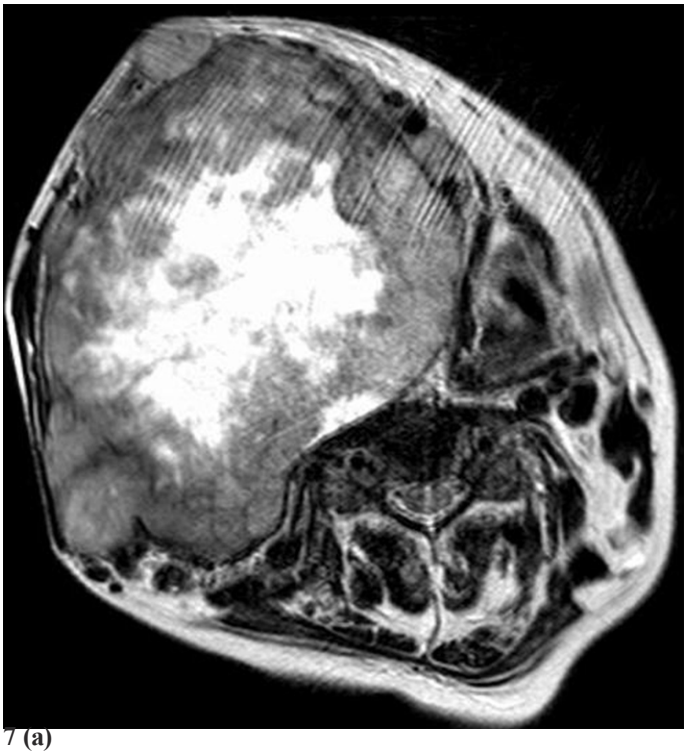
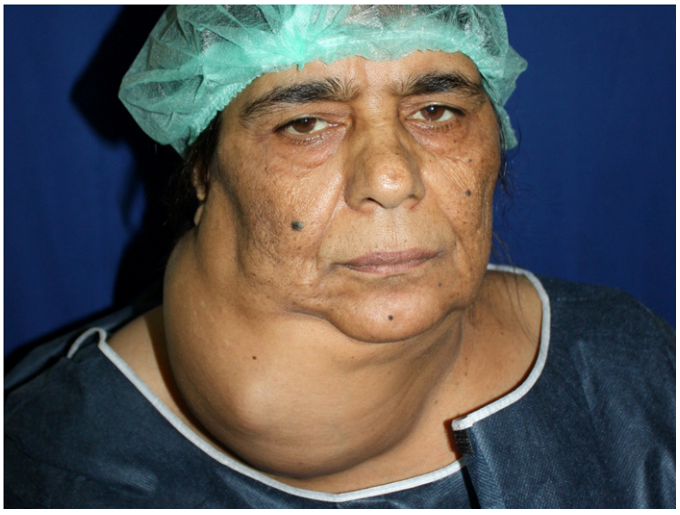


Figure 6: Left ICA and CCA ruptured during CB tumor surgery was reconstructed by using PTFE arterial graft.

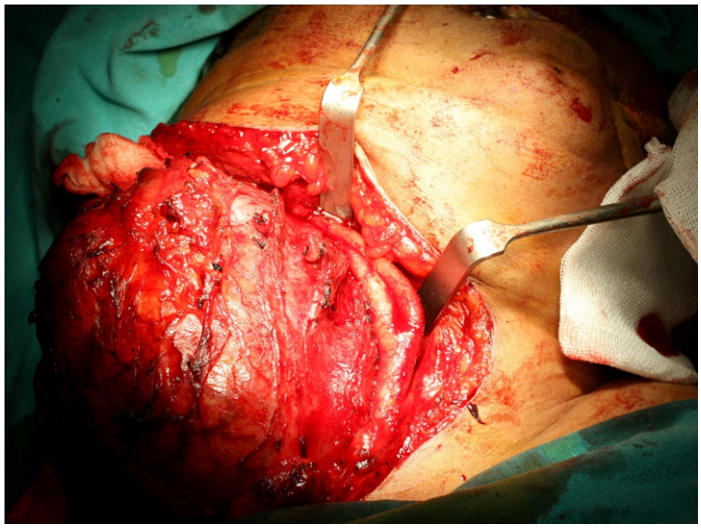


7 **(a)**



7 **(b)**

Figure 7: On MRI **(a)**, preoperative image of the mass with central necrosis which displaced CA and its branches to anteriomedial **(b)**; Sulci on the surface of mass are observed after dissection of CAs **(c)** - top of next page.



7 (c)

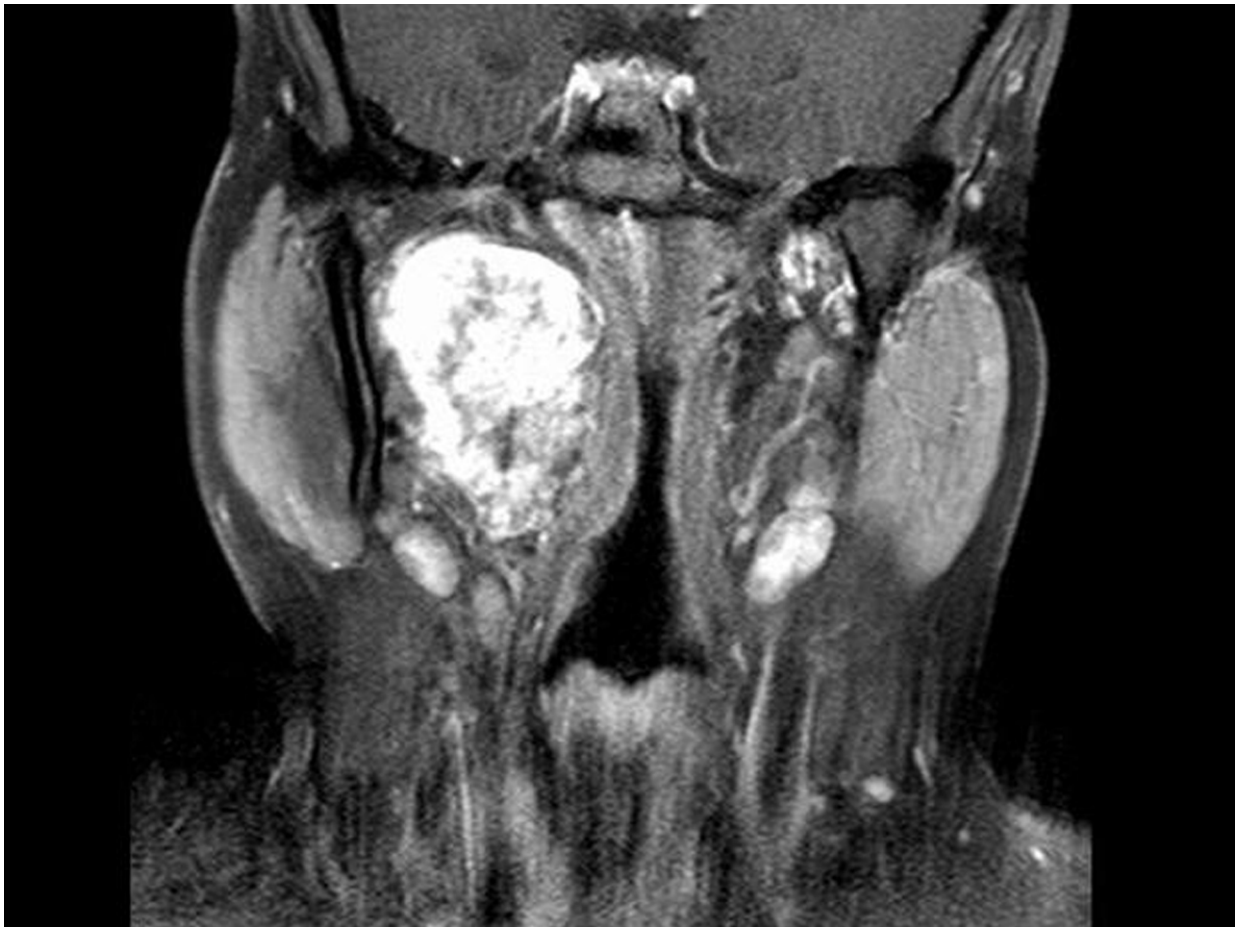
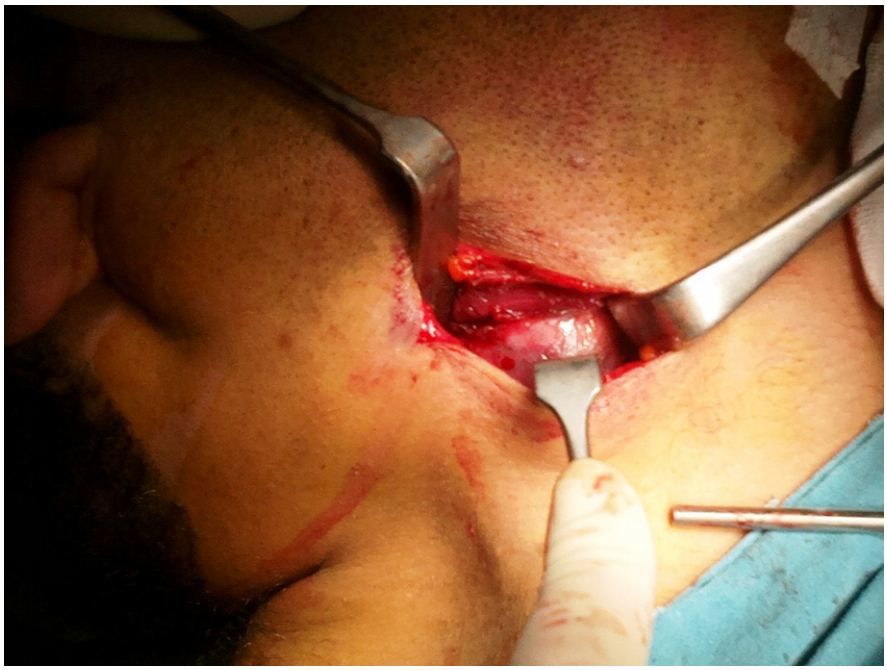


Figure 8: Dissection was rather difficult in the mass extending to skull base at right parapharyngeal region due to its localization which was adjacent to structures including ptergyoid muscles at anterior, parotid gland at lateral and nasopharynx at medial.

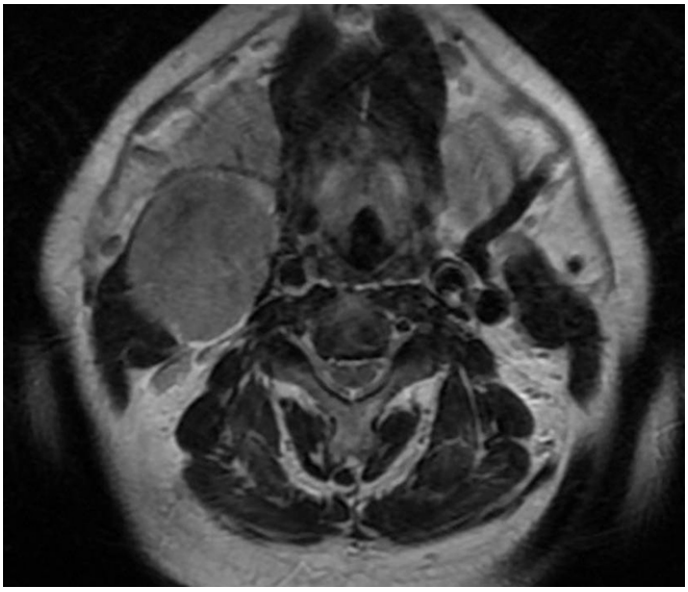
In another schwannoma case with higher localization at parapharyngeal region, dissection without harming neurovascular structures was hardly achieved (Figure 8). In a patient who presented with a mass at the third region of neck, it had been impossible to diagnose Ancient schwannoma and to identify that it hadn't originated from nervus vagus by clinical and radiological evaluations at preoperative period (Figure 1).

IJV rupture occurred due to probable invasion of IVJ because of aggressive course of tumor in the patient with Burkitt lymphoma scheduled to biopsy and underwent intervention at upper jugular region by 4 cm incision. In this patient, immediate exploration was performed and IJV was ligated at both proximal and distal parts (Figure 9).

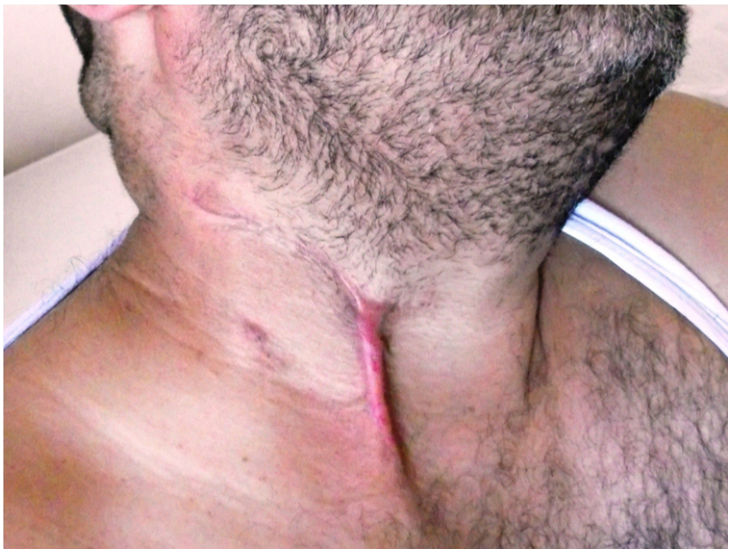
No systemic finding was detected in a girl in whom a conglomerate lymphadenomegaly was detected at neck. There was no specific finding in FNAC and radiological evaluations. The process of making a decision for incision biopsy was challenging. In this case, there was a risk for fistulization to skin and surrounding tissue in case of disrupting capsule integrity of lymph nodes (Figure 10).



9 (a)

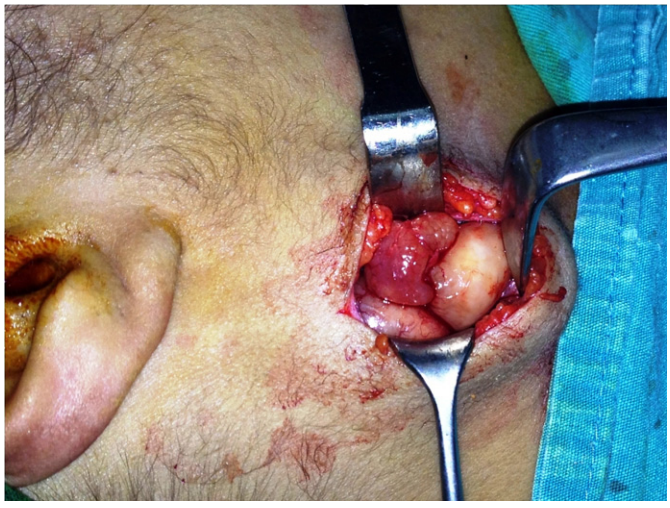


9 (b)

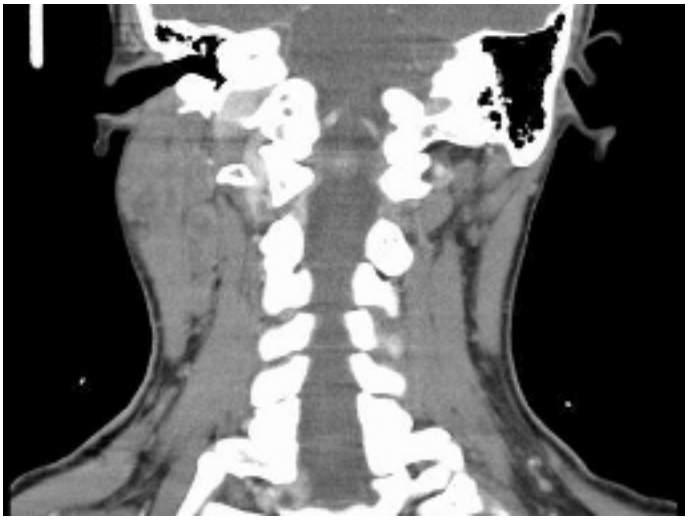


9 (c)

Figure 9: A biopsy was planned **(a)** by 4 cm incision due to normal appearance of major vascular structures on MRI **(b)**; however, IVJ rupture occurred during dissection. The appearance of defective and irregular incision scar caused by rapid exploration at the neck **(c)**.



10 (a)



10 (b)

Figure 10: Capsule integrity of lymphadenopathies was protected during excisional biopsy of the case with cervical tuberculosis **(a)** in which benign cytological findings in FNAC and conglomerated lymphadenopathy with central necrosis on CT scan were observed **(b).**

Mean follow-up was 25.70±28.36 (min.6 – max.84) months. Of the patients, recovery without complication was observed in 9 patients, whereas hemiplegia was present in one patient. One patient died during follow-up, while another patient had been receiving chemo-radiotherapy.

During the diagnostic process, the most challenging issue was failure of radiological data in terms of establishing invasion to paravertebral muscles or major vascular structures.

Discussion

The SCC of unknown primary in cervical lymph nodes are relatively rare, accounting for 3% of head and neck cancers [7]. ICA and/or CCA invasion is present in 5-10% of the cervical lymph node metastasis of SCC of unknown primary at head and neck region [8]. However, preoperative assessment doesn't provide conclusive information about degree of adhesion between tumor and CAs [9]. En bloc removal of tumor is recommended in case of CA invasion [8]. ECA invasion was observed in 1 of 3 cases with metastatic SCC of unknown primary in our series and radical neck dissection was performed by ligating the artery in this case. The tumor invaded paravertebral muscle but not CA in one of the remaining 2 cases. In this case, it failed to demonstrate paravertebral muscle invasion by radiological evaluations at preoperative period. The patient underwent a surgical intervention for dissection; however, paravertebral muscle invasion was observed during surgery. Biopsy was performed for histopathological evaluations and the patient was referred to radiotherapy after debulking. In the third case, there was no paravertebral muscle or CA invasion. In the SCCs of unknown primary, 5-years survival rises up to 74% [7]. It is difficult to achieve cure in advanced SCC of unknown primary by radiotherapy alone, although tumor volume can be reduced by radiotherapy at early stage. Surgical excision is recommended in these cases [10]. Thus, we treated these patients by surgery followed by radiotherapy. Complete remission was achieved by adjuvant radiotherapy in 2 cases other than the case considered as inoperable due to paravertebral muscle invasion detected during surgery.

The decision making process should be individualized in the treatment of paragangliomas by taking age, comorbid systemic diseases, tumor localization and diameter, presence of multiple tumors, cranial nerve deficit and surgeon's experience into account [11]. CB tumors can be malignant in 5-10% of the cases while 10% are hormonally active. Non-malignant CB tumors can also involve skull base, hypoglossal or vagal nerve by local invasion. Thus, CB tumors should be managed by surgery. The major handicaps of surgery include presence of marked blood supply and severe complications. Glomus tumors are supplied by hundreds of small arteries arising from adventitia of CA. Sparing integrity of adventitia and resection of tumor alone prolongs the surgery and the amount of bleeding is greater in this approach. However, it is safer in terms of rupture. On the contrary, rupture of muscular layer is more likely in the surgical interventions performed in subadventitial plane and total amount of bleeding is substantial than in the above-mentioned approach. In our study, rupture was observed in 2 cases but not in the remaining case. In the ruptured ICA and/or CCA, superficial femoral artery or saphenous vein can be used as graft material [8, 9]. Reconstruction was performed by using synthetic graft and patency was demonstrated by postoperative Doppler sonography in our case. If the ruptured ICA isn't dominant in terms of brain supply, no ischemia/hemiplegia occurs [12, 13]. Hemiplegia occurs if the dominant artery is ruptured as in our case. The dominant artery of cerebral blood flow can be identified by preoperative Positron Emission Tomography (PET) scan [14]. If grafting is impossible, ligation can be performed in the rupture of non-dominant CA.

Although there is contradiction in the management of VP, in a large series, Bradshaw et al. recommend to delay surgery until loss of vagal nerve function [2]. However, in the same study, the author indicated to a challenging decision making process in the management of giant VPs which extends to skull base without causing cerebral palsy or threats to other cranial nerves. In our case, there was no cranial nerve dysfunction including vagal nerve; importantly, there was respiratory distress due to compression. In the giant masses localized at neck, the most challenging task is to perform dissection by protecting neurovascular structures. Certainly, the most important vascular structure of neck is CAs. Giant masses not only adhere to these arteries but also displace them by changing their normal positions. The displacement is towards anterior in small tumors, while it is towards anteriomedial or anterolateral in larger tumors [15]. In our case, anteriomedial displacement and tortuous shape of CA as well as adhesion made dissection rather difficult (Figure 7c). However, the major distressing experience was that the case resulted in postoperative pulmonary thromboembolism. The prophylaxis for thromboembolism isn't a routine procedure in our clinic; but, the experience in this case indicated that surgery of giant masses is associated with risk of thromboembolism.

There are 5 variants of schwannoma including common, plexiform, cellular, epitheloid and ancient types [16]. The schwannoma of head and neck region mostly originates from vagal nerve or sympathetic nervous system [17]. Schwannoma with lateral localization at neck arises from cervical plexus or cervical sympathetic chain [5, 16]. MR imaging is excellent compared to CT scan in distinguishing it from tumors mimicking schwannoma and to identify relationship of tumor with CA [5]. In imaging modalities, it has been suggested that schwannoma arising between CA and IVJ can be originated from vagal nerve, while those displacing these structures to anterior can be originated from cervical sympathetic chain [17]. However, giant masses originating from vagal nerve can displace CA to anterior as in our case with vagal paraganglioma. Although schwannoma is benign in nature, it can occasionally cause Horner syndrome, which wasn't observed in our case. If a suspicion arises by preoperative radiological and cytological evaluations, patient should have to be informed about complications. But, the differential diagnosis of paraganglioma and schwannoma isn't feasible by preoperative radiological evaluations. In one case, histopathological evaluations revealed a schwannoma although radiological findings suggested paraganglioma. In this case, close localization of mass to skull base and

difficulty in resection of the mass without injuring CA were worrisome. Additionally, the close proximity to cranial nerves further complicated the dissection. Midline mandibulotomy is recommended rather than transcervical approach in the excision of the masses with higher localization that is closer to skull base [15]. In our case, resection was achieved via transcervical approach without causing cranial nerve deficit, despite difficulties.

IJV rupture may occur during neck dissection. Wide incision and dissection area allow control of bleeding and even to vein ligation. However, it is rather difficult when a small incision is performed for biopsy in case of concomitant IJV rupture. At this point, it is almost impossible to localize and ligate bleeding vein. Surgical site must be strongly compressed and neck must be opened as soon as possible and IVJ must be ligated at the point where it enters the mediastinum. This intervention doesn't allow control of bleeding but it may prevent pulmonary thromboembolism. Then, IJV should be ligated at a point close to skull base and collateral veins should also be ligated if needed. In fact, IJV rupture isn't common during biopsy. But, rapid growing aggressive tumors may invade this vein. Burkitt lymphoma is an aggressive one among lymphomas. It is difficult to consider Burkitt lymphoma by clinical evaluation or FNAC. It can be important to demonstrate whether tumor is either aggressive or sober. It is important to take all measures by considering that any complication may occur during biopsy and to take action immediately in case of complication.

Cervical lymph nodes are one of the most common localizations for extra-pulmonary Tbc [18]. Tbc is rarely seen in developed countries in contrast to developing ones. Thus, tuberculosis should be considered in the differential diagnosis of the cases presented with cervical lymphadenopathy. The definitive diagnosis is made by demonstration of bacteria on cultures from lymph node biopsy materials or bacterial growth in culture media. It is difficult to consider Tbc diagnosis according to FNAC or radiological findings, as non-specific inflammations or lymphoma are preferably considered in such cases because of their prevalence. In the biopsies for lymphoma, the disruption of capsule integrity isn't a major problem. However, there is a risk for cutaneous fistula development in case of tuberculosis. Thus, risk factors such as family history of tuberculosis should be meticulously questioned.

In a different series, FNAC has a sensitivity ranging from 81% to 92%, whereas specificity ranging from 86% to 98.9% [19]. In another study, a correlation of 95% was detected between FNAC and histopathological findings [20]. In our study, malign cytological findings were observed in 3 cases whereas benign cytological findings in 4 of 7 cases. All postoperative histopathological findings were in agreement with FNAC (Table 1). In this context, FNAC provides sufficient guidance in the preoperative surgical planning.

It is known that CT scan has a high specificity in identification of malignant/benign tumors [21]. However, in the context of surgery, it is important to predict presence or absence of invasion to major vascular structure and paravertebral muscles. Unfortunately, CT scan or MR imaging failed in this issue in our cases.

Conclusions

1. In the surgery of CB tumors, sparing adventitia of CA is important to prevent rupture.
2. In terms of maintaining cerebral blood flow, prolonged clamping of non-dominant CA or grafting after rupture don't cause ischemia or hemiplegia.
3. The cerebral blood flow can be restored via grafting ICA rupture by using synthetic materials.
4. It should be kept in mind that major vascular structures might be invaded by tumors when performing biopsy in aggressive tumor such as Burkitt lymphoma.
5. When a rupture occurs during neck biopsies performed through a small incision, a strong compression must be applied. Neck must be opened and bleeding must be controlled as soon as possible, and in case of bleeding from IJV, it must be ligated from distal part to protect lungs from air embolism or thromboembolism.
6. Prophylaxis for thromboembolism should be life-saving after neck surgery due to giant masses.
7. The surgery should be planned by keeping in mind that resection in SCC of unknown primary localized at neck should be challenging.
8. In the SCC of unknown primary localized at neck, adjuvant radiotherapy to the area between nasopharynx and upper mediastinum can achieve cure.
9. In the tuberculosis lymphadenitis at neck, the en bloc excision of lymph node with its capsule is essential in terms of cutaneous fistula.
10. The radiological imaging can be insufficient in the differential diagnosis between paraganglioma and schwannoma.
11. It can be difficult to detect invasion of paravertebral muscle and major vascular structures by radiology.

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Use of Tacrolimus ointment 0.1% for treatment of childhood localized Vitiligo: A prospective study.

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ABSTRACT

Background: Vitiligo is not contagious. It is not life-threatening, but Vitiligo can be life altering. It is characterized by the development of depigmented areas of skin. Phototherapy and corticosteroid are most commonly prescribed and are often not effective. Use of corticosteroid in the face may lead to cutaneous atrophy, telangiectasia and ocular complications.

Objective: The aim of this study is to show the efficacy and safety of Tacrolimus ointment in children with localized Vitiligo, particularly when located on the head and neck.

Method: The present study was based on newly diagnosed cases presenting to the outpatient clinic of pediatric dermatology clinic at King Hussein Medical Centre and Queen Rania children hospital between February 2008 and March 2013 who were reviewed respectively.

Their ages were from 2 to 15 years. The diagnosis was based on the history, clinical grounds and necessary investigations e.g. Complete Blood Count (CBC), Urinalysis, Liver and Renal Function Test, chest X-ray, thyroid function test and Wood's light, and skin biopsy for histopathology which were carried out whenever needed.

Patients were asked to apply topical Tacrolimus twice daily for at least 6 months with a regular visit each month to estimate the area of reduction and record the site effect.

Simple statistical analyses (mean, frequency, and percentage) were used to describe the study variables.

Result: A total of 210 patients were included in the study. The children's ages ranged from 2 to 15 years with a mean of 8.6 ± 7.4 years. There were 110 females (52.4%), 100 males (47.6%); with a male to female ratio of 1.1:1.

At least partial response to Tacrolimus ointment was noted on the head and neck in 182 (86.6%) , and on the trunk and extremities in 63.3%. Facial Vitiligo of the localized type showed the best response rate. The only reported side effect was initial burning on application in 35 (16.7%) patients.

Conclusion: Topical Tacrolimus is an effective and safe therapy in children with localized Vitiligo particularly involving the head and neck with fewer side effects.

Key Words: Tacrolimus, treatment outcome, Vitiligo, topical immunomodulator, protopic.

Introduction

Vitiligo is characterized by the development of depigmented areas of skin associated with a loss of melanin and melanocytes(1, 2, 10-12, 14- 17).

It is stated that it occurs in 1% of the world's population(1, 2, 9-12, 14-16,19,20). All races can be affected(1-3, 12, 14-16). The onset of Vitiligo is usually in childhood or young adulthood(16,19).

Approximately 25% of patients noted the onset of Vitiligo before the age of 10 years, 50% before the age of 20 years and 95% before the age of 40 years(1, 2, 10, 12, 15, 16).

Vitiligo can occur in infants as young as 4 months(1).

Localized or segmental Vitiligo affects preferentially the young(1, 11, 12). The peak of onset varies among different studies, with many authors stating that most cases are evident between 4 and 8 years of age(1, 16).

In most reported paediatric series, the majority of cases were in girls, but the frequency in the population is probably the same in both sexes (1, 2, 16, 19).

The etiology of Vitiligo is still largely unknown. Several theories have been proposed. The autoimmune hypothesis is the most important and popular (9, 11, 12, 15, 19, 20).

The goal of treatment is to suppress depigmentation and stimulate repigmentation (1, 11, 14). This is achieved by topical corticosteroids, immunomodulaters, vitamin D analogues, and phototherapy which is most widely presented (1,2,4,5,9-16).

Topical corticosteroids applied to the face, however, have local (e.g. atrophy, striae, telangiectasia and ocular) complications and systemic side effects (1, 2, 4, 10-13, 16, 17).

Among the established therapies, phototherapy (UVB-NB) and photochemotherapy (PUVA) have limited use for various reasons, difficult access to the sources of light and time spent during treatment(1, 5, 10, 16).

Phototherapy and corticosteroids have limited effectiveness particularly on the face (10, 16, 17).

Tacrolimus and pimecrolimus are a type of medicine called calcineurin inhibitors and are approved for treating atopic dermatitis in adult patients and pediatric patients over 2 years of age (1,6,7,8,10,13,16,19).

Tacrolimus (FK-506) is an immunosuppressant macrolide derived from the fungus streptomyces tsukubaensis, and can be used as an alternative to topical steroids in many other forms of skin disorders, like other kinds of eczema.

Tacrolimus appears to be quite safe, with no potential of skin atrophy, telangiectasia, or adverse ocular effects of topical steroids which have limited applications to the head and neck (4, 6, 7, 8, 10, 16).

Tacrolimus acts through the inhibition of phospholerylation depending of calcineurin which act on T cell and mast cells inhibiting T cell activation and the production of various inflammatory cytokines such as tumor necrosis factor (TNF) the level of which is higher in Vitiligo lesions and adjacencies. After the treatment with Tacrolimus (FK 506) decreased expression of TNF-a in the same areas, suggests that the suppression of these molecules would be involved in the repigmentation process (6-8, 10, 16).

The aim of the study is to evaluate the effectiveness and safety of Tacrolimus ointment 0.1% as a treatment modality in children with localized Vitiligo at King Hussein Medical Centre and Queen Rania Children Hospital.

Methods

The present study was based on newly diagnosed cases presenting to the outpatient clinic of pediatric dermatology clinic at King Hussein Medical Centre and Queen Rania children hospital between February 2008 and March 2013 who were reviewed respectively.

Their ages were from 2 to 15 years. The diagnosis was based on the history, clinical ground and necessary investigations e.g. Complete Blood Count (CBC), Urinalysis, Liver and Renal Function Test, chest X-ray, thyroid function test and Wood's light, skin biopsy for histopathology were carried out whenever indicated.

A detailed history was taken regarding the age, sex, residents, history of previous treatment, onset, duration of illness, aggravating factors like stress and sun.

Digital pictures of the lesions were taken to obtain an accurate measurement of the size of the lesions; the contours were traced on transparent sheaths at baseline and followed each visit.

Patients were asked to apply topical Tacrolimus twice daily for at least 6 months with a regular visit each month to estimate the area of reduction and record the site effect.

During spring and summer time the patients were advised to avoid sun exposure and put on sun block.

Each monthly visit clinical and Wood light assessment of repigmentation of the lesions was made; the outline of the lesion was drawn on transparent paper and the surface area of the lesions were measured each month.

The percentage of repigmentation of skin and hair, patterns of repigmentation and side effects were assessed subjectively. At the end of 6 months, pigmentation response was calculated

as the percentage of total lesional area of all the macules within the segment showing repigmentation and was graded as follows:

Grade 0: No response.

Grade I: Minimal response, when a quarter of the size of patch or less showed marginal or follicular repigmentation. 1-25%

Grade II: Mild response, when half the size of patch showed marginal or follicular repigmentation. 26-50%

Grade III: Moderate response, when more than half of the size of patch showed marginal or follicular repigmentation. 51-75%

Grade IV: Excellent 76-99%

Grade V: Complete 100%

Simple statistical analyses (mean, frequency, and percentage) were used to describe the study variables.

Result

A total of 210 patients were included in the study. The children's ages ranged from 2 to 15 years with a mean of 8.6 ± 7.4 years. There were 110 females (52.4%), 100 males (47.6%); with a male to female ratio of 1.1:1.

All patients had multiple patches on the head and neck, on the trunk and extremities, with duration ranging from 6-24 months; the size of the patches ranged from 1-5 cm² in diameter.

All patients were at least 2 years old with stable Vitiligo (not expanding during the last 1 year) on the head and neck and on the trunk and extremities.

Of these 182 (86.7%) patients showed some repigmentation, at the end of 6 months, of the head and neck. Of these patients, repigmentation was graded as complete in 56 (26.7%), excellent in 42 (20%), moderate in 63 (30%), mild in 14 (6.7%), and minimal in 7 (3.3%). The other 28 (13.3%) patients had shown no response.

On the trunk and extremities, 133 (63.3%) patients showed some repigmentation at the end of 6 months. Of these 133 (63.3%) patients, repigmentation was regarded as complete in 21 (10%), excellent in 21 (10%), moderate in 42 (20%), mild in 28 (13.3%), and minimal in 21 (10%). The other 77 (36.7%) patients had shown no response.

The only reported side effect was initial burning on application in 35 (16.7%) patients.

Table 1: Shows parameters and grades of response and side effect to treatment after 6 months:

Entity	Number		Percentage %	
Sex Males Females	100 110		47.6 % 52.4%	
	Head and neck	***Trunk and extremities***	***Head and neck***	***Trunk and extremities***
Grade 0: no response.	28	77	13.3%	36,7%
Grade I: minimal response 1-25%	7	21	3.3%	10%
Grade II: mild response, 26-50%	14	28	6.7%	13,3%
Grade III: moderate response, 51-75%	63	42	30%	20%
Grade IV: excellent 76-99%	42	21	20%	10%
Grade V: complete 100%	56	21	26.7%	10%
Total	182	133	86.7%	63.3%
Side effect (pruritis and burning sensation)	35		16.7%	

Discussion

Vitiligo is a common skin disorder characterized by loss of skin colour. It mainly affects a younger population and can cause serious cosmetic and social problems (1, 2, 14, 18).

The patients had no difference with regards to the age at presentation, age at onset, disease duration, percentage of patient with active disease, body surface area involvement and sites involved and leukotrichia. The percentage of the repigmentation of skin and hair, patterns of repigmentation and side effects were assessed subjectively (20).

Our study showed a predominance of female patients with a ratio of 1.1:1. This is in agreement with most pediatric series; the majority of cases were in girls. But the frequency in the population is probably the same in both sexes (1, 2).

High numbers of patients have limited areas of Vitiligo especially on the head and neck, trunk and extremities that do not need systemic treatment; in this area topical treatment like corticosteroids and other motilities of treatment are considered.

The use of topical steroids for a long time lead to many side effects like atrophy, telangiectasia, while this study showed that Tacrolimus is safe even if used for a long period with mild reversible side effects. In our study 35 (16.1%) patients developed side effects with Tacrolimus and the side effects were not severe enough to warrant withdrawal. The reported side effects like pruritis and burning sensation in patients was for only three days which then disappeared. This is agreement with other study 4, 7, 8, 10, 16, 17.

The present work was arranged to evaluate the effectiveness and safety of topical Tacrolimus ointment 0.1 in treatment of localized Vitiligo in children.

Topical FK 506 (protopic, Aatella) is approved for the treatment of atopic dermatitis in a growing number of case reports and small series that demonstrate that it can also induce repigmentation in Vitiligo especially on the face and neck (6,8,10,16).

The full role of autoimmune T-cell in Vitiligo remains unclear. Thus it is uncertain if the anti t-cell activity of the FK 506 underlies its mechanism in treating Vitiligo; recent studies have investigated how topical FK 506 alters the inflammatory environment on the skin (6,8,10,16).

Topical Tacrolimus may also act on keratinocytes to create signals that cause proliferation of melanocytes antigen and inhibition of the subsequent cytotoxic T lymphocytes reactions (6, 16).

In our study the result of topical Tacrolimus ointment in treatment of localized Vitiligo in head and neck, trunk and extremities is promising. From 210 patients it showed 182 (86.6 %) patients showed excellent response at the end of six months and of these 133 (63.3%) patients showed moderate response at the end of six months on the trunk and extremities. This is in agreement with other studies (3, 4, 10, 16, 17, 21, 22-24).

Conclusion

In conclusion topical Tacrolimus ointment 0.1% is an effective and safe alternative therapy for childhood localized Vitiligo particularly involving the head and neck, with fewer side effects.

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Seroepidemiological Study of Toxoplasma, Rubella, Cytomegalovirus and Herpes Simplex in Women with Bad Obstetric History

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ABSTRACT

Background: Most of the TORCH infections cause mild maternal morbidity but have serious fetal consequences.

Aim: To verify the prevalence of TORCH infections in women with BOH in Kirkuk Governorate.
Study design: Retrospective-descriptive study.

Materials and Methods: The information about the pregnant women was gathered from data available in the laboratory of Kirkuk General Hospital records.

Results: Of 2,566 women with Bad Obstetric History (BOH), 27 (1.05%) were anti-T.gondii IgM positive and 505 (19.68%) anti- T.gondii IgG positive. Anti-rubella IgM was detected in 238 out of 2,566 (9.28%) women with BOH, furthermore, anti- rubella IgG was positive in 2,186 out of 2,566 (85.19%) women with BOH. The CMV IgM sero-prevalence was 12.9% (331 / 2,566) in women with BOH, while CMV IgG seroprevalence was 88.58% (2,273/2,566).

Anti-HSV-2 IgM seroprevalence was 3.27 % (84/2,566) in women with BOH.

Conclusion: The present study has confirmed the significant association of TORCH and BOH. This study being retrospective and without controls has its limitations, still the observations obtained cannot be ignored. We recommend that all antenatal cases with BOH be routinely screened for TORCH complex.

Key words: TORCH, T.gondii, Rubella, CMV, HSV, BOH, IgM, IgG, Kirkuk, Iraq.

Introduction

Toxoplasmosis is a relatively widespread zoonosis parasitic infection caused by the intracellular parasite Toxoplasma gondii. The infection can be transmitted vertically, through placenta, to the fetus. [1]. Infection with T.gondii during early pregnancy may frequently lead to abortion, many intrauterine malformations or other serious complications [2,3]. The rate of transmission of Toxoplasma increases with the stage of pregnancy; in the first half of the gestation period it is 5-15%, which may reach 60-80% in the second half . In contrast, the serious complications may be 70-80% in the first half and reduced to less than 10% in the second half [4]. Thus determination of toxoplasmosis infection in pregnant women, due to risk of congenital toxoplasmosis is of particular interest in Iraq, as well as worldwide.

Rubella virus infection occurs worldwide, with a seasonal peak of infections in spring intemperate climates.[5]. Rubella has public health importance due mainly to the teratogenic potential of the virus [6]. However, the incidence of rubella has been substantially reduced in many countries through implementation of rubella vaccination strategies [6]. Rubella infection may result in miscarriage, fetal death, or congenital defects known as congenital rubella syndrome (CRS), when occurring just before conception and during early pregnancy.[7,8] Epidemiological and socioeconomic differences, and urban versus rural settings influence the rates of susceptibility to rubella among women of childbearing age, which may vary considerably among and within countries [6].

Before the introduction of rubella vaccine, the incidence of CRS varied from 0.1-0.2/1000 live births during endemic periods, and from 0.8-4/1000 live births during rubella epidemics.[9-12] During the past decade large-scale rubella vaccination has drastically reduced or practically eliminated rubella and CRS in many developed countries and in some developing countries.[13].

Sustained low coverage of rubella immunization in infants and young children can result in an increase in susceptibility among women of childbearing age that may increase the risk of CRS above levels prior to the vaccine being introduced.[6]. In the light of the remaining global burden of CRS and to avoid the potential risk of CRS, in countries including Iraq, a surveillance program should be implemented to clarify the immune status in women in the child bearing period.

Cytomegalovirus (CMV) is a virus that rarely causes disease in healthy individuals, however, primary infection of the mother during pregnancy presents risk of CMV infection of the fetus with resulting permanent disability [14] CMV is the most common congenital viral infection in certain global

areas and a leading cause of congenital hearing loss and neurological disability [15,16]. Fetal infection occurs when a CMV seronegative women develops a primary infection during pregnancy, latent infection reactivation from maternal infection acquired prior to pregnancy, or re-infection with a new CMV strain during gestation [16]. CMV risk rate of transmission from mother to fetus was higher among pregnant women with primary infection (30-40%) compared to those with non-primary (0.2-2%) infections (IgG positive prior to pregnancy, IgG positive at their 1st pregnancy visit, IgM positive with high IgG avidity).[17-19]

Primary CMV diagnosis is achieved with documented CMV IgG seroconversion, but in our community this documentation is rare since women are not routinely tested for CMV antibody. Currently, there is no recommendation for routine CMV screening during pregnancy [20], provider and public awareness of congenital CMV infection is low [21-24], and there is a high rate of CMV latent infection in the Iraqi population [25]. The extent to which prenatal screening or diagnostic testing for CMV is occurring in Iraq is unknown and there is currently little information on national practices around CMV testing during pregnancy. Identifying testing practices will provide useful information to monitor future screening and prevention program [16]. Here we used a public healthcare data base to explore current practices and rates of CMV testing and immunological status in women with BOH.

Herpes simplex virus (HSV) infections are caused by two strains, HSV-1 and HSV-2. Orolabial infection is mainly caused by HSV-1, however, this strain is responsible for up to 53% of primary genital herpetic infections [26]. HSV-2 genital infection is much more likely to recur than genital HSV-1 infection, thus the presence of antibody to HSV-2 and a compatible clinical history would be strong presumptive evidence that the disease is recurrent genital herpes [27-29]. In addition to agent factor, genetics may play a role in susceptibility to HSV infection [30].

Primary genital HSV-1 or HSV-2 infection in pregnant women can result in abortion, premature labor and congenital and neonatal herpes.[31-33] HSV-2 infections in the newborn are particularly severe and frequently involve the CNS. [34] Recent changes in HSV-1 and HSV-2 infection epidemiology have been reported, with type incidence changes and sequential genital infections with HSV-1 and HSV-2 [35,36].

Little is known about the risk factors associated with HSV seropositivity in pregnant Iraqi women. Identification of the risk factors may help to improve the control measures of HSV infection. Although there is improvement in the diagnosis and treatment of TORCH infections, still it represents a problem in developing countries. Clinical diagnosis of TORCH is difficult, since most of the maternal infections with adverse outcomes are initially asymptomatic. Routine TORCH complex screening during pregnancy is not recommended in Iraq and the extent to which it is performed is unknown. Using a healthcare database, seroprevalence of TORCH complex was determined among women with bad obstetric history (BOH). The aim of the present study was to verify the prevalence of TORCH infections in women with BOH in Kirkuk Governorate.

Materials and Methods

Data source:

The information about the pregnant women was gathered from data available in the laboratory of Kirkuk General Hospital records.

Study design:

Retrospective-descriptive study.

Study population:

The study was conducted in Kirkuk General Hospital. Women with bad obstetric history who were tested for TORCH during three years (2010, 2011 and 2012) were included in the study. Their age ranged from 15 to 45 years, with a mean age of 23.7 ±4.9 years. The total number of tested serum samples was 2,566; of them 884 for 2010, 828 for 2011 and 854 for 2012. The study was approved by the Tikrit University College Ethical Committee and approval from Kirkuk Health Authority Directorate was achieved.

IgG/IgM antibody testing:

Enzyme Linked Immunosorbent Assay was used for detection of IgM and IgG antibody in sera of women with BOH. The kits used IgG and IgM detection and were purchased from BioCheck Inc. The tests were performed according to manufacturer instructions.

Statistical analysis:

The data was presented as frequency and percent. The analysis was performed using Microsoft Excel 2007.

Results

Of 2,566 women with BOH, 27 (1.05%) were anti- T.gondii IgM positive and 505 (19.68%) anti- T.gondii IgG positive. The positivity rate for anti- T.gondii IgM decreased overtime (1.47% for 2010; 0.85% for 2011; 0.82 for 2012), but did not reach a significant level. However, anti- T.gondii IgG positivity was higher for 2011 (21.01%) as compared to 2010 (20.25%) and 2012 (17.8%), with a non significant difference.(Table 1 - opposite page).

Table 2 (page 24) shows the rate of detection for anti- T.gondii IgG and IgM over the months of year study. For the year 2010, anti- T.gondii IgG higher detection rate was in September (25.54%) and May (25%), while anti- T.gondii IgM higher detection rate was in October (3.7%) and September (3.64%). For the year 2011, anti- T.gondii IgG higher detection rate was in March (25.71%) and October (25.3%), while anti- T.gondii IgM higher detection rate was in January (5.95). for the year 2012, anti- T.gondii IgG higher detection rate was in July (30.6%) and June (29.31%), while the corresponding value for anti- T.gondii IgM was in September (4.71%). Statistical analysis did not reveal significant differences in

Table 1: Seroprevalence of IgM and IgG in women with Bad Obstetric History

Organism	Year (Number tested)								Year study trend			
	2010 (884)		2011 (828)		2012 (854)		Total (2566)		IgM		IgG	
	Number positive [Percent]		Number positive [Percent]		Number positive [Percent]		Number positive [Percent]		X^2	P	X^2	P
	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG				
Toxoplasma	13 [1.47]	179 [20.25]	7 [0.85]	174 [21.01]	7 [0.82]	152 [17.80]	27 [1.05]	505 [19.68]	4.28	0.118	3.03	0.22
Rubella	179 [20.25]	753 [85.18]	26 [3.14]	684 [82.61]	33 [3.86]	749 [87.70]	238 [9.28]	2186 [85.19]	189.00	**0.000**	8.65	**0.013**
CMV	173 [19.57]	823 [93.1]	74 [8.94]	722 [87.2]	84 [9.84]	728 [85.25]	331 [12.9]	2273 [88.58]	53.70	**0.000**	28.8	**0.000**
HSV-2	14 [1.6]	-	40 [4.83]	-	30 [3.51]	-	84 [3.27]	-	14.5	**0.001**	-	-

Month	Year (Number tested)								
	2010 (884)			2011 (828)			2012 (854)		
	Number positive [Percent]			Number positive [Percent]			Number positive [Percent]		
	No.	IgM	IgG	No.	IgM	IgG	No.	IgM	IgG
January	86	2 [2.33]	21 [24.42]	84	5 [5.95]	20 [23.8]	24	0	6 [25.00]
February	78	2 [2.56]	19 [24.36]	49	0	8 [16.3]	92	0	6 [6.52]
March	65	1 [1.54]	14 [21.54]	70	0	18 [25.71]	125	2 [1.60]	6 [4.80]
April	58	0	12 [20.69]	70	0	12 [17.1]	86	0	19 [22.10]
May	72	0	18 [25.00]	72	0	15 [20.8]	64	0	13 [20.31]
June	85	0	15 [17.65]	80	0	21 [20.8]	58	0	17 [29.31]
July	80	0	11 [13.75]	63	0	13 [20.6]	85	0	26 [30.60]
August	62	0	8 [12.90]	82	0	13 [15.8]	36	0	9 [25.00]
September	55	2 [3.64]	14 [25.54]	67	0	10 [14.9]	85	4 [4.71]	5 [5.90]
October	108	4 [3.70]	20 [18.50]	83	1 [1.20]	21 [25.3]	67	0	17 [25.40]
November	60	2 [3.33]	13 [21.67]	48	0	11 [22.9]	54	0	7 [12.96]
December	75	0	14 [18.67]	60	1 [1.67]	12 [20.0]	78	1 [1.28]	21 [26.92]
Total	884	13 [1.47]	179 [20.25]	828	7 [0.85]	174 [21.0]	854	7 [0.82]	152 [17.80]
X^2		14.46	8.65		31.88	7.81		21.61	56.85
P value		0.21	0.65		0.0008	0.73		0.027	0.0000

Table 2: Monthly frequency of IgM and IgG anti-Toxoplasma for years 2010-2012

seroprevalence for IgM (X2=14.46, P=0.21) and IgG (X2=8.65, P=0.65) between months of 2010, however, there were significant differences in IgM (X2=31.88, P=0.000) for 2011 and 2012 for both immunoglobulins (for IgM , X2=21.61, P=0.02; IgG, X2=56.85, P=0.0000).

Anti-rubella IgM was detected in 238 out of 2,566 (9.28%) women with BOH and the detection rate was significantly (X2=189, P=0.000) higher in 2010 (20.25%) than 2011 (3.14%) and 2012 (3.86%) years. Furthermore, anti- rubella IgG was positive in 2,186 out of 2,566 (85.19%) women with BOH, with a lower positivity in the year 2011 (82.61%). The proportion of rubella seronegativity was significantly different over the study period for IgM ((X2=189, P=0.000) and IgG ((X2=8.65, P=0.013). However, rubella IgG testing in this set of women indicated that 14.91% of them are prone to infection in their next pregnancy. This set of women were with a primary acute infection of 9.35%, and those are with hazard for rubella vertical transmission to their fetus.

Detection rate of rubella IgM was higher in March (100%) for 2010, while for 2011 was higher in January (28.57%), and for 2012 it was higher in September (29.41%). There was no significant difference between 2011 and 2012 anti-rubella IgM detection rate. However, the result of 2010 may indicate a pandemic pattern for this year. The seroprevalence was significantly different when analyzed on a monthly basis for years 2010, 2011 and 2012. (IgM , X2=161.1 to 391.11,

Month	Year (Number tested)								
	2010 (884)			2011 (828)			2012 (854)		
	Number positive [Percent]			Number positive [Percent]			Number positive [Percent]		
	No.	IgM	IgG	No.	IgM	IgG	No.	IgM	IgG
January	86	9 [10.47]	75 [87.21]	84	0	81 [96.43]	24	0	20 [83.33]
February	78	26 [33.33]	70 [89.74]	49	14 [28.57]	43 [87.76]	92	0	81 [88.04]
March	65	25 [38.46]	62 [95.38]	70	0	63 [90.00]	125	1 [0.80]	119 [95.2]
April	58	15 [25.86]	54 [93.10]	70	0	55 [78.57]	86	2 [2.33]	65 [75.58]
May	72	72 [100]	34 [47.22]	72	0	58 [80.56]	64	1 [1.56]	59 [92.19]
June	85	12 [14.12]	69 [81.18]	80	0	68 [85.00]	58	0	55 [94.83]
July	80	14 [17.50]	65 [81.25]	63	0	59 [93.65]	85	0	74 [87.06]
August	62	0	56 [90.32]	82	0	69 [84.15]	36	1 [2.78]	29 [80.56]
September	55	0	43 [78.18]	67	0	14 [20.89]	85	25 [29.41]	70 [82.35]
October	108	2 [1.85]	98 [90.74]	83	2 [2.41]	72 [86.75]	67	1 [1.49]	57 [85.07]
November	60	2 [3.33]	59 [98.33]	48	0	47 [97.92]	54	0	49 [90.74]
December	75	2 [2.67]	68 [90.67]	60	10 [16.67]	55 [91.67]	78	2 [2.56]	71 [91.03]
Total	884	179 [20.25]	753 [85.18]	828	26 [3.14]	684 [82.61]	854	33 [3.86]	749 [87.7]
X^2		391.11	110.14		161.10	211.39		168.03	28.27
P value		0.0000	0.0000		0.0000	0.0000		0.0000	0.002

Table 3: Monthly frequency of IgM and IgG anti-Rubella for years 2010-2012

P=0.0000; IgG , X2=28.27 to 211.39, P=0.002 to 0.0000) (Table 3).

The CMV IgM seroprevalence was 12.9% (331 / 2,566) in women with BOH, while CMV IgG seroprevalence was 88.58% (2,273/2,566). When the data was analysed on a yearly basis, there was a significant difference for the study years regarding CMV IgM (X2=53.7, P=0.000) and CMV IgG (X2=28.8, P=0.000). CMV IgM seroprevalence was higher in February for 2010 (50%) and 2011 (46.94%), while for 2012, the high seroprevalence was in March (35.2%). (Table 4). Concerning CMV IgG, the high seroprevalence was in July (97.5%) and February (97.44%) for 2010; while for 2011, the high rate was in March (98.57%) and July (98.41%). For the year 2012, CMV IgG seroprevalence was high in July (97.65%) and December (97.44%). The seroprevalence was significantly different when analyzed on a monthly basis for years 2010, 2011 and 2012. (IgM , X2=107.3 to 122.51, P=0.0000; IgG , X2=21.2 to 528.87, P=0.03 to 0.0000) (Table 4 - next page).

Anti-HSV-2 IgM seroprevalence was 3.27 % (84/2,566) in women with BOH; analyses on a year basis indicated a significant difference between study years (X2=14.5, P=0.001). The seroprevalence was higher for 2011 (4.83%) as compared to 2012 (3.51%) and 2010 (1.6%).(Table 1). Analysis on monthly basis indicated that anti-HSV-2 IgM seroprevalence was significantly different over months of the study periods (2010, X2=23.11, P=0.01; 2011, X2=122.19,

Month	Year (Number tested)								
	2010 (884)			2011 (828)			2012 (854)		
	Number positive [Percent]			Number positive [Percent]			Number positive [Percent]		
	No.	IgM	IgG	No.	IgM	IgG	No.	IgM	IgG
January	86	20 [23.26]	74 [86.05]	84	2 [2.38]	82 [97.62]	24	0	24 [100]
February	78	39 [50.00]	76 [97.44]	49	23 [46.94]	41 [83.67]	92	1 [1.09]	89 [96.74]
March	65	8 [12.31]	60 [92.31]	70	9 [12.86]	69 [98.57]	125	44 [35.2]	116 [92.80]
April	58	19 [32.76]	54 [93.10]	70	3 [4.29]	5 [7.14]	86	6 [6.98]	83 [96.51]
May	72	1 [1.39]	64 [88.89]	72	3 [4.17]	64 [88.89]	64	2 [3.13]	53 [82.81]
June	85	25 [29.41]	82 [96.47]	80	2 [2.50]	77 [96.25]	58	6 [10.34]	55 [94.83]
July	80	24 [30.00]	78 [97.50]	63	1 [1.59]	62 [98.41]	85	5 [5.88]	83 [97.65]
August	62	9 [14.52]	59 [95.16]	82	0	73 [89.02]	36	0	34 [94.44]
September	55	11 [20.00]	47 [85.45]	67	8 [11.94]	63 [94.03]	85	10 [11.76]	2 [2.35]
October	108	12 [11.11]	103 [95.37]	83	10 [12.05]	81 [97.59]	67	0	59 [88.06]
November	60	0	56 [93.33]	48	3 [6.25]	48 [100]	54	1 [1.85]	54 [100]
December	75	5 [6.67]	70 [93.33]	60	10 [16.67]	57 [95.00]	78	9 [11.54]	76 [97.44]
Total	884	173 [19.57]	823 [93.1]	828	74 [8.94]	722 [87.2]	854	84 [9.84]	728 [85.25]
X^2		107.3	21.10		119.44	472.32		122.51	528.87
P value		0.0000	0.03		0.0000	0.0000		0.0000	0.0000

Table 4: Monthly frequency of IgM and IgG anti-CMV for years 2010-2012

P=0.0000; 2012, X2=32.66, P=0.0006) (Table 5 - opposite page).

Discussion

Microbial agents such as Toxoplasma, rubella, CMV and HSV are important causes of infections during pregnancy. These infections often lead to mild or asymptomatic infection in the mother [37]. However, the infection during pregnancy may result in serious congenital abnormalities, intrauterine growth retardation and may cause foetal death [38,39,40].

Our results showed that T. gondii IgM and IgG seropositivity in women with BOH were 1.05% and 19.68% respectively. In addition, seropositivity for both IgM and IgG was not significantly different when analysed on a year study basis. T. gondii is higher in places consuming undercooked meat, which is not a tradition in our society, thus the seroprevalence is low in our study population. In Iraq, the seroprevalence of T.gondii varies greatly among different Iraqi regions ranging from 8.1% to 94%. The seroprevalence was higher in Baghdad (94%) as one study reported [41], however, another study performed in Baghdad [25] reported lower seroprevalence (8.1%). Our result was lower than reported for Baghdad [41], Babylon [42], Waset [43] and ThiQar [44], and higher to that reported by other groups in Baghdad [25]. The variation between different studies may probably be due to consuming of undercooked meat, raw vegetable and the number of stray cats [45]. However, the comparison between findings of different studies is not easy due to differences in study design, study population recruitment, methods for detection on immune response and researcher background; (e.g. an article published about Toxoplasma infection in women in Tikrit city was performed by a research specialist in computers).

Month	Year (Number tested)					
	2010 (884)		2011 (828)		2012 (854)	
	Number positive [Percent]		Number positive [Percent]		Number positive [Percent]	
	No.	IgM	No.	IgM	No.	IgM
January	86	5 [5.81]	84	0	24	0
February	78	2 [2.56]	49	0	92	1 [1.09]
March	65	3 [4.62]	70	4 [5.71]	125	1 [0.80]
April	58	0	70	4 [5.71]	86	2 [2.33]
May	72	0	72	3 [4.17]	64	1 [1.56]
June	85	0	80	0	58	1 [1.72]
July	80	0	63	3 [4.76]	85	11 [12.94]
August	62	2 [3.23]	82	1 [1.22]	36	3 [8.33]
September	55	0	67	1 [1.49]	85	3 [3.53]
October	108	2 [1.85]	83	2 [2.41]	67	2 [2.98]
November	60	0	48	2 [4.17]	54	1 [1.85]
December	75	0	60	20 [33.33]	78	4 [5.13]
Total	884	14 [1.6]	828	40 [4.83]	854	30 [3.51]
X^2		23.11		122.19		32.66
P value		0.01		0.0000		0.0006

Table 5: Monthly frequency of IgM anti-HSV-2 for years 2010-2012

Anti-T. gondii -IgM in our study seroprevalence was 1.05%, which is lower than that reported before for other regions in Iraq, as the range was 24.2% to 60% [25,41-44,46]. The 1.05% prevalence of anti-T.gondii-IgM among women with BOH points out to the importance of adequately diagnosing and monitoring congenital toxoplasmosis.

In the present study seroprevalence of toxoplasmosis (19.68%) was lower than that reported for other geographical areas such as 62.8% in Brazil [47], 43.8% in France [48], 44.8% in Libya [49], 35.1% in Qatar [50], 29.4% in Saudi Arabia [51], 41.9% in Yemen [52], 10.5% to 49.52% in India [53-55], 50% in Nepal [56], 24.6%-69.5% in Turkey [37,45, 57-61], 33% in Venezuela [62].

However, our toxoplasmosis seroprevalence was higher than that reported in the United Kingdom (7.7 - 9.1%) [63] and Norway (10.9%)[64]. The presence of elevated levels of Toxoplasma specific IgG antibodies indicates infection has occurred at some point, but does not distinguish between an infection acquired recently and one acquired in the distant past. In acute infection, IgG and IgM antibodies generally rise within 1 to 2 weeks of infection [2]. Timing of when T. gondii infection occurred in a pregnant woman is important because infection before conception poses little risk for transmission of infection to the fetus; however, infection after conception does pose such risk. [65].

Detection of Toxoplasma-specific IgM antibodies has been used as an aid in determining the time of infection, but IgM antibodies have been reported to persist for up to 18 months post-infection [66]. A negative IgM with a positive IgG result indicates infection at least 1 year previously. A positive IgM result may indicate more recent infection or may be a false positive reaction. Given the potential for false-positive results, the true value of IgM testing is in ruling out the presence of acute infection. In other words, negative IgM results are reassuring, whereas positive results should be interpreted carefully, confirmed in a toxoplasmosis reference laboratory, and followed by serial titers at least 3 weeks apart [66,67].

Rubella is an important virus for the first trimester maternal infections. In Iraq, rubella was incorporated into the national immunization program. However, no vaccination program is available for rubella after the age of 18 years. Previous studies from different regions of Iraq reported rubella seropositivity ranging between 5% and 73.9% in women with BOH [25, 42-46]. This reported range was lower than that of the present study (85.19%). However, the present study indicated that anti-rubella-IgM was lower (9.28%) than that reported for Mosul (16%)[46], ThiQar (20%) [44], Babylon (53.9%) [42] and Waset (62.3%) [43], but higher than that reported for Baghdad (4.8%) [25].

The present study finding concerning anti-rubella-IgM indicated that acute rubella infection still represents a health problem, which may be linked to increase in foetal rubella. However, the health impact of this problem was reduced with time as in this study IgM seroprevalence was lower than that previously reported in studies for Iraq [42-44]. In addition, presence of 85.19% IgG seropositivity indicated that the majority of women had protective immunity against rubella. However, the IgG seropositivity is lower than that reported for India 26.8 to 30.4%, [53-55], Turkey (93.5-96.1%) [57-61], Iran (96.2%) [68], Egypt (92.2%)[69] and Saudi Arabia (91.1%)[70].

Since the susceptibility of pregnant women to rubella was found to be 14.8% in our study, the necessity for rubella vaccination in the childbearing age group remain s controversial. Large studies from different age groups from urban and rural settings are needed to determine the necessity of such a national vaccination program [37] for Iraq. The alternative approach to solve such a problem in the meantime is to introduce rubella immune status testing as a routine one in antenatal clinics and not for suspected cases.

Analysis of anti-rubella-IgM on a yearly basis indicated that all samples tested for 2010 were seropositive for IgM. This may indicate an outbreak infection. Both anti-rubella-IgM and IgG seropositivity were significantly different over the study period and monthly analysis basis.

In the present study the seropositivity rate of women with BOH for CMV IgM and IgG antibodies were 12.9% and 88.58% respectively, with significant differences between years of study for both immunoglobulins. Concerning CMV- IgM antibodies seropositivity (12.9%) was lower than that reported for Baghdad (17.7%) [25], Mosul (24%) [46], ThiQar (45%) [44], Babylon (57.2%) [42] and Waset (60.2%) [43]. CMV-IgG seropositivity was higher (88.58%) than that reported for Baghdad (4.8%)[25], Waset (55.5%)[43] and Babylon (77.8%) [42].

The present study CMV-IgM seropositivity was higher than that reported for Turkey (1.2%) among pregnant women and USA (3%) in women of childbearing age [14]. CMV-IgG seroprevalence was within the range reported globally. The rate was reported in the range of 92.6% to 97.3% for Turkey [37, 57, 71-73], 48.8% in France [74], 56.3% in Finland [75], 78% in Russia [76], 84% in Spain [77], 92.1% in Saudi Arabia [78] and 8.4 - 34.7% in India [53-55]. Close contacts, poor hygiene and life style are highly associated with CMV infections [74], and this may reflect the variation in CMV seroprevalence reported by different studies.

The CMV testing of all pregnant women or restricted to high risk groups is still under debate in the scientific community [37]. A positive test for CMV IgG indicates that a person was infected with CMV at some time during their life but the IgG test cannot determine when a person was infected. However, if antibody tests of paired acute and convalescent phase serum samples show a fourfold rise in IgG antibody and CMV IgM is present or CMV is cultured from a urine or throat specimen, an active CMV infection is present [14]. The presence of CMV IgM is not solely indicative of primary infection. CMV IgM is detectable when a person, is newly infected, has been infected in the past but recently re-exposed to CMV, is undergoing reactivation of CMV infection that was acquired in the past, or has a false positive test result[79,80] and so CMV IgM is not unique to primary infection. Recently, IgG avidity assays, which measure antibody avidity, have been shown to reliably detect recent primary CMV infection. In contrast to IgM, low avidity IgG is present only with primary infection, increasing over 3 to 5 months to high avidity [81,82]. Thus IgG avidity has gained the diagnostic importance in identifying primary CMV infection, where several commercial CMV avidity tests are available [80, 83-86]. The presence of low CMV IgG avidity has been shown to be a unique and reliable serologic indicator of primary CMV infection [14]. Substantial improvements have been reported in the identification of at risk pregnancies using diagnostic algorithms that incorporate both IgG avidity and IgM measurements [86,87].

Contraction of HSV infection during pregnancy may be with a risk of foetal infection either intrauterine or during delivery. Therefore, screening of pregnant mothers for HSV is an important part in antenatal care. Geographical location influences HSV-2 seroprevalence [88]. Highest prevalence of HSV-2 was reported for Africa and America, while lowest prevalence has been seen in Asia [89,90,91]. Our study indicated that 3.27% (85/2566) seroprevalence for HSV-2- IgM, and it is significantly higher for 2011 as compared to 2010 and 2012, in addition, a significant difference demonstrated when the data was analyzed on monthly basis for the years of study. The seroprevalence of HSV-2-IgM

as this study (3.27%) indicated was lower than that reported for Baghdad (8.1%) [25], Mosul (11%) [46], Babylon (28.9%) [42] and Waset (73.9%) [43]. The reported HSV-2 IgM seroprevalence was 16.8% for India [88] in women with BOH, whereas that reported for Saudi Arabia was 0.5% [92].

Reported studies indicated a HSV-2 IgM seroprevalence of a range of 3.6% to 33.5% in India [53-55] and 1.8% for Bangladesh [93], 13.8% for Turkey [94].

In Iraq [Baghdad], Abdulmohymen [25] reported that there was a significant difference ($p<0.05$), in the serum level of Toxoplasma gondii specific IgM among the three investigated patients groups (Recurrent spontaneous abortion, non-recurrent spontaneous abortion, and successful pregnancy). A similar result was obtained by Abbas [95], who showed that 21.5% of women with first abortion have positive only IgM. Al-Fertosi [96] and Salman [97] showed that 19.17% of women had a single or repeated abortion. In addition, there is more than one T. gondii strain with difference in virulence among isolates in nature [98]. This strain's difference could be a potential explanation regarding the high prevalence of toxoplasmosis.

The relatively high frequency of toxoplasmosis in women with abortion as Abdulmohymen [25] reported could be due to the sample selection. Their samples were collected from Al-Kadhimyia Teaching Hospital which is a reference hospital for the surrounding rural areas where they have habits in favor of acquiring toxoplasmosis by eating unwashed raw vegetables or unpadded fruits. In addition, in the rural cities there is close contact with cats and consequent exposure to sporulated oocysts by ingestion of these oocysts that contaminate soil during gardening, or eating undercooked meat contaminated with cysts [25]. Moreover, the low level of education in the women about the risk factors for toxoplasmosis may play an important role in the high rate of infection [99].

Furthermore, Abdulmohymen's [25] study showed a highly significant difference between the women group with recurrent spontaneous abortion and the women group with successful pregnancy in acute infection of T. gondii, but showed no significant difference in the mean value between those with recurrent spontaneous abortion and with non recurrent spontaneous abortion (non-RSA) and non- RSA and those with successful pregnancy. It has been proposed that during pregnancy, systemic maternal immune response is biased in favor of Th2 cytokine [100,101]. Moreover,Th2 cytokines pattern of pregnancy induce the susceptibility to toxoplasmosis infection ,together with risk of placental infection and congenital transmission [102]. Evidence from murine and human pregnancy showed that due to Th1 type cytokine mediated pregnancy loss, a shift towards Th1-type immunity during T. gondii infection may help to explain pregnancy failure [103,104].Thus, a considerable amount of evidence suggests that Th1 cytokine might well be implicated in adversely affecting pregnancy, directly by interfering with trophoblast survival and function, and indirectly by activating cell-mediated immune effecters [105].

A significant difference between RSA and successful pregnancy group in acute infection of CMV was seen [25] in a Baghdad population. There are many confounding studies about the association between CMV infection and pregnancy loss; the studies showed that HCMV can result in abortion or stillbirth [106,107]. HCMV act as an immune modulator through elaborating an array of immune evasion strategies to avoid elimination from the host, and its viral proteins and is involved in the regulation of cellular gene expression and induction of pro-inflammatory cytokine [108]. It was reported that there was no significant difference in the serum level of HSV specific IgM among the three investigated groups [25]. Lutwick et al.,[109] reported, that in the world about one million pregnancies occur each year in women who have been infected with HSV-2, but complications occur in only .01% to .04% of all infected pregnant women [110].

In another study in Waset province, [43] Iraq, women with history of abortion IgM seropositivity were found as for Toxoplasma 54% ($P< 0.05$), Rubella_62.3% ($P< 0.05$), CMV_60.2% ($P> 0.05$) and HSV_73.9% ($P> 0.05$). A high percentage of repeated abortion (two and three or more) seen in women with seropositivity for CMV IgM and Rubella IgM 12.4% ($P> 0.05$) 5.7% ($P< 0.05$) respectively, CMV and HSV infection have a statistically significant correlation with the incidence of abortion in addition the significant role of CMV infection of repeated abortion. So routine screening of all women in child bearing age, shows the need to provide health education to pregnant women.

In Mosul [46], Iraq, Toxoplasmosis is with a high risk infection seropositivity rate of only 43% among women of child-bearing age; also 12% of them are already seropositive for cytomegalovirus (CMV) and therefore most cases of congenital CMV infection are likely to result from maternal reinfection. Rubella infections still occur each year and it appears in 16% in Mosul and in 9.28% in Kirkuk. Neonatal Herpes simplex virus (HSV) infection is also low in Mosul 11%. It is apparent that requests for TORCH screening have been over-ordered and clinicians should be encouraged to send appropriate specimens for specific tests depending on the clinical features of the individual case so as to reduce the adverse fetal outcome.

In Tikrit city, Iraq, detection of IgM antibodies demonstrate a significant correlation with history of abortion, but this study was performed by a researcher working in a computer center.[111] A study reported for Thi Qar Governorate, Iraq, in women with habitual abortion, indicated that 60 of 60 women (100%) had antibodies against CMV, 9 (15%) with IgM antibodies, 21 (35%) with IgG antibodies and 30 (50%) with both IgM and IgG antibodies[44]. In another study performed in Iraq, it was demonstrated that 43.7% of women with abortion have positive Toxoplasma IgM [112]. Salman in Baghdad [97] reported a detection rate of 19.17% for Toxoplasma IgM among women with abortion.

Rubella IgG antibodies in a study performed in Baghdad ,Iraq, were detected in 34.2% of aborted women [113]. The above findings indicated that about 2/3 of the population were

at risk for getting rubella infection during their pregnancy. These findings also highlight the need for rubella screening for pregnant women at their first prenatal visit, with standing orders for rubella vaccination after delivery together with reinforcement of the rubella vaccination program.

Strengths of the study include a large national sample size, which reflects the impact of TORCH in women with bad obstetric history. Our study limitations are that the information regarding gestational period, and pregnancy outcomes is not available.

In conclusion, the present study has confirmed the significant association of TORCH and BOH. This study being retrospective and without controls has its limitation, still the observations obtained cannot be ignored. We recommend that all antenatal cases with BOH be routinely screened for TORCH complex.

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Problem Based Learning Implementation Outcomes from Students' Perspectives

ABSTRACT

Background: Tikrit University College of Medicine is the only Iraqi medical college that adopted problem based learning curriculum since its establishment in 1989. The goals of problem based curriculum are to help the students develop flexible knowledge effective problem solving, self-directed learning, effective collaboration skills and intrinsic motivation.

Aim: To assess Iraqi medical student's perceptions of implementation of problem based learning as an educational approach to improve medical education quality.

Method: A cross sectional study was conducted on 40% of Tikrit University College of Medicine students. Data was collected from 215 students by using a questionnaire by simple quota sampling.

Results: The study indicated that 150 students (69.71%) chose problem based learning curriculum as a favored curriculum, while 65 students (30.2%) chose classical curriculum as a favored curriculum. In addition, the overall attribute scores were significantly higher (P<0.001) in the student group who chose problem based learning curriculum (208) as compared to the students who chose classical curriculum as a favored curriculum (189). Students who chose problem based learning curriculum reported higher scores in 13 items (50%) in the questionnaire than the students who chose classical curriculum. However, students who chose the classical curriculum as a favored curriculum, report higher attribute scores than students who chose problem based learning curriculum in 10 items (38.5%). Furthermore, equal scores in 3 items (11.5%) were reported.

Conclusion: The quality of learning and teaching by Problem based learning curriculum is better than that of the classical curriculum. The major limitation of this study is the lack of a control group.

Key words: Problem based learning, PBL, Curriculum, Outcomes, Medical education, Iraq, Innovative curriculum, Quality assurance.

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Introduction

The story of problem based curriculum started in 1899 when Sir William Osler"[1] the "father of new medicine" realized the complexity of medicine had already progressed beyond the ability of the teacher to teach everything that students would need to know. Osler recommended abolishing the lecture method of instruction and allowing students more time to study. He also emphasized the important role of teachers in helping students to observe and reason. Undergraduate medical education as with any other educational program, needs ongoing improvements to meet the changing demands of medical practice in the 21st century and improve teaching quality. Although the complexities of medical care have increased dramatically over the last century, the method of teaching medicine has changed little. Teachers need to learn about the latest techniques of medical education. Medical education should be given the same emphasis as research and patient care.[2]

McMaster University in Hamilton, Ontario, was the first undergraduate medical school to incorporate PBL methods into its curriculum. This approach was one of many innovations adopted when McMaster's medical school was founded in 1965; an admissions system that was not limited to grades and a non-traditional grading system were also implemented [3]. From this pilot program, interest in PBL methods grew and the approach was gradually implemented in other medical schools in Canada, as well as Schools in the United States, Europe, and Australia. The medical schools that adopted PBL did so in one of two ways: as an alternative track or throughout their entire program. The University of Limburg at Maastricht, Netherlands, embraced a wholly PBL

curriculum in 1974, and the University of New Mexico offered a concurrent PBL track in 1979 [4]. Since then, others, including Georgia's Mercer University School of Medicine in the United States, the University Medical School of Manchester, England, and the New South Wales Medical School in Australia, have adopted PBL methods in some or all of their courses [5]. Perhaps one of the strongest endorsements of PBL came in 1985 when Harvard University's medical school designated it as the standard method of instruction for all undergraduate students.

There has recently been widespread interest in the problem based learning curriculum (PBLC) since its adoption at McMaster university of Canada.[6]. The goals of problem base learning are to help the students develop flexible knowledge, and effective problem solving skills, self directed learning, effective collaboration skills and intrinsic motivation [7]. Working in groups, students identify what they already know, what they need to know, and how and where to assess new information that may lead to resolution of the problem [8]. The role of the instructor (known as the tutor in PBL) is that of facilitator of learning who provides appropriate scaffolding and support of the process, modeling of the process and monitoring the learning [9]. The tutor must build students' confidence to take on the problem, and to encourage the student while also stretching their understanding [10]. The six core characteristics of problem based learning are [11]: Consistent student centered learning, Learning occurs in small groups, Teachers act as facilitators or guides, A problem forms the basis for organized focus and stimulus for learning, Problem stimulates the development and use of problem solving skills, and New knowledge is obtained through means of self directed learning.

In Iraq, Tikrit University College of Medicine [TUCOM] is the first college to adopt the PBLC since its establishment in 1989. Educational activities such as lectures, professional skills, communication skills, clinical entrance, practicals, field studies, professional values and ethics, are structured to support PBL [12,13]. It is emphasized that the role of students, the importance of student satisfaction, the fact that students are reliable and valid sources of information in the assessment of different parameters of the education process, and the feedback and proposals of students can be used in curriculum evaluation [14-17]. There are studies on student's perceptions of their educational program and their gain during their medical education [18-20]. The present study aims to assess Iraqi medical students' perceptions for implementation of problem based learning as an educational approach to improve medical education quality.

Materials and Methods

A cross-sectional study was conducted in a period between the first of March and the end of May 2012 in Tikrit University College of Medicine. 215 students were chosen by quota sampling representing 40% of the total number of students in the college (40% were taken from each class), by applying the questionnaire of 26 items.

A questionnaire with 5 point Likert scale rating system was administered to students.

1. The option values were constructed as follows: for positive statement=strongly agree=+2 agree=+1, neutral=0, disagree= -1, strongly disagree= -2.
2. The frequency on each of the options for each attribute was computed. The frequency for each option was converted into percentage.

		Strongly Agree	Agree	Neutral	Disagree	Strongly disagree	
Option value		+2	+1	0	-1	-2	
Frequency Value	PBL	23.3% =3	51.3%=6	18.6%=2	1.3%=1	0.6%=1	
	Classical	38.4%=4	36.9%=4	15.3%=2	0%=1	0%=1	
Option Score	PBL	2x3=6	1x6=6	0x2=0	-1x1=-1	-2x1=-2	
	Classical	2x4=8	1x4=4	0x2=0	1x-1=-1	-2x1=-2	
Attribute Score	PBL	+6	+6	0	-1	-2	9
	Classical	+8	+4	0	-1	-2	9

Table 1: Gaining basic science knowledge

3. The following frequency values were assigned to the following percentage ranges:

   0_9% =1, 10_19%= 2, 20_29%=3, 30_39%=4, 40_49%=5, 50_59%=6, 60_69%=7, 70_79%=8, 80_89%=9, 90_100%=10 .

4. Multiply the frequency value by the option value to obtain an option score at each point.
5. Compute the sum of the option scores at each attribute to obtain an attribute score. The sum of all the attribute scores yields the Teaching Appraisal Score.
6. The sum of all the attribute scores in each item compared between group 1 (students who chose problem based learning curriculum) and group 2 (students who chose classical curriculum), to see which group reported a higher score in this specific item.
7. The overall attribute scores in all 26 items collected in group 1 and group 2 to assess the two systems.

Results

The study indicated that 150 students (69.71%) chose problem based learning curriculum as a favored curriculum, while 65 students (30.2%) chose classical curriculum as a favored curriculum, Figure 1. In addition, the overall attribute scores were significantly higher (P<0.001) in students group who chose problem based learning curriculum (208) as compared to the students who chose classical curriculum as a favored curriculum(189). Figure 2.

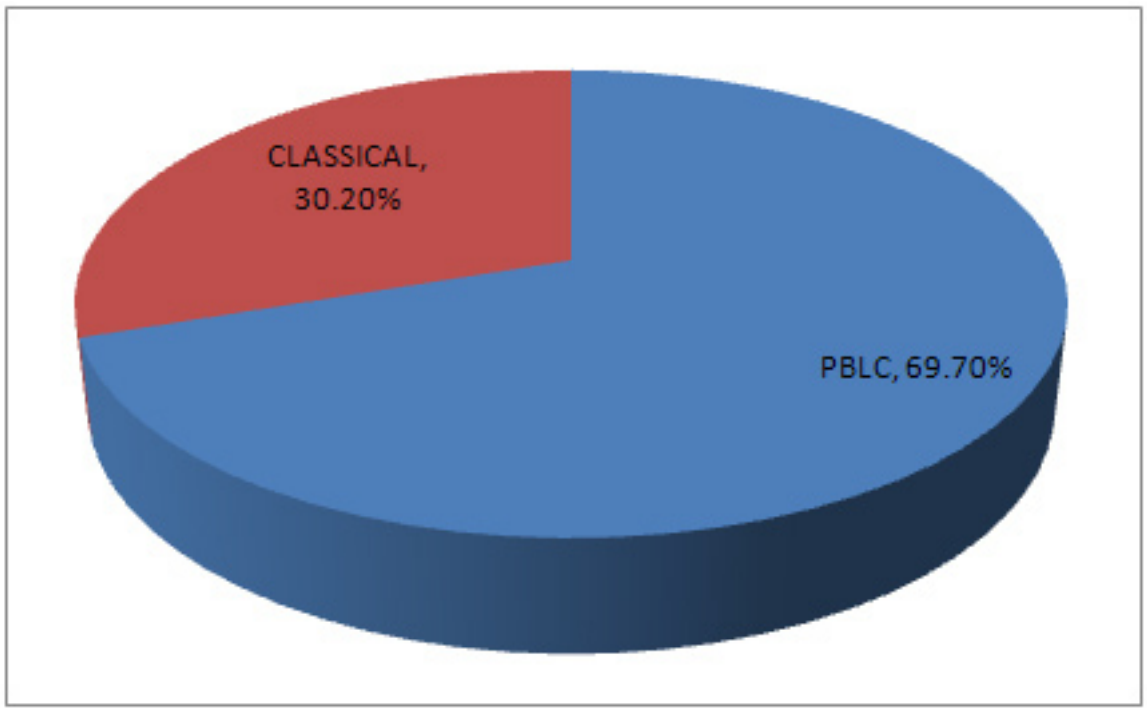

Figure 1: Students' perception of problem based learning

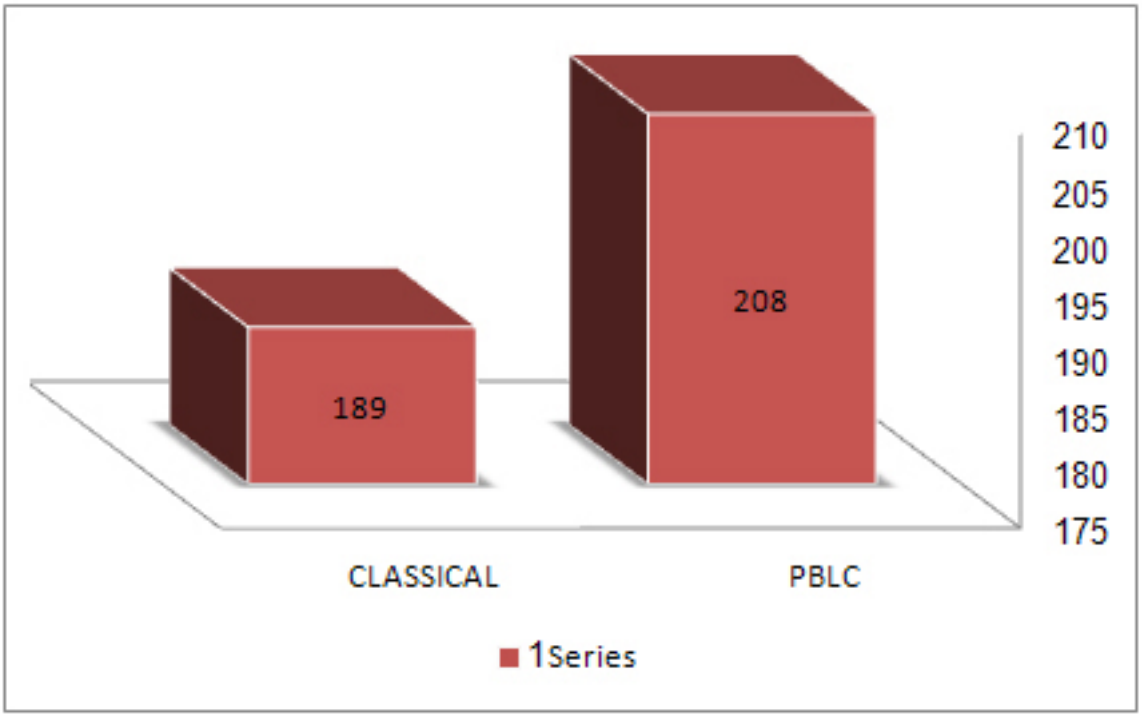

Figure 2 : Total attributes score for the two groups of students

No.	Item	Attribute score	
		PBL	Classical
1	Gaining basic sciences knowledge	9	9
2	Acquisition of clinical knowledge	9	11
3	Gaining clinical skills	8	10
4	Acquisition of community health knowledge and skills	**8**	5
5	Knowing basic concept of ethics and professionalism	8	10
6	Acquisition of clinical reasoning skills	7	8
7	Facilitation of problem solving skills	9	10
8	Facilitation of communication skills	7	7
9	Facilitation of self directed learning	**7**	4
10	Facilitation of integration of basics and clinical sciences	7	7
11	Increasing intrinsic motivation of students	**8**	6
12	Facilitation of development of self assessment skills	**8**	6
13	Facilitation of development of peer assessment skills	**8**	4
14	Using educational methods	**8**	7
15	Facilitation of sound communication with educators	8	11
16	Facilitation of examination performance	7	9
17	Positive effect on student confidence	**10**	9
18	Gaining discussion and presentation skills	**10**	4
19	Critical thinking and scientific analysis	**8**	5
20	Deep learning	7	8
21	Sound selection student career in the future	8	9
22	Gaining ability to work in team	**8**	6
23	Gaining ability for planning and implementing of scientific research	**8**	4
24	Knowing the managerial responsibilities	**8**	5
25	Facilitation of scientific writing	**6**	5
26	Facilitation of humanitarian behavior with colleagues and patients	9	10
	Teaching Appraisal Score	**208**	189

Table 2: Students' perceptions of problem based learning

Students who chose problem based learning curriculum reported higher scores in 13 items in the questionnaire than the students who chose classical curriculum (Table 2) and these include:

- Acquisition of community health knowledge and skills;
- Facilitation of self-directed learning;
- Increasing intrinsic motivation of students.
- Facilitation of development of self assessment;
- Facilitation of development of peer assessment skills;
- Using educational methods.
- The method has a positive effects on my self confidence;
- Gaining discussion and presentation skills;
- Prepares me for critical thinking and scientific analysis.
- Gaining ability to work in a team.
- Gaining ability for planning and implementing of scientific research;
- Knowing the managerial responsibilities;
- Facilitation of scientific writing.

However, students who chose the classical curriculum as a favored curriculum report higher attribute scores than students who chose problem based learning curriculum in 10 items (Table 2) and these include:

- Acquisition of clinical knowledge.
- Gaining clinical skills.

- Knowing basic concepts of ethics, professionalism.
- Acquisition of clinical reasoning.
- Facilitation of problem solving skills.
- Facilitation of sound communication with educators.
- Facilitation of examination performance.
- This method prepares me for deep learning.
- This method guides me to sound selection of my career in the future.
- Facilitation of humanitarian behaviors with my colleagues and patients.

Furthermore, equal scores in 3 items were reported (Table 2) and they include:

- Gaining basic science knowledge.
- Facilitation of communication skills.
- Facilitation of integration of basic and clinical science knowledge.

Students who chose problem based learning curriculum reported higher scores in 13 items (50%) in the questionnaire than the students who chose classical curriculum. However, students who chose the classical curriculum as a favored curriculum report higher attribute scores than students who chose problem based learning curriculum in 10 items (38.5%). Furthermore, equal scores in 3 items (11.5%) were reported.

Discussion

In the present study opinions of 215 medical students were assessed about PBLC and classical curriculum. The study shows that 150 students (69.7%) chose PBLC, whereas 65 students (30.2%) chose classical curriculum. This result agrees with that reported by others for other geographical areas with active or passive education culture [21-30]. The medical education that requires the active participation of students is more demanding and pushes the limits of personal resources more than classical techniques [31]. Thus the present study suggested that problem based learning curriculum may be helpful to predict medical student satisfaction and academic achievement with some centered instruction methods. In meta-analysis of studies that compare PBL with traditional methods of medical education, PBL was found to be significantly superior with respect to student's program evaluation [32]. Traditional education was superior for teaching factual knowledge of basic sciences [33].

In comparison with the acquisition of basic and clinical knowledge parameters, TUCOM students attributed higher scores to attainment of community health, self directed learning, intrinsic motivation of students, facilitation of self, peer assessment skills and using of educational methods objectives within the context of efficacy content. This finding agreed with that reported for other institutions [22] and it is accepted since PBLC focuses on clinical science from the first year [34]. In TUCOM with curriculum starting from the first year, the PBL scenarios are designed to include social and behavioral aspects, and they are supported by field, primary health care centers and small group activities [12,13].

From the perspective of curriculum content, TUCOM students gave lowest attribute score (6, Table 2) to facilitation of scientific writing. However, the score was higher in TUCOM students who chose PBL than those who chose traditional curriculum. Acquisition of basic science knowledge in our study population was with equal attribute scores for both who favor PBLC or classical curriculum. Reported studies indicated that PBL students get lower scores in basic science knowledge as compared to traditional education [22,30,32,34,35,36]. However, this study does not agree with the above reported studies. This is due to focusing of PBLC on clinical science more than basic science. [34]

In the evaluation of students regarding the efficacy of the education provided by TUCOM, the highest score was attributed to the items on ''Positive effect on student confidence'' and "Gaining discussion and presentation skills''. This finding reflects the positive role of the student in PBL. Regarding acquisition of clinical knowledge skills, students who favor traditional education record higher attribute scores than students in favor of PBL. This result disagrees with an evaluation at Harvard Medical School about PBLC students, in which PBLC students gained slightly higher scores at NBME part 2 than classical students.[37]

Students, who prefer traditional education, report higher score for ''knowing basic concept of ethics and professionalism'' and ''Facilitation of problem solving'' than the PBL group. This result disagrees with reported studies [2], where PBLC graduates report a high score due to higher focusing on ethical problems during undergraduate studies. These findings could be due to the implementation of the program rather than its contents. After 2003, the college administration does not pay attention to small group discussion sessions in years 1-3 and community field work. These do influence the outcome and students' satisfaction about the education approach. These findings should be taken seriously and reform of implementation of the TUCOM program must be considered. A more detailed study for program evaluation is warranted. In addition, a number of faculty members joined TUCOM, but they are not well trained in innovative medical education and some of them not believe in this education method. They form an opposing group that resists any implementation of innovative educational strategies. Furthermore, the worst behavior of the Ministry of Higher Education is the acceptance of students from other colleges who pass the 1st year and that from outside the country that is with low secondary school scores.

Students in favor of traditional education, report a higher score for ''acquisition of clinical reasoning skills'' than the PBL group and this finding is agreed with by a study from the national university of Singapore. [2]. Both groups report equal attribute scores to ''facilitation of communication skills''. This result disagrees with a study reported for Dokuz Eylul university school of medicine [30]. This is due to improper training on communication skills in our college as a result

of not sending students to primary health care centers and reduction of discussion hours from 6 to 2 hours. Students in favor of PBL show a higher attribute score for ''facilitation of self-directed learning'' than those who are in favor of traditional education. This result agrees with a study from Dokuz Eylul university school of medicine. [22]

Both student groups in this study show equal attribute scores for ''facilitation of integration of basic clinical knowledge''. The reported studies [38] suggest that facilitation of integration was better in PBLC rather than the traditional education. The variation in reported scores may be due to improper implementation of the TUCOM PBL curriculum. In reported literature, the evaluation of students regarding the efficacy of the education provided by medical schools that adopted PBLC, a high score was attributed to item on ''communication with educators'' [30, 39]. However, in the present study, the students in favor of PBL attributed a lower score (8, 40%) than who were in favor of traditional education 11, (55%). This is due to improper application of PBLC in TUCOM where small group systems allow direct contact between students and educators. In the success of educational programmes, the importance of positive communication of tutors with students and positive learning environment are emphasized [40]. It is thought that the faculty development programmes in TUCOM, which aimed to improve educators adaptation to the system, was not established well. In addition, the social sactorization is with negative impact on communication between faculty members and students. There is a gap between them due to fear and possibility of force used by students. The faculty training programmes had a positive impact on the development of sound communication skills between students and faculty members [41].

Regarding the percentages attributed by TUCOM students to the efficacy of each year, the students in favor of PBL was higher for all years (2,3,4,5, and 6 years) involved in the study and the highest was attributed by year 5 (83.8% for PBL, 16.2% for classical education). TUCOM students attributed a higher score for traditional education in regard to item of '' facilitation of examination performance''. This student opinion is based on that in traditional education the examination schedule is easily arranged, while in PBL programs there is a formative and summative assessment,and consequently, these add burden on students. In addition, newly appointed faculty members, who are not well trained, deepen this problem. Furthermore, in Iraq and may be in other countries, there is an appearance of a new challenge to medical education, that is clinical educators are interested mainly in their private work rather than public. The present study finding does not agree with that reported by others [31].

The PBL group report higher ability of critical thinking, scientific analysis than those in favor of the classical group. This result agreed with that reported for other medical schools. [2]. As expected, a high score for deep learning was attributed by students in favor of traditional education than that of PBL group. However, this result was in contrast to that reported in Turkey [30]. The fact that the average of scores attributed to most of the public health, facilitation of the self directed learning, increasing intrinsic motivation of the students, facilitation for the development of self and peer assessment skills, using of educational methods, development of students' self confidence, gaining of discussion and presentation skills and critical thinking and scientific analysis, ability to work in team ; and ability for planning and implementing of scientific research, managerial responsibilities and scientific writing, were rated higher for PBL. These findings agree with that reported by others [2,22,30,32].

These parameters are among the curricular objectives of TUCOM. They have a crucial importance in community based medical education [42]. The overall attribute score was significantly higher ($P<0.001$) in the student group who chose problem based learning curriculum (208 students) as compared to the students who chose classical curriculum as a favored curriculum (189 students). In addition, the mean of the attribute score was higher (8) for the group in favor of PBL than those in favor of classical education (7.27).

The information regarding PBL effectiveness mainly comes from undergraduate medical education studies [31]. Two of the most interesting findings from the literature concern how students structure and retain new knowledge. David, Dolmans, Patel, and van der Vleuten [43] believed that students using PBL are more successful at integrating their knowledge of basic science concepts into clinical problems and retain this knowledge better than students in conventional curricula. By activating prior knowledge in their discussions, learners can begin to construct explanatory models, which in turn facilitate the processing of new information. The finding of the present study agreed with their belief. Dolman and Schmidt [44] concluded that students can retain new information better if they have opportunities to elaborate on it during group discussions. Known as "contextual learning," this term is often used in discussions of PBL. Key features of contextual learning are that it stimulates learners' prior knowledge, encourages them to create explanatory models for relevant problems, and provides opportunities for group discussions. Our study indicated that PBL has a positive effects on student self confidence, prepares them for critical thinking and scientific analysis, things that represent a solid structure for building professionalism of medical graduates.

It is thus reasonable to conclude that PBL is also an interactive intervention for improvement of community health because it provides physicians with opportunities to acquire community health knowledge and skills and interact and practice their skills. These acquired skills will consequently improve health care delivery in developing countries since the majority of our society is seeking their medical care from primary health care centers. Davis et al. [45] argued that PBL designs that have breaks between sessions allow participants the opportunity to learn-work-learn. Under these circumstances, students can implement what they learned in one session and then discuss their experiences in a later session with their peers. The present study does confirm this assumption as 50% of the questionnaire items were in favor of PBL compared to 38.5% of items in favor of traditional education.

Perhaps the most common criticisms of PBL are the extra time and the extra expense required to create such courses [46,47]. These criticisms are more complicated than would first appear and require a closer look. Besides considering whether the study involves undergraduate education, it is essential to determine whether it was an in-person or a distance course because these factors, not PBL per se, may be responsible for some of the extra cost [48]. Most of the current information about the cost of PBL comes from studies of undergraduate and postgraduate medical education. For instance, Smits et al. [46] found that creating a problem-based, postgraduate medical training program costs 15% more than a lecture-based program, while Albanese and Mitchell [47] concluded that for 100 or fewer students, the time spent preparing and delivering lectures was equal to or greater than the time spent tutoring PBL groups and that students in PBL programs covered materials only 82% as fast as students in conventional, lecture-based courses. Yet, although these findings are interesting, it is difficult to compare them or to draw conclusions from them because the researchers considered the issue of costs from different points of view [48]. Smits et al. focused on the total cost of creating a problem-based course, and Albanese and Mitchell broke the cost into categories, such as faculty and support-staff time, instructional efficiency (how long students took to cover content), instructional media (textbooks and non-print media), and physical supports (rooms and buildings) [48]. What these studies clearly show, however, is that many variables must be considered when trying to determine the costs of creating problem-based courses. More research is needed to understand the costs of implementing PBL in undergraduate programs and how these figures compare to other course designs.[48] Two other criticisms of PBL in undergraduate medical education relate to the difficulty of creating suitable problems and the importance of having effective facilitators [48]. However, these two challenges for PBL can be overcome by faculty member training.

Conclusion

Based on the survey of the students, PBL was found to be effective in developing and enhancing general skills in students. The quality of learning and teaching by Problem based learning curriculum is better than that of the classical curriculum. The study indicated that 150 students (69.71%) chose a problem based learning curriculum as a favored curriculum, while 65 students (30.2%) chose classical curriculum as a favored curriculum. In addition, the overall attribute scores were significantly higher ($P<0.001$) in student groups who chose problem based learning curriculum (208 students) as compared to the students who chose classical curriculum as a favored curriculum (189 students).

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Investigation of the effect of atorvastatin (Avas) on oxidative stress marker, lipids, Atherogenic indices and liver function enzyme in serum of Iraqi postmenopausal women

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ABSTRACT

Background: Menopause is the time in a woman's life when her periods (menstruation) eventually stop and the body goes through changes that no longer allow her to get pregnant. It is a natural event that normally occurs in women aged 45 - 55. Changes in estrogen and progesterone hormones cause menopause symptoms. The hormonal changes associated with menopause play an important role in most cardiac related disorders associated with menopause.

Objective: This study was designed to evaluate the utility of Atorvastatin (Avas) (20 mg) as a part of the program for prevention measures against cardiovascular disease for Iraqi postmenopausal women who suffer increased oxidative stress and lipid and lipoprotein levels as a natural result for estrogen deficiency.

Methods: This study included 50 samples of sera of Iraqi premenopausal women with an average age 25-45 years, in (follicular phase) between day 3 and 5 after menstruation as a control group and 50 samples of sera of Iraqi postmenopausal women with an average age of 46-52 years who did not take any hormonal replacement therapy.

The postmenopausal women were given Atorvastatin 20 mg per day at bed time for 12 weeks and followed up its effect on oxidative stress marker malondialdehyde (MDA), lipid and lipoprotein levels, Atherogenic indices and safety indicators (liver function enzymes) every four weeks and the results were compared for the purpose of determining the effectiveness of Atorvastatin on these variables.

Results: This study demonstrated that Atorvastatin (Avas) (20 mg) for 12 weeks were effective in lowering of MDA, TC, LDLC, TG and Atherogenic indices such as Cardiac Risk Ratio, Atherogenic Coefficient, Atherogenic Index of Plasma (CRR, AC, AIP) and increased HDL in Iraqi postmenopausal women, also Atorvastatin did well on safety indicators.

Conclusion: That it is possible to propose Atorvastatin as a therapy for primary prevention of cardiovascular diseases that postmenopausal women can be exposed to.

Key words: Atorvastatin (Avas), postmenopausal, oxidative stress, malondialdehyde Atherogenic indices, lipids, liver function enzyme

Menopause is a time in a woman's life when her periods (menstruation) eventually stop and the body goes through changes that no longer allow her to get pregnant. It is a natural event that normally occurs in women aged 45 - 55. Changes in estrogen and progesterone hormones cause menopause symptoms (1).

Estrogen plays a role in the increased production of neurotrophic growth factors, which modulate neuronal growth survival and aging. Menopause is a natural step in the process of aging. Free oxygen radicals have been proposed as important causative agents of aging. Aging increases because of free radical damage. Hence menopausal women develop oxidative stress (OS) because of estrogen deficiency and advancing age, accompanied by age related changes (2).

The accumulation of fat in intra-abdominal depot is more common in postmenopausal women than their premenopausal counterparts and hence postmenopausal subjects have a greater risk of developing metabolic complications such as type 2 diabetes, hypertension, atherosclerosis and coronary artery disease (CAD) as well as obesity-related cancers(3).

The effect of the hormonal changes associated with menopause on the serum lipid levels play an important role in most cardiac related disorders associated with menopause (4). This increased risk may be associated with alterations in the lipid profile characterized by changes in low density lipoprotein particle size and buoyancy (5). Low-density lipoprotein has been implicated in the development of coronary heart disease(CHD) and has been observed to be increased in postmenopausal women until they become similar to the corresponding rates in men of similar age (6) . This has been attributed in part to adverse changes in plasma lipids and lipoprotein levels due to reduced estrogen levels (7).

Postmenopausal women commonly show elevated plasma levels of LDL-C and total cholesterol (TC), and moderately increased triglycerides (TG), whereas high-density lipoprotein-cholesterol (HDL-C) decreased moderately (8). Clinical studies have demonstrated that lowering LDL-C significantly decreases coronary events in patients with menopause (9)

Dobiasova and Frohlich proposed the term Atherogenic index of Plasma (AIP) defined as log (TG/HDL-C), on the basis that people with high AIP have a higher risk for CHD than those with low AIP, that AIP is positively correlated with the fractional esterification rate of HDL(FERHDL), and that AIP is inversely correlated with LDL particle size. Because FERHDL predicts particle size in HDL and LDL, which in turn predicts CHD risk, the simultaneous use of TGs and HDL-C (both readily available in a plasma lipoprotein profile) as AIP may be useful in predicting plasma atherogenicity(10).

Expert guidelines recommend treating hypercholesterolemia in postmenopausal women as a part of the coronary prevention strategy. The adherence to a step I cholesterol-lowering diet is the first-choice therapy for dyslipidaemia management, which applies for postmenopausal women, but such measures alone are often not enough to reach a desirable control. Then, cholesterol-lowering drugs should be indicated for these patients; Hydroxy-3- methyl- glutaryl Coenzyme A reductase (HMGCoA reductase ; EC 1.1.1.88) inhibitors being a first-choice alternative for lowering serum LDL-C in postmenopausal women (11).

Atorvastatin is a statin that, across its dosage range (10 - 80 mg/d), induces reductions of serum LDL-C. It has been shown as effective and safe for treating hypercholesterolemia in postmenopausal women (12).

As with other statins, atorvastatin is a competitive inhibitor of HMG-CoA reductase. Unlike most others, however, it is a completely synthetic compound. HMG-CoA reductase catalyzes the reduction of (HMG-CoA) to mevalonate, which is the rate-limiting step in hepatic cholesterol biosynthesis. Inhibition of the enzyme decreases de novo cholesterol synthesis, increasing expression of low-density lipoprotein receptors (LDL receptors) on hepatocytes. This increases LDL uptake by the hepatocytes, decreasing the amount of LDL-cholesterol in the blood. Like other statins, atorvastatin also reduces blood levels of triglycerides and slightly increases levels of HDL-cholesterol (13).

The objective of this study was to assess the effect of atorvastatin (Avas) which was manufactured by (micro LABS LIMITED) INDIA at 20mg/day for 12 week on the serum of oxidative stress marker malondialdehyde (MDA), lipids, lipoprotein levels, atherogenic indices, and on safety indicators such as transaminase enzymes and alkaline phosphates enzyme in Iraqi postmenopausal women with dyslipidemia.

Subjects

Blood samples were collected from coronary center unit / Baghdad teaching hospital and classified into two groups as the following :

A. Fifty postmenopausal women (age 46-52 years) at least one year of persistent amenorrhea with documented dyslipidemia (abnormalities in blood lipid levels). None of the postmenopausal women were taking, or had ever taken, hormone replacement therapy. Women who had undergone surgical menopause (oophorectomy) were excluded from the study.

B. Fifty apparently healthy premenopausal women (age 25-45) years in (follicular phase) between 3rd and 5th day after menstruation, served as control subjects.

Design of study

Design of this study included the following:

- Estimate the levels of MDA, lipids, lipoproteins, atherogenic indices, transaminase enzymes ALT & AST, and alkaline phosphates enzyme ALP in sera of groups A and B before treatment.

- A group take medication of Atorvastatin (Avas) (20 mg) once a day at bed time for 12 weeks.
- Measure the level of MDA, lipoproteins, account indicators of atherosclerosis and measure the effectiveness of the above mentioned enzymes every four weeks from the time period specified for use of Atorvastatin.
- Compare test results and determine the effect of Atorvastatin(Avas) at the level of the measured variables to the time interval to be used.

Laboratory analysis

Blood samples were drawn after a 12 hour fast and aliquots taken from the postmenopausal & premenopausal (especially between 3rd and 5th day after menstruation (follicular phase) women for laboratory determinations. The serum was separated within 30 minutes and stored at -20° C until analyzed. Luteinizing hormone (LH), Follicle stimulating hormone (FSH) and estradiol (E_2) are an automated quantitative test for use on the VIDAS instruments for the quantitative measurement of LH, FSH and E_2 in human serum using the ELFA technique (Enzyme linked fluorescent assay) (14). Oxidative stress marker (MDA) was estimated according to the method described by (Fong et.al 1973) (15).

Serum total cholesterol (TC), HDL- cholestero l(HDLC) and triglyceride (TG) were assayed enzymatically with commercial test kits (Biolabo SA, France) (16-18) serum LDL-cholesterol was calculated using the Friedewald equation (19) as follows :

LDLC= TC-HDLC-TG/2.2

VLDLC=TG/2.2

The Atherogenic indices were calculated as follows:

Cardiac Risk Ratio (CRR)=TC/HDLC

Atherogenic Coefficient (AC)=(TC-HDLC)/HDLC

Atherogenic Index of Plasma (AIP)=log(TG/HDLC) (20)

Other laboratory safety indicators Alanine aminotransferase (ALT;EC 2.7.6.1), Aspartate aminotransferase (AST;EC 2.6.1.1) and Alkalin phosphatase (ALP;EC 3.1.3.1) were assessed through routine enzymatic methods and reagent kits from (Biomerieus, SA, France and Biolabo SA, France) (21-22) respectively.

Statistical Analysis

Data presented were the means and standard deviation Student's t-test was used to compare the significance of the difference in the mean values of any two groups; P value equal to or less than 0.05 was considered statistically significant.

The overall predictive values for the results in all studied groups were performed according to program of office XP 2007.

Results

Table 1 (opposite page) showed the levels of LH, FSH, and E_2 in sera of post and pre menopausal women groups (Group A) and (Group B) respectively. A significant increase of LH and FSH levels in sera of group A compared to group B (17.675 ± 6.01), (6.29±5.18), (21.79±4.32), (8.20±11.09)ng/ml respectively while there was a significant decrease in E_2 level in sera of group A compared to group B.

The Results are presented in Table 2 which shows the mean SD of MDA, total cholesterol and its sub-fractions and atherogenic indices (CRR, AC, AIP) for groups A & B. There was a statistically significant increase ($P \leq 0.05$) in MDA, TC, TG, LDLC and VLDL, CRR, AC, AIP and a statistically significant decrease ($P \leq 0.05$) in HDLC in sera of group A compared with group B.

In the present study, we estimated the serum level of ALT, AST, and ALP levels in post menopausal and pre menopausal women (Table 3). From this study we found that, the activity of AST was elevated significantly in post menopausal women as compared to pre menopausal women ($P \leq 0.05$), but, the activity of ALT was increased non significantly in post menopausal women.

Table 4 (page 52) shows the effect of medication atorvastatin on Mean SD of MDA, lipid profile and safety indicators in sera of postmenopausal women for 12 weeks.

Groups	No. of samples	LH ng/mL mean±SD	P	FSH ng/mL mean±SD	P	E_2 ng/mL mean±SD	P
A	50	17.675±6.01		21.79±4.32		33.25±6.65	
B	50	6.29± 5.18		8.20±11.09		138± 5.79	
			P≤0.05		P≤0.05		P≤0.05

Table 1: Levels of LH , FSH and E2 in sera of post & pre menopausal women

	NO. of samples	GROUP A	GROUP B	P
MDA(nmol/dl)	50	20.5± 5.9	15.12±3.6	P≤0.05
TC(mmol/L) mean±SD	50	7.015±0.20	4.41±0.564	P≤0.05
HDLC(mmol/L) mean±SD	50	0.97±0.186	1.1±0.30	P≤0.05
TG(mmol/L) mean±SD	50	3.01±0.30	1.76±0.36	P≤0.05
LDLC(mmol/L) mean±SD	50	4.68±1.92	2.51±0.31	P≤0.05
VLDL(mmol/L) mean±SD	50	1.36±0.09	0.8±0.28	P≤0.05
CRR mean±SD	50	7.23±2.1	4 ±1.5	P≤0.05
AC mean±SD	50	6.23±2.5	3±0.9	P≤0.05
AIP mean±SD	50	0.49±0.12	0.20±0.08	P≤0.05

Table 2: Oxidative stress marker MDA, lipid profile, Cardiac Risk Ratio, Atherogenic Coefficient and Atherogenic Index of Plasma in sera of post & pre menopausal women groups

	No. of samples	GROUP A	GROUP B	P
ALT(U/L) mean±SD	50	17.1±3.2	17.3±3.5	P>0.05
AST(U/L) mean±SD	50	20.5±7.3	18.3±4.8	P≤0.05
ALP(U/L) mean±SD	50	78.37±22.09	70.72±20.4	P≤0.05

Table 3: ALT , AST, ALP activities in sera of post & pre menopausal women groups

After twelve weeks atorvastatin 20 mg/d significantly (P≤0.05) lowered, MDA (16.3±3.3) ,TC(4.2±1.6), TG(1.7±0.09), LDLC(1.13±0.02), VLDL(0.77±0.001), CRR(1.82±0.03) , AC (0.82±0.2) and AIP (-0.13±0.02), whereas they significantly increased (P≤0.05)HDL (2.3±0.06). Also atorvastatin significantly (P≤0.05) increased safety indicators ALT, AST, ALP(21.8±3.7), (26.4±5.5), (98.1±34.0) respectively but this increase did not exceed the normal range.

Discussion

In females, physiology is complicated by variations in function during the normal menstrual cycle. FSH produces growth and development of ovarian follicles during the first 14 days after the menses. This leads to a gradual increase in oestradiol production from granulose cells, which initially suppresses FSH secretion (negative feedback) but then, above a certain level, stimulates an increase in both the frequency and amplitude of gonadotrophin - releasing hormone , (GnRH) pulses , resulting in a marked increase in LH Secretion (positive feedback). he mid cycle “surge” of LH induced ovulation. After release of the ovum the follicle differentiates into a corpus luteum which secretes progesterone. Withdrawal of progesterone results in menstrual bleeding (23).

The cessation of menstruation (the menopause) occurs; and the hormonal changes associated with menopause e.g low plasma levels of estrogen and marked increase in luteinizing and follicle stimulating hormone levels (24). These hormonal

	NO	Group (A) After 4 weeks	Group (A) After 8 weeks	Group (A) After 12 weeks
MDA(nmol/dl)	50	18.6±3.2	17.2±2.9	16.3±3.3
TC (mmol/L) mean±SD	50	6.2±2.1	5.6±1.8	4.2±1.6
HDL (mmol/L) mean±SD	50	1.2±0.03	1.9±0.08	2.3±0.06
TG (mmol/L) mean±SD	50	2.8±0.09	2.0±0.06	1.7±0.09
LDLC (mmol/L) mean±SD	50	3.73±0.06	2.8±0.04	1.13±0.02
VLDL (mmol/L) mean±SD	50	1.27±0.01	0.90±0.002	0.77±0.001
CRR (mmol/L) mean±SD	50	5.16±0.1	2.94±0.02	1.82±0.03
AC (mmol/L) mean±SD	50	4.16±1.3	1.94±0.01	0.82±0.2
AIP (mmol/L) mean±SD	50	0.36±0.02	0.022±0.0001	-0.13±0.02
ALT(U/L) Mean±SD	50	18.8±2.8	19.5±3.3	21.8±3.7
AST(U/L) Mean±SD	50	21.2±5.8	25.2±6.8	26.4±5.5
ALP(U/L) Mean±SD	50	86.6±8.6	90.4±30.7	98.1±34.0

Table 4: Effects of Atorvastatin (20mg/d) on lipid profile and safety indicators for 12 weeks

changes associated with menopause affect on the metabolism of serum lipids and lipoproteins levels and play an important role in most cardiac related disorders associated with menopause(4).

The deficiency of estrogen in postmenopausal women develops oxidative stress, due to release of free radical or reactive oxygen species (ROS) and becomes the cause of various pathologies like development of hypertension (25).

The results in this study are similar to reports from Nigeria and other parts of the world (26-27) and also agree with findings of (Berg et.al 2004) (28) who demonstrated higher TC, HDLC and TG in postmenopausal women in comparison with premenopausal women.

Data on HDLC have been consistent with studies and have shown that menopause is associated with low HDLC level and inconsistent with other studies that suggest that HDLC is unaffected (29).

Alterations in lipid profile have also been associated with age. The TC, TG, LDL-C and atherogenic index were significantly higher and HDL-C lower in women above 45 years when compared to those of women aged between 25-45 years. Increasing age has been associated with higher plasma LDL-C and Apo B levels in women, where significantly higher LDL-C and Apo B levels were observed in postmenopausal women than in premenopausal women (30).

Haarbo et al. (1990) (31) also observed high total cholesterol, LDL-C and VLDL-C as well as triglycerides levels with increasing age.

The elevated TC, LDL-C and atherogenic index in postmenopausal women and women greater than 45 years has been attributed to hormonal changes and failure of follicular development, whereas the plasma estradiol levels that reduces the risk of coronary heart disease falls below the levels seen in premenopausal women (32).

The lower LDL-C levels of the premenopausal women and women between 25 and 45 years in this study could be explained by the increased HDL-C which scavenges cholesterol esters, reducing its availability for LDL-C formation. A lower atherogenic index indicates a greater proportion of HDL-C, and is a measure of risk for coronary heart disease. Thus premenopausal women and women between the age ranges of 25 to 45 years used in this study satisfy the criteria for reduced risk of coronary heart disease by the revised guidelines of American National Cholesterol Education Programme (33).

Increased atherogenic indexes after menopause have been reported by (Pascot et al., 1999) (34). These results are compatible with the results of this study.

Atherogenic index of plasma which is a mathematical relationship between TG and HDL-C has been successfully used as an additional index when assessing cardiovascular (CV) risk factors(35). Indeed, it has been suggested that AIP values of -0.3 to 0.1 are associated with low, 0.1 to 0.24 with medium and above 0.24 with high CV risk (36).

Atherogenic indices (CRR, AC, AIP) are powerful indicators of the risk of heart disease: the higher the value, the higher the risk of developing cardiovascular disease and vice versa(37).

Outcomes of this study are in agreement with Sucheth akumari ,et al (2010) (38) who demonstrated the activity of AST was elevated significantly in post menopausal women as compared to pre menopausal women, but, the activity of ALT was increased non significantly in post menopausal women.

ALT is the enzyme produced within the cells of the liver. The level of ALT abnormality is increased in conditions where cells of the liver have been inflamed or undergone cell death. As the cells are damaged, the ALT leaks into the bloodstream leading to a rise in the serum levels. Any form of hepatic cell damage can result in an elevation in the ALT. AST also reflects damage to the hepatic cell. AST level was elevated in liver and heart diseases. This indicates that postmenopausal women are more prone to liver damage and exhibit altered liver function, as the age advances.

Serum alkaline phosphatase is the most commonly used marker of bone formation. ALP is a ubiquitous enzyme that plays an important role in osteoid formation and mineralization. The total ALP serum pool consists of several dimeric forms which originate from various tissues such as liver, bone, intestine, spleen, kidney and placenta. In adults with normal liver function, approximately 50% of the total ALP activity in serum is derived from the liver, whereas 50% arises from bone(39).

In our study it was observed that Serum ALP levels were significantly increased in postmenopausal women compared to premenopausal women($P \leq 0.05$). This shows that the bone mass continues to decline with age (40). Annual change in ALP indicates that bone resorption prevails on bone formation in the early postmenopausal period (41).

Atorvastatin showed a significant reduction in the MDA level. This result agrees with a previous study by Koter M. et al(42). The antioxidant mediated effect of atorvastatin results from inhibition of mevalonate pathway leading to the reduction in the synthesis of important intermediates including isoprenoids (farnesyl pyrophosphate & geranylgeranyl pyrophosphate) which serve as lipid attachments for intracellular signaling molecules in particular inhibition of small GTPase binding proteins (Rho, Rac, Ras and G proteins) whose proper membrane localization and function are dependent on isoprenylation. These proteins modulate a variety of cellular processes including signaling, differentiation and proliferation. (43-44).

Atorvastatin attenuates endothelial ROS formation through attenuating endothelial superoxide anion production by inhibition of NADPH oxidase activity via a Rho protein dependent mechanism. Some of the antioxidant effects of atorvastatin may be due to its metabolites such as hydroxyl metabolites which have direct antioxidant effect. Atorvastatin improves and preserves the level of vitamin C, E and endogenous antioxidants such as reduced glutathione.(45)

Also the findings of this study are compatible with a previous study which showed that atrovastatin at 10mg/d for eight weeks, favourably changed the lipid profile in postmenopausal hypercholesterolemic women and was well tolerated (46).

This study demonstrated that atorvastatin (Avas) (20 mg) was manufactured by (Micro Labs Limited) India for 12 weeks was effective in lowering MDA, TC, LDLC, TG and Atherogenic indices (CRR,AC,AIP),and increased HDL in Iraqi postmenopausal women. Also atorvastatin fared well on safety indicators so that it is possible to propose atorvastatin as therapy for primary prevention of cardiovascular diseases that can be exposed to postmenopausal women.

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