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The Concept of Evidence-Based Medicine: How Far is My Practice from the Standard?

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Abstract

Clinical judgment and long experiences, reading textbooks and scientific journals, in addition to conferences and personal beliefs are generally sources for physician management plan, however; errors in clinical reasoning and gaps in evidence were described since long time. For which evidence based strategy had been applied in medical practice aiming to improve the decision-making skills of individual physicians to individual patients. Evidence base medicine (EBM) is a systematic approach to clinical problem solving which make use of the best research evidence in term of clinical expertise and patient values.

Keywords Evidence-based medicine
DOI: 10.22578/IJMS.14.4.1

List of abbreviation: EBS = Evidence-based medicine

Physicians are treating their patients according to the clinical judgment and long experiences that were gained from clinical practices, reading textbooks and scientific journals, attending conferences and personal beliefs. It is not unusual to rely on the experience of older and wiser colleagues as well. During the busy daily practice; physicians in most of the time have no opportunity to ask if there is another and a better option for this particular patient. Is there a better medication than the one that I gave? Is there a better surgical technique than the one that I used to perform? Why there are different strategies of management between different clinics and hospitals for the same medical problem? It is very often that we see differences in management between the colleagues of the same department. Each physician (according to his / her clinical judgment) thinks that he or she is providing a good service to the patients.

In the second half of the last century, a lot of work and publications focused attention on the role of clinical judgment and identified biases that can affect it [(1)]. In 1973, the existence of wide variations in how physicians practiced was documented [(2)]. The errors in clinical reasoning and gaps in evidence were described clearly in the 1980s [(3-6)]. These publications increased awareness of the weaknesses in medical decision making and paved the way for the introduction of evidence-based methods.

The term "evidence-based" was first used by David M. Eddy in a work shop in 1987 and published in 1990 [(7)]. Gordon Guyatt is the first one who used the term “Evidence-Based Medicine (EBM)” in an unpublished description of a program at McMaster University for prospective or new medical students [(8)]. Guyatt and others first published the term two years later (1992) to describe a new approach to teaching the practice of medicine [(9)]. In 1996,

David Sackett clarified the definition of evidence-based medicine as "the conscientious, explicit and judicious use of current best evidence in making decisions about the care of individual patients" [(10)]. Every part of this sentence is important. Consider the words themselves:

Conscientious: being careful and thorough in what you do.

Explicit: being open, clear and transparent.

Judicious: using good judgment and common sense.

According to this definition; we are looking for current best evidence, which is not perfect but simply, the best available one. It should not be old or out-of-date evidence; we need to find modern, up-to-date current evidence.

If you are going to practice in this way, you have to be able to find evidence from scientific studies that are relevant to your patients. You then have to understand those studies and be able to appraise them (not all studies will be relevant to your patient and even if they are, they may not be good studies). And finally, you have to apply those results when making decisions about your patient. This means being able to integrate the evidence with your patients' personal needs, their values and beliefs and their wishes.

There are five key steps in EBM that summarize how it works with this concept:

Asking the right question: Converting the need for information (about prevention, diagnosis, prognosis, therapy, causation…etc) into an answerable question.

Accessing and searching for the evidence: Tracking down the best available evidence with which to answer that question.

Appraising the evidence: Clinically appraising the evidence for its validity (closeness to the truth), impact (size of the effect) and applicability (usefulness in clinical practice).

Acting on the evidence: Integrating the clinical appraisal with your clinical expertise and with the patient's unique circumstances.

Assessing your practice: Evaluating the effectiveness and efficiency of the mentioned 1-4 steps and seeking ways to improve them for the next time.

Finally, there is a hierarchy for the quality of evidence. This is presented in a pyramid below: [(11)]. In developing countries like Iraq, the introduction of any advanced approach in the education and practice of medicine needs a lot of efforts and time. There are few Arab countries that succeeded to introduce EBM concepts into their medical curricula. EBM helps clinicians adopt interventions that are more likely to benefit their patients and, like other developing countries, Iraq faces many problems, including an old infrastructure and buildings, modest healthcare system; fragmented healthcare providers; interventions supported by weak evidence; and inconsistent quality of care. Multiple wars and unstable political situation since the 1980s, has further aggravated the situation, adding misery to the already deplorable situation. However, such challenges cannot be used as an excuse for failing to promote the use of reliable evidence to inform decisions in health care. Our strong belief is that in Iraq, we need practices based on the best available evidence for the same reasons that EBM is needed elsewhere.

Initial steps should be taken to attract professionals with an interest in EBM. This included conducting a series of lectures and workshops to medical students and practicing doctors. Establishing an EBM unit is mandatory to help organizing such workshops and follow the outcomes.

Take home message of this editorial

To improve the decision-making skills of individual physicians to individual patients, an approach called evidence-based medicine has been practiced. This approach developed with time and its current improved definition is that: "EBM is a systematic approach to clinical problem solving which allows the integration of the best available research evidence with clinical expertise and patient values" [(10)].

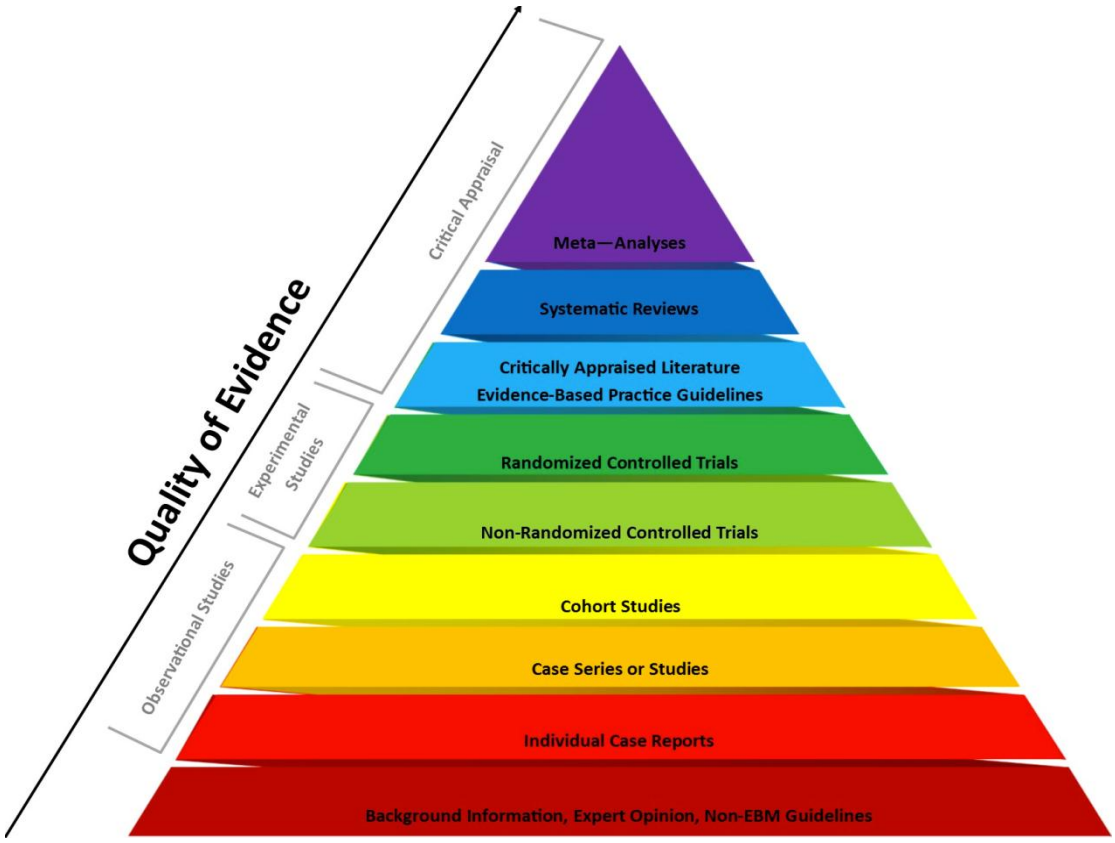

Figure 1. Evidence Base Pyramid

It must be kept in mind that external clinical evidence can inform but not replace individual clinical expertise. This is a lifelong and self-directed problem-based learning approach.

Because of the growth of scientific and medical knowledge, a doctor must remain up-to-date with the latest articles and researches, with a critical eye to be able to evaluate. Nevertheless time is a limit and a doctor is not able to read every research, so researches must be chosen selectively and effectively [(12)].

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Peroperative Factors which Achieve Successful Patellar Tracking in Primary Total Knee Replacement

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Abstract

Background Primary total knee arthroplasty (TKA) is technically demanding surgery, which if it is done perfectly can give excellent patient satisfaction and pain relief. Still abnormal patellar tracking is the most common complication of primary TKA (24%) and it is the most common cause of revision.

Objective To emphasize on the effectiveness of adherence to certain surgical steps (peroperatively) and to which extent these steps can reduce the incidence of peroperative abnormal patellar tracking in primary TKA.

Methods A prospective descriptive study was conducted in Al-Imamein Al-Kadhimein Medical City from May 2011 to December 2014; it involves 67 patients who underwent unilateral total knee replacement for different pathology and variable knee deformity. Step wise surgical procedure for primary TKA insisting on certain technique and tricks to achieve perfect TKA and finally checking the patellar tracking preoperatively using no thumb test and towel clips test.

Results NEXGENR posterior cruciate substitution design used in 62 patients (92.5%) and condylar constrained knee in 5 patients (7.5%) and abnormal patellar tracking occurred preoperatively only in 1 patient (1.4%).

Conclusion Strict adherence to surgical procedures keeping in mind certain factors, which should be fulfilled is essential for successful peroperative patellar tracking, while postoperative assessment required different investigation like computed tomography or dynamic radiographs to ensure this good patellar tracking and improving the outcome of primary TKA in our country.

Keywords primary TKA, peroperative patellar tracking

DOI: 10.22578/IJMS.14.4.2

List of abbreviation: TKA = Total knee arthroplasty, PS = Posterior substitution, CCK = condylar constrained knee, LPS = Legacy posterior substitution

Introduction

Primary total knee arthroplasty (TKA) is a technically challenging procedure that if performed with reasonable skill, can provide significant pain relief and patient satisfaction. The procedure is commonly done in most of specialized hospitals in Iraq in the last 4 years. The most frequent complications in primary total knee arthroplasty involve abnormal patellar tracking [1-6], up to 24% and most common cause of revision 50% [7].

Optimizing patellar tracking in TKA requires attention to several peroperative factors, which include:

- Mechanical alignment of the femur and tibia.
- Femoral component rotation and sizing.
- Tibial component rotation.
- Femoral component coronal positioning.
- Tibial component coronal positioning.
- Patellar component positioning.
- Patellar height in Patellar resurfacing.
- Malposition of the tibial component in an internally rotated position increases the Q angle by moving the tibial tubercle laterally. The increased Q angle leads to lateral

subluxation. The tibial component should be centered on the medial border of the tibial tubercle, with any deviation into slight external rotation. Similarly, internal rotation and medial translation of the femoral component move the trochlea more medial relative to the extensor mechanism, leading to lateral subluxation [3-5].

The intraoperative evaluation of femoral component rotational alignment is based on anatomical landmarks. The posterior femoral condyles, epicondylar axis, and anteroposterior axis, all are useful in the primary knee arthroplasty setting. In revision arthroplasty, the position of the previous component and the epicondylar axis are usually the only landmarks available for this assessment. Malpositioned patellar, femoral, or tibial components also may lead to patellofemoral instability. Excessive lateral patellar facet resection is possible because of the normal asymmetry of the medial and lateral patellar facets. Often, the level of the lateral facet resection must be much shallower than the medial facet resection to avoid tilting of the patellar component. Lateral placement of the patellar component on the cut surface of the patella fails to reproduce the normal median eminence of the patella and can lead to lateral subluxation of the patella in extension. Studies by Hofmann et al and Lewonowski et al showed a reduction in lateral release rates in patients who had centralized patellar components.

By taking care of all these factors peroperatively and checking the patella tracking peroperatively by no thumb test (putting the knee through full range of motion with patella in its position without any pressure from the lateral side which should remain central with no tilt or shift) and reattachment of the patella to the medial retinaculum by towel clips and check patellar tracking, we can avoid any per operative lateral release of patellar retinaculum and decrease the incidence of patellar necrosis [8].

So the aim of this study was to assess the effectiveness of different surgical factors, which should be done perfectly during surgery to decrease the incidence of patellar maltracking which include (Mechanical alignment, femoral component rotation and sizing, tibial component rotation, femoral component coronal positioning, tibial component coronal positioning, Patellar component positioning and Patellar height in Patellar resurfacing.

Methods

This prospective study was conducted in Orthopedic Unit in Al-Imamein Al-kadimein Medical City from May 2011 to December 2014. It involved 67 patients who underwent unilateral total knee replacement. Their age were between 45 years and 70 years (60 female and 7 male) (Figure 1). The indications for TKA were osteoarthritis in 62 patient and rheumatoid arthritis in 5 patients (Figure 2).

All these knees replaced using the NEXGENR posterior cruciate substitution (PS) high flexed design and condylar constrained knee (CCK) system of the prosthesis.

Inclusion criteria

1. Male or female patient aged between 35-80 years.
2. Patients with osteoarthritis and rheumatoid arthritis.
3. Varus deformity less and more than 15 degree.
4. Valgus deformity less and more than 15 degree.
5. Patients with range of motion more than 80 degrees with or without flexion contracture.
6. All patients have their first total knee replacement.
7.

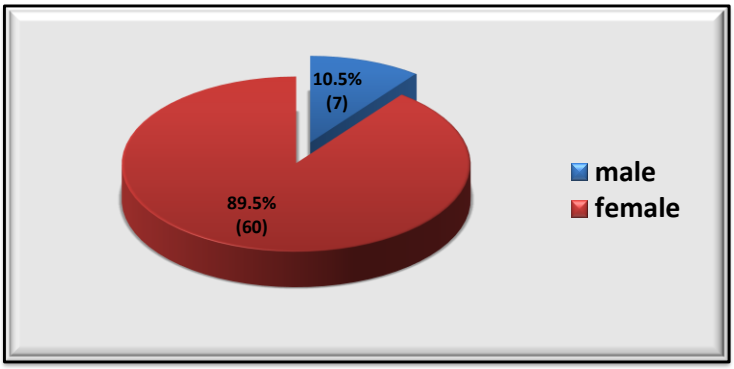

Figure 1. Pie chart shows distribution of the sample according to the gender

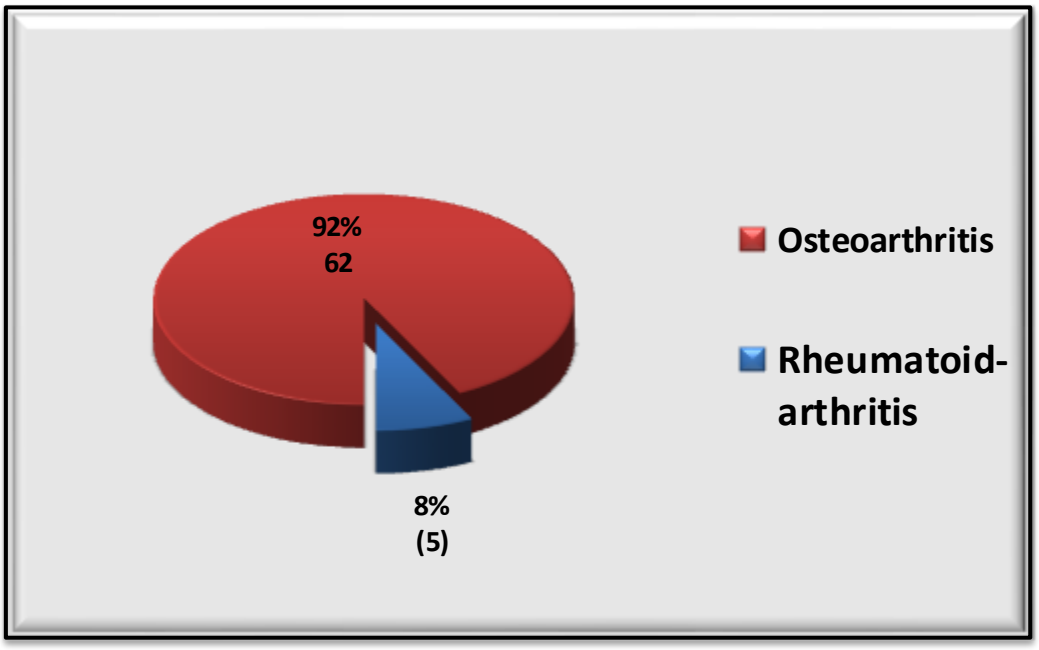

Figure 2. Pie chart chows distribution of the sample according to the underling pathology

Exclusion criteria

1. Patients with previous knee infection.
2. Patients with previous knee trauma or osteotomy around the knee or bony defect more than 5 cm.
3. Previous patellar fracture.
4. Patients with range of motion less than 80 degrees.
5. Patients with neuromuscular or sever traumatic knee instability.
6. Any patient with revision.

Osteoarthritis is the main indication in 62 patients (92%), and rheumatoid arthritis in 5 patient (8%).

The left knee replaced in 35 patient (52%) and 32 (48%) right knee (Figure 3).

The deformity was varus in 58 (86.5%), 12 of them had sever deformity (more than 15 degree), and valgus in 8 (11.9%), 3 of them had severe deformity (more than 15 degree) and recarvatum in one patient (1.5%). Six patients

had flexion contracture more than 30 degree all are with sever varus deformity, and the others were easy primary total knee replacement (varus and valgus deformity less than 15 degree, range of motion more than 110, flexion contracture less than 15 degrees, no infection or trauma or knee instability or previous osteotomy (Figure 4) (Table 1).

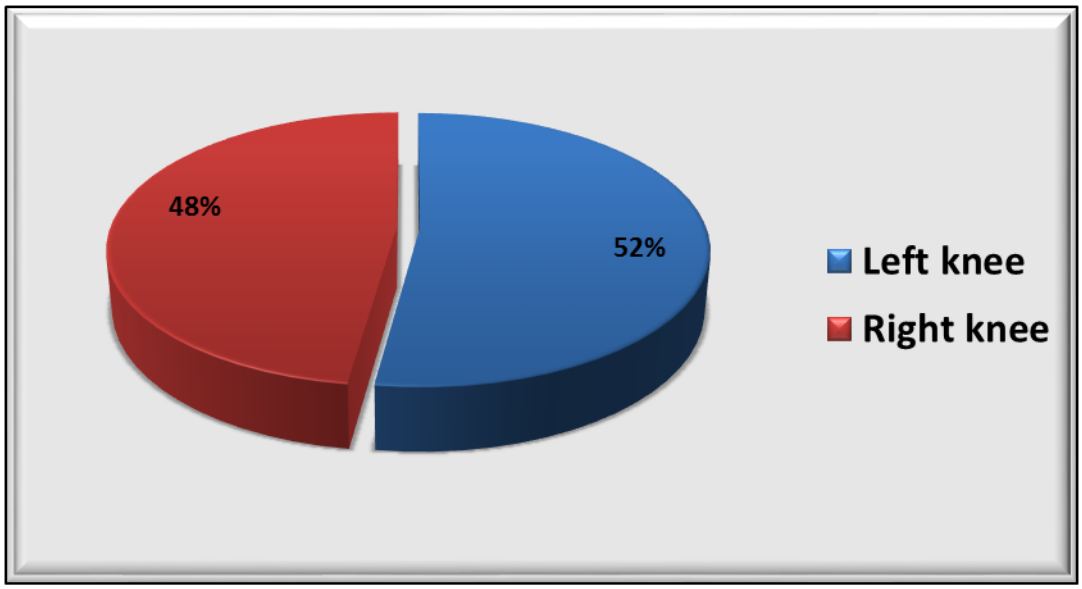

Figure 3. Pie chart shows distribution of sample according to the side affected

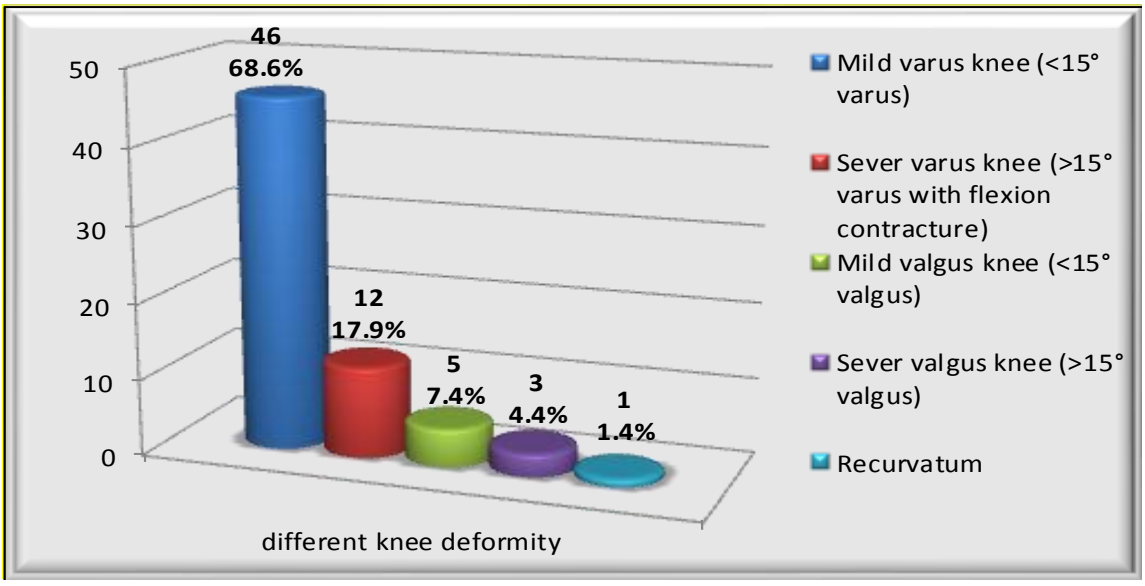

Figure 4. Bar chart show distribution of patients according different knee deformity

Table 1. Frequency distribution of the patients according to the different knee deformity (n=67)

Different knee deformity	Patients	
	NO.	%
Mild varus knee (<15° varus)	46	68.6
Sever varus knee (>15° varus with flexion contracture)	12	17.9
Mild valgus knee (<15° valgus)	5	7.4
Sever valgus knee (>15° valgus)	3	4.3
Recurvatum	1	1.4
Total	67	100

Legacy posterior substitution (LPS) posterior cruciate substitution was used in 62 (92.5%) and CCK condylar constrained knee in 5 (7.5%) cruciate retaining was not used in any patient.

All the patients prepared well preoperatively regarding medical fitness and proper examination of the limb and the knee.

Long leg standing radiograph of lower limb was done with measurement of anatomical mechanical axis, lateral distal femoral axis, medial proximal tibial angle; varus or valgus angle.

All patients approached through anteromedial line skin incision and medial parapatellar incision. With the use of leg holder, and the use of pneumatic tourniquet is optional during the procedure.

Surgical procedure

1. proper distal femoral and proximal tibial bone cut fallowed by checking the mechanical axis of the cut from the femoral head to the center of ankle using special device passing through knee spacer.
2. Accurate femoral sizing using special jig provided by the set depending on the superolateral portion of femoral condyle, and lateralization of four in one cutting block.
3. Accurate external rotation of femoral component depending on the epicondylar axis mainly and to lesser extent the whitesite line and post condylar axis. IN most of our patient femoral component was implanted in 3 degree external rotation and few in 5 degree non in 0 degree.
4. Accurate tibial tray implantation by accurate rotation through putting the center of the tibial tray with the junction of lateral 2/3 and med 1/3, and accurate sizing by putting the curve of the tray on the ant curve of the tibial cut and avoiding medial and lateral displacement or over hang.
5. Only 3 patients in our series had patellal resurfacing (two of them with rheumatoid arthritis and one with sever patellar damage and abnormal shape by long standing history of osteoarthritis), in the others patellar denervation by cauterization of surrounding tissue up to 4 mm depth and denution of abnormal osteophyte was done.
6. In between the steps repeated trial to check each step was done using different trial devices.
7. Multiple checks of the patellar tracking by insuring that the patellar tendon covering the middle of tibial tuberosity and the middle of tibial tray.
8. Finally patellar tracking in trial run and after implantation of final implant is checked by NO THUMB TEST {the position of patella is checked through full range of motion from full extension to full flexion with the patella in its position in front of knee and no any pressure applied to lateral side of the patella by thumb the patella should track smoothly with no any lateral shift or subluxation or dislocation or tilt with or without reattachment of the patella to

medial retinaculum by towel clips} and rechecking of this tracking after towel clips application.

9. Proper closure of the arthrotomy and skin over two drains.
10. Final checking of range of motion, stability, alignment, and patellar tracking is done in every case.

Results

LPS posterior cruciate substitution was used in 62 (92.5%) and CCK condylar constrained knee in 5 (7.5%), while cruciate retaining was not used in any patient (Figure 5) (Table 2).

Table 2. Frequency distribution of the patients according to the types of knee prosthesis (n=67)

Different knee deformity	Patients	
	No.	%
LPS	62	92.6
CCK	5	7.4
Total	67	100

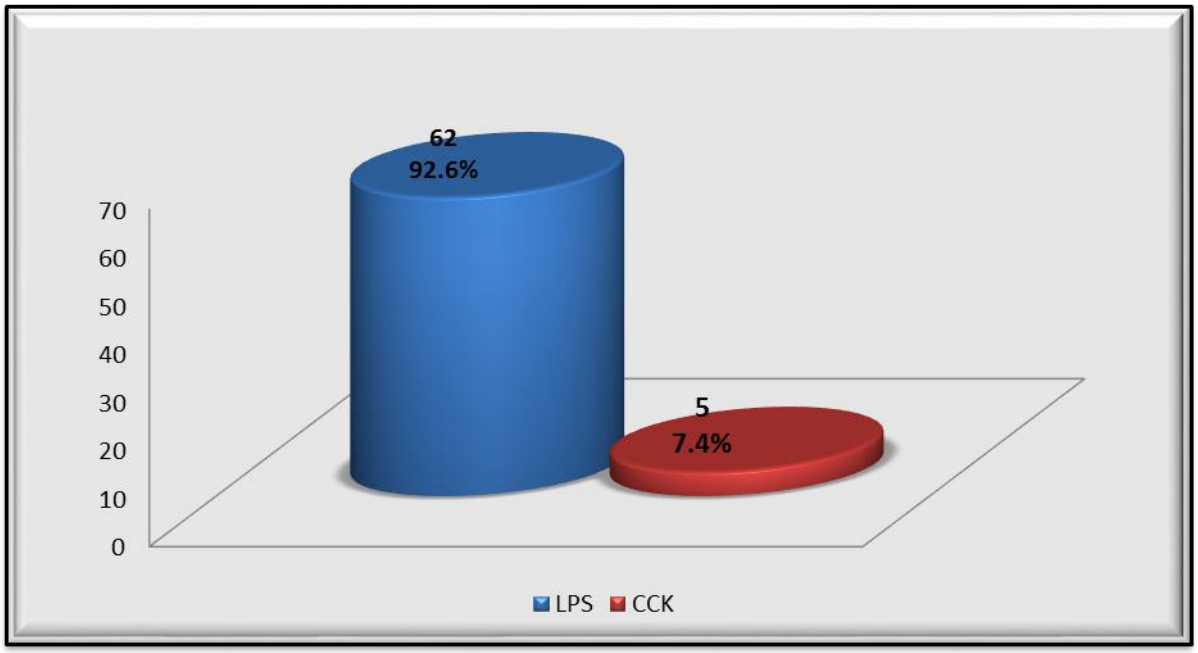

Figure 5. Bar chart shows distribution of patients according to the types of knee prostheses

CCK was used for the following conditions: 2 cases due to severe flexion extension gap mismatch, 2 cases due to sever varus deformity and incompetence of the collateral ligament, 1 case due to recarvatum and severe valgus deformity.

Fallowing the strict surgical steps during each operation of primary TKA result in: abnormal patellar tracking occur in 1 patient (1.4%), which has recurvatum knee and required lateral release, and in 66 patient (98.6%) with different types of prosthesis and different types and degrees of deformity have no abnormal patellar tracking peroperatively as checked by no thumb test and towel clips test (Figure 6).

Discussion

The patella with or without replacement plays essential role in success of TKA and most of patellofemoral complications related to the

patient, surgical technique or the design of the component [9].

Patellar instability after TKA is serious complication, its etiology can be related to the surgical technique and component position, extensor mechanism imbalance [10], Which was insisted on in this study.

The presence of anterior knee pain, especially during stressful activity is indication of patellar instability [10].

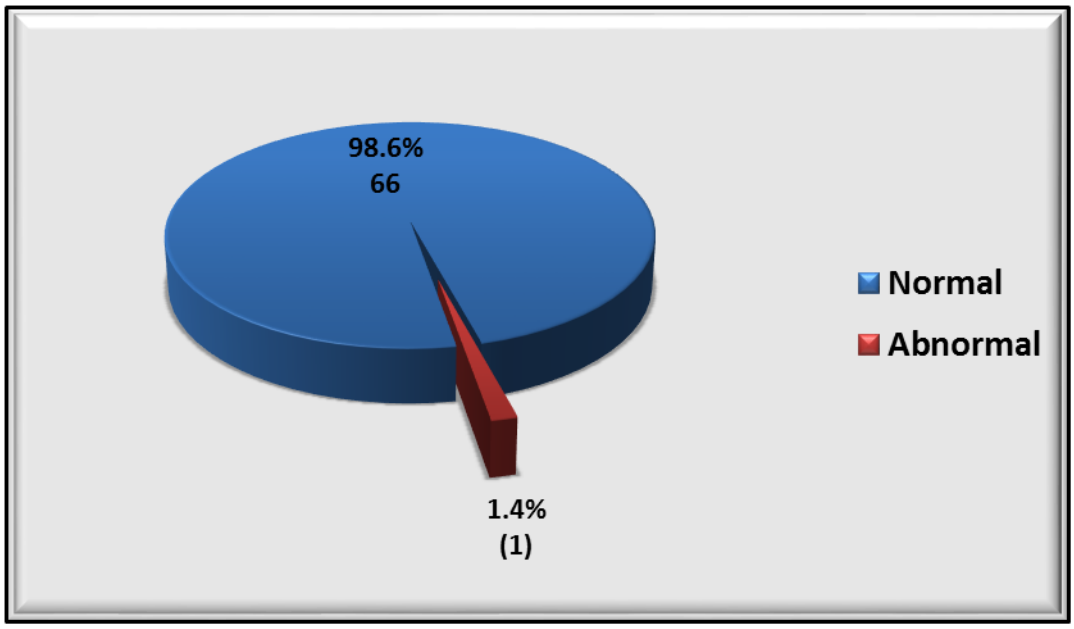

Figure 6. Pie chart shows distribution of the sample according to the patellar tracking

Assessing intraoperative tracking during TKA is important to avoid postoperative patellar complication, no thumb test, the towel clips test and vertical patellar test, which is novel technique to assess lateral retinaculum release [11], the use of no thumb test and reevaluation with towel clips test may reduce unnecessary lateral retinaculum release [12] and so in this study no thumb test and towel clips test were used as the most dependable peroperative tests to assess patellar tracking.

Patellofemoral instability result from component malposition and limb malalignment, prosthetic design, improper patellar preparation and soft tissue imbalance; nonsurgical treatment is generally unsuccessful [13].

Although patellofemoral radiograph may display lateral subluxation, only computed tomography can quantify rotational malalignment of femoral and tibial components [13].

Patellar instability may occur after TKA with or without patellar resurfacing, subluxation is more common than dislocation the incidence of symptomatic instability leading to revision is (0.5%- 0.8%) [14,15]. In this study, the incidence of patellar instability is (1.4%), the case is recarvatum knee complicated by negative proximal tibial slope which lead to abnormal extensor mechanism.

Anglin et al [16] stated that the major determinant of patellar tilt and shift were patellar component medicalization, patellar resection angle and femoral component rotation, neither tibial component rotation nor patellar thickness should be adjusted to improve patellar tracking [16]. However, it was insisted that accurate positioning of all component, mechanical limb alignment accurate bone cuts, and soft tissue balancing

lead to decrease abnormal patellar tracking in TKA and reduce the postoperative knee pain and the need for revision.

This study concluded that strict adherence to every step in the surgical procedure and accepting nothing less than perfect in every step whether on the femur or tibia or patella and whole alignment and soft tissue balancing, can achieve perfect patellar tracking peroperatively and reduce the need of lateral retinaculum release, which by itself increase post-operative complication.

This study recommend further studies to assess the importance of this perfect patellar tracking during surgery and its effectiveness in reducing the postoperative patellofemoral complication and by then reducing the incidence of revision. Also it is recommended that next studies fallow the patients for several months to assess how peroperative patellar tracking affect post-operative patellofemoral and knee function clinically and with the use of post-operative knee computed tomography scan and special dynamic radiological views to assess patellar tracking.

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Conflict of interest

The author declares no conflict of interest.

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Clinical Characteristics and Outcomes of Acute Coronary Syndromes in a Group of Iraqi Patients

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Abstract

Background Coronary artery disease (CAD) is one of the most common diseases in the world. Acute coronary syndromes (ACS) represent the acute life-threatening phase of CAD. Epidemiology and management of ACS patients differ a lot between countries and there is a wide gap between guidelines and clinical practice.

Objective To assess contemporary data on clinical characteristics and outcomes of patients with ACS in the Medical City Complex and to evaluate adherence to the guidelines' recommended treatment.

Methods This is a descriptive study registry, started on January 2014 to June 2014, and involved 348 patients with ACS in the Medical City Complex.

Results The mean age of this study's population was (60.3±11.2 years), ranging between 29 to 90 years old. Most of patients were males (61%). Only 233 (67%) patients have typical angina. Symptom onset-to-admission time was delayed (≥ 12 hours) in 65% of patients. The final diagnosis was: ST elevation myocardial infarction (STEMI) in 126 (36.2%), non ST elevation myocardial infarction (NSTEMI) in 40 (11.4%), and unstable angina (UA) in 182 (52.3%) of patients. Electrocardiography was normal in 29% of patients with UA and 10% of patients with NSTEMI. Anterior territory was the most common location of ischemia (77%). Hypertension (47.9%) was the most significant risk factor followed by diabetes mellitus (41.6%) and smoking (31.8%). Reperfusion therapy for patients with STEMI was applied in 73(57%) patients: 56 (44.4%) by thrombolytic therapy, 24 (19%) by percutaneous coronary intervention (PCI) and 3 (2.3%) by emergency CABG. Overall in-hospital mortality was 7.7% (15%, 7.5% and 2.7% for STEMI, NSTEMI, and UA respectively).

Conclusion There is lack of awareness of ischemic symptoms among our patients. There is high incidence of risk factors that can be modified by primary and secondary measures. There is underutilization of invasive management. We have a high mortality rates in patients with ACS.

Keywords Acute coronary syndromes, registry, in-hospital outcome

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List of abbreviation: CAD = Coronary artery disease, ACS = Acute coronary syndromes, STEMI = ST-elevation myocardial infarction, NSTEMI = Non-ST-elevation myocardial infarction, UA = unstable angina, ECG = Electrocardiography, NSTE-ACS = Non-ST-elevation acute coronary syndromes, CCU = cardiac care unit, BTH = Baghdad Teaching Hospital, ICHD = Iraqi Center for Heart Diseases, PCI = Percutaneous coronary intervention, CAG = Coronary angiography, CABG = Coronary artery bypass grafting

Introduction

Coronary artery disease (CAD) begins indolently as a fatty streak in the lining of the artery that soon progresses to narrow the coronary arteries and impair myocardial perfusion. It may present as chronic stable angina or acute coronary syndromes (ACS) in which, the atherosclerotic plaque ruptures and causes sudden thrombotic occlusion and acute ST-elevation myocardial infarction (STEMI), non-ST-elevation MI (NSTEMI) or unstable angina (UA). In STEMI, the occlusion is total, myocardial necrosis ensue with elevation of cardiac biomarkers and ST segment in the surface electrocardiography

(ECG). In the other two syndromes, the occlusion is partial or involves a minor vessel, transient or permanent ST depression and or T inversion may occur with or without elevation of cardiac biomarkers in NSTEMI or UA respectively. Because NSTEMI and UA are indistinguishable at initial evaluation, and the entity of UA is receding as the sensitivity of biomarkers of myocardial injury increases, they are often described together as NSTE- ACS [(1)].

Worldwide, the number of CAD deaths increased by 31% between 1990 and 2010. In 2010, CAD accounted for 13.3% of all deaths worldwide [(2)]. Hospitals morbidity data provided by Iraqi Ministry of Health in 2004 demonstrates a 65% increase of the hospital admission due to CAD and stroke and more than a fivefold increase in outpatient visits with the same diagnoses between 1989 and 1999 [(3)]. Iraq is in the top 25 countries in Middle East with age-adjusted CAD mortality rates of about 214 per 100,000 [(4)]. It has been projected that between 1990 and 2020, CAD mortality in the Middle East countries will increase by 146% for women and 174% for men [(5)].

Much of what we understand about risk and likelihood of cardiovascular disease, its incidence and prevalence are derived from 'western' data. However, there is now an increasing awareness of ethnic variations and risk, socio-cultural and socio-economic influences as well as geographical variations. In our country, because we are fighting the consequences of CAD rather than attacking its cause we may never success. We need to adopt a pro-active strategy based upon information and evidence. We initiated a limited ACS registry hoping that it would be a nidus for a national registry.

The aim of the study was to assess contemporary data on clinical presentation, risk factors, management and outcomes of patients with ACS in the Medical City Complex, and to evaluate adherence to the guidelines' recommended treatment.

Methods

The reported study was a descriptive study. It included 348 consecutive patients having ACS admitted to the cardiac care unit (CCU) of Baghdad Teaching Hospital (BTH) and Iraqi Center for Heart Diseases (ICHD) over 6 months from January 2014 to June 2014. BTH is a general hospital with daytime PCI facilities but no primary PCI program (PPCI). ICHD is tertiary center with PPCI facilities. Patients were subjected to full history and clinical examination, ECG, and echocardiography. Presenting symptoms were recorded as typical angina, atypical angina, dyspnea, cardiogenic shock, palpitation and/or cardiac arrest. Cardiac biomarkers were tested by "DIAQUICK" Cardiac Combo Cassette, which is a rapid visual immunoassay for the qualitative detection of human myoglobin, CK-MB, and cardiac troponin I; the test is positive if cTn I is ≥ 1 ng/mL, CK-MB is ≥ 5 ng/mL, and myoglobin is ≥ 50 ng/mL. Quantitative cardiac biomarkers assay was not available. Lipid profile tests were available for 27 patients only and were incomplete and therefore excluded from the study. The initial diagnosis was made by the attending-physician and patients were then classified as having STEMI, NSTEMI, or UA. The diagnosis of different type of ACS and definitions of data variables were based on American College of Cardiology (ACC) clinical data standard [(6)]. Patients were followed for complications and death during hospital stay.

Statistical Analysis

Continuous variables were presented as mean±standard deviation. Categorical variables were described as frequency and percentages. Continuous variables were assessed by t- test and Categorical variables by Chi-square test. The level of statistical significance was set at P value ≤ 0.05.

Results

Initial diagnosis

The study included 348 patients, 219 (63%) admitted to the CCU of BTH and 129 (37%)

admitted to the CCU of ICHD. Of the 348 patients, 126 had STEMI (36.2%), 40 had NSTEMI (11.4%) and 182 had UA (52.3%).

Baseline demographic and clinical characteristics are presented in Table (1) and (2) according to diagnosis and age groups respectively. Mean age of patients was 60.3±11.2 year. The youngest patient in the study was 29 yrs old and the oldest was 90 yrs old. Men constituted 61% of patients. Hypertension (47.9%) was the most significant risk factor followed by diabetes mellitus (41.6%) and smoking (31.8%).

Patients with NSTE–ACS were older than STEMI patients, although statistically not significant. UA and NSTEMI patients were of similar age. Patients with STEMI had lower prevalence of the traditional cardiovascular risk factors: diabetes and hypertension compared with NSTE–ACS patients.

The patients were divided into three categories by age: < 45 years, 45-54 years and ≥ 55 years. Men predominate all age group especially the young. Risk factors differed significantly among the three age groups (table 2). In patients < 45 years old, 57% were smokers and this percentage decreased with increasing age (p = 0.001). Younger patients were more likely to have a family history of CAD (p = 0.001) and were less likely to have diabetes (P value NS) or hypertension (P = 0.022).

Table 1. Distribution of clinical characteristics according to diagnosis

Risk factor	STEMI n=126	NSTEMI n=40	UA n =182	Total n = 348	P value
Median age	58.9 ± 11.6	60 ± 12.8	61.2±10.5	60.3±11.2	0.863
Male	81 (64.2%)	26(65 %)	108 (59.3%)	215 (61%)	0.616
DM	39 (30%)	19 (47%)	87 (47.8%)	145(41.6%)	0.009
HTN	45 (35.7%)	25 (62.5%)	106 (58.2%)	176(47.9%)	0.001
Smoking	33 (26.2%)	16 (40%)	66 (36.2%)	111(31.8%)	0.11
h/o CAD	17 (13.4%)	9 (22.5%)	78 (42.8%)	104(29.8%)	0.001
Family history	3 (2.3 %)	2 (5 %)	3 (2.3%)	8 (2.2%)	0.761
h/o PCI	2 (1.5%)	5 (12.5%)	14 (7.6%)	22 (6.3%)	0.016
h/o CABG	0	0	4 (2.1%)	4 (1.1%)	0.452

DM: diabetes mellitus, HTN: hypertension, h/o: history of, CAD: coronary artery disease, PCI: percutaneous coronary intervention, CABG: coronary artery bypass grafting

ECG was normal in 29% of patients with UA and 10% of NSTEMI. Anterior location of ischemia was the commonest (77%) (Table 3).

Time to presentation: Accurate timing cannot be assured, however, 15% of patients with STEMI presented within < 6 hours, 20% within 6 – 12 hours, and 65% ≥ 12 hours. Median time was 11.5 hr (1-24 hr). Sixty per cent of patients were admitted after 2:00 PM.

Reperfusion therapy for STEMI was applied in 73(57%) patients: 56 (44.4%) by thrombolytic therapy and 24 (19%) by PCI (10 patients by primary PCI and 14 patients by rescue PCI) and three (2.3%) by emergency coronary artery bypass grafting (CABG). No patient was referred for primary or rescue PCI in BTH.

Invasive management: CAG was done in 58% of all patients. It was negative in 6 patients labeled as UA. PCI was done in 108 (31%) patients. See tables 5. IABP was used in three STEMI patients who were suffering from mechanical complications before referral for CABG.

In hospital mortality: 7.7% died in hospital (27 patients) due to cardiac arrest or cardiogenic shock (Table 5).

Table 2. Distribution of clinical characteristics according to age groups

Risk factor	< 45 yr n=21 (6%)	45-55 yr n=105 (30%)	> 55 yr n=222 (63%)	Total n=348	P value
Male	17 (80.9%)	77 (73%)	121 (54%)	215(61%)	0.001
DM	7(30%)	38 (36.1%)	107 (48.1%)	145(41.6%)	0.059
HTN	5(23.8%)	45 (42.8%)	126 (56.7%)	176(47.9%)	0.022
Smoking	12 (57.14%)	48 (45.7%)	51 (22.9%)	111(31.8%)	0.001
h/o CAD	3 (14.2%)	22 (20.9%)	79 (35.5%)	104(29.8%)	0.007
Family history	3 (14.3%)	5 (4.7%)	0	8 (2.2%)	0.001
h/o PCI	0	7 (6.6%)	15 (6.7%)	22 (6.3%)	0.486
h/o CABG	0	0	4 (1.8%)	4 (1.1%)	0.317

DM: diabetes mellitus, HTN: hypertension, h/o: history of, CAD: coronary artery disease, PCI: percutaneous coronary intervention, CABG: coronary artery bypass grafting

Table 3. ECG changes

ST elevation	126 (36.2%)			
ST depression	158 (71%) of all NSTE- ACS			
LBBB	7 (2%) one diagnosed as STEMI, the rest as UA			
Normal	53 (29%) of UA and 4 (10%) of NSTEMI			
location	Anterior 77%	Inferior 7.4%	Lateral 3.2%	Posterior 2.3%

Table 4. Invasive management

Procedure	STEMI N = 126	NSTEMI n = 40	UA n = 182	Total n = 348
CAG	32 (25%)	14 (35%)	124(68%)	202 (58%)
PCI	24 (19%)	6 (15%)	78 (42%)	108 (31%)
CABG	4 (3%)	8 (2%)	40 (21.9%)	52 (14%)

STEMI: ST elevation myocardial infarction, NSTEMI: non-ST elevation myocardial infarction, UA: unstable angina, CAG: coronary angiography, PCI: percutaneous coronary intervention, CABG: coronary artery bypass grafting

Table 5. In-hospital outcomes in medical city complex

Complications	STEMI n = 126	NSTEMI n = 40	UA n = 182	Total n = 348	P value
Arrhythmias	14	3	6	23 (6.6%)	0.024
Heart Failure	10	2	13	25 (7.2%)	0.821
Cardiogenic shock	11	0	3	14 (4%)	0.02
Mechanical	3	0	0	3 (0.8%)	0.264
Stroke	0	2	0	2 (0.5%)	0.036
Death	19 (15%)	3 (7.5%)	5 (2.7%)	27 (7.7%)	0.001

Discussion

Results are compared with that of GRACE study (the largest global registry that involved 6073 cases from 234 hospitals in 13 countries from Europe, North and South America, Australia, and New Zealand [7], as well as various registries (table 6).

Table 6. Comparison distribution of risk factors with other studies

Item	Current Study	GRACE (7)	EHS-ACS II (8)	Cairo (9)	UAE (10)	Czech (11)	Brazil (12)	Polish (13)
Pt no.	348	60723	6385	401	1840	1221	860	10093
Age	61	66.3 **	64.7 **	54.4 **	51 **	68 **	62.6 *	65.6 **
Male	63.2	67 NS	70.1 **	79 **	93.1 **	63.4 NS	58.3 NS	61.4 NS
HTN (%)	49.7	60 **	57.3 **	55.36 *	34.6 **	70.2 **	78.1 **	68.7 **
DM (%)	41.1	25 **	24 **	37.66 *	38.9 NS	35.6 *	31.6 **	24.1 **
Smoking (%)	31	58 **	36.8 *	61 **	46.4 **	30.9 NS	26 *	28.4 NS
CAD (%)	29.9	59 **	22.5 NS	41.8 **	23.7 **	25 *	47.6 **	24.7 *
Death (%)	7.7	5 *	4 *	4.2 *	3.3 **	5.7 NS	4.8 *	5.6 NS

NS = no significant difference ($p > 0.05$), * = Significant difference ($p \leq 0.05$), ** = Highly significant difference ($p \leq 0.001$).

Pt no.: patients number, HTN: hypertension, DM: diabetes mellitus, CAD: coronary artery disease, GRACE: global registry of acute coronary events, EHS-ACS: Euro Heart Study of acute coronary syndromes, UAE: United Arab Emirates

In this study, the median time from onset of symptoms to CCU arrival (11.9 hr) was the much later than in UAE registry (127 minutes), GRACE (140 minutes) [7], Cairo (165 minutes) [9] and even Russia (4.33 hrs for STEMI and 7.42 hrs for NSTE-ACS) [14] or India (6 hrs) [15]. This indicates lack of awareness of alarming symptoms of ischemia and shows the need for more public health education.

The mean age of our patients is significantly lower than that in European studies and higher than the Gulf and Egypt studies and so cannot be explained on economic base only; genetic and cultural factors need to be studied.

Hypertension is the most prevalent risk factor in this study. According to a population-based survey conducted in 1979 by Alwan, hypertension comprises 12% of the Iraqi population [3]. This increased to 40.4% on 2006 according to a large national survey by the Ministry of Health; the prevalence of self-reported hypertension was 19% indicating low awareness and 8.7% of patients were taking herbal or traditional remedy [16]. On 2012, a small report from selected primary health care centers in Nasiriya city reported a similar prevalence (46.1%) [17]. In a local study of 110 patients with hypertension and myocardial infarction the salt-free diet and drug

noncompliance rate was seen in 69% and 71% of patients respectively [18]. All these studies indicate increasing prevalence of hypertension over time among Iraqi population but with poor patient awareness and compliance and thus less control and more complications. However, hypertension was less prevalent compared to other international studies. Whether this is related to sample volume and methodology or ethnicity, which is known to plays a major role in the prevalence of hypertension, its response to treatment and related mortality; needs to be clarified by further study [19-22].

Diabetes has the highest incidence in our patients compared to other studies. According to Ministry of Health survey on 2006 diabetes affects 10.4 % of Iraqi population with 15.6% of them were taking herbal or traditional remedy for treatment [16]. A local study in Basrah involved 5,445 persons on 2014 showed that 19.7% had diabetes with 70.3% had a body mass index ≥ 25 kg/m² [23]. In another local study in Basrah included 1079 patients the mean glycated hemoglobin (HbA1c) was 9.46% ± 2.0% and only 5.5% achieved the target of HbA1c of < 7% [24]. These studies confirm that we are still lagging in diabetic control from guidelines.

Smoking is less prevalent in our study compared with international studies [7-13]. As supported by some of the aforementioned local studies, smoking ranges between 16.9 to 25%, is a finding that depends on the history given by the patient.

The final diagnoses (STEMI 36.2%, and NSTE-ACS 63.7% in the form of NSTEMI 11.4% and UA 52.3% are comparable to that in the largest global registry GRACE [7] (STEMI 34% and NSTE-ACS 59% in the form of NSTEMI 30% and UA 29%), but the proportion of UA is higher than NSTEMI within our NSTE–ACS category. The use of insensitive rapid visual immunoassay for detection of cardiac biomarkers, and rare use of serial testing for biomarkers may be responsible for this overlap between NSTEMI and UA. In addition, it is possible that some of the patients were overdiagnosed because the criteria for UA are rather liberal and do not require ECG changes. Typical ischemic ECG abnormalities were absent in about 29% of UA and 10% of NSTEMI patients.

In GRACE study, CAG was performed in 62% of STEMI (followed by PCI in 45% and by CABG in 4%), 57% of NSTEMI (followed by PCI in 32% and by CABG in 7%), and in 49% of UA patients (followed by PCI in 23% and by CABG in 6%) [7]. In Euro Heart Study-ACS I on 2001 and ACS II on 2004 there is significant shift from fibrinolytic therapy to primary PCI (PPCI). The use of PPCI rose from 37 to 59% [8]. In the Czech Republic, only 21% of the patients with STEMI presented to a regional hospital; the majority were referred directly to a cardiocenter by emergency medical personnel [11]). In comparison, we have underutilization of PCI in ACSs in general and especially in STEMI due to limited availability of 24 hrs PCI facilities with expert staff and poor referral from general hospitals. In some high-income countries like in Gulf registry (Gulf RACE) [25] primary PCI was performed in 7% of cases and in a large US registry primary PCI was performed in 22% of STEMI patients [26], suggesting the effect of administrative other than financial factors.

Mortality in current study is higher than in most other studies except for Russia [14] and India [15], which showed a similar high mortality rates (9.7 % and 6.5% respectively), similar long time to presentation and underutilization of invasive management (PCI in 18.7% of STEMI and 11.4% of NSTE- ACS in Russia registry and 8% primary PCI in India registry). While the very low mortality in Taiwan (1.3%) [27] and UAE (3.3%) [10] registries are related to exclusion of patients with > 12 hrs to presentation in the former and the high percentage of young foreign patients in the later.

Limitations

The participating two hospitals are teaching hospitals, one with primary PCI facility and one without. Thus, the present findings may not be

generalized to patients of other hospital types. Lipid profile and body mass index were not checked. Only conventional coronary disease risk factors were identified. The study reports only in-hospital outcome, which may be inadequate to capture the true burden of CAD and the impact of our practice on the outcome on long term basis.

This study concluded that there is a long time delay from onset of symptoms to hospital admission among our patients indicating lack of awareness of ischemic symptoms and it seems to be the most detrimental factor. There is high incidence of risk factors that can be modified by primary and secondary measures. There is underutilization of invasive management. We have a high mortality rates in patients with ACS secondary to the aforementioned factors.

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Conflict of interest

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Detection of Hepatitis C Virus in Iraqi Patients with Oral Lichen Planus

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Abstract

Background Oral Lichen planus (OLP) is chronic mucocutaneous disorder with or without involvement skin, of unknown etiology. Lichen planus (LP) is T-cell-mediated chronic inflammatory disease of the stratified squamous epithelium. It can affect oral mucosa, skin, esophagus, nasal mucosa, larynx, genitalia, urinary tract, hair follicles and the eyes. Latterly has been focused on presence association between OLP and hepatitis C virus (HCV) infection, but this relation is not stable, since the prevalence of this virus in patients varies depended on studies, ranging from 0% to 67.8%.

Objective This study was established to investigate the relationship between the OLP and HCV infection in Iraq and to detect the virus by Real-time polymerase chain reaction (RT-PCR).

Methods Blood samples were collected from 62 patients with OLP and controls to detect the HCV in those patients by using one step anti-HCV test, ELISA test and RT-PCR.

Results The current study revealed that the early detection of HCV by one step Anti-HCV for all OLP patients was negative and by ELISA showed that four patients (12.5%) of OLP were positive for HCV. While using RT-PCR found that only one patient (3.1%) was positive for the presence of the virus.

Conclusion There was not relationship found between OLP and HCV infection in Iraqi patients.

Keywords Oral lichen planus, HCV, RT-PCR, ELISA

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List of abbreviation: ELISA = Enzyme linked immunosorbant assay, HCV = Hepatitis C virus, LP = Lichen planus, OLP = Oral lichen planus, RT-PCR = Real-time polymerase chain reaction, RNA = Ribonucleic acid

Introduction

Oral lichen planus (OLP) considered as a disease of stratified squamous epithelia [(1)]. The clinical estimate of the oral lesions is depended on the six clinical shapes: atrophic, bullous, erosive, papular, plaque and reticular [(2)] that usually cause bilateral white striations, papules, or plaques on the buccal mucosa, tongue, and gingiva. Erythema, erosions, and blisters may or may not be present [(3)]. OLP occurs more frequently than the cutaneous form and tends to be more persistent and more resistant to treatment, oral lesion accompanies, precedes or follows cutaneous lesion [(4)]. Theoretically some genetic change may facilitate the development of OLP in a subgroup of patients with hepatitis C results to geographical heterogeneity [(5)] or epitopic similarities between hepatitis C virus (HCV) and keratinocytes could explain the relationship between lichen planus (LP) and HCV, but this could not be demonstrated in any studies [(6)]. The first case of LP relationship to HCV was reported from France in 1991 [(7)]. The ratio of HCV infection in patients with OLP appear to be high in Japan, Italy and Brazil [(8)] and not detected in British, Nordic countries, and Germany. Whereas the results in USA,

India and Saudia Arabia are rescaled [9]. Although the pathogenesis of OLP is unknown and several studies have been suggesting its association with chronic hepatitis C [6,10] but the correlation of OLP with HCV infection remains controversy. After acute HCV infection, the ratio of patients who may stay chronically infected is estimated to be as high (85-90%). A large proportion of these chronically infected patients without symptom for HCV infection and becomes carriers. OLP in certain individuals can be used as a marker for diagnosis of HCV infection in asymptomatic patients, leading to early therapy and best prognosis [11]. It has been estimated that HCV-infected patients have at least twice the risk of progress LP than the general population. The reports of correlation the LP with HCV suggest marked geographic differences [12]. During the past years, different studies from Brazil, Iran, Israel, Saudi Arabia, Thailand, Taiwan and Turkey showed statistically significant association between presence of OLP and HCV infection [13], another two Italian studies, two Indian studies, two Iranian studies, one Brazilian study, one Turkish study, one Serbian study and one from UK were not able to find any correlation between chronic HCV infection and OLP [13]. There are no study present between oral lichen planus and HCV in Iraqi patients.

This study was established to investigate the relationship between the OLP and HCV infection in Iraq and to detect the virus by Elisa test and Real-time polymerase chain reaction (RT-PCR)

Methods

Patients and control groups

Thirty-two patients attending the Dermatological Outpatient Clinic at Medical City in Baghdad from June 2014 till February 2015, were eligible in this study. A specialist physician based on the clinical pictures diagnosed them all. Excluded criteria: patients without chronic periodontitis, gingivitis and without systemic diseases. Current study included thirty apparently healthy volunteers were considered as control group, their age and gender matched with patients group, received no treatment with no complaint of other chronic or systemic diseases, no history or clinic evidence of malignant disease.

Blood Samples

3 ml of Blood was collected from each patient and control, put in clot activator tubes for serum separation by centrifugation at 2600 g for 15 minutes at 4°C, and then stored at -80˚C. The Ethical Committee of College of Medicine, Al-Nahrain University approved this study, and all samples were obtained with informed consent in accordance with Medical City in Baghdad declaration.

Detection of Antibodies to HCV infection:

One-step Rapid test: One-step Anti-HCV test is a rapid direct binding test for the qualitative detection of anti HCV antibody in serum (Blue Cross Bio-Medical, Beijing, China).

Detection of antibodies (both IgG and IgM) of HCV by ELISA: (CTK BIOTECH, USA, E0511).

Detection of RNA of HCV by RT-PCR:

Viral RNA extraction: The ExiPrepTM (BIONEER, Korea, K3525) automated viral RNA Kits are suitable to extract of viral RNA from serum samples.

Detection of HCV by RT-PCR: The artus HCV RG RT-PCR Kit (QIAGEN, Germany, 4518265) constitutes a ready to use system for the detection of HCV RNA using polymerase chain reaction (PCR) on rotor-gene Q instruments.

The statistical analysis of this prospective study performed with the statistical package for social sciences (SPSS) 21.0 and Microsoft Excel 2013. Chi-square test used to describe the association of these data. Numerical data were described as mean, standard deviation of mean. Mann-Whitney test used for comparison between two groups while kruskal wall is test used for comparison among more than two

groups. The lower level of accepted statistical significant difference is bellow or equal to 0.05.

Results

Distribution of patients and control group according to age was listed in table (1), most patients (43.8%) were at age group (>50 years), however, there is no significant difference between this group and other age groups (p=0.102). There was female predominance among patients, as 20 (62.5%) of OLP patients were females, while only 12 (37.5%) of patients were males figure (1).

While the distribution of patients according to clinical types the OLP patients were classified to three subgroups, (14 patients were reticular form and 11 of patients were with erosive form while 7 patients with plaque like form). In addition, there were no significant differences (p>0.05) in types of disease according to gender, table (2).

Table 1. Distribution of OLP patients and healthy controls according the age groups

Age groups		Study groups		Total
		Healthy control	OLP	
20-30 years	Count	2	4	6
	%	6.7%	12.5%	9.7%
31-40 years	Count	11	4	15
	%	36.7%	12.5%	24.2%
41-50 years	Count	10	10	20
	%	33.3%	31.3%	32.3%
>50 years	Count	7	14	21
	%	23.3%	43.8%	33.9%
Total	Count	30	32	62
	%	100.0%	100.0%	100.0%
p value			0.102^{NS}	

NS: non-significant

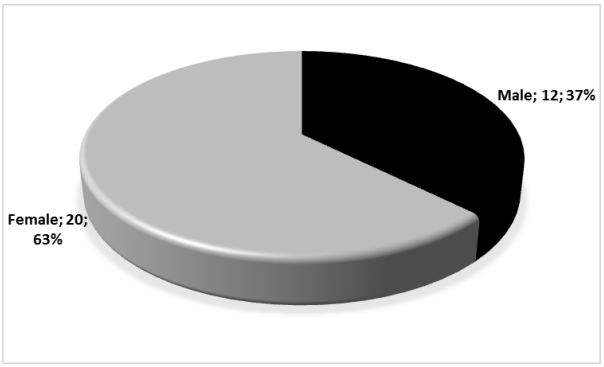

Figure 1. Distribution of OLP patients according to gender

Table 2. Distribution of OLP patients according the clinical types and gender

		Erosive	%	Plaque like	%	Reticular	%	p value
Gender	Male	2	18.18	2	28.57	8	57.14	0.116^{NS}
	Female	9	81.82	5	71.43	6	42.86	

NS: non-significant

Detection of HCV

One-step Anti-HCV: The primary detection (serological screening) of HCV by one step Anti-HCV strips showed no antibodies against this virus neither in serum of OLP patients nor in serum of healthy controls as clearly shown in table (3).

Table 3. Anti-HCV serological screening by one-step Anti-HCV strips in OLP patients and healthy control

			Study groups		Total
			Healthy control	OLP	
One-step Anti-HCV	Negative	Count	30	32	62
		%	100	100	100
	Positive	Count	0	0	0
		%	0.0	0.0	0.0
Total		Count	30	32	62
		%	100	100	100

ELISA: The current results revealed that only four patients (12.5%) of OLP were positive to anti-HCV Ab, whereas 28 (87.5%) of patients were negative to anti-HCV Ab. Moreover, there were no significant differences (p>0.05) in percentages of Ab between patients and control group, table (4). There was no significant differences (p>0.05) in mean serum level of anti-HCV Abs among three types of OLP, as shown in table (5).

Table 4. Anti-HCV screening by Elisa in OLP patients and healthy control

			Study groups		Total
			Healthy control	OLP	
ELISA	Negative	Count	30	28	58
		%	100	87.5	93.5
	Positive	Count	0	4	0
		%	0.0	12.5	6.5
Total		Count	30	32	62
		%	100	100	100
p value			0.064^{NS}		

NS: non-significant

Table 5. Serum level of anti-HCV Abs according to clinical types of OLP

		Type of lesion			P value
		Erosive	Plaque like	Reticular	
HCV ELISA	Mean	0.59	0.22	0.49	0.207^{NS}
	Median	0.13	0.16	0.19	
	Percentile 25	0.10	0.14	0.12	
	Percentile 75	0.38	0.22	0.46	

NS: non-significant

RT-PCR: The concentration and purity of nucleic acid samples has been measured by nanodrop, and only those samples with a purity ranged from 1.80 to 2.0 have been enrolled in this study. Only one patient (3.1%) of 32 OLP patients was positive for HCV and the virus was not detected in control group figure (2&3), table (6). It is worthy to mention that through this work not relationship found between HCV infection, which detected by RT-PCR and clinical types of OLP with p-value (0.373), table (7).

Table 6. Detection of HCV–RNA by RT-PCR in OLP patients and healthy controls

			Study groups		Total
			Healthy control	OLP	
HCV RT-PCR	Negative	Count	30	31	61
		%	100	96.9	98.4
	Positive	Count	0	1	1
		%	0.0	3.1	1.6
Total		Count	30	32	62
		%	100	100	100
p value				0.516^{NS}	

NS: non-significant

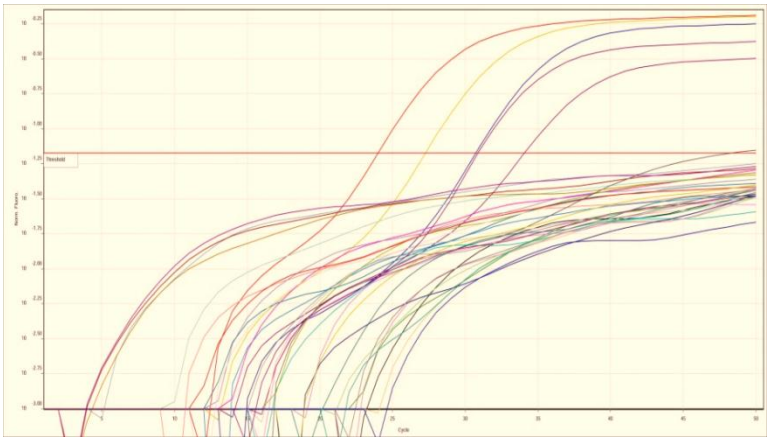

Figure 2. Detection of HCV infection in OLP patients

Table 7. Relationship between clinical types of OLP with RT-PCR in HCV infection

		Erosive	%	plaque like	%	reticular	%	p value
HCV RT-PCR	Negative	10	90.91	7	100	14	100	0.373^{NS}
	Positive	1	9.09	0	0.00	0	0.00	

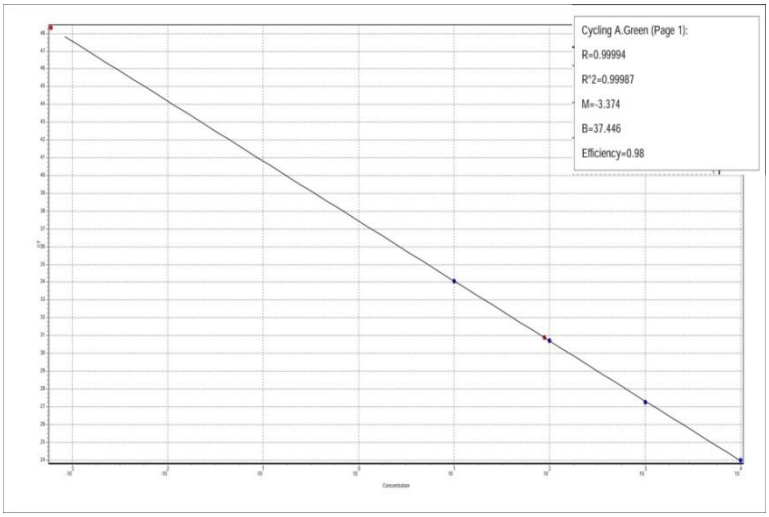

Figure 3. Standard curve of one positive sample in OLP patients with 4 standard positive controls (cyclic green)

Discussion

It is well known that OLP is a chronic inflammatory oral mucosal disease of unknown etiology; however, environmental and genetic factors are known to be involved in the development of this disease [14]. OLP has been reported as a complication of chronic HCV infection, and found that HCV-infected patients have at least twice the risk of developing OLP than the general population [12]. The present result showed that the OLP is more prevalent in patients with age more than 50 years with mean age (48.16) and this result is consistent with other study reported by Munde et al. [15]. Also the current results denoted a predominance of OLP among females than males which is comparable with other study conducted by Yu et al., who indicated that LP occurs more commonly in females [16]. The ratio of female to male was 2:1 and this ratio is agreement with other broad results in Iran [17], Saudi Arabia [18], Turkey [19] and in India [10]. The higher incidence in females may be referred to the hormonal differences between them and in part, their effects on the immune system. Those hormones make the females tend to mount more robust immune Th2 responses and may enhance the development of autoimmune disease [20]. In the present study, the reticular type was with highest frequency among patients and this finding agreement with previous reports which suggest that the reticular types was predominance types in OLP [10,12,14]. Although the pathogenesis of OLP is unknown, it is recognized as a T-cell-mediated immune disease, and several studies have been suggesting its association with chronic hepatitis C [6,10]. The relationship between the HCV and OLP remains a controversial subject [10]. Mokni et al. in 1991 was the first found the presence of association between OLP and chronic liver disease in France [21]. On the other hand,

Lavanya et al. 2011 mentioned that epidemiological evidences strongly suggest that HCV may be an etiologic factor in OLP [22]. The virus RNA was present in saliva, serum, skin lesions and oral tissues of involved HCV patients, this can suggest a cause-effect relationship between LP and HCV [23,24]. Several studies showed 15-35% of OLP patients have hepatic disease or disorders [25]. Halawani et al. 2010 showed that among 51 patients with OLP in Saudia Arabia, 5 (9.8%) were anti-HCV positive, of which 2 (3.9%) were HCV-RNA-PCR positive [25]. Similarly, El-Rifaei and colleagues take serum from 34 patients with OLP and found that 5 out of 34 (14.70%) were positive to HCV infection [18]. Many studies showed no correlation between chronic HCV infection and OLP [10,12,17,26-28]. Gerayli et al. showed that there is no correlation between OLP and HCV in Iranian patients [24], in the Mashhad [25] and in Tabriz [29]. Anyway, Strak pointed out to that the prevalence of HCV antibodies in patients with OLP was found to be significantly higher than that in the control group in Iran, Jordan, Kingdom of Saudi Arabia and Turkey [30]. In Japan, where the prevalence of HCV infection was the highest in the country, which observed that 62% of the patients were with LP [31]. Also another study showed the prevalence of anti-HCV and HCV RNA in Japanese patients were 67.80% (40/59) and 59.32% (35/59), respectively in OLP [32]. Of all 232 Chinese OLP patients, the antibody of HCV infection was detected positive in 4 patients (1.72%) using ELISA [33]. In India, only two studies proving presence relationships between OLP and HCV [34,35]. The current study revealed that the early detection of HCV infection in OLP patients.

The present study concluded that there was no relationship found between OLP and HCV infection in Iraqi patients.

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Author contribution

Dr. Hassan H.: collection of samples, data and writing the draft of the manuscript. Dr. Abbas: supervised data collection, study design and revising the manuscript. Dr. Ahmed helped in RT-PCR technique. Dr. Hassan S. helped in diagnosis of OLP patients.

Conflict of interest

The authors declare no conflict of interest.

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Effect of Betahistine and Metformin on Lipid Profile in Obese Females in Iraq: A Randomized, Placebo-Controlled Clinical Trial

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Abstract

Background Obesity has become a major worldwide health problem and therefore, the associated morbidity, mortality and both medical and economical costs are expected to increase as well. Obesity increases cardiovascular risk via risk factors such as triglycerides (TG), high LDL cholesterol, low HDL cholesterol, elevated plasma glucose and insulin concentrations.

Objective To investigate the effect of metformin and betahistine along with lifestyle change on lipid profile in obese women in Iraq.

Methods This study was carried out on 78 female patients with age range of 18-50 years who were allocated into three groups: Group 1: treated with oral metformin 850 mg twice daily with lifestyle change for 12 weeks. Group 2: treated with betahistine 32 mg 3 times daily with lifestyle change for 12 weeks. Group 3: treated with placebo 500 mg twice daily with lifestyle change for 12 weeks to serve as control. Complete history was taken, in addition to clinical examination to meet inclusion criteria. Serum transaminases (ALT+AST) and estimated glomerular filtration rate (GFR) were estimated at baseline to exclude hepatic or renal abnormalities.

Results Each metformin and betahistine, along with lifestyle intervention highly significantly reduced total cholesterol level, LDL-C level, TG and VLDL level, and increased plasma level of HDL after 12 weeks in obese women with disturbed lipid profile compared to pre-treatment values, and the changes elicited by metformin and betahistine (plus lifestyle change) were highly significant compared to placebo (lifestyle change alone).

Conclusion The results obtained in this study clearly demonstrated the beneficial effect of using metformin or betahistine to obese women with dyslipidemia and confirmed the role of pharmacotherapy in targeting the lipid metabolism changes accompanying obesity.

Keywords Obesity, dyslipidemia, lifestyle change, betahistine, metformin

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List of abbreviation: ALT = Alanine aminotransferase, AMP = Adenosine mono phosphate, ANCONA = Analysis of co-variance, ANOVA = one way analysis of variance, ASP = Acylation-stimulating protein, AST = Aspartate aminotransferase, ATP = Adenosine triphosphate, BAT = Brown adipose tissue, BMI = Body mass index, CKD = Chronic kidney disease, EDTA = Ethylene diamine tetra-acetic acid, e-GFR = Estimated glomerular filtration rate, FDG-PET scans = Fluorodeoxyglucose Positron_emission_tomography scan, FFA = Free fatty acids, HDL = High-density lipoprotein, HRH1= Histamine receptor H1, HRH3 = Histamine receptor H3, HSL = Hormone-sensitive lipase, IDL Intermediate-density lipoprotein, IL = Interleukin, Kcal = Kilo calories, LCAT = Lecithin-cholesterol acyltransferase, LCD = Low-calorie diet, LDL = Low-density lipoprotein, LPL = Lipolipase enzyme, mRNA = Messenger ribo-nucleic acid, NHLBI = National heart, lung and blood institute, PPAR = Peroxisome-proliferator-activated-receptor, PUFA = Polyunsaturated fatty acids, SREBP = Sterol regulatory element-binding protein, TC = Total Cholesterol, TG = Triglyceride, TNF-α = Tumor necrosis factor-alpha, VLDL = Very Low-density lipoprotein, WAT = white adipose tissue, WHO = World Health Organization

Introduction

Obesity has become a major worldwide health problem. In every single country in the world, the incidence of obesity is raising continuously and therefore, the associated morbidity, mortality and both medical and economical costs are expected to increase as well [(1)]. The prevalence of obesity

reported by the WHO for the Iraqi population in 2005 was 8.3% and 19.1% for males and females respectively [2]. In 2008, another study concluded that obesity affects about 30% of adult population, with higher prevalence in women [3].

Obesity threatens to become the primary cause of non-communicable diseases over the world [4], with high health and social costs [5]. The medical expenditure regarding the obesity treatment in the USA was $147 billion in 2008, which has twice of that of the last ten years [6].

Obesity increases cardiovascular risk via risk factors such triglycerides (TG), higher low density cholesterol (LDL), lower high density (HDL) cholesterol, elevated plasma glucose and insulin and high blood pressure [7].

Hypertriglyceridemia might be the main cause of the other lipid abnormalities, as it leads to delayed clearance of the TG-rich lipoproteins and creation of small dense LDL, and decreased HDL-C concentrations [8]. The higher TG levels is regarded as a hallmark of dyslipidemia in obesity; partly due to the enhanced free fatty acid (FFA) entry to the liver, leading to hepatic accumulation of TG, leading to an increased hepatic synthesis of large amount of very low density lipoproteins (VLDL), which hinders the lipolysis of chylomicrons, due to competition mostly at the level of lipoprotein lipase (LPL) and increased remnant TG being conveyed to the liver [1].

Lipolysis is further compromised in obesity by lowered mRNA expression levels of lipolipase (LPL) in adipocytes and decreased LPL activity in skeletal muscle [9].

Current treatment of obesity-associated dyslipidemia concentrates on lifestyle changes including increased physical activity and a healthy diet [10]. Weight loss has been shown to markedly decrease TG concentrations, which can be caused by an increase in LPL activity [11], and an enhanced catabolism of TG-rich lipoproteins [12].

Statins are the drugs of first choice of all pharmacological agents. They do not fully correct the characteristic dyslipidemia associated with obesity, which may contribute to the residual risk after initiating statin therapy [13]. Combinations of statin with ezetimibe, can inhibits the intestinal cholesterol absorption. Fibrates are primarily indicated for hypertriglyceridemia [14]. Nicotinic acid hinders the lipolysis of adipocytes, which results in reduced FFA levels, reduced VLDL synthesis, a slight elevation in HDL production rate and lower catabolism of HDL [12,13]. Omega-3 fatty acids had also been found to increase the conversion of VLDL into IDL, suggesting an extra benefit from combining omega-3 fatty acids with statins via increased catabolism of VLDL, IDL and LDL [12].

Metformin is an anti-hyperglycemic medication for the treatment of type 2 diabetes mellitus. Its anti-hyperglycemic effect is by potentiation of insulin action via reducing insulin resistance, increasing peripheral glucose uptake and reducing gluconeogenesis. Patients on metformin therapy do not gain weight, and could lose weight [15,16]. Other effects of the use of metformin are lowering of systolic and diastolic blood pressure [17], improving glucose and lipid metabolism, and reducing blood pressure in hypertensive, obese females [18].

Betahistine is a histamine receptor H1 (HRH1) agonist and HRH3 antagonist [19] that has been used to treat Meniere disease since the early 1960s [20]. Being a histamine HRH3 auto-receptor antagonist, it increases the release of histamine. H3 hetero-receptors are present in non-histaminergic neurons, regulating release of neurotransmitters like dopamine, norepinephrine and serotonin [21]. A clinical study had shown that, in a subgroup post hoc analysis, weight loss happened in non-Hispanic women [22]. Betahistine, administered as an open-label fashion, had been reported to reduce olanzapine-associated weight gain, and improved lipid profile in patients with schizophrenia, compared with control subjects [23].

This study aims were to study the effects of betahistine and metformin along with lifestyle intervention compared to control (lifestyle

intervention alone) on dyslipidemia in obese Iraqi females.

Methods

Obese females, aged 18-50 years old, had been primarily enrolled in this study, and were divided into 3 groups. Medical history was taken from each patient. Body mass index (BMI), lipid profile, blood pressure, glomerular filtration rate (GFR) as renal professionals consider the (GFR) to be the best overall index of kidney function [24], liver transaminases (AST and ALT) were measured (as mentioned in details below). A written consent from each patient to be involved in the study was obtained.

Inclusion criteria

1. Female obese patients.
2. BMI equals or more than 30.
3. All are aged between 18-50 years, and pre-menopause.

Exclusion criteria

1. Patients with renal insufficiency: GFR less than 60 represents CKD.
2. Hepatic impairment: Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) higher than two-folds the reference range.
3. Heart diseases (including unstable angina, myocardial infarction, transient ischemic attacks/stroke, clinically significant arrhythmia, congestive heart failure (increased risk of lactic acidosis), or cardiac valve abnormalities.
4. Patients with a history of seasonal allergy, asthma, or peptic ulcer.
5. Known allergy to one of the medications used in the study.
6. Pregnant or lactating women
7. Known cases of type 1 or type 2 diabetes mellitus.
8. Patients with known history of thyroid dysfunction, Cushing's syndrome or Addison's disease.

Treatment Arms

Group 1: obese patients on metformin + lifestyle intervention (1700 mg/d) for 12 weeks
Group 2: obese patients on betahistine + lifestyle intervention (96 mg/d) for 12 weeks
Group 3: control group: obese patients on Placebo + lifestyle intervention for 12 weeks

Lifestyle intervention (Diet program and physical activity)

Diet Program

The patients were instructed to follow the diet regimen adapted by the Obesity Unit At Alkindy Medical College (at which, the study was done), which provides a low-calorie diet (LCD) of 1000-1200 kcal/day. The National Heart, Lung, and Blood Institute (NHLBI) Obesity Education Initiative recommended 1,200 to 1,600 kcal/day for men, and 1,000 to 1,200 kcal/day for women as a Low-calorie diet (LCD) [25].

Physical activity

The patients were instructed to practice a medium intensity exercise for 60-90 minutes/day. The Dietary Guidelines for Americans recommend that to lose weight, obese people have to participate at least 60 to 90 minutes of moderate- to vigorous-intensity daily physical activity, along with caloric intake restrictions [26].

Lipid profile analysis

Fasting serum Total Cholesterol (TC), High-density lipoprotein (HDL) and Triglyceride (TG) level were measured. Low-density lipoprotein (LDL) and the Very low-density lipoprotein (VLDL) were derived using the Friedwalds equation.

Total cholesterol (TC)

Principle (Reflotron cholesterol): The cholesterol esters are cleaved into the corresponding fatty acid and cholesterol, which are then oxidized to cholestenone and hydrogen peroxide in the presence of oxygen. In a further reaction step catalyzed by the

enzyme peroxidase, the hydrogen peroxide oxidizes a redox indicator, resulting in a blue dye, which is proportional to the cholesterol concentration in the sample. The cholesterol is measured at a wavelength of 642 nm and at 37°C in mg/dl or mmol/l.
Device and tools: Reflotron (Roche)

Triglyceride (TG)
Principle (Reflotron triglycerides): The triglycerides are cleaved in an enzymatic reaction. Various reaction steps then lead to the formation of H_2O_2. This oxidizes a redox indicator to a blue dye in a reaction catalyzed by the enzyme peroxidase at a temperature of 37 °C the dye formed is measured at a 642 nm and the triglyceride concentration in mg/dl or mmol/l depending on how the instrument has been set.

HDL: Reflotron HDL cholesterol
Principle: The Reflotron HDL Cholesterol test (Boehringer Mannheim GmbH) directly separates and analyzes high-density lipoprotein (HDL) cholesterol in plasma collected with EDTA in an integrated dry-reagent system suitable for alternative site testing of lipoprotein. The cholesterol concentration of this HDL is then determined enzymatically. The cholesterol esters are cleaved into the corresponding fatty acid and cholesterol, which is then oxidized to cholestenone and hydrogen peroxide in the presence of oxygen. At a temperature of 37 °C the dye formed is measured at a 642 nm and the HDL cholesterol concentration displayed in mg/dl or mmol/l depending on how the instrument has been set.

LDL and VLDL
These two lipoproteins were derived using the Friedewalds equation.
Friedewald Formula (1972) :
LDL = TC - HDL - TG/5 (mg/dL)
VLDL= TG/5 (mg/dL)

The Friedewald formula (FF) is an estimation of LDL-c level that uses the following levels of total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-c): LDL-c (mg/dL) = TC (mg/dL) − HDL-c (mg/dL) − TG (mg/dL)/5. This formula became the standard method for LDL-c assessment because it is economical and simpler than direct assays, the most accurate LDL-c measurement methods [27].
BMI was calculated by dividing body weight (kg) by the square of the height (meters), an on-line calculator was used.
eGFR was calculated making use of an on-line calculator provided by the National Kidney Foundation.

Statistical analysis
The results represented as the mean ± standard deviation. Data were fed to the computer program. Statistical Package for Social Science SPSS version 16.0 under Windows Seven, was used for analysis.
Normality test (Shapiro-Wilk test) was performed first, student t-test (paired) with p-value less than or equal 0.05 ($P \leq 0.05$) were used to compare between pre- and post-treatment values within the same group. ANOVA test was used for comparing pre-treatment values among groups, and pre-intervention values were used as covariates for adjustment when comparing post-intervention results, using ANOVA test [28,29].

Results
Baseline data (table 1) of the enrolled females showed no statistically significant difference among different treatment arms concerning participants number, age, and BMI. Liver transaminases and e-GFR results showed normal liver and kidney functions, with no statistically significant difference among obese women in this regard. Baseline serum levels of TC, HDL, LDL, TG and VLDL showed also no statistically significant difference among different treatment arms.

Table 1. Baseline characteristics comparison for enrolling patients

Characteristic (Baseline)	Group 1 Metformin (n=26)	Group 2 Betahistine (n=27)	Group 3 Control (n=25)	P-value
Body Mass Index (kg/m^2)	38.2±3.30	37.7±4.0	37.08±3.28	0.875
Age years (mean ± SD)	35.81±11.47	35.39±10.04	36.32±8.85	0.966
Serum AST (i.u/ L)	22.69±7.2	21.3±8.0	22.32±5.8	0.975
Serum ALT (i.u/L)	22.88±7.3	21.41±6.15	22.52±5.2	0.674
e-GFR (ml/min/1.73m^2)	84.69±12.9	84.70±11.3	84.80±8.3	0.999
TC (mg/dl)	197.4 ±7.78	198.9 ± 9.17	196.2±6.22	0.825
HDL (mg/dl)	36.50±2.14	37.07 ± 2.16	36.04±1.88	0.559
TG (mg/dl)	156.65±13.69	157.8±10.94	156.3±11.11	0.989
LDL (mg/dl)	128±6.52	130±8.85	129±6.02	0.800
VLDL (mg/dl)	31.3±2.73	31.40±2.18	31.36±2.34	0.989

-Results represented as mean±standard deviation

- P-value >0.05 means that there is no significant difference between the groups data using ANOVA test, TC= total cholesterol, HDL= High density lipoprotein, TG= Triglycerides, LDL= Low density lipoprotein, VLDL= Very low density lipoprotein, NS= Non-significant

Table (2) showed that administration of metformin (with lifestyle intervention) to obese women with dyslipidemia (Group 1), caused highly significantly reduction in serum TC, TG, LDL and VLDL levels, and at the same time highly significantly raised serum HDL level after 12 weeks compared to pre-treatment value. Rates of change were: -14.44% for TC, -21.04% for LDL, -18.9% for TG, and -18.9 for VLDL. HDL change rate was +9.7%.

While the administration of betahistine (with lifestyle intervention) to obese women with dyslipidemia (Group 2), caused highly significantly reduction in serum TC, TG, LDL and VLDL levels, and at the same time highly significantly raise in serum HDL level after 12 weeks compared to pre-treatment value. The rates of change were: -15.28% for TC, -22.42% for LDL, -19.7% for TG, and -19.7% for VLDL, whereas HDL change rate was +10.4%.

On the other hand; placebo treated group with lifestyle intervention (group 3), revealed a significant reduction of serum TC and LDL, with a significant elevation of HDL. No statistically significant change on pre-treatment values of TG and VLDL happened after 12 weeks. The rates of change were: -3.26% for TC, -4.6% for LDL, -0.77% for TG, and -0.77% for VLDL, whereas HDL change rate was +2%.

Table 2. Lipid profile changes

Treatment	TC	P-value	LDL	P-value	TG	P-value	VLDL	P-value	HDL	P-value
Metformin	-14.44	0.000	-21.04	0.000	-18.9	0.000	-18.9	0.000	+9.7	0.000
Betahistine	-15.28	0.000	-22.42	0.000	-19.7	0.000	-19.7	0.000	+10.4	0.000
Control	-3.26	0.020	-4.6	0.010	-0.77	0.056	-0.77	0.056	+2.0	0.010

Data represent the percent of change adjusted for baseline values (ANOVA test is used). P-value of the paired t-test is used. P-value less than 0.05 means a significant difference. P ≤ 0.001 represent a highly significant difference

Pairwise comparison between each two groups demonstrated no statistically significant difference between metformin and betahistine group effects on all measured parameters (p-values are more than 0.05), whereas a highly statistically significant difference between each metformin group and betahistine group effects were found when compared to control group (p-values are less than 0.001), as shown in table (3).

Table 3. Pairwise comparisons

Treatment	TC	LDL	TG	VLDL	HDL
Metformin vs. Betahistine	0.637	0.130	0.687	0.837	0.151
Betahistine vs. Placebo	0.000	0.000	0.000	0.000	0.000
Metformin vs. Placebo	0.000	0.000	0.000	0.000	0.000

Data represent the p-values of the pairwise comparisons. P ≤ 0.001 represent a highly significant difference

Discussion

Several studies concluded that the combination of diet and physical activity was effective in normalizing the lipid profile and overwhelming obesity [30,31]. Exercise seems to enhance the ability of skeletal muscles to make use of lipids as opposed to glycogen, hence reducing plasma lipid levels [32]. The mechanisms include increasing lecithin-cholesterol acyltransferase (LCAT); an enzyme that transfers ester to HDL cholesterol, which has been found to increase following exercise training [33], and lipoprotein lipase hyperactivity, which depend upon energy expenditure [34].

Metformin, betahistine and the control groups caused a highly statistically significant reduction in TC, LDL, TG and VLDL, with a highly significant elevation in HDL. Both Metformin and Betahistine treatment groups achieved a highly statistically significant difference compared to the control group, whereas no statistically significant difference between Metformin and Betahistine treatment regarding all of the above mentioned parameters.

It is well documented that plasma FFA are raised in obese people as a consequence of an elevated fatty acid release from adipose tissue and a lowered plasma FFA clearance [35,36]. The increase in FFA and obesity-associated inflammation play a central role in the development of insulin resistance [37]. There are only 2 sources where plasma FFA can be derived from; first: lipolysis of TG-rich lipoproteins inside the circulation; and second: intracellular lipolysis within adipose tissue [1]. Several fatty acids are cytotoxic and this cytotoxicity depend on their type [38]. Polyunsaturated fatty acids (PUFA), e.g. arachidonic acid and linoleic acid, might mediate a diet-induced inflammation [39]. They can encourage the synthesis of pro-inflammatory cytokines like IL-1, IL-6 and TNF-α [40].

An “escape mechanism” should exist in order to remove FFA from the microenvironment in which they are formed. In this mechanism, both insulin and the acylation-stimulating protein (ASP) play an essential role in peripheral fatty acid trapping [1].

It has been shown that ASP mRNA expression in visceral adipose tissue is decreased by nearly 40% in obese subjects with or without insulin resistance, compared to lean subjects [9]. Furthermore, obese subjects also showed less uptake of dietary fat by adipose tissue, resulting in an increased delivery of chylomicron remnants to the liver, and consequently enhanced VLDL-TG being delivered to the peripheral adipocytes [41].

Treatment of insulin resistance has been shown to reduce plasma FFA concentrations by lowering fasting FFA levels [42].

The postprandial elevation of insulin results in an effective inhibition of hormone sensitive lipase, which is the main enzyme for hydrolysis

of intracellular lipids. FFA are up taken by adipocytes and myocytes, yet; a fraction of FFA remains in the plasma compartment where they are bound by albumin and transported to the liver [43]. When the supply of FFA for energy expenditure is inadequate like in the fasting state, FFA are mobilized from adipose tissue for oxidation in energy demanding areas like cardio myocytes [44]. Insulin hormone is also an essential regulator of FFA mobilization from adipose tissue. The scavenger receptor CD36 is the best described FFA transporter, and is abundant in muscle, fat tissue and the capillary endothelium [45]. Insulin and muscle contractions enhance the CD36 expression, therefore; facilitate FFA uptake [46]. Therefore, insulin resistance has an important influence on the metabolism of TG-rich lipoproteins and FFA [1].

Metformin inhibits glucose, lipid and protein synthesis as well as cell growth, while stimulates fatty acid oxidation and glucose uptake [47]. A study reported that the pleiotropic actions of metformin are closely related to the activation of AMP-activated protein kinase (AMPK). AMPK is viewed as a fuel gauge checking systemic and cellular energy status, which plays a vital role in protecting cellular functions during energy-restricted conditions [48]. Activated AMPK shifts cells from the anabolic to the catabolic state, shutting down the ATP-energy-consuming pathways, restoring energy balance. This control involves phosphorylation of basic metabolic enzymes and transcription factors/co-activators, and modulating gene expression by AMPK [49]. Moreover, metformin treatment of insulin resistance has been shown to lower plasma FFA concentrations by lowering fasting FFA plasma levels without any effect on catecholamine mediated lipolysis of adipocytes [42].

Betahistine has been proved to ameliorates dyslipidemia caused by chronic olanzapine treatment in rats through modulating the hepatic AMPK-SREBP-1 (sterol regulatory element-binding protein) and peroxisome-proliferator-activated-receptor (PPAR) - gamma-dependent pathways [50].

Another possible mechanism through which betahistine could improve dyslipidemia is via norepinephrine's action on β3-adrenergic receptor. Being a histamine HRH3 antagonist, it can increase norepinephrine's release, which might appear to be a useful therapeutic target for activating the brown adipose tissue (BAT), considering evidence from studies using a selective β3-agonists (CL-316,243) and knockout rodent models [51].

Catecholamines might also 'brownify' the white adipose tissue (WAT), 2 case reports of widespread brown fat deposits in omental and mesenteric areas, detected via human FDG-PET scans, point out a possible role for catecholamines in the 'browning' of WAT (52). Therapeutically, catecholamine may trans-differentiate WAT into Beige AT, but this approach need to avoid the associated sympathomimetic effects to be safe [53].

Data from several animal studies have established that through BAT activation, dyslipidemia improves and triglyceride stores inside white adipose tissue (WAT) can be exploited to generate heat via modulation of adaptive thermogenesis [54].

Catecholamine mediated lipolysis of adipocytes is another possible mechanism. Hormone-sensitive lipase (HSL) is a primary lipase for catecholamine-mediated lipolysis. The mobilization of fat stored in adipose tissue is stimulated by hormone-sensitive lipase [55].

Taken together; there may be multiple pathways that might explain how betahistine and metformin can improve lipid profile in obese women with dyslipidemia.

The results obtained in the present study clearly demonstrates the beneficial effect of using metformin or betahistine for obesity-associated dyslipidemia and confirms the role of pharmacotherapy in targeting the lipid metabolism changes accompanying obesity.

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Author contribution

Dr. Al-Anbari conducted the study, collected the data and performed the statistical analysis and drafting of the article. Dr. Al-Zubaidy contributed in the designing, organization and finalization of the protocol, and Dr. Khazaal participated in the physical examination of the patients throughout the period of study.

Conflict of interest

The authors declare no conflict of interest concerning this work.

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The Efficiency of Molecular and Conventional Methods in Detection of *Candida albicans* Isolated from Immunocommpromsed Patients with Pulmonary Symptoms

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Abstract

Background *Candida albicans* (*C. albicans*) has emerged as a potentially pathogenic fungus rather than benefit mucosal commensal in patients with pulmonary diseases. Although respiratory candidiasis secondary to pulmonary tuberculosis has been reported in the past, it has gained more relevance recently due to increased use of broad spectrum antibiotics and immunosuppressive drugs.

Objective To detect *C. albicans* in sputum samples from patients with pulmonary diseases using conventional and molecular methods.

Methods One hundred sputum samples obtained from patients with pulmonary symptoms were included in this study. Sputum samples were dispensed into three specimen parts; the first one was applicated for cultured on Sabouraud dextrose agar at 37 °C for 48 hrs and then the purified colony of Candida underwent biochemical tests including API Candida strips, and germ tube. The second part was undergone direct gram stain, while the third part was applicated for DNA extraction and then molecular diagnosis with PCR technique using specific primers.

Results Culture result revealed 43 positive samples for Candida species out of 100 samples. Among these positive samples, 23 (53.5%) were positive for *C. albicans* in each of culture, germ tube and API. Molecular test revealed an amplicon with 538bp fragment of phospholipase gene from the same 23 samples.

Conclusion *C. albicans* is highly prevalent among patients suffering from bronchopulmonary symptoms. The molecular and conventional methods gave concomitant results as detection tools for the diagnosis of such microorganisms.

Keywords *Candida albicans*, phospholipase B gene, sputum.

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List of abbreviation: C = Candida, PLB = Phospholipase B, SAS = Statistical analysis system, SDA = Sabouraud's Dextrose agar

Introduction

Candidiasis is secondary mycotic infection caused by members of the genus Candida. Chiefly, *Candida albicans* (*C. albicans*) is responsible for about 70 to 80% of all Candida infections. Infection with *C. albicans*, which is an opportunistic yeast pathogen, increases predominantly in patients with predisposing condition including immunodeficiency such as human immune deficiency virus infections, prolong used of broad-spectrum antibiotics, corticosteroids therapy, diabetic patients and infections with other debilitated disease [(1)].

In immunocompromised patients, the clinical appearance of the *C. albicans* infection is often very complex and identification of the

organism is difficult. Therefore, speedy diagnosis and management of candidiasis are crucial for these patients [2]. C. albicans has the ability to form germ tube and chlamydospore, which is a characteristic feature of the yeast. *C. albicans* produces germ tubes when inoculated in serum and incubated for 30 min at 37 °C. The yeast cells have the ability to form germ tube in their initial stages when the hyphae are still attached to the yeast cell looking like sprouts [3]. Furthermore, this fungus secretes many enzymes such as proteinase, which has the ability to degrade a number of important defensive host proteins, particularly immunoglobulin and complement [4].

Candida pneumonia is one of the most challenging infections of all the Candida infection. Pneumonia due to infection with Candida spp. is extremely rare, but because of contamination with oral flora, these organisms are frequently cultured from respiratory secretions [5,6]. Non-culture-based methods, such as DNA detection by PCR, have been developed in order to assist in the rapid diagnosis of fungal infections, allowing for the initiation of species-oriented therapy as early as 6 h after the onset of disease [7].

This study was carried out to detect *C. albicans* in sputum samples from patients with pulmonary diseases using conventional and molecular methods.

Methods

Sputum samples have been collected from 100 patients of age group ranged from 10-90 years old, with a mean age 47.23±19.51. Some of these patients were suffering from systematic disease such as such as tuberculosis, diabetes mellitus, leukemia, while other were with immunocompromised status. Those patients were attending and admitting to Al-Yarmouk Teaching Hospital, Imamein Kadhimein Medical City and Chest and Respiratory Diseases Institute, Baghdad City Hospital during the period from September 2015 to February 2016. Each sputum sample was dispensed into three specimen parts the first one was applied for culture on Sabouraud dextrose agar at 37 °C for 48 hrs. Purified colonies from this culture were underwent biochemical tests including API Candida strips and germ tube. The second part was used in direct gram stain, while the third one was applied for molecular method. Standard strains of *C. albicans* ATCC 10231, was obtained from the National Institute of Health in Baghdad, which was used as a positive control.

Isolation and identification of *C. albicans*

Gram stain method was applied to each fresh sputum specimen and examined microscopically for detecting Candida species. Sputum samples were streaked on Sabouraud's Dextrose Agar (SDA) and incubated at 37 °C for 24-48 hrs. The isolates were re-identified by using API 20 C AUX and germ tube production. API 20 C AUX was performed according to the manufacturer's instructions (Biomuriex, France) for the confirmatory identification of the *C. albicans* and other species. Germ tubes production is a diagnostic characteristic method for *C. albicans*. A small part of yeast colony to be tested was emulsified with 0.5 ml of mammalian serum in a small test tube. The tube was incubated aerobically at 37 °C in an incubator for 2 hrs. A drop of the serum was removed to a slide and examined microscopically using the x10 and x40 objective lenses. A cylindrical filament originating from the blastoconidium without any constriction at the point of origin and without obvious swelling along the length of the filaments indicates a germ tube positive yeast [7].

Molecular method for diagnosis of *C. albicans*

DNA was extracted from each sample using PreplT. MAX, DNA genoTek, purification kit (Canada) with modification by mixing 200 µl of the sputum sediment with the 40 µl of MAX lysis Reagent. The resultant suspension was undergone repeat freezing-thawing by subjecting the samples to liquid nitrogen for 5 minute followed by boiling for 3 min for five cycles [8]. The primers set used for the

amplification of PLB genes of Candida was Forward 5'-TTGTGTTGCTACATCACCAAC-3' and reverse 5'-TTTGCTGGCAACTTGATTACC- 3' [9] to produce a DNA fragment of 538 bp . The thermocycling conditions were as follows: after initial denaturation at 94 °C for 5 min, 30-cycle amplification profile consisted of 95 °C for 30 s, 63 °C for 35 s and 72°C for 1 min was adapted. Final elongation was carried out at 72 °C for 10 min. PCR products were processed into a 1.5% (wt/vol) agarose gel (Merck-Germany) at 120 mV for 30 min. A molecular marker (100 pb DNA ladder; Bioneer/Korea) was run concurrently. DNA bands were visualized and photographed under UV light after the gel was stained with ethidium bromide.

Statistical analysis

Statistical Analysis system (SAS) software was used for all statistical analysis continuous variables were expressed in mean ± standard deviation (SD). The Pearson's Chi-square test or Fisher exact test was used for comparing the categorical variable. A two-sided significant level of 0.05 was considered to indicate a statistically significant difference.

Results

Demographic and clinical characteristics of patients:

The characteristics of the study population are shown in table (1).

***C. Albicans* from patients with pulmonary manifestation and other underlying diseases:**

C. albicans was isolated from 4 (18.1%), patients with hematological malignancies, 8 (28.5%), patients with solid tumor, 1 (4.3%), patients with asthma, 6 (23.07%) patients with diabetes mellitus and 4 (17.4%) patients with tuberculosis.

Cultivation and Gram stain

A total of 100 sputum samples were cultivated on Sabouraud's Dextrose agar and incubated for 2 days at 30 ˚C. Forty three samples (43%) were positive for Candida species the colonies were mucoid and have a creamy color (Figure 1). Gram stain confirmed this result in that the 43 samples were gram positive.

Table 1. Demographic and clinical features of patients

Variables	Value
Age (mean ±SD)	(47.23 ±19.51)
Sex M:F (No.)	64:36
Clinical Features (No, %)	
Hematological malignancies	22 (22%)
Solid tumors	28 (28%)
Asthma	5 (5%)
Diabetes mellitus	26 (26%)
Tuberculosis	33 (33%)

Figure 1. Candida species colonies on Sabouraud's agar media

Germ tube formation

A total of 43 culture samples were examined for germ tube. The result revealed that 23 (53%) were positive for *C. albicans*, as shown in Figure 2.

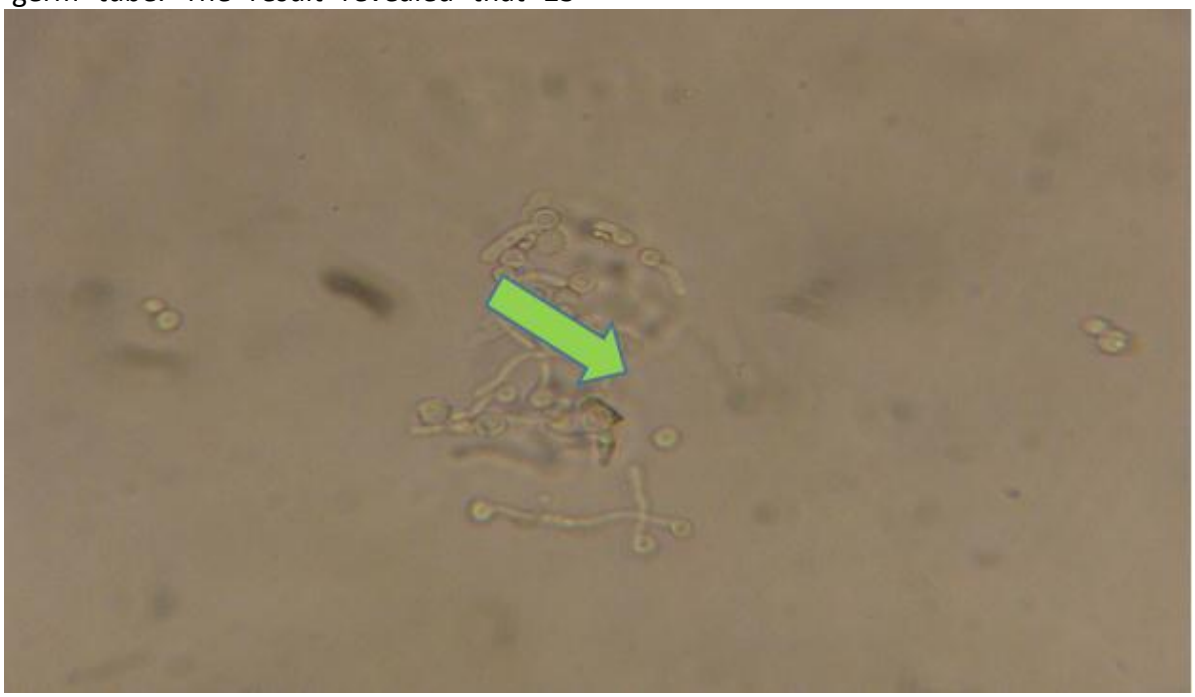

Figure 2. Germ tube formation by *Candida albicans*

API Candida kit

A total of 23 positive cultures were examined by API 20 AUX Candida strips. It was found that all samples represent *C. albicans* 23 (23%). Using PCR as a golden standard to evaluate both germ tube and API Candida system, the specificity and sensitivity of both API Candida system and germ tube are 100%.

Molecular detection

Conventional PCR was done for the amplification of PLB gene, which by using specific set of primers sequences. The results showed that, this gene (PLB gene) was present in twenty three out of one hundred sputum samples (23%). PCR product of this gene was 538 bp. (Figure 3).

Discussion

In the present study, relatively high percentage of *C. albicans* infection was found among patients with hematological malignancies, solid tumor, asthma, diabetes mellitus and from patients with tuberculosis. These results are in accordance with those obtained by Ansari, et al [10] who showed that a fungal infection represents a growing problem in patients with hematologic malignancies. In particular, with chemotherapy-induced neutropenia, the majority of the infections were referred as *C. albicans* (74.7%). 11. Ramirez-Garcia, et al [11] reported that, the opportunistic fungus *C. albicans* increases the risk of carcinogenesis and metastasis. In another's study, *C. albicans* was isolated from sever patients with diabetes mellitus [12], while Kali et al [13] mentioned that Candida co-infection was observed in 40% of patients with pulmonary tuberculosis.

Many factors are accused for this increment in fungal infections, the most important of which is massive and prolong use of immunosuppressive drugs. Most of these drugs are chemotherapies intended to treat malignancies. However, they reversely affect the immune system and eventually enhance the opportunistic microorganisms, to invade the body [14]. Beside chemotherapies, the

invasive method for diagnosis or treatment in intensive care unit may facilitate the contamination with *C. albicans.* Other factors, like personal hygiene, also have a role [14].

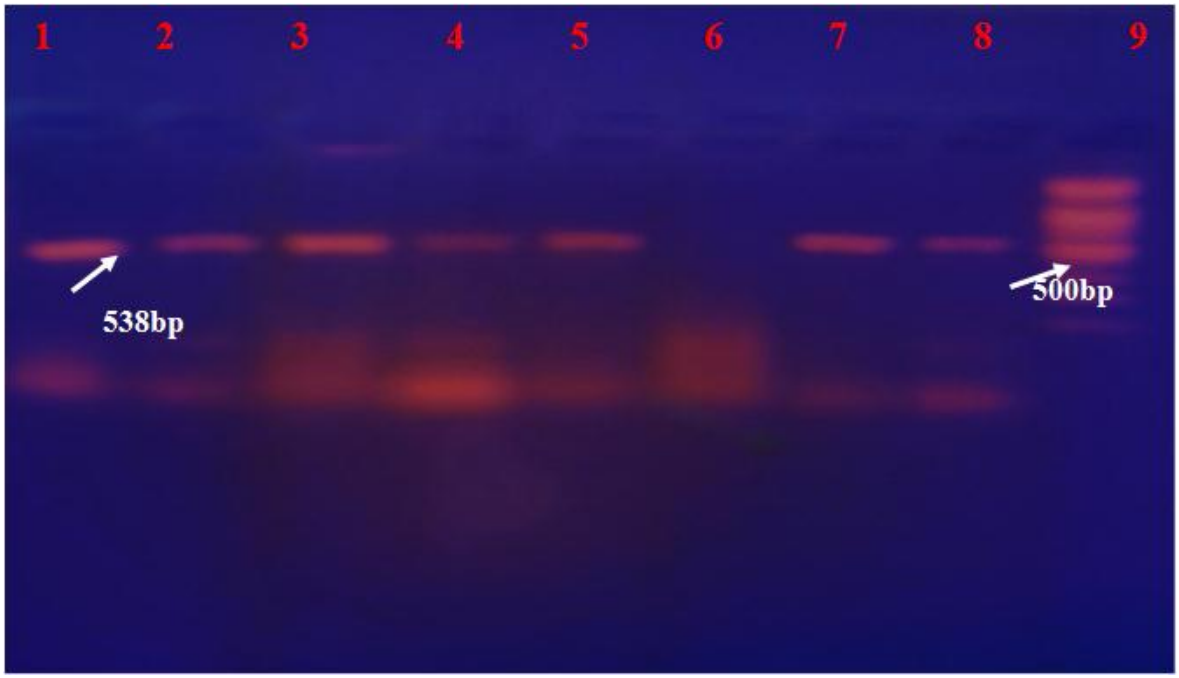

Figure 3. Gel electrophoresis (2% agarose, 7v/cm2, 1.5hrs) of the PCR products, lane 9(MW): 100 bp DNA ladder; lane (1-5 &7) Positive sample for (PLB genes of Candida albicans gene538 bp); lane 6: Negative control. Lane 8: Positive control

Microscopic examination of sputum using staining methods remains popular in the diagnosis of pulmonary infection especially in low-income countries, due to its rapidity, low cost, relatively easy to perform and high positive predictive value [15]. On the other hands, culture is considered to be the "gold standard" method for the diagnosis of pulmonary infections but require 20 to 100 viable organisms per sample and this is a cumbersome in partially treated patients. Culture also labor intensive and time-consuming [16].

In this study, positive cultures were tested by germ tube and biochemical API 20 AUX. Results of germ tube revealed that 53.4% of positive culture were *C. albicans*. All germ tube positive samples were positive for *C. albicans* by API 20 AUX. The API 20 AUX, and germ tube technique provides a convenient and reliable methods for identification of *C. albicans*.

Twenty three samples were positive for PLB gene, which is specific for *C. albicans*. The PLB gene of Candida species is a novel target, which shows a high variability of sequences among Candida. The nucleotide sequence variability between the different species of Candida can reach 95% [17]. Thus, it is possible through designing specific set of primers to target the unique sequence of PLB gene.

Being used the same set of primers as in the current study Harmal et al [9] proved that species-specific PCR assay could identify and differentiate between the four most common Candida species isolated from clinical specimens namely, *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. tropicalis*. Distinctive product size for each of these 4 species allow specific identification directly from the gel electrophoresis without the need for further genotyping.

Based on molecular weight of amplicon product from that PCR product of this gene, it

was 404 bp in Candida glabrata and 252 bp in *C. parapslosis* [18,19].

In conclusion, *Candida albicans* is an important fungal infection formed a high percentage among patients suffering from bronchopulmonary symptoms. The molecular and conventional methods gave concomitant results as detection tools for the diagnosis of such microorganisms.

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Author contribution

Dr. Al-Attraqchi made the drafting of the article and revising it critically for important intellectual content. Dr. Dawood was responsible for samples and patients selections. Dr. Hassan made the DNA extractions, molecular, conventional methods diagnosis, analysis and interpretation of results and statistical analysis, while Hadab was responsible for the design and acquisition of data, molecular material supplementation.

Conflict of interest

The author declares that they have no competing interests.

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Molecular Characterization of the Oncogenic Potential and Mechanisms of Cytomegalovirus Infecting MRC-5 Cells

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Abstract

Background Human cytomegalovirus (HCMV), a member of herpes virus family, is associated with different cancers in humans, including breast and colorectal cancers.

Objectives To shed light on the possible oncogenic mechanisms exerted by HCMV on tissues at the genetic level.

Methods HCMV was propagated in medical research council-5 (MRC-5) cell line and cytopathic effects of HCMV were checked for confirming infection. Oncogenic mechanism that exerted by HCMV was screened using high-throughput real-time qPCR microarray to investigate the mRNA expression of 983 genes in 16 gene families associated with oncogenesis in human cells.

Results Twenty differentially expressed genes. Upregulated genes (SCGB1A1, MAP2K3, ITGB3, TGFB1, PTN, MMP2, AKT1, AKT2, BCL2L10, CKS1B, SERPINB4, CASP4, TOLLIP, BCL-2, SERPINB5) were mostly of oncogenic or oncopromoting activity, involved mainly in MAPK, PI3K/Akt, inflammation, and angiogenesis pathways while downregulated genes (STAT3, BAK1, BLM, RB1, and IGF2R) were mostly tumor suppressor, proapoptotic, and DNA damage repair genes.

Conclusion The current study revealed that HCMV might have an oncogenic or oncomodulating activity on normal infected cells by different intracellular pathways.

Keywords Cytomegalovirus, oncogenic viruses, mrc-5, Waffergen, SmartChip Real-Time PCR System.

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List of Abbreviation: HCMV =Human cytomegalovirus, MRC-5 = Medical research council-5

Introduction

Human cytomegalovirus (HCMV) is a ubiquitous herpes virus that leads to a life-long persistence. The frequency of infection ranges from 50-100% in the community [(1)]. HCMV, species *Human herpes virus 5*, belongs to genus *Cytomegalovirus*, subfamily *Betaherpesvirinae*, family *Herpesviridae*. HCMV is a leading agent of complications of organ transplantation, congenital malformations, non-heterophil infectious mononucleosis, and life-threatening morbidities in immunocompromised individuals [(2)]. In addition, active and/or latent infection of HCMV has been recently linked with different human cancers including breast, colorectal, prostate, and malignant glioma cancers [(3)].

In vivo, HCMV is capable of infecting many cell types including fibroblasts, endothelial cells, smooth muscle cells, hepatocytes, macrophages and lymphocytes, and tumor cells [(4)]. Medical research council-5 (MRC-5) cells are human diploid fibroblast cell line approved for vaccine production and favored for the routine propagation of HCMV [(5)]. MRC-5 cells have been used as a standard for over 30 years in basic research for HCMV infection. Moreover, MRC-5 cells have the ability of 46 cells doublings, enabling prudent exploit of these cells in assessing intracellular changes after exposure to HCMV [(5)].

The relationship between HCMV infection and cancer has been investigated for decades. Presence of HCMV DNA, mRNA, and its proteins in tumor tissues indicated a probable role of HCMV infection in the causation of several human malignancies [2]. Moreover, sero-epidemiologic evidence added more linkage between HCMV and human cancers [6]. It was shown that HCMV can transform normal human embryonal cells in vitro [7]. Other studies reported that HCMV infection in normal rodent cells affects cellular genes that are critical for malignant transformation [8,9]. However, there is controversy on the carcinogenic effect of HCMV on normal cells. It was suggested the oncomodulation concept of HCMV, which refers to the capability of HCMV to preferentially infect tumor cells and enhance their malignant profile lead to a shift to a more malignant phenotype of tumor cells and tumor progression [10]. Several studies showed that the presence of HCMV in tumor cells but not in adjacent normal tissue was more than 90% of patients with certain malignancies, such as colon cancer, malignant glioma, prostate carcinoma, and breast cancer [11-14]. Nevertheless, in the context of the oncogenic or oncomodulation scenarios, by all means, HCMV, seems to have a direct or indirect role in carcinogenesis.

There have been numerous studies on the oncogenic and oncopromoting potential of HCMV on tumor cell lines such as neuroblastoma cell lines [15-17]; however, no such coverage was done on the oncogenic potential of HCMV, if any, on normal cell lines such as MRC-5 cells using high throughput quantitative PCR microarray. Therefore, this study aimed at covering a different aspect of HCMV relationship with cancers as this study focused on the changes at genetic expression of normal cells, MRC-5 cell line, as a response to HCMV infection. This approach is believed to shed light on HCMV oncogenic/ oncomodulating potential on normal cells rather than previously researched tumor cells. Hence, this study was designed to investigate the mechanisms of oncogenesis, or oncomodulation, if any, by HCMV on normal diploid cells by using a high throughput SmartChip quantitative real-time PCR microarray. This study explored 983 genes that may have a role in carcinogenesis and transformation of human cells and tried to give a complete map for the target genes which might be up- or down- regulated by this virus. This will facilitate the understanding of the possible oncogenic and/or oncomodulating mechanisms exerted by HCMV on human cells.

Methods

This study was conducted in multidiscipline laboratories of Kent State University. It is a single authored project funded by Kent State University, Ohio, USA and Fulbright organization and was conducted during the period from July 5th to September 15th 2012.

Cells and viruses

MRC-5 human diploid fibroblasts passage 6 was received from American Tissue Culture Collection (ATCC CCL-171, USA). Cells were cultured in Eagle's minimum essential medium (EMEM) supplemented with Earle's salts containing 10% (v/v) fetal calf serum, 1x10^6 IU/l penicillin, 120 mg/l streptomycin and 2 mM glutamine. Subcultivation ratio of CCL-171 was 1:2 to 1:5 and the medium renewal once every 5-6 days. Flasks of 75 qcm were used in propagation of MRC-5 cells and incubated at humid 37 °C with 5% CO_2 incubator.

MRC-5 cells were subcultured by removing and discarding culture medium. Briefly by rinsing the cell layer with 0.25% (w/v) Trypsin 0.53 mM EDTA solution to remove all traces of serum, which contains trypsin inhibitor. Then, 2.0 to 3.0 ml of Trypsin-EDTA solution were added to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Cells that are difficult to detach may be placed at 37 °C to facilitate dispersal. Afterwards, 6.0 to 8.0 ml of EMEM complete growth medium were added and resultant cell suspension was

aspirated by gently pipetting and appropriate aliquots of the cell suspension were added to new culture vessels and incubated at 37 °C until next subculturing [18].

HCMV infections were performed using high-passage strain AD169, obtained from ATCC. About $1x10^5$ cells of MRC-5 were suspended in 1 ml of cell-free AD169 suspension ($1x10^5$ pfu/ml) for 3 hours at multiplicity of infection (MOI) equal to 1. Afterwards, infected MRC-5 cells were centrifuged at 3500 g for 5 minutes and pellet was resuspended in fresh EMEM medium; washing step was repeated 3 times. Then, HCMV-infected MRC-5 cells were plated onto Corning® T75flasks 75 sq.cm flask for 4 weeks with weekly renewal of EMEM medium and cells subculturing. Cytopathic effects of HCMV were screened routinely for the confirmation of the persistence of HCMV infection.

Two sets of MRC-5 cells were grown for 4 weeks, the first set was infected with HCMV, AD169, virus and the second set was not infected. Stringent measures were pursued to separate two sets apart in order not mix infection. Vital cell count was performed at every freshening step for both sets. The vital count difference between two sets was not more than 7%, being HCMV-infected cells of lower count. So, 7% difference in cell vitality was not considered significant factor that could alter genetic comparison between the two sets of cells.

Flow cytometry analysis

After 4-week interval of growth and viral propagation, HCMV- infected and noninfected MRC-5 cells were harvested and transferred to 15 ml tubes. All of the tubes were centrifuged at 300 g for 8 minutes. The supernatants were discarded and the pellets were washed two times by cold PBS. Then, pellets were resuspended in 70% ice-cold ethanol with PBS, 1:10 v/v. Then, all the supernatants were aspirated after centrifugation at 500 g for 8 min. The washing step by PBS was repeated and the supernatants were aspirated. A cell count was performed and the number of cells adjusted to $1x10^5$ cell/ml with cold PBS and then, 100% methanol was added and then stored at -20 °C until staining. The method used was according to Jacobbergar and Lehman [19]. Briefly, after removal of fixative, the cells were resuspended in flourochrome-labeled antibody to CMV early nuclear antigen using case-customized kit (Dako, Denmark, No. 1284) diluted in PBS-130 mM NaCl, 15 mM phosphate (pH 7.4), and 1:1 volume of heat-inactivated normal goat serum with 0.004% Triton X-100 and 0.2% sodium azide. The antibody was incubated with the cells for 90 min at 37 °C and washed 3 times in cold PBS. Then, MRC-5 cells were incubated for 30 minutes at 37 °C in 0.5 ml of RNase A (Qiagen, Germany) and samples were then stored in the dark at 4 °C overnight. The assay was measured in duplicates for each sample. The percentage of CMV-infected cells was measured using CyAn ADP apparatus (BECKMAN COULTER, USA). The software Summit (V4.3) was used to analyze the flow cytometry results.

SmartChip Real-Time PCR System

SmartChip Human Oncology Gene Panel version 1.5.1 from (WaferGen, BIOSYSTEMS, Fremont, CA, U.S.A.) enables gene expression profiling of 4,128 reaction wells on a single sample using the SmartChip Nanodispenser (WaferGen, BIOSYSTEMS, Fremont, CA, USA). SmartChip panels are preloaded with pre-optimized PCR primers that have been validated in both microliter- and nanoliter-scale SYBRGreen real-time PCR experiments. The primers used to investigate 983 human oncogenesis-related genes in quadruplicate belonging to 16 functional groups (Table 1), 9 endogenous controls (Table 2) and 6 exogenous yeast controls. The SmartChip Human Oncology Gene Panel was used to measure differential gene expression from 0.5 µg of RNA of HCMV-infected MRC-5 cells versus that of non-infected MRC-5 cells; only

significantly up- and down-regulated genes are displayed in this study.

Table 1. The gene families covered by high throughput Real-Time qPCR System using WaferGen SmartChip Human Oncology Gene Panel

Gene Family
ADME*
Apoptosis
Cancer
Cell Cycle/Proliferation
Cardiovascular Disease
DNA damage repair
Drug Target
G-protein coupled receptor
Growth factor
Homeostasis/Metabolism
Inflammation
Kinase
Proteinase
Signal Transduction
Transcription Factor
NeuroDisease/Phosphotase

*Drug absorption, distribution, metabolism and elimination genes.

Table 2. Endogenous internal control (reference genes) used in SmartChip Real-Time PCR System: all of reference genes were amplified and used successfully for normalization

Primer	Functional	failed	Total	Ct	Ct SD	Tm	Tm SD
TAF10	4	0	4	25.34	0.359	79.0	0.155
GUSB	4	0	4	30.58	0.320	82.2	0.099
TAF11	4	0	4	28.38	0.763	81.4	0.164
HPRT	4	0	4	26.69	0.193	81.9	0.101
PPIA	4	0	4	21.43	0.078	85.0	0.068
HMBS	3	0	3	29.66	1.25	83.2	0.088
ACTB	4	0	4	25.33	0.750	87.2	0.178
B2M	4	0	4	21.94	0.129	85.4	0.085
TAF1L	4	0	4	29.00	1.10	83.1	0.058

RNA extraction

RNA extraction was done according to the guidelines and protocols provided by Wafergen Bio, USA. 1×10^6 MRC-5 cells were harvested and transferred to 15 ml tubes. After centrifuging the tubes at 300 g for 5 minutes, the supernatants were discarded. The pellets were resuspended in PBS and were washed for 3 times. Total RNA was isolated using RNeasy Mini Kit (Qiagen, Germany: Cat No./ID: 74104) Briefly, according to the manufacturer's protocol for RNA isolation from cell culture, cells were resuspended in 700 µl of lysis buffer (Buffer TR) with vigorous vortexing to inactivate cellular RNases together with cell lysis. Later, the lysed cells were transferred to homogenization columns. The columns were centrifuged at 10.000 × g

for 2 min. The flow-though was saved and equal volume of80% ethanol (700 µl) was added. The lysed cells were transferred into RNA binding columns that were centrifuged at 10.000 × g for 1 min and the flow-through was discarded. Columns were washed using washing buffer and then DNA fragments were removed by DNase I treatment. Further centrifugation at 10.000 × g for 1 min was done to remove any traces of used buffers. Total RNA was collected by placing the columns into new 1.5 ml microtubes with 60 µl RNase-free water addition and standing for 1 min. The microtubes were centrifuged at 10.000 × g for 1 minute.

RNA quality and quantity were determined by Life Science UV/Vis Spectrophotometer, DU Series 700 (BECKMAN COULTER, USA). RNA concentration used in downstream experiments ranged from 503 to 524 ng/µl. Moreover, total RNA quality was checked by Agilent 2100 Bioanalyzer (Agilent, USA) and RNA integrity number (RIN) was measured. RIN of total RNA was 9.1 and passed the quality control limit for running downstream experiments.

Synthesis of cDNA

The reverse-transcription of up to 0.5 µg of the isolated MRC-5 cell's RNA was done using iScriptTM cDNA Synthesis Kit (BIO-RAD, Hercules, Canada). According to the manufacturer's protocol, 4 µl of 5 × iScript reaction mix were mixed with 1 µl iScript reverse transcriptase and 15 µl of RNA template in 1.5 ml microtubes to give final volume of 20 µl per reaction. The complete reaction mix was incubated for5 min at 25°C then for 30 min at 42 °C. The incubation temperature was increased to 85°C for 5 min. Finally, cDNA was stored at −80 °C for qRT-PCR reaction.

Real-time quantitative RT-PCR

Real-time quantitative PCR reaction was conducted according to the procedure validated by (Wafergen Bio, USA) using the SmartChip human oncology panel version 1.5.1. These chips were preloaded with primer content optimized for performance with the high throughput Real-Time PCR System. The SmartChip Nanodispenser (Wagergen Bio, USA) was used to dispense the sample, combined with master mix and controls, under vacuum into the SmartChip. Five hundred (500) ng of starting sample of cDNA yielded cDNA equivalent of 96 pg of sample per well. Once loaded with sample, the SmartChip was placed into the WaferGen SmartChip Cycler Nanodispenser (Wagergen Bio, USA). The SmartChip Real-Time PCR system employs a qPCR reaction compatible with SYBR green I DNA binding dye. Results were reported in the form of Ct (threshold cycles), Tm (melting temperatures), and amplicon melting curve analyses.

Primers used are based on criteria such as specificity, insensitivity to sequence polymorphisms, amplicon size, and minimization of primer artifacts. Primer specificity was determined using (a) melting curve analysis of amplicon product, (b) gel electrophoresis analysis of amplicons and (c) sequence verification of the amplicons. The up- and down- regulation of genes were analyzed by SmartChip Software (Wafergen Bio, USA). The sensitivity of real-time PCR is 20-30 copies of RNA per well with specificity of genes discrimination <90% homology. To keep precision of readings, standard deviation <0.25 Ct between replicates was used. For valid runs, all primers shared the same or close-by Tm and amplicon size and no primer dimers or primer secondary structures were allowed. Thermal cycling of primers was 95C for 180 sec once, and for 40 cycles: 95C for 60 sec, and 60 C for 70 sec with amplicons size ranged from 80 to 120 nucleotides. Automatic screening for low efficiency wells turned out to be zero and PCR efficiency for all primers was measured and taken into account for calculating fold changes in genes expression. All-means normalization method, where the means of all expressed genes are employed, was used in

the current study. In addition, six exogenous yeast controls were used (WGBS-YCF1-6); at least four of them should be detected and be linear in proportion. At each run two negative tissue controls (NTC), chip no. 34565 and 35935, and two positive tissue controls, brain tissue (PTC), chip no. 34576 and 35939, were used.

The fold change calculation used was based on the Comparative Ct Method. The comparative Ct Method is also known as the 2-ΔΔCt method and as follows: ΔΔCt = ΔCt treated – ΔCt untreated, where: ΔCt treated = Ct treated – All Mean treated and ΔCt untreated = ΔCt untreated – All Mean untreated. The significant up/down regulated genes are those genes with at least two fold change.

Data analysis

The data in the current study are shown as mean±2SD.The data analysis was conducted by using SPSS software version (11.0.0.1). Simple percentage calculation was used to obtain the % of HCMV-infected cells by Flow cytometry assay. For real-time qPCR, SmartChip qPCR software exerted all data analysis. P values less than 0.05 were considered significant.

Results

Percentage of MRC-5 cells infected with HCMV

After 4 weeks propagation of HCMV, AD-169 strain, in MRC-5 cells, the percentage of infected cells was measured by using flow cytometry method. The percentage of HCMV-infected MRC-5 cells was shown to be 94.1%. This finding indicated that MOI 1 of MRC-5 cells with AD-169 strain of HCMV is sufficient to attain near complete infection of all exposed cells after 4 weeks of viral propagation. This step is essential to confirm the homogeneity of real-time qPCR results of gene expression profiling.

SmartChip Real-Time PCR System

After the completion of real-time PCR assay, descriptive statistics of SmartChip panel technical results are shown in Table 3 and Figure 1. In addition, the setting values used for SmartChip RT-PCR panel are shown in Table 4.

Table 3. The technical results of the SmartChip oncogenic panel used in the current study

Name	Value
Total wells	4128
No amplification	1648
Curve fit failed	232
With curves	2248
Bad R2	0
Low efficiency	0
High efficiency	0
Ct is small	0
Ct is large	136
Low saturation	0
Saturation/Baseline ratio is low	1
Multiple peaks	62
Multimodal Tm's	14
Informative	302
Replicates without curves	347

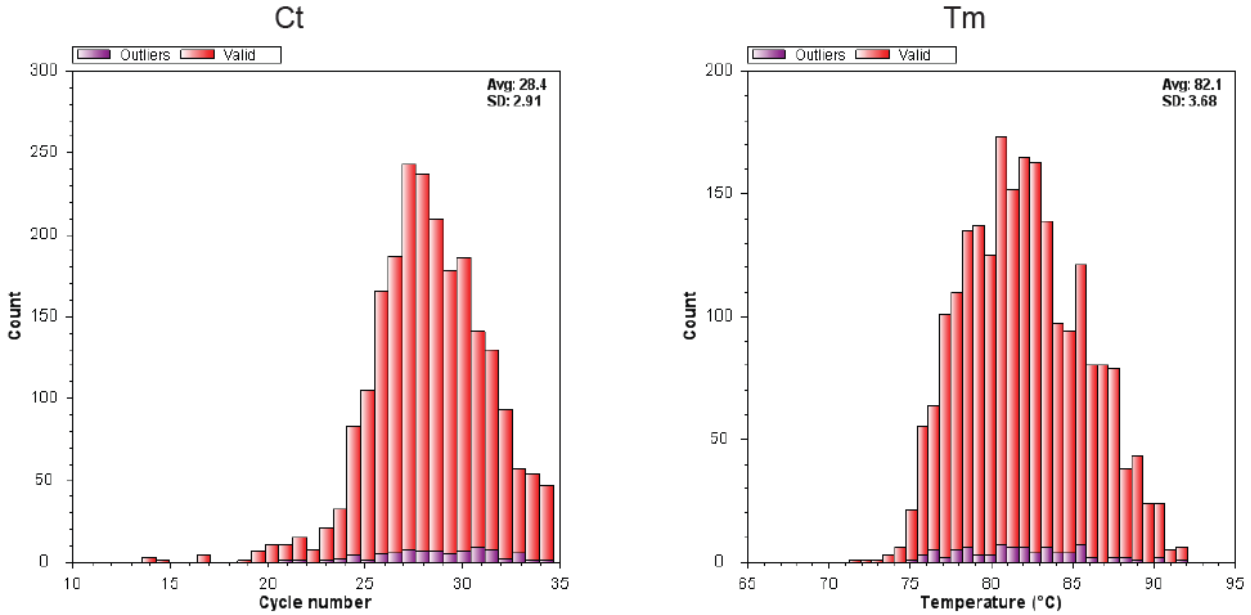

Figure 1. Histogram of the frequency of Ct and Tm for SmartChip RT PCR showing that Ct 25 to 30 cycles and Tm 75 to 85 C were dominant among different primer sets used for both HCMV infected and non-infected SmartChiponcopanels

Table 4. The settings and values of the RT-PCR SmartChip assay

Threshold	Value
Ct max deviation	0.5
Fail curve based on R2	0.99
Grubbs confidence	0.05
Grubbs Min SD	0.25
High Ct	33
High efficiency	3
Informative replicates SD	0.6
Low Ct	5
Low efficiency	1.5
Low saturation	1000
Low saturation/baseline ratio	1.8
Max Baseline drift	21
Max Ct	35
Max efficiency	3.5
Melt multimodel	1
Melt peak left	0.6
Melt peak right	0.3
Min amplification	500
Min efficiency	1.5
No amplification ratio	2
Point used for curve fit	15
R2	0.99

Detection of the up- and down-regulation of gene expression by SmartChip Real-time PCR System

The quantitative analysis by real-time PCR revealed a complete picture regarding the up- or down-regulated genes in HCMV-infected MRC-5 cells compared to that of non-infected cells. The results showed that almost all genes were expressed in a very close level (less than 2 folds) while only 15 genes were upregulated and 5 genes were downregulated (Table 5; Figure 2). Although the number of genes that showed differential expression was totally 20, this variation has its own importance due to the association of the affected genes to critical cell functions including cancer, apoptosis and cell cycle regulation. It is noteworthy to mention that most of the genes up- or down-regulated in the current study belong to gene families of multiple functions (Table 6), for example a gene family is related to inflammation, cancer, signal transduction and apoptosis at the same time.

Discussion

MRC-5 cell line was chosen and AD-169 strain of HCMV was used in order to propagate HCMV in normal diploid cells for 4 weeks ensuring long-term infection that is necessary to observe any variation in genes expression. The limitation of this study is the same as its advantage, namely MRC-5 cells. Breast, colon and prostate tissue cells are more prone to cancers where HCMV is implicated. However, CMV grows slowly in other cell lines and the initial trials of this study for infecting HCMV in cell lines other than MRC-5 turned out with shorter periods of HCMV propagation as well as much higher cell death [data not shown]. Hence, MRC-5 cells were preferred over other types of cells.

Table 5. Fold change and Log2 Fold change of the up- and down-regulated gene expression in HCMV-infected MRC-5 cells compared to HCMV non-infected cells

Primer	ΔΔCt	Fold Change	Log2(Fold Change)
SCGB1A1	-3.927	15.211	3.927
MAP2K3	-3.215	9.285	3.215
ITGB3	-2.287	4.879	2.287
TGFB1	-2.253	4.767	2.253
PTN	-1.890	3.708	1.890
MMP2	-1.889	3.704	1.889
AKT1	-1.770	3.410	1.770
AKT2	-1.757	3.381	1.757
BCL2L10	-1.756	3.378	1.756
CKS1B	-1.400	2.639	1.400
SERPINB4	-1.311	2.482	1.311
CASP4	-1.164	2.240	1.164
TOLLIP	-1.108	2.156	1.108
BCL-2	-1.078	2.111	1.078
SERPINB5	-1.003	2.004	1.003
STAT3	1.020	0.493	-1.020
BAK1	1.031	0.489	-1.031
BLM	1.031	0.489	-1.031
RB1	1.152	0.429	-1.223
IGF2R	1.194	0.211	-2.248

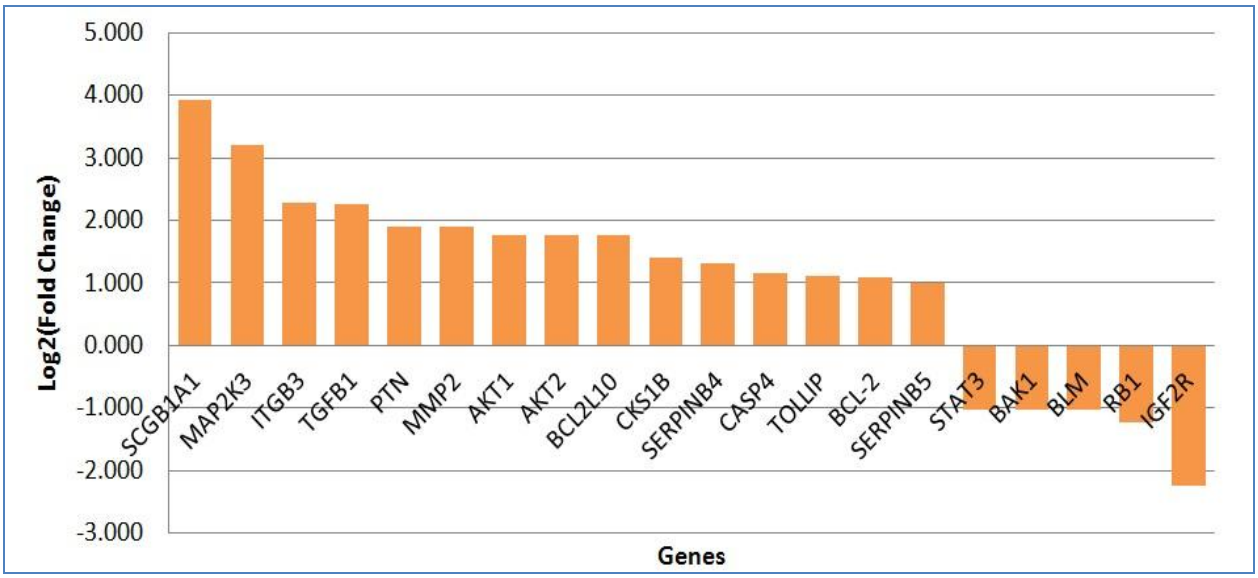

Figure 2. A diagram showing 15 up-regulated and 5 down-regulated gene expression in HCMV-infected MRC-5 cells compared to HCMV non-infected cells

Table 6: Gene families and gene functions/pathways involved in the differentially expressed genes between HCMV-infected and HCMV non-infected MRC-5 cells

Up-regulated Gene	Gene family	Gene product related function/pathway/ By Genecards [21]	Down-regulated gene	Gene family	Gene product related function/pathway/ By Genecards [21]
SCGB1A1	Inflammation	SCGB1A1 (Secretoglobin, Family 1A, Member 1. Among its related pathways are FOXA1 transcription factor network and Prostaglandin Synthesis and Regulation. this gene product binds to phospholipase A2 inhibitor activity	STAT3	Cancer; Inflamation; Signal Transduction; Transcription Factor;	STAT3 (Signal Transducer And Activator Of Transcription 3 (Acute-Phase Response Factor)) is a Protein Coding gene. Diseases associated with STAT3 include hyper ige syndrome and hyper-ige recurrent infection syndrome. Among its related pathways are Immune System and Signaling by GPCR
MAP2K3	Kinase; Signal Transduction;	MAP2K3 (Mitogen-Activated Protein Kinase Kinase 3) is a Protein Coding gene. Among its related pathways are MAPK signaling pathway and Immune System. GO annotations related to this gene include transferase activity, transferring phosphorus-containing groups and protein tyrosine kinase activity	BAK1	Apoptosis	BAK1 (BCL2-Antagonist/Killer 1) is a Protein Coding gene. Diseases associated with BAK1 include benign mammary dysplasia and chronic gonococcal salpingitis. Among its related pathways are Apoptotic Pathways in Synovial Fibroblasts and ERK Signaling
ITGB3	Cancer; Cardiovascular Disease	ITGB3 (Integrin, Beta 3, Glycoprotein IIIa). Among its related pathways are PI3K-Akt signaling pathway and Immune System	BLM	DNA Damage Repair	BLM (Bloom Syndrome, RecQ Helicase-Like) is a Protein Coding gene. Diseases associated with BLM include bloom syndrome and Werner syndrome. Among its related pathways are Cell Cycle, Mitotic and Integrated Pancreatic Cancer Pathway. GO annotations related to this gene include nucleic acid binding and ATPase activity
TGFB1	ADME; Apoptosis; Cancer; Cardiovascular Disease; Inflamation; Signal Transduction;	TGFB1 (Transforming Growth Factor, Beta 1) is a Protein Coding gene. Diseases associated with TGFB1 include camurati-engelmann disease and cystic fibrosis. Among its related pathways are MAPK signaling pathway and Signaling by GPCR	RB1	Cell cycle checkpoint	RB1 (Retinoblastoma 1) is a tumor supressor protein Coding gene. Diseases associated with RB1 include retinoblastoma and familial retinoblastoma. Among its related pathways are Pathways in cancer and Glioma.

PTN	Cancer; Cardiovascular Disease; Inflamation;	PTN (Pleiotrophin) is a Protein Coding gene. Among its related pathways are NF-KappaB Family Pathway and Apoptotic Pathways in Synovial Fibroblasts.	IGF2R	Growht factor	IGF2R (Insulin-Like Growth Factor 2 Receptor) is a Protein Coding gene. Diseases associated with IGF2R include hepatocellular carcinoma. Among its related pathways are Apoptotic Pathways in Synovial Fibroblasts and GPCR Pathway.
MMP2	Cancer; Cardiovascular Disease	MMP2 (Matrix Metallopeptidase 2) is a Protein Coding gene. Among its related pathways are Developmental Biology and Pathways in cancer.			
AKT1	ADME; Apoptosis; Cancer; Cardiovascular Disease; G-Protein Coupled Receptor; Inflamation; Kinase; Signal Transduction;	AKT1 (V-Akt Murine Thymoma Viral Oncogene Homolog 1) is a Protein Coding gene. Diseases associated with AKT1 include breast adenocarcinoma and proteus syndrome, somatic. Among its related pathways are PI3K-Akt signaling pathway and PI3K/AKT Signaling in Cancer			
AKT2	Signal Transduction	AKT2 (V-Akt Murine Thymoma Viral Oncogene Homolog 2) is a Protein Coding gene. Among its related pathways are PI3K-Akt signaling pathway and PI3K/AKT Signaling in Cancer. An important paralog of this gene is AKT1.			
BCL2L10	Apoptosis	BCL2L10 (BCL2-Like 10 (Apoptosis Facilitator)) is a Protein Coding gene. Diseases associated with BCL2L10 include prostatic cyst. Among its related pathways are Apoptosis (WikiPathways) and Apoptosis and Autophagy.			
CKS1B	Kinase	CKS1B (CDC28 Protein Kinase Regulatory Subunit 1B) is a Protein Coding gene. Among its related pathways are Pathways in cancer and Cell Cycle, Mitotic.			
SERPINB4	Angiogenesis;	SERPINB4 (Serpin Peptidase Inhibitor, Clade B (Ovalbumin), Member 4) is a Protein Coding gene. Diseases associated with SERPINB4 include inverted papilloma and tongue squamous cell carcinoma. Among its related pathways are Amoebiasis			
CASP4	Apoptosis, Drug target	CASP4 (Caspase 4, Apoptosis-Related Cysteine Peptidase) is a Protein Coding gene. Diseases associated with CASP4 include hepatitis c virus. Among its related pathways are Immune System and Apoptotic Pathways in Synovial Fibroblasts			
TOLLIP	Inflamation; Signal Transduction	TOLLIP (Toll Interacting Protein) is a Protein Coding gene. Diseases associated with TOLLIP include lymphoproliferative syndrome, x-linked, 1. Among its related pathways are Immune System and Interleukin receptor SHC signaling.			
BCL-2	Apoptosis	BCL2 (B-Cell CLL/Lymphoma 2) is a Protein Coding gene. Diseases associated with BCL2 include follicular lymphoma and intravascular large b-cell lymphoma. Among its related pathways are PI3K-Akt signaling pathway and Immune System.			
SERPINB5	Angiogenesis	SERPINB5 (Serpin Peptidase Inhibitor, Clade B (Ovalbumin), Member 5) is a Protein Coding gene. Diseases associated with SERPINB5 include mandibular cancer and syringoma. Among its related pathways are MicroRNAs in cancer and Angiogenesis (CST)			

Using high throughput platforms of real-time qPCR is superior on using gene expression microarrays. The reason is that high throughput real-time qPCR is highly quantitative and precise, unlike that in gene expression microarrays which are precise but not quantitative [20]. The current study screened which genes are differentially expressed when MRC-5 cells are infected with HCMV. Results showed that 15 genes were upregulated in HCMV-infected cells belonging to collectively 11 gene families namely, inflammation, kinase, signal transduction, cancer, cardiovascular disease, ADME, apoptosis, cancer, G-protein coupled receptor, angiogenesis, and drug target. In addition, expression of 5 genes were downregulated in HCMV-infected cells belonging collectively to 9 gene families namely, kinase, apoptosis, DNA damage repair, cell cycle checkpoint, cancer, inflammation, signal transduction, transcription factor, and growth factor.

Taking each gene family separately for the upregulated genes, five genes have inflammation downstream effect including SCGB1A1, TGFB1, PTN, AKT1, and TOLLIP and six genes showing kinase/signal transduction effect namely, MAP2K3, TGFB1, AKT1, AKT2, CkS1B, and TOLLIP and five genes belong to cancer promoting family of genes including ITGB3, TGFB1, PTN, MMP2, and AKT1 and five genes related to apoptosis including TGFB1, AKT1, BCL2L10, CAPS4, BCL-2 and two genes related to angiogenesis namely SERPINB4 and SERPINB5.

Gene function analysis of the upregulated genes shows an important observation that the majority of the gene functions are oncogenic and/or oncopromoting directly or indirectly. SCGB1A1 gene is a Secretoglobin, Family 1A, Member 1; among its related pathways are FOXA1 transcription factor network and Prostaglandin Synthesis and Regulation [21]. So, this gene is pro-inflammatory and was upregulated more than 15 folds indicating HCMV induce inflammation through this gene. It is well known that inflammation is one mechanism of cancer promotion and initiation [22] especially in long lasting infections [23]. Another gene is associated with inflammation and cancer, PTN which is a Pleiotrophin; among its related pathways are NF-KappaB Family Pathway and Apoptotic Pathways [21]. Pleiotrophin, PTN, gene was found to elicit pro-tumorigenic effects through its receptor, protein tyrosine phosphatase receptor, Z1 (PTPRZ1) [24]. Moreover, mitogen-activated protein kinase (MAPK) family were found to be likely effectors of PTN signaling and is associated with ovarian cancer [24]. Another gene, TOLLIP (Toll Interacting Protein), is also associated with inflammation and signal transduction; among its related pathways are immune system and interleukin receptor SHC signaling [21]. TOLLIP has been linked strongly with lung, prostate, breast cancers and melanoma [25]; and related to activation of interleukin-1 (IL-1) receptor (IL-1R), Toll-like receptor 2 (TLR2), and TLR4 which trigger NF-kappaB and MAPK-dependent signaling, thereby initiating immune responses and maybe tumors [26].

MAP2K3 gene is a MAPK, Kinase 3; involved in MAPK signaling pathway and immune system. MAPK pathway has a clear role in carcinogenesis [21]. This kinase is activated by mitogenic and environmental stress, and participates in the MAPK-mediated signaling cascade [27,28]. Expression of K-ras oncogene is found to result in the accumulation of the active form of this kinase, which thus leads to the constitutive activation of MAPK14, and confers oncogenic transformation of primary cells [29]. It is well known that K-ras expression is strongly associated with carcinogenesis [30]. Therefore, HCMV might acts like a K-ras oncogene in inducing transformation. Another upregulated gene involved in MAPK pathway was TGFB1, which also triggers local immune suppression [31] and induces cancer [32]. Enhanced expression of TGFB1 was found to enhance several cancer-related events such as

cell division, cell motility, and the development of new blood vessels, angiogenesis. The TGFβ-1 protein is abnormally overexpressed in certain types of prostate, breast, colon, lung, and bladder cancers [33].

One of the interesting findings of the current study is that three upregulated genes are involved in PI3K/Akt pathway, which has a well-known role in carcinogenesis [34]. These genes are ITGB3, AKT1 and AKT2. Phosphatidylinositol 3 kinase, or PI3K activates Akt, which modulates the function of numerous substrates involved in the regulation of cell survival, cell cycle progression and cellular growth [21,34]. It has been shown that PI3K/Aktsignalling pathway components are frequently altered in human cancers [35]. It was found that survival signals counteracting apoptosis induced by several receptors are mediated mainly by PI3K/Akt; hence this pathway was found to decisively contribute to the resistant phenotype of many cancers [34,36]. The PI3K/Akt pathway is involved in many of the mechanisms targeted by new anticancer drugs such as Everolimus by Novartis, which is an immunosuppressant and recently approved in treatment of hormone therapy-resistant estrogen receptor-positive breast cancer by blocking some links in the activated PI3K/Akt pathway [37].

Regarding apoptosis-related genes that were upregulated after HCMV infection, the picture is complex and not so clear. Two upregulated genes induce apoptosis process, which are CASP4 and BCL2L10 while the third one is anti-apoptotic, BCL-2. This needs further investigations by separate experiments in order to elucidate the net effect of apoptosis exerted by HCMV on normal cells.

Another upregulated gene was Matrix Metallopeptidase 2 (MMP-2) gene encoding type IV collagenase. Among its related pathways are developmental biology and pathways in cancer [21]. MMP-2 and MMP-9 secretion is elevated in several types of human cancers and their elevated expression has been associated with poor prognosis [38]. The major implications of MMPs in cancer progression is their role in extracellular matrix degradation, which allows cancer cells to migrate out of the primary tumor to form metastases [39].

Serpin Peptidase Inhibitor, SERPIN, B4/B5 are two genes mildly upregulated by HCMV infection and both are related to enhanced angiogenesis. SERPINB4 is associated with inverted papilloma and tongue squamous cell carcinoma. SERPINB5 (Serpin Peptidase Inhibitor, Clade B Member 5) is associated with mandibular cancer. Among its related pathways are MicroRNAs in cancer and angiogenesis [21]. In addition, elevated levels of these two genes are associated with inflammatory reactions in atopic dermatitis and psoriasis [40]. Hence, these genes exert two mechanisms that might promote cancer status namely induction of inflammation and angiogenesis.

The last of the upregulated genes is CKS1B which is CDC28 protein kinase regulatory subunit 1B; among its related pathways are pathways in cancer and cell cycle, and mitosis [21]. The expression of CKS1B is elevated in multiple cancers, including breast cancer, lymphoma, myeloma, colon cancer, prostate cancer, and lung cancer [41]. Amplification and overexpression of CKS1B are strongly associated with lymph node metastasis and poor prognosis in breast and salivary cancer [42]. Generally, CKS1B is an essential factor in facilitating Skp2-dependent degradation of p27 [41].

There are only 5 downregulated genes after exposure to HCMV infection, which are: STAT3 (cancer, inflammation, signal transduction and transcription factor), BAK1 (pro-apoptotic), BLM (DNA damage repair), RB1 (cell cycle checkpoint), and IGF2R (growth factor) [21].

Downregulation of expression of all above mentioned genes except for STAT3 is of oncogenic, oncopromoting or oncomodulating effect [21]. Downregulation of BAK1 (BCL2-antagonist/killer 1) lowers the antagonism of BAK1 to BCL-2 leading to less apoptotic potential of the cells favoring cell

carcinogenesis indirectly [43]. For RB1 gene, it encodes Retinoblastoma 1which is an essential tumor suppressor protein that regulates cell cycle progression; lowered expression of RB1 product is strongly associated with cancer [44] as cell proceeds to mitosis without rate limiting control by the tumor suppressor protein, retinoblastoma. For BLM gene, it provides instructions for making RecQ helicases, enzymes that attach to DNA and unwind the double helix of the DNA molecule. This unwinding is necessary for several processes in the cell nucleus, including copying DNA in preparation for cell division and repairing damaged DNA [21]. Because RecQ helicases helps maintain the structure and integrity of DNA, they are known as the "caretakers of the genome". Hence, decreased expression of BLM helps damage DNA molecule and facilitates carcinogenesis in long term infection of HCMV [45,46]. For IGF2R (Insulin-Like Growth Factor 2 Receptor), diseases associated with IGF2R include hepatocellular carcinoma and in breast cancer [21]. IGF2R is a negative regulator of cell growth and a putative tumor suppressor gene and the regulation of M6P/IGF2r levels is critical in breast physiology and lowered expression of IGF2R is associated with various aspects of breast cancer [47]. The role of the differential expression of IGF2R between HCMV infected and non-infected seems important as it was downregulated by more than 4 folds and because of its important role in normal physiology of breast tissue [47] where HCMV is strongly linked to breast cancer.

In regard to STAT3 gene (signal transducer and activator of transcription 3), it comprises a family of cytoplasmic transcription factors that mediates intracellular signaling and nucleus transcriptional activity [21]. Constitutive activation of STAT3 was shown in a wide variety of human tumors, including hematological malignancies, head and neck, breast, lung, and gastric cancers [48]. Unlike other differentially expressed genes of this study, lowered expression of this gene seems of protective role against cancer. However, what is the exact role of the lowered STAT3 in HCMV infection is not yet clear. This needs further investigation. Nevertheless, one gene with anticancer activity versus numerous genes with cancerous activity seems of inferior effect.

So taken together, this study has concluded for the first time, that the effect of HCMV infection on the level of expression of 983 genes related directly or indirectly to oncogenesis was studied in normal non-cancerous cells. In this study, HCMV infection stimulated expression of several genes that might trigger or promote cancer by different mechanisms. Most important mechanisms observed were activating MAPK and PI3K/AKT pathways, inducing inflammation, inhibiting apoptosis, inducing angiogenesis, and inhibiting tumor suppressor proteins. Activation of MAPK and PI3K/AKT pathways and lowered tumor suppressor activity implies to tumor initiating more than tumor promoting effect, while inducing angiogenesis, inflammation, inhibiting apoptosis, and inducing extracellular matrix proteases implies to tumor promoting/modulating effect more than tumor initiating effect. This study is believed to direct the coming studies on investigating deeply the role of differentially expressed genes by HCMV infection. This can shed light on the exact mechanisms and pathways that HCMV might exert to initiate or promote/modulate human cancers.

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Conflict of Interest

The author declares that there is no any kind of conflict of interests regarding the publication of this paper.

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Brachial Artery Diameter as a Predictor of Endothelial Dysfunction in Sickle Cell Disease

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Abstract

Background Sickle cell disease is hematological disease that affect the endothelial function. The hemoglobinopathy in this disease triggers erythrocyte polymerization and the sickling process leads to vascular occlusion, tissue hypoxia and subsequent reperfusion injury, thus inducing inflammation and endothelial injury.

Objective To assess the value of brachial artery diameter measurement as a predictor of the state of endothelium in sickled individuals.

Methods Thirty patients with sickle cell disease (15 females and 15 males) with a mean age of (27.0±8.9 yr) and 30 healthy controls (18 females and 12 males) with a mean age of (29.7±9.1 yr) participated in the study. Assessment of endothelial function done by studying physiological parameters, which included flow-mediated dilatation and endothelial-independent dilatation of the brachial artery depending on the measurement of the diameter of the blood vessel.

Results Endothelial independent dilatation was significantly lower in sickle cell disease patients (21.71±6.96) in comparison with that of the control group (26.81±6.31) despite the findings that both base line brachial artery diameter and intima media thickness were not significantly different between both groups.

Conclusion Brachial artery diameter assessment is a useful noninvasive predictor of endothelial dysfunction in patients with sickle cell disease. The reproducibility of the test in addition to its low cost and being free of biological hazards makes it optimum for assessing the state of the endothelium and may be used to monitor the response to treatment.

Keywords Sickle cell disease, flow-mediated dilatation, endothelial-independent dilatation, brachial artery diameter.

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List of abbreviation: ED: Endothelial dysfunction, EID: Endothelial-independent dilatation, FMD: Flow-mediated dilatation, Hb: Hemoglobin, NTG = Nitroglycerin, NO = Nitrous oxide, SCD = Sickle cell anemia

Introduction

Sickle cell disease (SCD) is an inherited disorder in which, the adult hemoglobin (HbA) is replaced by sickle hemoglobin (HbS) due to a single base-pair change. This alteration leads to chronic hemolytic anemia, pain, and later to organ failure [1,2]. HBS usually polymerizes when deoxygenated to form a gelatinous network of fibrous polymers that stiffen the erythrocyte membrane, increase viscosity, and cause dehydration due to potassium leakage and calcium influx. These changes also produce the sickle RBCs [3].

The endothelium, the largest organ in the body, is the inner most layer of the blood vessels. It senses mechanical stimuli, such as pressure and shear stress, and, hormonal stimuli, such as vasoactive substances. In

response, it releases agents that regulate vasomotor function, trigger inflammatory processes, and affect hemostasis [4]. The vascular endothelium is an active paracrine, endocrine, and autocrine organ that is indispensable for the regulation of vascular tone and the maintenance of vascular homeostasis [5,6]. The endothelium also plays a pivotal role in regulating blood flow, generating an active antithrombotic surface that facilitates transit of plasma and cellular constituents throughout the vasculature. Thus, dysfunction or damage to the endothelium causes a vasodilator response of the blood vessels [7]. Several vasomotor tests have been proposed to assess the state of the endothelium. These tests are based on the concept that certain stimuli trigger the release of nitrous oxide (NO) from the endothelium to mediate vascular relaxation. Quantitative coronary artery diameter assessment at angiography before and after infusion of vasoactive substances and strain-gauge venous impedance plethysmography for the forearm arteries are considered as sensitive tools. However, these tests are rather invasive procedures and reproducibility is questionable [5].

An alternative method for assessment of endothelial function is the measurement of flow mediated dilation (FMD) of the brachial artery in response to increased shear stress during hyperemia, using high-resolution ultrasound; the technique was introduced in 1992 by Celermajer and associates as a non-invasive endothelial function test [8]. Accordingly, it was demonstrated that flow-dependent dilation of the radial and brachial arteries is largely sustained by NO synthase [9]. This stimulus provokes the endothelium to release nitrous oxide (NO) with subsequent vasodilation that can be imaged and quantified as an index of vasomotor function. This technique is attractive because it is noninvasive and allows repeated measurements and therefore, provides a valuable “read-out” of vascular NO availability. The amount of vasodilatation is directly proportional to the amount of NO released by the endothelium and this allows us to evaluate endothelial function. The increase in flow and vasodilatation is measured by high-resolution ultrasonography of the brachial artery [10].

The aim of this study was to assess the value of brachial artery diameter measurement as a predictor of the state of endothelium in sickled individuals.

Methods

The study was conducted at Ultrasound Unit at Al-Nahrain College of Medicine after being approved by the Research Ethical Committee at Al-Nahrain College of Medicine. A written consent was obtained from each participant after explaining the details of the procedure. The patients were recruited from Hematology Unit of Al-Imamein Al-Kadhimein Medical City, Department of Inherited Blood Diseases in Al-Karama Teaching Hospital, and the National Center for hematology (NCH). All patients were previewed and examined by specialist or consultant hematologist to define eligibility to be enrolled in this study at the aforementioned centers. Patients were confirmed to have SCD clinically and by laboratory measures. The examination, assessment and the investigations needed for the research were done in the Central Laboratory of Al-Imamein Al-Kadhimein Medical City and in Al-Nahrain College of Medicine.

The study had been conducted from April 2013 to November 2014. A total of 60 subjects were included in this study; thirty of them (15 females and 15 males) were patients with homozygous sickle cell disease proved by the result of hemoglobin electrophoresis, and 30 (18 males and 12 females) were apparently healthy individuals with normal blood film findings and were considered as control, the latter subjects included college staff and medical personnel. Both the patients and the controls were of a comparable age group.

The inclusion criteria for the patients group were a homozygous Hb SS disease patient proved on clinical and laboratory basis aged 15

years old or above with Hb > 6 g/dL. Those SCA patients with severe anemia (Hb ≤ 6 g⁄dl), recent crises within the last week or recent blood transfusion in the last 4 weeks were excluded from the study. Other exclusion criteria were pregnancy, intake of tonic supplement within the last week or on any statin drug within the last four weeks, patients with significant cardiac disease and/or ECG abnormalities. In addition, other conditions that may independently affect endothelial function, i.e., diabetes mellitus, cigarette smoking, and hypertension (diastolic blood pressure > 90 mmHg) were not included in the study.

Detailed history and general physical examinations were carried out for each individual included in this study, an assessment of vital signs, and systemic examination performed to assess any possible complications. The height and the weight of the patients were estimated to calculate the body mass index (BMI)

For each individual included in this study, the physiological parameters of the endothelial function assessment were done after an over-night fasting.

The ultrasound study was performed with a multi-frequency (5-13 MHz) linear array probe LOGIQ P6 PRO, GE healthcare, Austria. All ultrasound scans were performed by a single radiologist experienced in vascular sonography. The ultrasound assessment was performed in the morning between 8 and 10 A.M.

The brachial artery diameter was assessed using Gray scale and spectral doppler sonography (Figure 1).

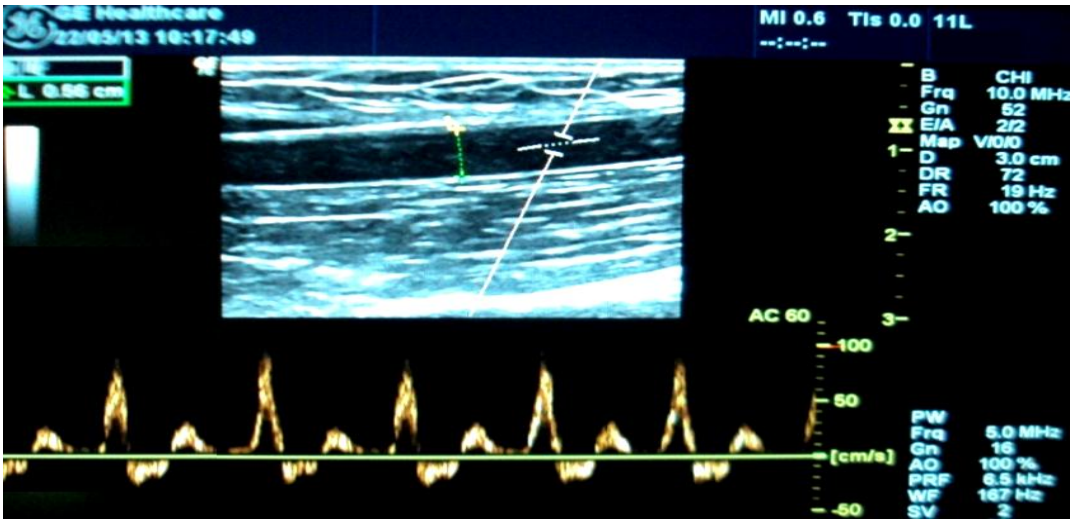

Figure 1. The ultrasound assessment of the diameter of the brachial artery

The patient/subject was asked to rest in a supine position for at least 10 min (for blood pressure stabilization), the right brachial artery was imaged 2-5 cm above the elbow. A segment with clear anterior and posterior intimal interfaces between the lumen and the vessel wall was selected for continuous gray scale imaging in longitudinal plane. During image acquisition, anatomical landmarks such as veins and fascial planes were noted to help maintain the same image of the artery throughout the study and the scanned area was marked to measure the same segment of brachial artery repeatedly i.e. at rest, after reactive hyperemia, and after administration of nitroglycerin (NTG). The Brachial artery diameter was measured at the same time in the cardiac cycle, the latter was achieved by using the spectral Doppler to define the image of interest at the peak systolic time. At the sites where luminal areas were measured, the intima-media thickness (IMT) was defined as

the distance between the blood-intima interface (the intimal leading edge) and the media-adventitia interface (the leading edge of the next bright layer). All recorded values represent the average of three consecutive measurements

A baseline rest image was acquired; the vessel diameter was measured with ultrasonic calipers from the leading edge of the anterior wall to the leading edge of the posterior wall of the brachial artery. Thereafter, arterial occlusion was created by sphygmomanometer cuff inflation to at least 50 mmHg above systolic pressure for 5 min to occlude arterial inflow.

The same measurements were repeated within 1 min after cuff deflation to assess FMD. Brachial artery diameter percent change was calculated and recorded as the FMD of the patient. The variability of the diameter measurement was minimized by calculating the average derived from 3 diameter measurements determined along the longitudinal segment of brachial artery. At least 10 min of rest is needed after reactive hyperemia (i.e., FMD) before another image is acquired to reflect the re-established baseline condition. Then a single dose of (0.4 mg) of NTG sublingual tablet had been given for 5 minutes to determine the maximum obtainable vasodilator response, and to serve as a measure of endothelium-independent vasodilation (EID) reflecting vascular smooth muscle function. After 5 min the NTG tablet was discarded and the same protocol for measurement of the diameter of the brachial artery was used as mentioned previously.

The changes after both interventions i.e. after stress and after NTG were expressed as percentage change from pretreatment value. Percentage increase in lumen diameter during post ischemic hyperemia as compared to basal lumen diameter, labeled as flow-mediated dilation; a marker of endothelium dependent dilation, is calculated as the maximum change in diameter from baseline, expressed as a percentage according to following equation:- FMD = (d2-d1) x 100/d1; where d1 is the brachial artery baseline diameter and d2 is brachial artery diameter at 1 min of cuff release. The percentage increase in lumen diameter after administration of NTG tablet as compared to basal lumen diameter was labeled as NTG%. As a marker of endothelium independent dilation, is calculated as the maximum change from baseline, expressed as a percentage change in diameter, using the same equation to assess the EID, as follow: EID = (d3-d1) x 100/d1; where d1 is the brachial artery baseline diameter and d3 is brachial artery diameter after 5 min of administration of NTG tablet.

Analysis of data was carried out using the available statistical package of SPSS-22 (Statistical Packages for Social Sciences- version 22). Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values). The significance of difference of different means (quantitative data) were tested using Students-t-test for difference between two independent means. The significance of difference of different percentages (qualitative data) were tested using Pearson Chi-square test (X^2-test) with application of Yate's correction or Fisher exact test whenever applicable. Statistical significance was considered whenever the P value for the test of significance was equal or less than 0.05.

Results

The study enrolled 30 patients with SCD (age range 15-50 years, mean±SD= 27.0±8.9 years) and 30 apparently healthy subjects (age range 18-49 years, mean±SD= 29.7±9.1). Significant difference was found between the patients` group and the control group regarding the BMI (22.9±4.0 kg/m^2 for the SCD patients versus 26.1±4.7 kg/m^2 of the control group). Categorization of BMI was done according to the WHO criteria into 4 groups: Underweight (BMI: < 18.5), Normal (BMI: 18.5-24.9), Overweight (BMI: 25-29.9) and Obese (BMI: ≥

30). In general, SCD patients appear to have lower BMI when compared to the control subjects (P = 0.006), and even when they were categorized (P = 0.022). Table 1 summarizes the distribution of BMI of both studied groups.

Table 1. Body mass index of the sickle cell disease patients and control subjects

BMI (kg/m^2)	SCD patients		Control subjects		P value
	No.	%	No.	%	
Underweight (<18.5)	4	13.3	1	3.3	0.022
Normal (18.5 - 24.9)	20	66.7	12	40.0	
Overweight (25 - 29.9)	4	13.3	13	43.3	
Obese (≥ 30)	2	6.7	4	13.3	
Mean BMI ± SD	22.9±4.0		26.1±4.7		0.006

Concerning the FMD, no significant difference was found in value of FMD% of the brachial artery when comparing the SCD patients and the control (13.02 vs. 15.91 respectively), however, the EID% in SCD patients (mean= 21.71) was significantly lower (P < 0.05) than that of controls (mean = 26.81) (table 2). On the other hand, no significant differences was found in the basal brachial artery diameter and intima media thickness between both groups (Table 2).

Table 2. Vascular physiological parameters for SCD patients and the control subjects

Physiological Parameters	SCD patients (Mean±SD)	Control subjects (Mean±SD)	P value
FMD%	13.02±4.59	15.91±6.60	0.054
EID%	21.71±6.96	26.81±6.31	0.004*
BAD (mm)	3.41±0.52	3.55±0.43	0.27
BAIMT (mm)	0.33±0.06	0.35±0.07	0.49

BAD = brachial artery diameter, BAIMT = brachial artery intima media thickness, FMD = flow mediated dilatation, EID = endothelial independent dilatation, * = significant difference

Positive correlation had been demonstrated between the FMD and EID in SCD patients (r = 0.752; p = 0.0001, figure2). However both parameters were negatively correlated with the base line diameter of the brachial artery in SCD patients (Figures 3,4).

Discussion

Sickle cell disease (SCD) is the commonest structural Hb variant; the outlook for patients with SCD continues to be poor. Today there is no doubt that general survival is improving with more medical care and improvement of environmental and social factor [11].

This study had revealed that SCD patients have lower body mass index as compared to controls. This finding is in agreement with many previous studies [12,13], this finding could be a consequence of the increased resting energy expenditure caused by the increased erythropoietic and cardiac activities [14], or it could reflect the greater increase in height than weight usually seen in adolescents with SCD [11-15].

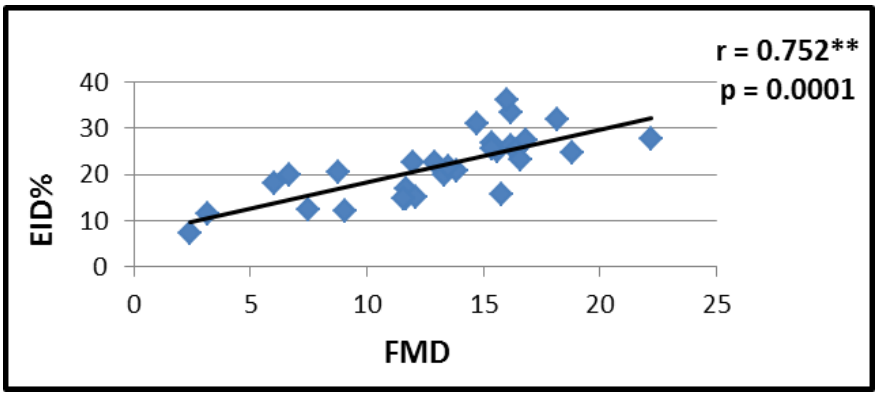

Figure 2. Correlation between flow mediated dilatation and endothelial-independent dilatation

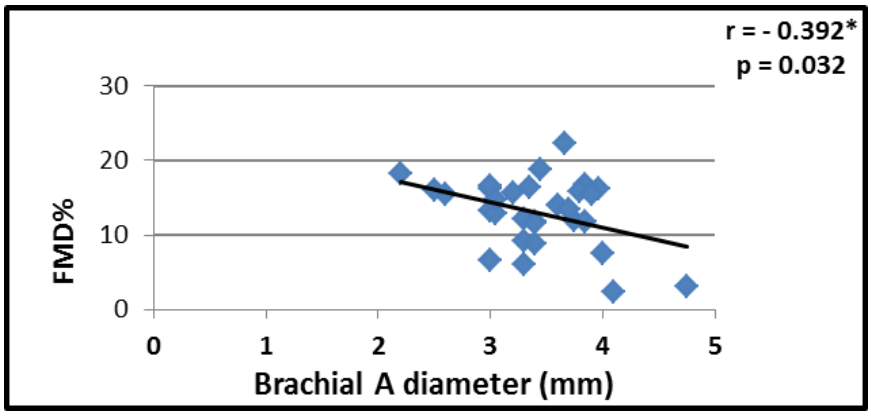

Figure 3. Correlation between flow mediated dilatation and brachial artery diameter in sickle cell disease patients

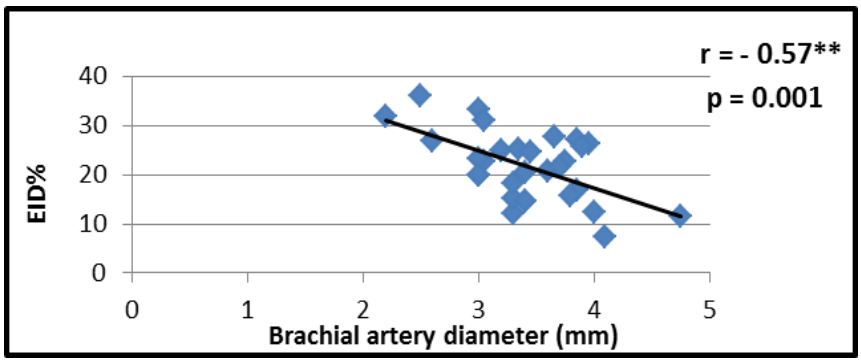

Figure 4. Correlation between endothelial- independent dilatation and brachial artery diameter in sickle cell disease patients

Decline in endothelial function was observed with increasing duration of symptoms and vaso-occlusive crisis/year in SCD cases. While others had mentioned that FMD was decreased or impaired in SCD patients in steady states [18-20]. One possible explanation for the insignificant difference in FMD could be due to fact that the control subjects enrolled in this

study had have higher BMI than patients. Other possible explanation is that the impaired FMD in the patients suggest failure of muscular arteries to adjust their internal diameter in response to mechanical stimulation. Overall, these results strongly suggest that systemic arteries in patients with SCD fail to adjust appropriately to chronic or acute increases in wall shear stress, which reflects mechanical forces exerted on endothelial cells.

Flow-mediated and nitroglycerin-induced dilation were both impaired in SCD compared to controls. The impaired response raises the possibility that there is a generalized loss of the bioavailability of nitrous oxide or impairment of the function of vascular smooth muscle to response to nitrous oxide, which is produced either by the endothelium or by sublingual nitrate administration.

This study showed no difference in brachial artery diameter and IMT in patients and control, this is in concordance with Eberhardt, 2003 [18] and Belhassen, et al 2001 [21]. Interestingly, baseline brachial artery diameter was similar in the patients and controls. It had been shown that, at a normal state, a sustained increment in the wall shear stress induces NO dependent increase in arterial diameter that normalizes wall shear stress regardless of blood flow. Therefore, in SCD, failure of vessels to adjust their diameter to wall shear stress changes probably reflects an impairment in the basal or stimulated release of nitrous oxide [21].

The present study conclusion was brachial artery diameter assessment is a useful non-invasive predictor of endothelial dysfunction in patients with sickle cell disease. The reproducibility of the test in addition to its low cost and being free of biological hazards makes it optimum for assessing the state of the endothelium and may be used to monitor the response to treatment.

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Author contribution

Dr. Al-Janabi collects the data and interprets the results, Dr. Al-Saadi did the Doppler study, Dr. Hamdan interprets the results and finalizes the writing the article and Dr. Al-Tammemi examined the patients and interprets the results.

Conflict of interest

The author declares that they have no competing interests.

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Review of the Causes of Obstructive Jaundice and the Role of Endoscopic Retrograde Cholangiopancreaticography (ERCP) in the Management

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Abstract

Background Obstructive jaundice poses diagnostic and therapeutic challenges to practicing physician gastroenterologist and general surgeons.

Objective To highlight the etiological spectrum, treatment outcome of obstructive jaundice endoscopically by Endoscopic Retrograde Cholangiopancreaticography (ERCP).

Methods It is a cross sectional case series study included 140 patients who presented with obstructive jaundice and dilated biliary system. They managed at Gastroenterology and Hepatology Center in Al-Imamein Al-kadhimein Medical City, Baghdad, Iraq from April 2012 to April 2014. All the patients were offered abdominal sonagraphy (U/S), magnetic resonance cholangiopancreaticography (MRCP) and upper endoscopy, as well as other laboratory work up, in order to prepare them for either palliative or curative treatment by the ERCP. Both success rate and complications were reported.

Results Common bile duct (CBD) stones were confirmed in 100 patients (71.4%) (56 female and 44 male) while pancreatic-biliary tumors found in 25 patient (17.8%) (15 male and 10 female) as pancreatic tumor in 11 patients, periampulary and ampulary tumor in 5 patients and cholangiocarcinoma in 4 patients while the rest due to metastasis. Benign causes as biliary fibrosis was seen in (10.8%). The success rate of ERCP treatment was 87.2%, but complications represented 6.4%. All of them were mild and reported within 24 hours.

Conclusion CBD stones are the predominant cause of benign cause of surgical obstructive jaundice, while the carcinoma of head of pancreas is the commonest concerning malignant causes. ERCP is very safe and effective procedure in the management.

Keywords CBD stone, Obstructive jaundice, ERCP, gallstone.

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List of abbreviation: CBD = Common bile duct, CDC = Choledochal cyst, CT = Computerized tomography, ERCP = Endoscopic retrograde cholangiopancreaticography, EUS = Endoscopic ultrasonography, INR = International normalized ratio, MRCP = Magnetic resonance cholangiopancreaticography, OGD = Esophagogastroduodenoscopy, PT = prothrombin time, PTT= Partial thromboplastin time, SEMS = Self-expandable metallic stent, SOD = Sphincter of Oddi dysfunction.

Introduction

Obstructive jaundice is a condition in which, there is blockage of the flow of bile out of the liver with a consequence of incomplete bile excretion from the body, which is may be due parenchymal diseases like in hepatitis (viral, immune or drug induced). This type of jaundice is termed cholestasis or medical jaundice, or due to surgical jaundice in which, there is dilation of biliary system. The imaging study will show dilatation of biliary canal either with internal filling defect or external compression to the extrahepatic biliary system. In United States, the incidence of biliary obstruction is approximately 5 cases

per 1000 people, and the majority of cases are attributable to cholelithiasis (gallstones) [1-3]. Other commonest causes of obstructive jaundice including: pancreatic-biliary tumor (pancreatic or cholangiocarcinoma and ampullary tumors) [4,5], benign biliary stricture, sphincter of Oddi dysfunction, primary sclerosing cholangitis, or previous surgery of the liver and/or biliary system [6,7]. Other less common etiologies like congenital structural defects and cholidochal cyst of the bile duct or even Lymph node enlargement, as well as pancreatitis and Parasitic infection [1].

Clinical features of obstructive jaundice are jaundice, dark-colored urine, steatorrhea, easy bruising and itching. These are usually seen more in patient with progressive painless obstructive jaundice due to biliary fibrosis or tumor, while biliary colicky abdominal pain (typically occurs in the right upper quadrant) is suggestive of gall stones. Unintentional weight loss is a red flag of malignancy. Cholangitis is expected whenever biliary obstruction is complicated by infection [8,9].

Diagnosis of the obstructive jaundice depends on clinical features and laboratory finding e.g. increase in the bilirubin in addition to imaging studies (the corner stone in diagnosis) like abdominal U/S, abdominal MRI, MRCP. The latter is very sensitive in the diagnosis the dilation of biliary system and the site and cause of pancreatic-biliary obstruction [10].

Treatment pptions for obstructive jaundice depend on the exact cause of the jaundice and on the severity of the disease.

Endoscopic retrograde cholangio-pancreatography (ERCP) is a sophisticated interventional procedure that combines upper gastrointestinal (GI) duodenoscope and X rays to treat problems of the bile and pancreatic ducts as well as used for diagnosis also. ERCP is used when it is suspected a person's bile or pancreatic ducts may be narrowed or blocked due to tumors, or stone stuck in the common bile ducts, pancreatitis due to impacted common bile duct (CBD) stone in ampulla, sphincter of Oddi dysfunction, scarring of the ducts (fibrosis). Stent insertion in benign cases (plastic type) or in malignant pancreatic-biliary obstruction (SEMS) for inoperable patient [9-11].

ERCP complications may include pancreatitis, perforation and bleeding. It is recommended to check prothrombin time (PT), partial thromboplastin time (PTT) and platelet count before this procedure [2,6]. For inoperable cases, surgical treatment whether laparoscopic or laparotomy may be indicated [10,11].

In order to highlight the role of ERCP in defining the etiological spectrum as well as the treatment outcome for obstructive jaundice, this study performed.

Methods

Total patient were 140 (78 female, 62 male). They had been referred for GIT Department in Al-Imamein Kadhimein Medical City because of obstructive jaundice from April-2012 to April-2014.

Before any intervention, international normalized ratio (INR) must below 2 to avoid bleeding during ERCP. Whenever, it is above 2, correction can be achieved using vitamin k 10 mg daily for 3 days.

For all patients, hemoglobin should equal or above 12 g\dl and platelets count of at least 50,000/mm^3 or more.

Pre ERCP requisite included oxygen saturation above 90% and forced vital capacity more than 75% of predictive value in addition to ejection fraction by echocardiography study more than 50%.

Pentax video system EPK 1000, Pentax Duodenoscope ED with functional channel 4.2 mm was used in this study.

Fluoroscope machine model 9800 C-arm used for screening the biliary system.

The contrast used is (Omnipaque 240 mg/ml) equal to lohexol 518 mg + tromtamol 1.2 mg + sodium. Calcium editate 0.1 mg/ml

Boston scientific and Wilson's cock accessories (dream guide wire, Sphincterotome, balloon extractors, balloon dilators and stents) used in this setting.

The procedures performed while the patient is conscious under sedation using 5 mg of midazolam and 50 mg of pethidine intravenously, with continuous monitoring of by oximetry. Admission for all the patients post- ERCP procedure was considered up to 24 hours for follow up to manage and notice any possible complications.

After explanation and discussion the value and complication of ERCP for patients or their companions, the patients consent were taken.

Chi squared test was run to determine the significance considering P value < 0.05.

Results

Female patients were 78 and their ratio to male patients was 1.25:1, the age range was 25-80 yrs with a mean age of 52 yrs.

All patients had increased bilirubin level and most of patient presented with mild to moderate increase in the liver enzymes (Table 1).

The sensitivities of diagnosis to the cause of obstructive jaundice (by abdominal U/S, MRCP and ERCP) were 71.4%, 85.7% and 96.4% respectively (Table 2).

Benign obstructive jaundice (e.g. cholidocholithiasis, biliary fibrosis and Sphincter of Oddi dysfunction (SOD)) is more common than malignant causes (82.2% vs. 17.8% respectively). Diagnosis of CBD stone was found in 100 patient and represents 71.4%, which is mainly seen in female patients (56) with the mean age was 45 year (Table 2).

Table 1. Characteristics of patients with obstructive jaundice

Female/male ratio	Total case No (%)	Biocheical parameters				
		TSB G/DL	SGOT IU/L	SGPT IU/L	ALP IU/L	INR
55/45	100 (71.4)	1-2	30-100	30-100	120-360	1.5-2
20/15	35 (25.0)	2.1-4	101-300	101-300	361-600	2.1-2.5
3/2	5 (3.6)	> 4	> 300	> 300	> 600	> 3
78/62	(140) 100%					

INR=international normalized ratio, TSB = Total serum bilirubin, ALP = Alkaline phosphatase, SGPT = Serum glutamic pyruvic aminotransferase, SGOT = Serum glutamic oxaloacetic transaminase, Reference values INR = 1-1.5, TSB = 1 mg/dl, ALP = 40-120 IU/L, SGPT = 20-30 IU/L, SGOT = 20-30 IU/L [1-3]

Table 2. Imaging criteria for 140 patient with obstructive jaundice

Imaging	Biliary dilation	Stone	Tumor	CDC	SOD and FIBROSIS	No DX	Sensitivity of DX
U\S	140	85	15	-	-	40	71 .4%
MRI, MRCP	140	98	20	2	-	20	85.7%
ERCP	140	98	22	2	13	5	96.4%

Biliary dilation= CBD diameter more than 8-10 mm, CDC = Choledochal cyst, SOD= sphincter of Oddi dysfunction

Other benign causes of obstructive jaundice apart from stones seen in 15 patient representing (10.6%) of all cases of the obstructive jaundice, which include the followings: distal biliary fibrosis in 10 patient where some of those patients had history of

cholecystectomy but 2 patients had previous sphincterotomy. SOD was suspected in 3 patients according to criteria for diagnosis (age, female, recurrent jaundice) but it couldn't prove as there is no manometry study in our center. Sphincterotomy and follow up gave excellent result for above groups.

Diffuse choledochal cyst (type 1) diagnosed in 2 female patients who presented with features of obstructive jaundice and mass in the right upper quadrant. Their diagnosis confirmed by imaging study and ERCP.

Tumors were seen in 25 patient in this study (15 males and 10 females, M:F was 1.5:1), which represent (17.9%). Their mean age was 62 year. Pancreatic tumor was seen in 11 patients, periampulary and ampulary tumor is second tumor in order seen in 5 patients and the least is cholangiocarcinoma in 4 patients only. Other 5 patients had obstructive jaundice due to metastasis from breast carcinoma, ovarian cancer or lymphoma due to external compression to biliary system. For those were inoperable, treatment by biliary SEMS deployment were performed (Table 3).

Table 3. Causes of obstructive jaundice in 140 patients

Cause	Total cases No (%)	Male No (%)	Female No (%)	Mean age
Benign	115 (82.1)	47 (75.8)	68 (87.2)	48 yrs
Cholidocholithiasis	100 (71.4)	44 (71.0)	56 (71.7)	45 yrs
Fibrosis	10 (7.1)	2 (3.2)	8 (10.3)	
SOD	3 (2.1)	1 (1.6)	2 (2.6)	
CDC	2 (1.4)	0 (0.0)	2 (2.6)	
Malignant	25 (17.9)	15 (24.2)	10 (12.8)	62 yrs
Ca pancreas	11 (7.9)	7 (11.3)	4 (5.1)	66 yrs
Periampullary tumor	5 (3.6)	4 (6.5)	1 (1.3)	
Cholangiocarcinoma	4 (2.8)	3 (4.8)	1 (1.3)	
Metastasis	5 (3.6)	1 (1.6)	4 (5.1)	
Total cases	140	62	78	52 yrs

Success of ERCP treatment reported in 122 patient (87.2%) while 18 patients (12.8%) patients failed to get response to ERCP. Fifteen of them have periampullary diverticulum with subsequent difficult biliary cannulation to extract CBD stone, two patients proved to have pancreatic tumor that obstruct the lower CBD while one patient with fibrosis and very small ampulla (Table 4) (Figure 1).

Complications were seen in 9 patient representing 6.4%, as one female patient developed perforation of ampulla during the use of precut knife for cannulation that necessitate treatment by laparotomy later on. Five patients developed mild to moderate pancreatitis that managed conservatively.

The other 3 patients developed minor to moderate bleeding during the procedure which treated conservatively by injection of adrenaline and normal saline (Table 4).

Discussion

In this study, all patient had an increase in the direct bilirubin level and most of patient had increased liver enzymes, which is in agreement with study by Siddique et al and others [12-15].

ERCP was superior in the diagnosis of the cause of obstructive jaundice compared to other imaging tools in term of sensitivities as follows: (abdominal U/S, MRCP, and ERCP 71.4%, 85.7% and 96.4% respectively) in close to study by Verma et al who described sensitivities of

same modalities as 87.3%, 90% and 100% respectively [13]. The benign conditions were more frequent than malignant causes with mean age of presentation as 48 year. This is in agreement with Assi et al study done in Thi-Qar governorate that found the benign conditions forming 93.4% of total causes the CBD stone was the most predominant (75.8%), which was more in young female under 55 year too [14].

Table 4. Outcome of ERCP procedure in treatment of 140 patient with obstructive jaundice

Disease spectrum	F/M	ERCP success	ERCP procedure	Failure	Total	Complications
Stone	56/44	85	ERS+ S.EXT	15	100	4 pancreatitis 2 bleeding 1 perforation
Tumor	10 /15	23	SEMS	2	25	--------
Biliary fibrosis	8/2	9	ERS	1	10	1 pancreatitis
Type 1 CDC	2/0	2	no ERCP	-----	2	
SOD	2/1	3	ERS		3	1 pancreatitis.
Total	78/62	122 (87.2%)		18 (12.8%)	140	9 (6.4%)

ERS = Endoscopic retrograde sphincterotomy, S.EXT= Stone extraction, SEMS = self-expandable metallic stent, CDC = Cholidochal cyst, SOD = Sphincter of Oddi

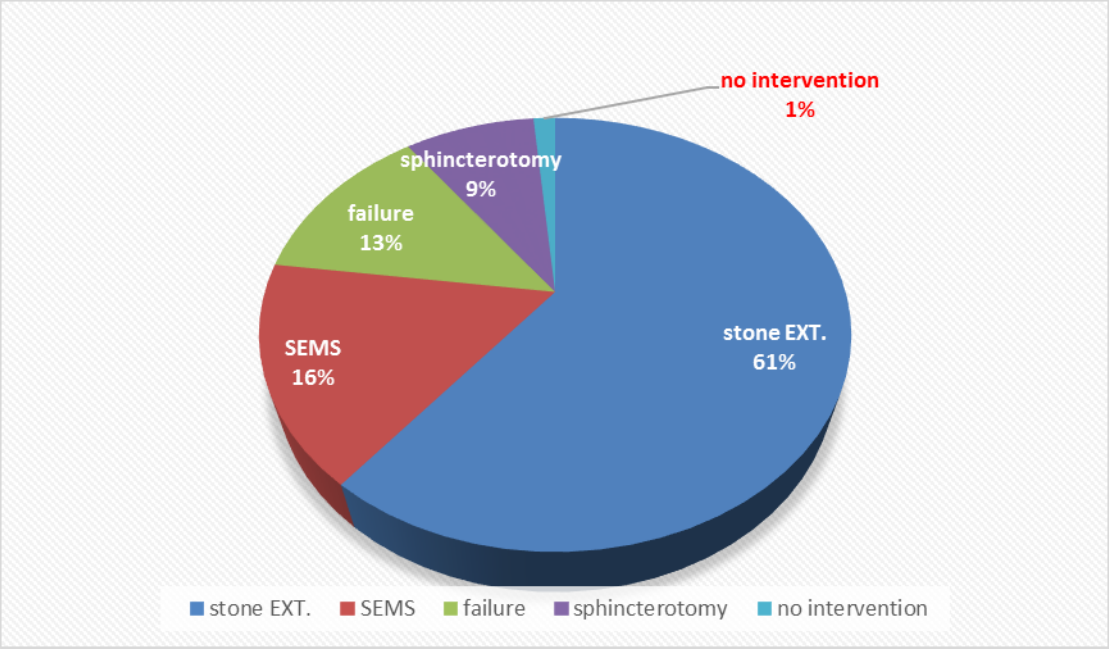

Figure 1. Outcome ERCP procedure

Table 5. Comparative studies of type obstructive jaundice in different centers

Study	Malignant	Benign
Khanzada et al [11]	57%	43%
Siddique et al (Pakistan) [12]	56.66%	43.33%
Verma et al (India) [13]	52.73%	47.27%
Assi et al (Thi-Qar, Iraq) [14]	6.6%	93.4%
Lobo et al (Croatia) [17]	27%	73%
This study (Baghdad, Iraq)	17.8%	82.2%

The CBD stone in this study was the commonest cause of benign obstructive jaundice (mean age 45 yrs and more in the female), but the second cause was the fibrosis. This is consistent with study of Prat et al [15].

Another study was carried out in Croatia by Kujundzić et al, showed 83% due to benign causes with 54.1% secondary to CBD stone, which was higher among young female while malignant causes reported in 27% of condition. These result are close to this study [16].

There is disagreement between this study and study published by Khanzada et al in Karachi [11] as well as Siddique et al study [12] who found that malignant obstructive jaundice was more common than benign causes [12]. The possible explanation for this difference may be due to high incidence of CA gall bladder.

This study shows cholangiocarcinoma as the least common tumor, which agreed with studies by Saddique et al, Khanzada et al and by Verma et al [11-13,16].

In this study, the success rate of ERCP was 87.2% and failure was 12.8%, while success rate by of Prat et al, was 96% [15]. Stone extraction in this study done for 92% while by Prat et al was 100%. The difference is due more expert endoscopist, and general anesthesia and surgical backup that contribute in increasing the success rate [15].

Failure of ERCP in this study (15 patients from 18 patients) was due to peri-ampullary diverticulum. This cause is the commonest cause in failure of ERCP as mentioned by Lob et al [17]. The complications was mild pancreatitis (4 patients (2.8%)), moderate pancreatitis (2 patients (1.4%)), simple bleeding (2 patients (1.4%)), which is close to Prat et al [15]. In conclusion, benign causes of obstructive jaundice are more frequent than malignant causes.

The commonest cause of the benign obstructive jaundice in this study is the CBD stone, which is more in female patients. The next in frequency is biliary fibrosis. In malignant obstructive jaundice, the pancreaticobiliary malignancy and pancreatic tumor are the commonest type of tumor, which is more in middle and old age male patients.

ERCP was invaluable procedure in treatment of obstructive jaundice with very high success rate low cost and less traumatic to the patient.

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Conflict of interest

The author declares no conflict of interest.

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Standard Disscectomy versus Microdiscectomy: Short Term and Long Term Outcome Comparison in Treatment of Lateral Lumbar Disc Hernaiation

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Abstract

Background Despite the high incidence of coincident spinal degenerative changes due to the high dynamic interplay between adjacent spinal elements leading to the clinical pain syndromes, yet the diagnostic approach and therapeutic options are still diverse and often inconsistent.

Objective To evaluate the short and long term outcome of two different surgical approaches in the treatment of lateral lumbar disc prolapse associated with spondylosis.

Methods Twenty patients presenting with a comparable complaints of radicular low back pain falling in the age group of 40-50 who attended the outpatient clinic in Science and Technology Hospital in Sanaa from 1st January 2008 to 1st of June 2009 and who were diagnosed to have lumbar lateral disc prolapse with mild spondylotic changes in need for surgery were divided into two groups. Group A offered microdiscectomy while group B offered standard discectomy. They were followed up and evaluated both clinically and radiologically at fixed postoperative intervals (day of discharge, three months, and one year post operatively).

Results It has been revealed that most of cases showed improvement of their presenting complaints due to the acute decompression offered to the neural tissue by either approach though was initially much higher with the minimally invasive microdiscectomy. However, the picture changed at three months interval where (30%) of patients from group A had complaints, two cases (66.6%) of the incompletely responding cases presented with new symptoms mostly due to incompletely treated spondylotic changes and 1 patient (33.3%) of the incompletely responding cases presented with persistence of symptoms due to incomplete disc removal. In contrast, only one case from group B had the persistence of symptoms, which was due to the effect of spondylosis. With further follow up at 1 year interval 40% of cases from group A had complaints mostly in form of bilateral radiating pain due to incompletely treated spondylotic changes, while only (20%) from group B had complaints either as ipsilaterally radiating pain due to incomplete disc resection or as bilaterally radiating pain due to postoperative adhesions.

Conclusion Treatment with the first modality though has the advantages of a shorter duration of surgery, less invasion, less postoperative stay at hospital and comparable clinical response on short term follow up to that of second group, yet data at long term follow up showed that it is associated with a higher incidence of recurrence or incomplete resolve of the presenting complaint as well as evident evolving radiological complications in contrast to treatment by the second modality.

Keywords Lateral disc prolapse, spondylosis, micro discectomy, standard discectomy.

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List of abbreviation: LBP = Low back pain

Introduction

Low back pain (LBP) affects approximately 60–85% of adults during some point in their lives [1-3]. Fortunately, for the large

majority of individuals, symptoms are mild and transient, with 90% subsiding within 6 weeks [4]. Chronic back pain which is defined as pain symptoms persisting beyond 3 months affects an estimated 15-45% of the population [5,6] is particularly important being responsible for completely disturbing patient's day activities with subsequent burdens on a family and community basis [7]. Lateral disc herniations as one of the causes of back pain constitute 7-12% of all disc herniations. They may be purely far lateral or extraforaminal in location, located beyond the pedicles, or may include intraforaminal and even intracanalicular components. Lateral disc herniations are occurring predominantly at the L4-L5 and L3-L4 levels in almost equal numbers. Clinical syndromes reflect compression of the superiorly exiting nerve root and ganglion; i.e. a L4-L5 far lateral disc produces a L4 root syndrome [8]. Intervertebral discs are believed to undergo what Kirkaldy Willis and Bernard [9] first coined a ''degenerative cascade'' of three overlapping phases that may occur over the course of decades. Phase I (Dysfunction Phase), Phase II (Instability Phase) [10] and Phase III (Stabilization Phase) (11). On the other hand, spondylosis of the lumbar spine is considered mechanically, as the hypertrophic response of adjacent vertebral bone to disc degeneration (although osteophytes may infrequently form in the absence of diseased discs) [13]. In another word, spondylosis may be applied nonspecifically to all degenerative conditions affecting the discs, vertebral bodies, and/or associated joints of the lumbar spine [12,13]. For purposes of this review, we will use this final, broad definition of spondylosis, recognizing the high incidence of coincident degenerative changes, and the dynamic interplay between adjacent discs, vertebra, and nerves that create the clinical pain syndromes within the axial spine and associated nerves. Elective lumbar discectomy is regarded as a good treatment option for lumbar disc herniation if sciatica or neurological deficits occur and still persist after 6 weeks of conservative therapy [14-16]. Mixter and Barr first described herniated disc as a cause of neural compression in the lumbar spinal canal in 1934 [17]. They described a surgical approach to the problem that involved partial hemilaminectomy and partial removal of the disc (standard open discectomy). In 1977, a new technology was introduced by Yasargil [18] and Caspar [19] that involved the use of an operating microscope for the surgical removal of the disc. They independently described microsurgical techniques that provided excellent lighting and magnification of the operative field. Compared with the standard open discectomy, the microdiscectomy enabled the use of smaller incisions of the skin and fascia and facilitated a less traumatic surgical procedure. The first follow-up report of Williams et al. in 1978 showed encouraging results following lumbar micro discectomy [20]. Since that time, these two procedures have been considered the gold standard for the surgical treatment of lumbar disc herniations. However, there is a little consensus with regard to a definitive treatment approach for disc herniation associated with spondylosis and seldom comparative studies focusing on the short and long term outcome have been attempted. Thus, the current study was conducted to evaluate the short and long term outcome for standard and micro discectomy procedures in the treatment of symptomatic lateral lumbar disc prolapse associated with mild degree of spondylosis.

Methods

This is a descriptive study comparing the short and long term outcome of 2 different surgical approaches in the treatment of lateral lumbar disc prolapse associated with spondylosis, using simple statistical measures in the description as numbers and percentages. The samples were included twenty patients of both sexes falling in the age group of 40-50 years, who attended to the outpatient clinic in the University of Science and Technology Hospital in Sanaa from 1st January 2008 to 1st of June 2009. The patients participated in the study were examined clinically and radiologically

preoperatively and were diagnosed to be in need for surgical treatment. An informed consent was taken from all the patients to participate in this study.

Patient's inclusion criteria were as follows:

1. All of the patients had a comparable affected level of activities according to Oswestry Low Back Pain Scale.
2. All of the patients had a comparable intensity of back and radicular pain according to Oswestry Low Back Pain Scale. In which, all of them had clinical complaints including moderate to severe radicular pain accompanied by very positive mechanical signs; LaseÂgue and reverse LaseÂgue (femoral stretch test) maneuvers. Neurological deficit signs and symptoms including motor, reflex, and sensory findings were consistent with their radiological findings.
3. Radiologially, all of them having the diagnosis of lateral disc prolapse and mild degree of spondylosis in the lumbar vertebrae.
4. All of the patients were operated by the same surgeon.

All the patients who had similar complaints due to other pathologies or had not been subjected to accurate conservative treatment or had previous back surgical interventions were excluded.

For the purpose of this study, the patients were allocated into two groups each of ten:

1. Group A: patients were operated by unilateral microdiscectomy.
2. Group B: patients were operated by standard discectomy which includes: hemi-laminectomy and total ligamentum flavum resection and bilateral foraminotomy.

After surgery the patients were followed up by the researcher himself (specialist neurosurgeon) both clinically at fixed postoperative intervals (day of discharge, three months, and one year postoperative) and radiologically at 3 months and 12 months interval. These follow up dates are set with the patients before the operation as part of the treatment strategy in the department. For aim of clarification, we will use the term incompletely responding cases to those who either did not show clinical response to surgical treatment or had improvement and then recurrence of complaint or had newly evolving but related complaints.

Results

Our study showed that, with regard to clinical response in terms of resolve of the presenting chief complaint, the majority of patients in both groups showed response (clinically and with the aid of Oswestry Low Back Pain Scale) at the three follow up intervals as shown in figure 1.

With regard to incompletely responding cases they were further analyzed at 3 months and 12 months postoperative date.

First of all, according to their clinical picture (through reviewing their signs and symptoms with the aid of Ostwestry back pain scale to assess their pain intensity and site and improvement together with clinical examination):

At three months postoperative date, the incompletely responding cases from group A fell in three different clinical presentations in equal percentages which was either radiating pain at similar preoperative side or on contralateral side or bilaterally radiating, while the only incompletely responding case in group B fell in the ipsilateral radicular pain sector as shown in table 1.

At 1 year postoperative follow up date other cases were added to the incompletely responding sector of both groups (emphasizing that those who showed incomplete response at 3 months follow up date were still showing no further response at 1 year postoperative period). The additional two incompletely responding cases (one from group A and another one from group B) fell into the category of bilaterally radiating lower back pain (stressing that one incompletely responding case from group B showed resolve of complaint) as apparent in table 2.

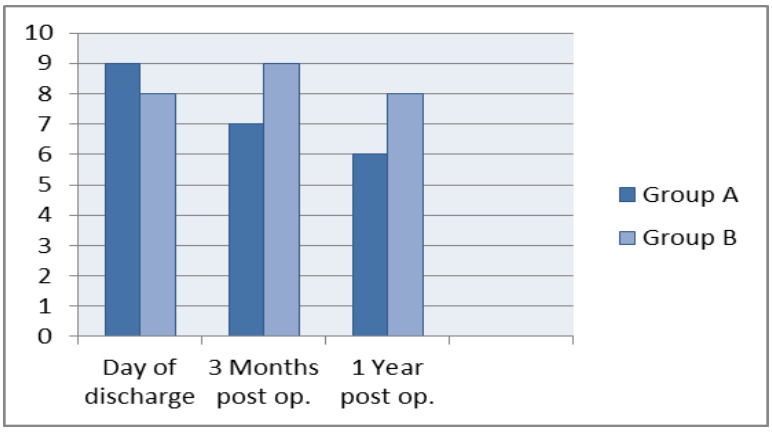

Figure1: Histogram showing the relationship between the type of surgery and pain resolution at three time intervals of follow up

Table 1. Distribution of the incompletely responding cases of both groups in respect to clinical picture 3 months postoperatively

Clinical complaints	Cases			
	Group A		Group B	
	No.	%	No.	%
Pain at similar side	1	33.3%	1	100%
Pain on contralateral side	1	33.3%	0	0%
Bilateral pain	1	33.3%	0	0%
Total	3	100%	1	100%

Table 2. Distribution of the incompletely responding cases of both groups in respect to clinical picture one year postoperatively

Clinical complaints	Cases			
	Group A		Group B	
	No.	%	No.	%
Pain at similar side	1	25%	1	50%
Pain on contralateral side	1	25%	0	0%
Bilateral pain	2	50%	1	50%
Total	4	100%	2	100%

Thereafter, the patients were analyzed according to their relevant radiological findings through obtaining MRI to the lumbar spine with emphasis on the operation site and comparing that with the preoperative images: At three months postoperative date, the incompletely responding cases from group A were mostly having progressive spondylotic changes with an effect on the nearby neural tissue (66.6%) and to a lesser extent the presence of incomplete disc removal was evident in the other case (33.3%). In group B the MRI finding for the still incompletely responding case was that of spondylosis (100%) as shown in table 3.

Table 3. Distribution of incompletely responding cases of both groups in respect to MRI findings three months postoperatively

Clinical complaints	Cases			
	Group A		Group B	
	No.	%	No.	%
Spondylosis	2	66.7%	1	100%
Incomplete disc removal	1	33.3%	0	0%
Total	3	100%	1	100%

At 1 year, postoperative follow up date MRI picture of the incompletely responding cases in group A were that of spondylotic changes effect (50%), the presence of Incomplete disc removal (25%) and contralateral disc bulge (25%), while the picture in group B was different with a spondylosis (50%) and adhesions (50%) as shown in table 3.

It is worthy to mention that the clinical picture of the responding cases in both groups was stable throughout the follow up period and their MRI picture did not show significant changes just like those in the incompletely responding group.

Table 4. Distribution of incompletely responding cases of both groups in respect to MRI findings one year postoperatively

Clinical complaints	Cases			
	Group A		Group B	
	No.	%	No.	%
Spondylosis	2	50%	1	50%
Adhesions	0	0%	1	50%
Contralateral disc bulge	1	25%	0	0%
Incomplete disc removal	1	25%	0	0%
Total	4	100%	2	100%

Discussion

In this study, it has been revealed that although at time of discharge the majority of patients in both groups showed improvement regarding their chief complaints, which can be attributed to relief of the neural tissue compression offered by surgery with either modality, however, three months later, the picture was changed in such a way that certain number of patients in group A showed either recurrence or newly evolving related symptoms, in contrast to group B where the number of the responding cases raised up. This could be explained by reviewing the radiological findings for the incompletely responding cases in both groups in which, 2 cases out of the 3 incompletely responding cases within group A showed the picture of persistent not treated well nearby spondylotic degenerative changes that usually accompany the disc herniation process and thus persistent some degree of neural tissue compression, which was the cause for patients complaints in the form of contralaterally and bilaterally radiating back pain. Furthermore, one case out of the three incompletely responding cases showed persistent ipsilaterally radiating back pain due to the incomplete herniated disc removal. These results were the fact that limited access micro discectomy offered only

partial removal of the herniated disc giving a temporary relief of symptoms but much less access to treat the associated spondylotic changes and suboptimum treatment for the herniated disc. While the only non-responding case in group B was due to the progressive spondylotic changes. This disagrees with a study held by Porchet et al. [21], who claimed that there was no difference between the classical macroscopic approach to lumbar disc herniation and the more modern micro discectomy.

On the other hand, it is believed that the cause behind the crescendo improvement in group B was due to the more extensive decompression offered for the compressed neural elements by the second approach through extensive removal of the herniated disc together with the associated compromising osteophytes and the thickened ligamentum flavum and the better access to neural foramina bilaterally, that was clear in the postoperative MRI pictures of those patients as compared to the MRI findings in group A. When additional analyses were carried out to examine whether outcome could change over 1 year period of follow up, it was obvious that additional number of patients in both groups had recurrence of symptoms especially in Group A were the symptoms mostly were bilateral due to incomplete and suboptimum neural tissue freeing from the associated spondylotic changes whether at the side of the surgery or on the contralateral side and the development of a contralateral disc bulge with their effect collectively on the nearby neural tissue, yet none was attributed to postoperative adhesions as revealed in the postoperative MRI finding of this group and this is the usual occurrence with microdiscectomy were the wound is small and the postoperative adhesions are less. In contrast with the ordinary discectomy where the chance to remove all the causative pathological factors is much higher and more optimum due to the wide field offered by that approach even with the higher chance to get adhesions still the number of the responding cases is higher and the main cause between the incompletely responding cases was distributed between ongoing Spondylotic changes and postoperative adhesions [22]. As the vast majority of patients undergoing disc surgery in our Spine Centre do so in connection with degenerative disorders (spondylosis), it is obvious from all above that the early result was highly predictive of the longer-term outcome. This has been reported before, in relation to surgery for degenerative diseases of the lumbar spine [23-26], and as surgery typically serves a ‘‘mechanical’’ purpose, aiming to relieve pain by removing all the causes of physical obstruction including disc and associated spondylotic effects we can see that the limited access micro discectomy was deficient in this aspect in comparison with ordinary discectomy.

This study concluded that treatment with the first modality though has the advantages of a shorter duration of surgery, less invasion, less postoperative stay at hospital and comparable clinical response on short term follow up to that of second group, yet data at long term follow up show that it is associated with a higher percentage of recurrence or incomplete resolve of the presenting complaint as well evolving radiological complications. On the other hand, patients treated with the more extensive surgery, despite having the draw backs of a longer duration of surgery, being more extensively invasive, and requiring a relative longer postoperative stay at hospital, however, they had their preoperative complaints resolving, more rapidly than in the 1st group and the resolution seeming to be more permanent with less radiological complication on long term basis.

Therefore, our recommendations are that well patients with longer life expectancy, patients without medical diseases that stand against longer period of anesthesia to be operated on by the second more extensive approaches (without disturbing the stability) for longer lasting and better outcome. Anyhow, this is a

descriptive study of observations from our practice, and we do recommend further analytic studies in this respect.

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Conflict of interest

The author has no conflicts of interest.

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Allergic Fungal Rhinosinusitis in Patients with Nasal Polyposis

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Abstract

Background Nasal polyposis and chronic rhinosinusitis is much debated subject. Generally speaking, nasal polyposis should probably be regarded as one form of chronic inflammation in the nose and sinuses, which is indeed part of the spectrum of chronic rhinosinusitis. Nasal polyposis is the ultimate form of inflammation for unknown reasons; polyps preferentially develop in subtypes of inflammatory diseases. Allergic fungal rhinosinusitis (AFRS) is defined the consequence of immunocompetent patient whenever there is allergy to fungus.

Objective To determine the frequency of allergic fungal rhinosinusitis among patients having nasal polyposis.

Methods A prospective study of 60 selected patients with nasal polyposis was studied at Al-Imamein Al-Kadhimein Medical City. After thorough history and full ENT examination, all patients sent for computed tomography (CT) scan of the nose and paranasal sinuses. Patients who needed surgical treatment, the specimens were sent for histopathological analysis to identify the fungi. The diagnosis of allergic fungal sinusitis was based on analysis of clinical, radiological, and laboratory investigations.

Results The mean age was (41.3±13.7) years; male to female ratio was (1.7:1). Both nasal obstruction 54 (90%) and nasal discharge 51 (85%) were the commonest clinical presentation. CT scans opacities were found in all 60 patients (100%). Fungal elements were detected by histopathological study in 7 (11.7%) of them.

Conclusion Allergic fungal rhinosinusitis is a significant cause of nasal polyposis.

Keywords Allergy, fungal infection, nasal polyposis.

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List of abbreviation: ESS = endoscopic sinus surgery, CT = Computed tomography, CRS = Chronic rhinosinusitis, AFRS = Allergic fungal rhinosinusitis, ABPA = Allergic bronchopulmonary aspergillosis, EMCRS = Eosinophilic mucin chronic rhinosinusitis, H&E = hematoxylin-eosin, MRI = Magnetic resonance imaging, CN = Cranial nerve

Introduction

Nasal polyps are always associated with paranasal sinus pathology. There is hyperplasia of the maxillary mucous membrane and the ethmoidal cells are filled with polypoid mucous membrane. This pathology may not necessarily result in symptoms, but it can cause a feeling of congestion and may also increase the tendency to bacterial infection, especially following a common cold. When polyps develop in children, it can cause widening of the ethmoidal cells, and flattening and broadening of the nasal bridge (frog nose) [(1)]. Patients with severe nasal polyposis and blood eosinophilia often have, or will develop, asthma (30 percent) and/or non-steroidal anti-inflammatory drugs (NSAID) intolerance (15%). Most patients develop asthma before polyps. When ingestion of NSAID within minutes to a few hours has resulted in rhinitis, asthma, skin itching or urticaria, then the diagnosis is made.

In case of doubt, a challenge test with acetylsalicylic acid may be necessary [1]. Large polyps can be identified by simple rhinoscopy. Rigid scope is the preferred examination, as it can diagnose small polyps in the middle meatus and give a superior assessment of the extent of the disease and of anatomical abnormalities [1] (Table 1).

Table 1. Endoscopic staging of nasal polyposis [1]

Endoscopic appearace	Score
No polyp	0
Restricted to middle meatus	1
Below middle turbinate	2
Massive polyposis	3

Computerized tomography (CT) scan of the nose and paranasal sinuses gives an excellent demonstration of the anatomy and pathology. It is indicated in all cases before endoscopic surgery. In addition, it is used for staging of the disease [1] (Table 2).

Table 2. CT scan staging of nasal polyposis [1]

Sinus site	Right	Left
Maxillary	0-2	0-2
Anterior ethmoid	0-2	0-2
Posterior ethmoid	0-2	0-2
Sphenoid	0-2	0-2
Frontal	0-2	0-2
Ostiomeatal complex	0-2	0-2
Total	0-12	0-12

0= no opacity; 1= some opacity; 2= total opacity

Fungi can cause both acute and CRS disorders, and can occur as either tissue-invasive or noninvasive conditions as delineated by deShazo and colleagues, 1997 [2]. Invasive disorders include: (i) Acute fulminate necrotizing form is the classic fungal infection epitomized by 'Mucormycosis'. Patients typically are immunosuppressed, and the infection leads to widespread facial and paranasal tissue necrosis that has high morbidity and mortality, (ii) Chronic invasive fungal rhinosinusitis. It has often been reported in diabetics and commonly leads to periorbital tissue invasion and the 'orbital apex syndrome. Surgical resection and systemic antifungal drugs are required, but the infection may recur and is difficult to treat and (iii) Granulomatous invasive fungal sinusitis, indolent fungal sinusitis and primary paranasal granuloma [2]. Here, the fungal infection is more localized to the superficial sinus mucosa and is well contained within a robust granulomatous inflammatory process. Sinus mucosal resection may be curative, but systemic antifungal drugs are commonly used postoperatively to assure complete resolution of fungal infection once the histopathological diagnosis is available [2]. The second type is noninvasive fungal rhinosinusitis. This includes fungal ball 'sinus mycetoma' and allergic fungal rhinosinusitis (AFRS). (i) Fungal ball is a multitude of fungal hyphae that compressed into a thick exudate within a sinus lumen. (ii) Allergic fungal rhinosinusitis. It is defined as an immunocompetent patient with an allergy to fungus. Since initial publications, approximately 7% of all chronic rhinosinusitis cases requiring surgery have been diagnosed as AFRS; the fungi, which are the cause of the hypersensitivity reside in the mucin and provide continued stimulation [2].

This study designed to determine the frequency of AFRS among patients having nasal polyposis.

Methods

A prospective study of 60 selected patients was conducted in the ENT Department at Al-Imamein A-Kadhimein Medical City, Baghdad from December 2012 to December 2013. All patients were with nasal polyposis between 11 to 60 years old. Immuncompromised patients were excluded from this study.

Thorough history, full ENT examination including nasal endoscopy under local anesthesia using 0° and 30° rigid Hopkins

telescope, radiological examination and laboratory investigations were done to all of them.

CT scans of the nose and paranasal sinuses axial and coronal (3 mm) slice, over the osteomeatal complex, revealed a mass in the nasal cavity, osteomeatal complex obstruction, anterior ethmoid sinus opacification, posterior ethmoid sinus opacification, frontal sinus opacification, sphenoid sinus opacification, concha bullosa, septal, agger nasi, erosion in lamina papyracea, erosion base of skull, Haller cell, Onodi cell, supra- orbital cell, carotid canal dehiscence, mucocele, abnormal uncinate process were detected. All patients had chest x-ray. All patients were sent for skin prick test. Total IgE was measured for all of them.

Routine investigations were done for all patients who underwent nasal surgery under general anasthesia.

Patients treated medically two week prior to surgery, with:

1. Oral Amoxicillin/clavulanic acid, 40 mg/kg/day, (tds). with maximal adult dose of 500/125 amoxicillin/clavulanic acid (tds).
2. Penicillin allergic patients were treated with clarithromycin 250 two times daily.
3. Oral steroid (prednisolone 5 mg tablet) in a dose of 0.5-1 mg/kg body weight (tds) maximal dose of 60mg /day (if there is no contraindication).
4. Intranasal steroid: Budesonide (Rhinocort R) for adults and children above 12 years in a dose of 64 micrograms (1 spray) each nostril twice daily.

All patients were underwent endoscopic sinus surgery under general anesthesia. Fifteen minutes before the patient enter the theater, a cotton pledge soaked with 50/50 of 1% xylometazoline and 2% lidocain, introduced into nasal cavity. Using rigid Hopkins rod endoscope (0° and 30°) with camera connected to the monitor, the nasal cavity was examined. Mucin collected initially by large syringe with a suction tube. The inflamed tissue and polyps was removed and collected in a saline-moistened sterile bottle.

By gentle medialization of the middle turbinate, the middle meatus exposure improves. If this was not enough, the tissue on the lateral side of the middle turbinate was removed using tissue shaver.

Next, uncinectomy was performed via an incision with the sharp end of freer elevator or a sickle knife. The incision was placed at the most anterior portion of the uncinate process, which is softer on palpation in comparison to the firmer lacrimal bone, where the nasolacrimal duct is located. Then, a Blakesley forceps is used to grasp the free uncinate edge and remove it. The remaining parts of the uncinate were then removed with great care to avoid unnecessary damage to the mucosa. After that 30° endoscope is used to identify the maxillary ostium then middle meatal antrostomy was done if required. The ethmoidal bulla was opened, identification of the basal lamella of the middle turbinate and then opening of the posterior ethmoid cells if it was indicated by CT scan. Entering the frontal recess or the sphenoid sinuses was not done unless these sinuses were involved in CT scan. All tissues were removed, kept in 10% formalin and sent for histopathological examination using H&E, revealed mucinous background &often took a chondroid appearance, while eosinophils and Charcot- Leyden crystals are heavily stained and become easily detectable. Histopathological study remains the most reliable indicator of AFRS even better than positive fungal culture as a positive fungal culture does not confirm the diagnosis of AFRS, nor does a negative culture exclude it. For example, fungi may proliferate as saprophytic growth in diseased sinuses. Again, fungal culture was not done in our setting due to the lack of mycology department in the hospital.

Results

Concerning the age factor which was range from (11-60) years. The highest number with nasal polyposis was seen in patients aged 50-60

years old with mean age (41.3) and SD (±13.7) (Fig. 1). There were 22 females (36.7%) and 38 males (63.3%). The male:female ratio was 1.7:1 with nasal polyposis while it was 1.3:1 with rhinosinusitis.

The most common symptom was nasal obstruction (90%) (fig. 2).

Endoscopically, mucosal congestion is the commonest finding while septal spur is the least presentation 13.3% (fig. 3).

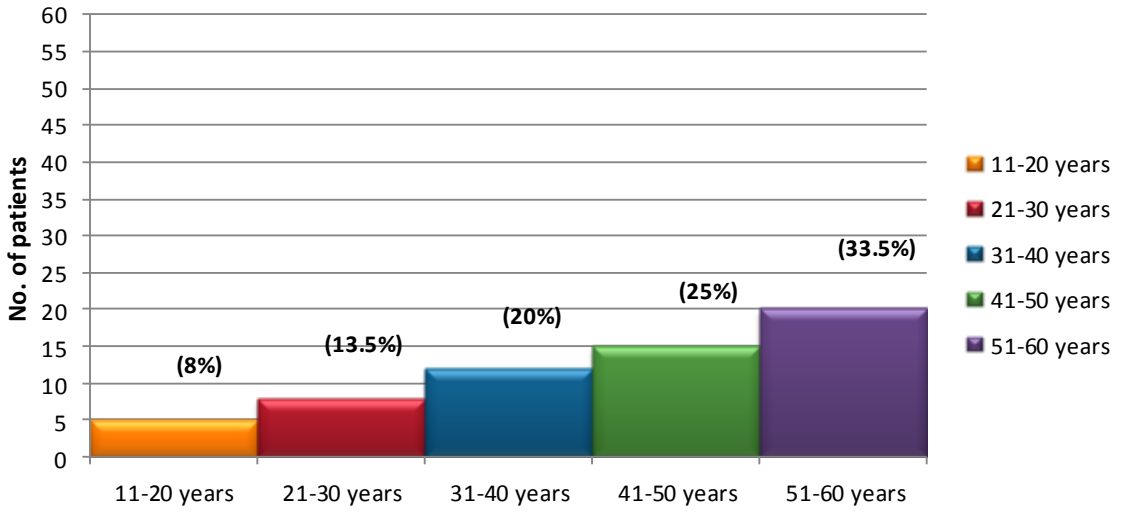

Figure 1. Age distribution in nasal polyposis patients

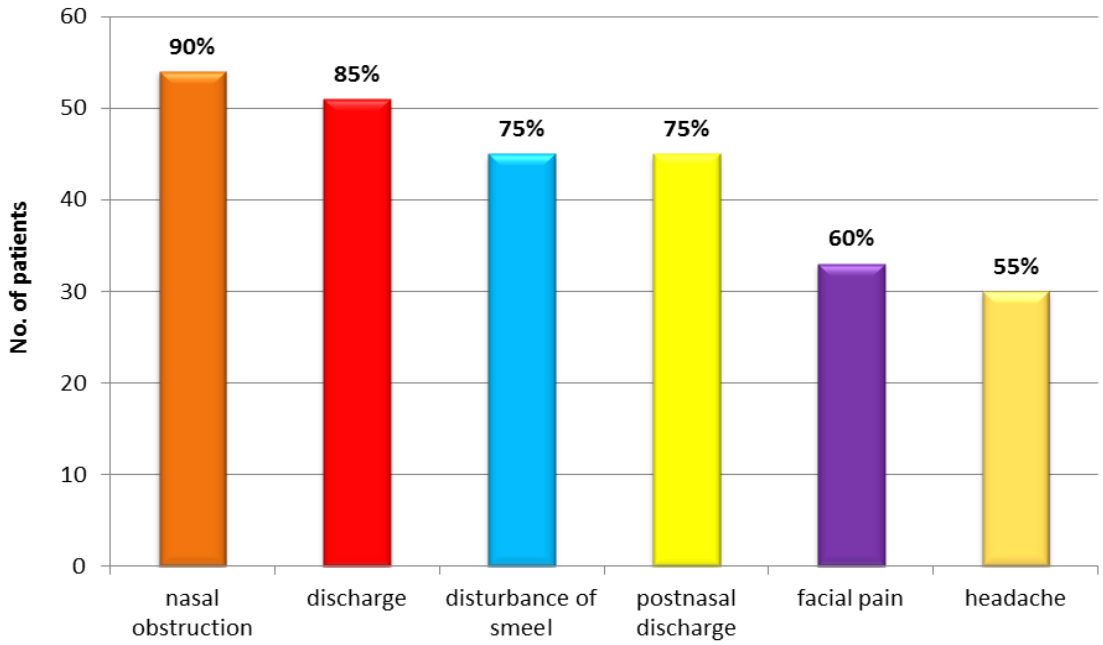

Figure 2. Clinical features of nasal polyposis

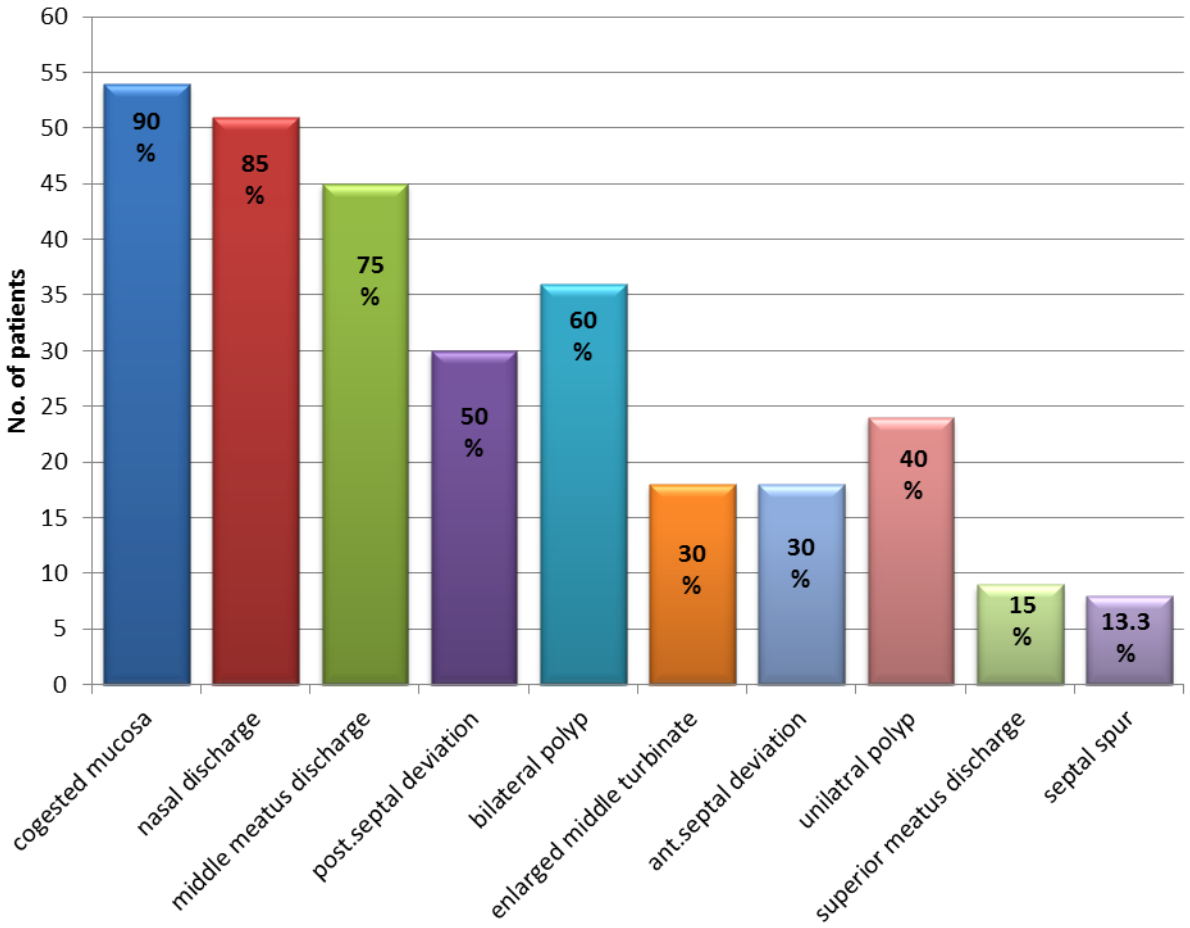

Figure 3. Endoscopic findings

On CT scan, mucosal thickening, osteomeatal complex obstruction and diffuse opacities were found in all cases (100%). Forty-eight (80%) of patients had more than three sinus involvement sites in patients with sinus polyposis (fig. 4), while mucosal thickening with sinus expansion in majority of AFRS patients but the least as bone erosion (fig. 5).

Concerning the laboratory diagnosis, fungal elements were found in 7 patients (11.7%) in histological specimens (If sample is positive for fungal element in histopathology, it was considered as positive mycological criteria). Total IgE was elevated in 22 (36.6%) patients as shown in figure (6).

Total IgE level is statistically not significant for the diagnosis of AFRS (P=0.1) (fig. 7).

For histopathological study that is shown in fig. (8), it is of excellent significance statistically in the diagnosis of bilateral nasal polyposis and AFRS (P=0.007), and of no significance with unilateral polyp (P=0.1).

Only 5% patients show association of nasal polyposis with Samter's triad (nasal polyposis with aspirin intolerance and asthma) (fig. 9). Therefore; only 7 patients of had findings consistent with AFRS.

The characteristics of patients with AFRS is summarized in table 4.

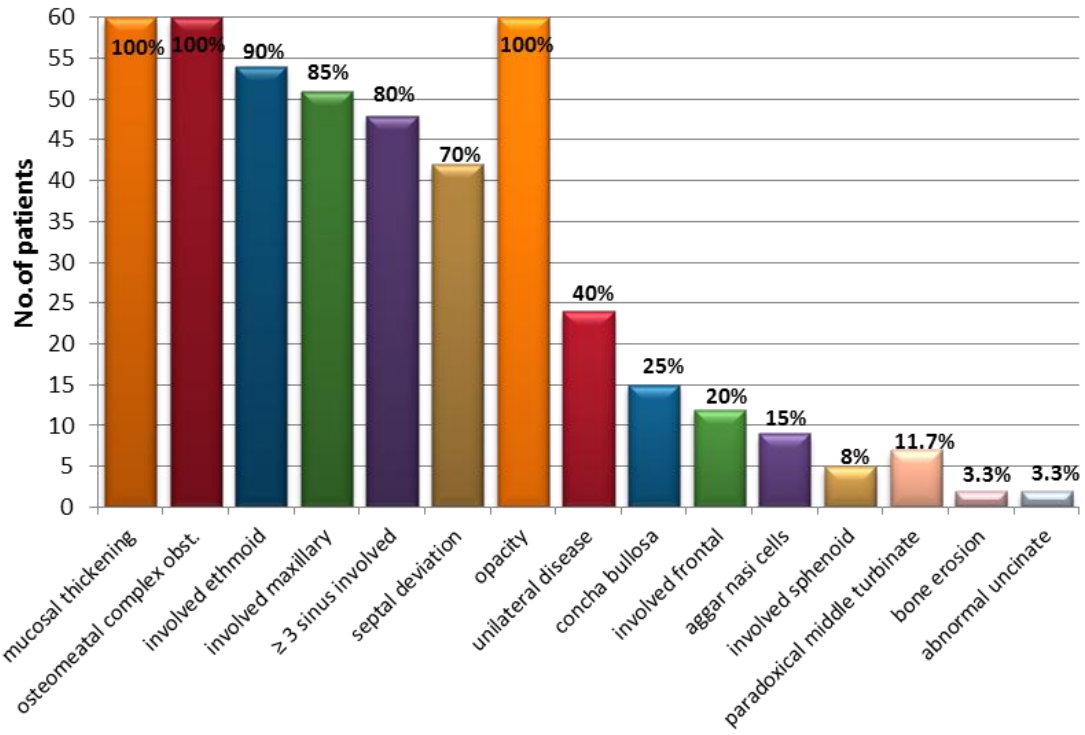

Figure 4. CT findings in nasal polyposis patients

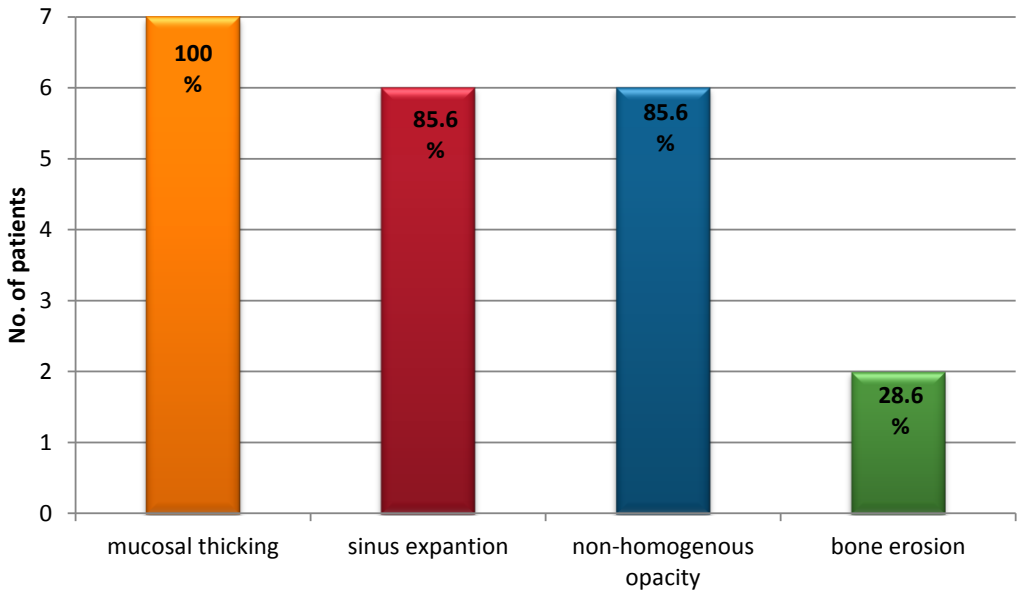

Figure 5. CT findings in AFRS patients

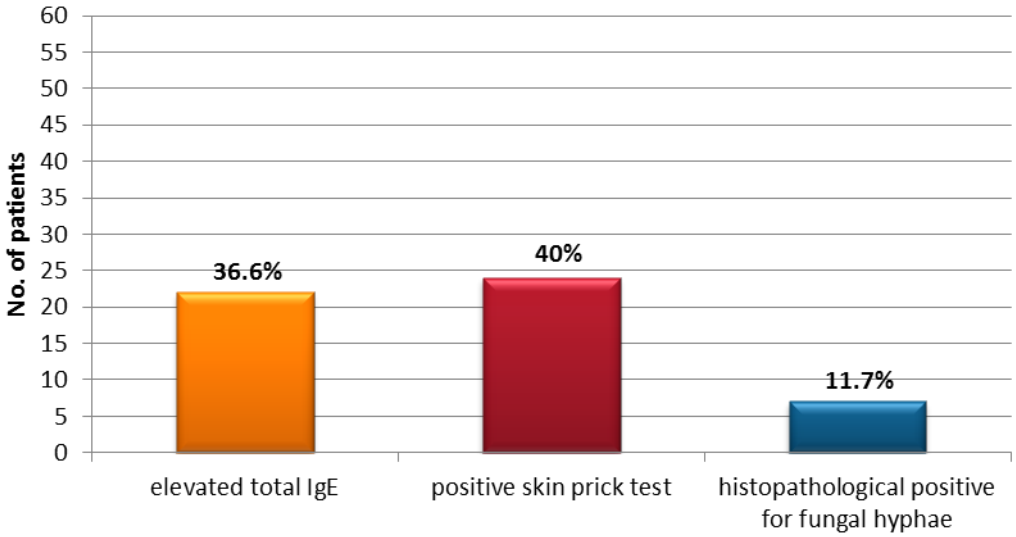

Figure 6. Laboratory findings in nasal polyposis patients

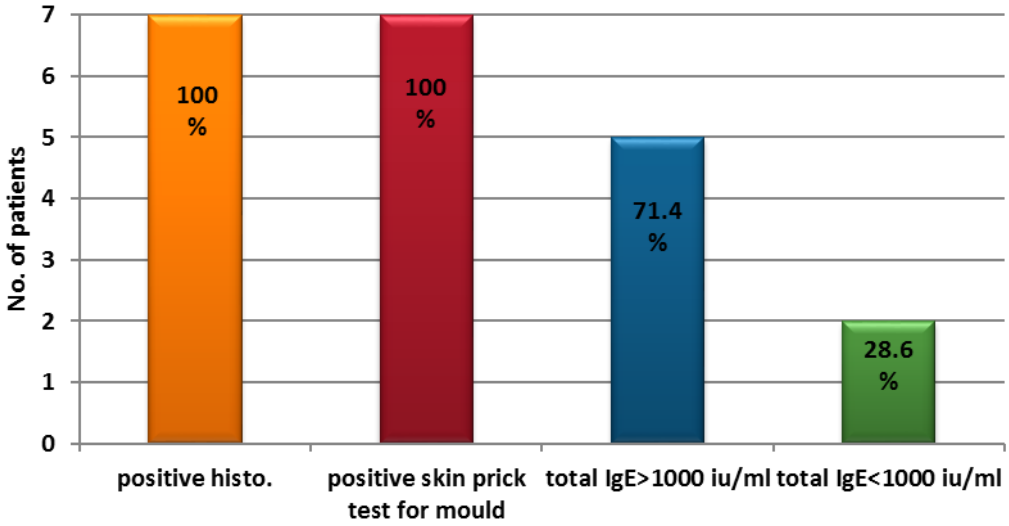

Figure 7. laboratory findings in AFRS patients

Discussion

All patients in this study were immunocompetent with greatest number of patients with AFRS occur in the 3rd decade of life, which is similar to the findings of Zakirullah et al [3].

In this current study, male:female (M/F) ratio in patients with AFRS is 1.3:1, the same male predominance as founded by Thahim et al [4]. However, the M/F ratio may be age dependent. A study done on UT Southwestern' children, male predominance were found (M/F ratio 2.1:1; average age=13 year) while in adults, females were predominant (M/F ratio 1:1.4; average age 36 year) [5].

Nasal obstruction, nasal congestion, nasal discharge and postnasal drip are the major clinical presentation in those patients. Same finding were observed by Hedayati et al [6] and Zakirullah et al [3] who found that 96% of his patients complain of nasal obstruction.

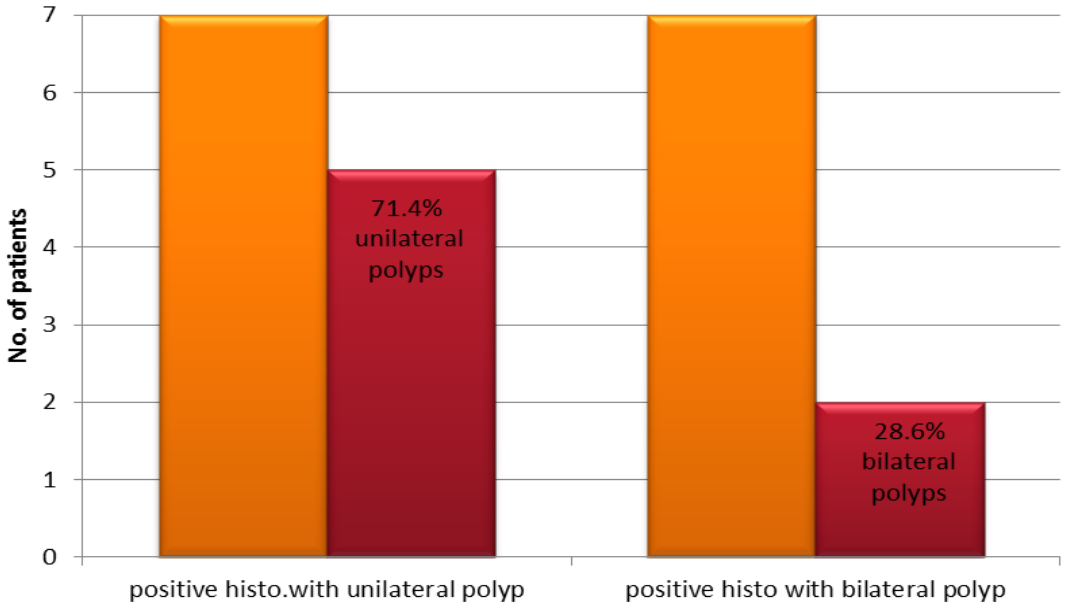

Figure 8. Correlation between positive histopathlogical findings and presence of nasal polyposis

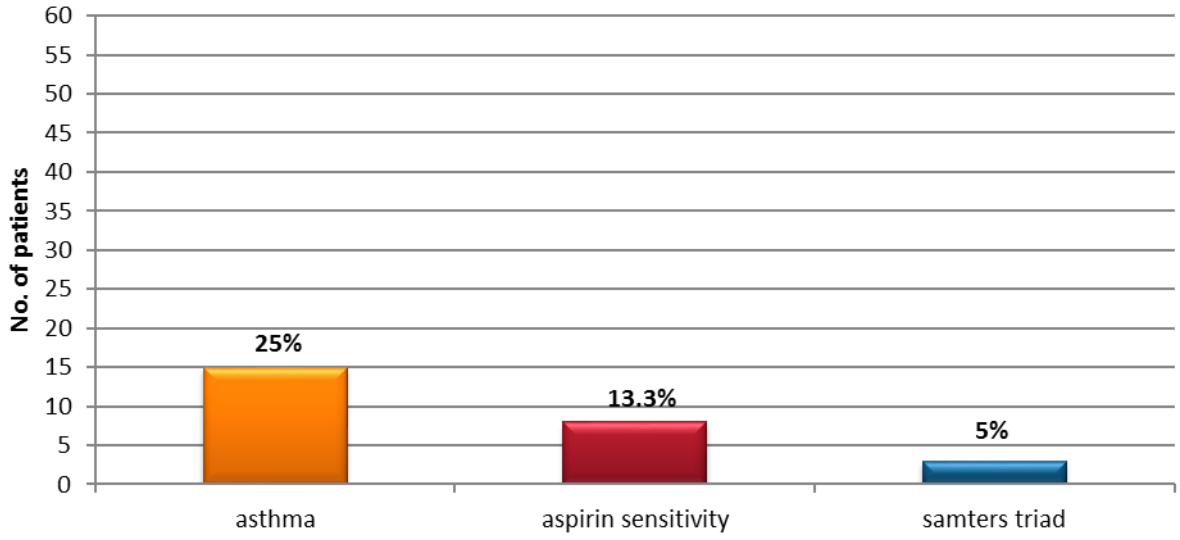

Figure 9. Association between nasal polyposis, asthma and aspirin sensitivity

In the current study, 7 patients had finding consistent with AFRS, the disease was unilateral in 5 (71.4%) of them and bilateral in 2 (28.6%). Bent and Kuhn [7] and Thahim et al [4] also reported unilateral predominance of the disease. On the other hand, Marple found 51% bilateral disease in 45 patients [8].

Operative details showed extensive polyposis and characteristic thick peanut-buttery tan to dark-green allergic mucin in all cases. Similar findings are reported by Bent and Kuhn [7] and by Schubert [9]. Samter's triad (the association of nasal polyposis, aspirin intolerance and asthma) was found in 5% of our patients; Kim JE report that 4.8% of patient undergone functional endoscopic sinus surgery had Samter's triad [10].

Table 4: The distribution of patients with allergic fungal rhinosinusitis according to age, sex, site of polyposis & CT scan findings

AFRS patients	Number		Number	%
Age	7	2nd decade	1	14.3
		3rd decade	5	71.4
		4th decade	1	14.3
		5th decade	-	-
Gender	7	Female	3	42.9
		Male	4	57.1
Polyp	7	Bilateral polyp	2	28.6
		Unilateral polyp	5	71.4
CT finding	7	Non-homogenous opacity	6	85.6
		Bone erosion	2	28.6
		Mucosal thickening	7	100

Fungal elements were detected by histopathological examination in 7 (11.7%) of our patients, Kupferberg et al found positive histopathological finding in 12.1 of her 91 patients [(11)].

This variation in the results between the current study and the others may be due to geographic variation in the prevalence of AFRS.

The most common CT findings in current study were mucosal thickening and osteomeatal complex obstruction both appeared in all patient 100%. Same findings reported by Mukherji et al [(12)] who found mucosal thickening in all the 45 studied patients.

Also CT scan showed that unilateral disease was found in 5 of 7 patients diagnosed as having AFRS (71.4%) and 85.6% had double density sign, although these findings are not specific for AFRS, they remain relatively characteristic of the disease and may provide preoperative information supportive of a diagnosis of AFRS.

Expansion or thinning of involved sinus walls is common in AFRS and is caused by the expansible nature of the accumulating mucin and polyps.

Bone erosion found in 2 (3.3%) of patients. Bakhshaee et al [(13)] found 2 (5.26%) of his 60 patients were with bone erosion, this actually not due to direct invasion by the fungus but it results from long standing pressure by polyps and accumulation of mucin on the adjacent bone.

Other laboratory investigations showed increased level of total IgE in 22 cases, however, not all of them were diagnosed as having AFRS because other immune mediated conditions are associated with high level of IgE e.g. allergic rhinitis. These results are matching with the reports of Schubert [(14)].

Total IgE values generally were elevated in AFRS, often to more than 1,000 IU/mL. Total IgE level traditionally has been used to monitor the clinical activity of allergic bronchopulmonary fungal disease. On the basis of similar IgE behavior associated with recurrence of AFRS, and therefore; it is proposed as a useful indicator of AFRS clinical activity, Schubert [(14)].

Thus, it can be concluded that AFRS is a significant cause of nasal polyposis. It is recommended that all patients with nasal polyposis biopsy should be taken and send for histopathology because AFRS is significant cause of nasal polyposis. Further studies should be done to detect the causative agents, the level of genu species, fungi, by doing culture method.

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Author Contribution

All results were collected by the three authors, reviewed and written by Dr Al-hassani.

Conflict of interest

No publication conflict of interest

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The Role of Atorvastatin in the Treatment of Chronic Obstructive Pulmonary Disease with Elevated High Sensitive C-Reactive Protein

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Abstract

Background There is a growing interest in the potential beneficial effects of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors drugs (statins) in chronic obstructive pulmonary disease (COPD) as anti-inflammatory agent. The basis of the systemic inflammation in COPD comes from two possibilities: spill-over effect or inherent systemic-based pro-inflammatory state conferred by a genetic disposition. The inhaler-based therapy for COPD aims to reduce symptoms, improve quality of life and reduce hospitalization, but does not substantially change disease progression or reduce mortality.

Objective To assess the efficacy and safety of statin therapy in COPD patients with evidence of inflammatory markers.

Methods Ninety patients were included in the study, aged 40 years or more, who visit the outpatient private clinic in Babylon government, Iraq from September 2012 to April 2016. They were divided randomly for 3 groups (receiving 40 mg, 10 mg atorvastatin or placebo, respectively), in addition to their baseline treatment. Severity reassessment performed after 6 months' duration of treatment as well as hospitalization frequency and mortality.

Results Statin therapy showed a significant improvement in the both doses treated groups regarding the HsCRP, CAT (chronic obstructive airway disease assessment test) score and forced expiratory volume in first second after 6 months of treatment. This improvement fails to be reported significant effect on CAT score when compared to placebo group. Thus, statin treatment doesn't show any symptomatic improvement as measured by CAT score over placebo treatment.

Conclusion The statin treatment in patient with chronic obstructive pulmonary disease can be useful in form of improvement of hospitalization, number of exacerbations but not mortality.

Keywords Atorvastatin, statins, COPD, high sensitive C-reactive protein.

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List of abbreviation: HMG-CoA = 3-hydroxy-3-methyl-glutaryl-coenzyme A, COPD = Chronic obstructive pulmonary disease, FEV1 = Forced expiratory volume in 1 second, CRP = C-reactive protein, HsCRP = High sensitivity C-reactive protein,

Introduction

Over the last 20 years, there has been a growing interest in the potential beneficial effects of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) in chronic obstructive pulmonary disease (COPD) [1-5].

The clinical studies suggest that statin therapy may confer a number of benefits in COPD such as reducing both the frequency of, and mortality from, infective exacerbations, reducing mortality from pneumonia, reduced decline in lung function and reduced risk of lung cancer [1,2,4]. While the benefits of statin therapy in COPD can be rationally explained by the known pharmacological effects of statins on the lungs [5,6] and justified where co-morbid cardiac disease commonly exists [1,7].

The basis of the systemic inflammation in COPD comes from two possibilities; either 'spill-over' effect from inflammation driven primarily in the lungs in response to aero-pollutants chiefly cigarette smoke exposure, neutrophilic inflammation and recurrent infection [8] or inherent systemic-based pro-inflammatory state conferred by a genetic disposition [1,9]. Smoking significantly enhances (possibly unmasking) this inflammatory disposition by being a recurring pro-inflammatory stimulus to the pulmonary and immune systems. This possibility is supported by data showing that poor lung function (reduced forced expiratory volume in 1 second (FEV1)), independent of smoking, predicts poor vascular and respiratory outcomes [10,11]. Conversely normal lung function, even after decades of smoking exposure, confers a greater 'degree of protection' from cardiorespiratory outcomes than that observed in those with poor lung function who have been lifelong non-smokers. These observations raise the possibility that systemic inflammation could be a secondary driver of inflammation in the bronchial epithelium alongside that derived from smoke exposure ('reverse' effect) [11,12].

Given that statins have been shown to lower systemic inflammation through inhibition of the inflammatory pathways mediated by NF-κB and IL-6 [13]. It is no surprise that statins are now considered effective anti-inflammatory agents, lowering systemic markers (IL-6 and C-reactive protein (CRP)) by over 50% in a matter of days [13,14]. It should not be forgotten that statins also possess important anti-apoptotic, anti-oxidant and anti-proliferative effects, whether these are independent of their anti-inflammatory effects is not known [14].

In the treatment of COPD, while currently recommended therapy for COPD is primarily inhaler-based, where the aim is to reduce symptoms, improve quality of life and reduce hospitalization, this approach does not substantially change disease progression or reduce mortality [15].

More importantly, these treatments do not improve the many and varied systemic manifestations of COPD. The only oral medication for COPD is roflumilast, which is limited to severe disease characterized by recurrent acute exacerbations of COPD [16,17].

This study is a clinical trial to assess the efficacy and safety of statin therapy in 2 different doses (high vs. low dose vs. placebo) for COPD patients with evidence of inflammatory markers as high sensitivity CRP (HsCRP) in term of severity, hospitalization and mortality.

Methods

Ninety patients from both genders were included in the study, whom age from 40 years and above. They were treated at the outpatient private clinic in Babylon government, Iraq, during the period from September 2012 to April 2016.

Inclusion criteria considered any patients with diagnosis of COPD by consistent history of smoking, clinical manifestation with spirometric criteria and high resolution computerized tomography (HRCT) findings who don't have any other diseases like ischemic heart disease, diabetes mellitus, hypertension or dyslipidemia neither before nor at time of diagnosis. For all enrolled patient, there is evidence of inflammation detected by positive HsCRP. All patients were in stable state maintained using inhaled budesonide /formeterol (160/4.5) twice daily and/or long acting anticholinergic and/or phyllocontine (225 mg) at night.

Those who are excluded from the trial included any patient refused the participation in the study or those with no obvious diagnosis of COPD or patients presenting during exacerbation episodes and patients not under regular treatment.

The 90 patients were divided equally and randomly into 3 groups:

Group 1: Thirty patients receive atorvastatin 40 mg at night

Group 2: Thirty patients receive atorvastatin 10 mg at night
Group 3: Thirty patients receive placebo treatment in addition to their baseline treatment for all 3 groups.
All patients are assessed at zero time for severity using chronic obstructive airway disease assessment test (CAT) score (clinical questions used to assess the control briefly by the patients word), spirometry in addition to their initial liver function tests, creatinine phosphokinase and HsCRP. A second assessment performed at 6 months' time for all the above initial evaluation as well as inquiring for COPD–induced hospitalization, exacerbations attacks and over-all mortality, in addition to adverse effect like myopathy or hepatitis picture necessitate medical seek. Primary end-points were reduction in HsCRP (inflammatory markers), FEV1 changes (physiological markers) and CAT score changes (clinical markers for diseases control). Secondary end-points were frequency of hospitalization, exacerbation and over-all mortality. Safety issues were also assessed like GIT effect, CNS effect as dizziness and amnesia, liver effect and myopathy.
Statistical analysis was carried out using SPSS version 17. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as (Mean ± SD). Paired t-test was used to compare means between paired numerical readings when difference between readings was normally distributed. Wilcoxon Signed Ranks Test was used to compare means between paired numerical readings when difference between readings was not normally distributed. A p-value of ≤ 0.05 was considered as significant.

Results

Patients' demography

As shown in table 1, the majority of patients were males and old age as the risk factor is smoking, which have a stronger effect in male and in elderly.

Table 1. The Distribution of patients with COPD according to age gender

Age (years)	Mean (64.52 ± 7.14)	Range (45-78)
Gender	**No.**	**%**
Male	51	56.7
Female	39	43.3
Total	90	100.0

Regarding frequency of hospital admissions in the 6 months of study; the majority of the patients does not need hospitalization as most of them could be treated as an outpatient as shown in (Figure 1).

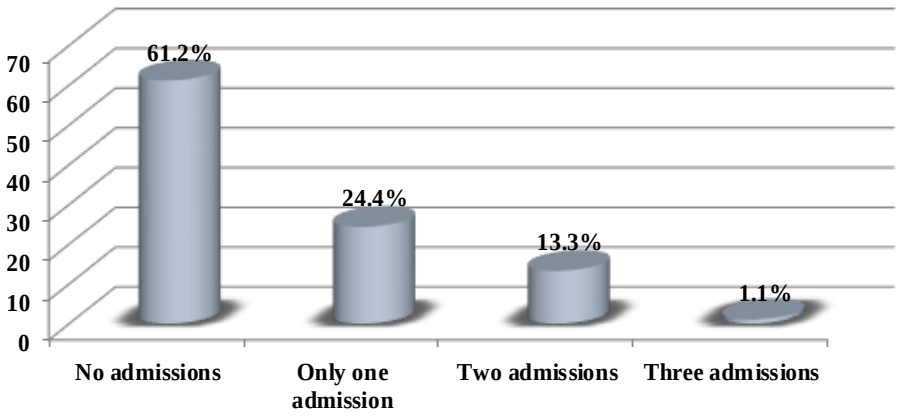

Figure 1. Distribution of patients according to frequency of hospital admissions

The distribution of patients with COPD according to frequency of exacerbations in the 6 months of the study is shown in (Figure 2) where the majority showed 1-2 attacks of exacerbation in a relatively short period, which represent the heavy burden of this disease on the health budget.

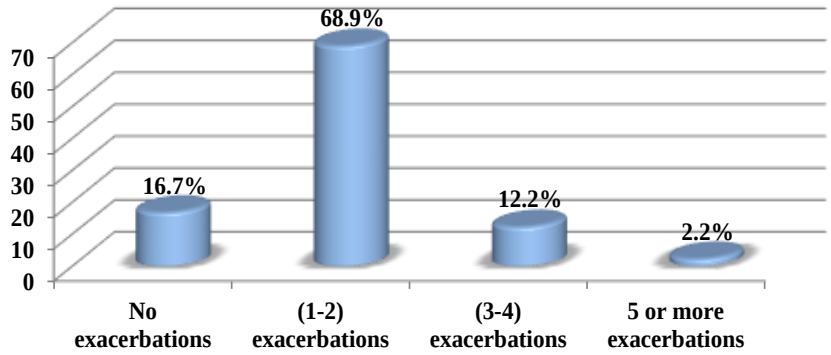

Figure 2. Distribution of patients according to frequency of exacerbations.

The distribution of patients with COPD according to side effects developed in the study duration: Both groups showed side effects, which mean that these effects may be not related to the drug treatment alone but also to the disease itself as shown in (Table 2).

Table 2. Distribution of patients according to side effects

Clinical side effects	Number	%
Nervous system		
Yes	28	31.1
No	62	68.9
Total	90	100
Liver abnormalities		
Yes	3	3.3
No	87	96.7
Total	90	100
Gastrointestinal system		
Yes	44	48.9
No	46	51.1
Total	90	100
Myopathy		
Yes	10	11.1
No	80	88.9
Total	90	100

Figure 3 shows the distribution of patients with COPD according to death in the study duration where death occurs in about (10%) of patients.

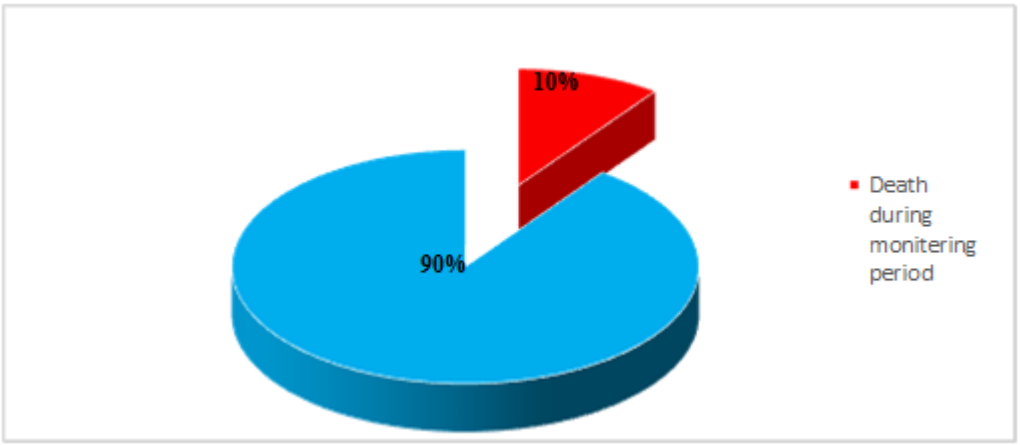

Figure 3. Distribution of patients according to death occurrence

The Study Groups and Study Variables analysis
There were no significant differences between means of age & gender in the three study groups as shown in (Tables 3 and Table 4) which indicate matching of the three groups.

Table 3. The mean differences of age by study groups

Gender	Group 1 No. (%)	Group 2 No. (%)	Group 3 No. (%)	P-value
Male	17 (56.7)	14 (46.7)	20 (66.7)	
Female	13 (43.3)	16 (53.3)	10 (33.3)	0.295
Total	30 (100)	30 (100)	30 (100)	

*p value ≤ 0.05 was significant
**p value ≤ 0.01 was highly significant

Table 4. The Distribution of patients with COPD according to age with each study groups

Study groups	No.	Age Mean ± SD	P value
Group 1	30	65.06 ± 6.52	
Group 2	30	65.23 ± 5.56	0.502
Group 3	30	63.26 ± 8.97	

Table 5 shows mean differences of HsCRP, CAT-score and FEV1 after treatment by study groups, there were significant differences between means of HsCRP and FEV1 by study groups, while there were no significant differences between means of CAT- score by study groups.

Table 5. Mean differences of CRP, CAT-score and FEV1 after treatment by study groups

Variable	Study groups	No.	Mean ± SD	P-value
CRP	Group 1	28	2.98 ± 1.05	<0.001*
	Group 2	28	3.32 ± 1.02	
	Group 3	25	4.57 ± 0.67	
CAT-score	Group 1	28	18.92 ± 5.44	0.53
	Group 2	28	17.60 ± 5.43	
	Group 3	25	18.96 ± 4.05	
FEV1	Group 1	28	47.96 ± 7.63	0.033*
	Group 2	28	47.57 ± 8.46	
	Group 3	25	42.76 ± 7.25	

Note: 9 patients from all groups died during follow up, *p value ≤ 0.05 was significant

The mean differences of HsCRP level, CAT-score and FEV1 before and after high dose of statin using (40 mg/day) is illustrated in table 6; were statistically significant.

Table 6: The mean differences of HsCRP, CAT-Score and FEV1 before and after high dose of statin

Variable	Categories	No.	Mean	P value
HsCRP	Before treatment	28	4.37 ± 0.94	< 0.001*
	After treatment	28	2.98 ± 1.05	
CAT-score	Before treatment	28	23.82 ± 5.38	< 0.001*
	After treatment	28	18.92 ± 5.44	
FEV1	Before treatment	28	38.28 ± 6.96	< 0.001*
	After treatment	28	47.96 ± 7.63	

Note: Two patients from this group died during follow up, *p value ≤ 0.05 was significant

The mean differences of CRP level, CAT-score and FEV1 before and after high dose of statin using (10 mg/day) is shown in table 7; were also statistically significant.

There was a statistically significant association between use of statin, frequency of hospital admissions and frequency of exacerbations among patients with chronic obstructive airway diseases as shown in table 8.

Regarding mortality, there was no significant association between use of statin and patient's mortality as shown in table 9.

There was significant association between use of statin and gastrointestinal upset and myopathy; while there was no significant association between use of statin and other system effects as shown in table 10.

Table 7. The mean differences of HsCRP, CAT-Score and FEV1 before and after low dose of statin

Variable	Categories	No.	Mean	P value
HsCRP	Before treatment	28	4.53 ± 0.84	< 0.001*
	After treatment	28	3.32 ± 1.02	
CAT-score	Before treatment	28	22.46 ± 5.29	< 0.001*
	After treatment	28	17.60 ± 5.43	
FEV1	Before treatment	28	39.35 ± 5.95	< 0.001*
	After treatment	28	47.57 ± 8.46	

Note: Two patients from this group died during follow up, *p value ≤ 0.05 was significant

Table 8. Association between patients' groups and frequency of exacerbation or hospital admissions

Study variables		Study Groups High dose No. (%)	Low dose No. (%)	Placebo No. (%)	P value
Number of admission	No	23 (76.7)	19 (63.3)	13 (43.3)	0.029*
	One or more	7 (23.3)	11 (36.7)	17 (56.7)	
Number of exacerbations	No	6 (20.0)	7 (23.3)	2 (6.7)	< 0.001*
	1 or 2	24 (80.0)	21 (70.0)	17 (56.6)	
	≥ 3	0 (0.0)	2 (6.7)	11 (36.7)	

*p value ≤ 0.05 was significant

Table 9. Association between study groups and mortality

	Study Groups High dose No. (%)	Low dose No. (%)	Placebo No. (%)	P-value
Dead during follow up	2 (6.7)	2 (6.7)	5 (16.7)	0.494
Still alive after follow up period	28 (93.3)	28 (93.3)	25 (83.3)	

*p value ≤ 0.05 was significant

Discussion

The majority of patients (61.2%) does not need hospitalization despite that 68.9% show single exacerbation, which means that much patients with mild COPD exacerbation can be treatment as outpatients, this finding is consistent with most of the clinical practice data [18]. Both the overall side effects seen in the study groups (statin or the placebo groups) are more in the GI upset and muscular effect, this was expected finding as the both can be induced by the drugs or the disease itself [19]. The overall mortality is 10% over 6 months, 5 patients of them in the placebo arm but the difference is not statistically significant, this finding is higher than Kirchmayer et al, which may be due to small sample size. The effect on all-cause mortality is a gray area because it is possible that there are potential beneficial effects of statins on cardiovascular comorbidities as it is known that smoking is a causative factor in the majority of patients with COPD and in the

development of coronary artery disease. Cardiovascular comorbidities are common in COPD patients [20].

Table 10. Association between study groups and side effects

Clinical manifestations	Study Groups			P value
	High dose No. (%)	Low dose No. (%)	Placebo No. (%)	
GIT upset				
Present	22 (73.3)	11 (36.7)	11 (36.7)	
Absent	8 (26.7)	19 (63.3)	19 (63.3)	0.005*
CNS				
Present	11 (36.7)	10 (33.3)	7 (23.3)	
Absent	19 (63.3)	20 (66.7)	23 (76.7)	0.510
Liver effects				
Present	3 (10.0)	0 (0.0)	0 (0.0)	
Absent	27 (90.0)	30 (100.0)	30 (100.0)	0.104
Myopathy				
Present	7 (23.3)	3 (10.0)	0 (0.0)	
Absent	23 (76.7)	27 (90.0)	30 (100.0)	0.015*

*p value ≤ 0.05 was significant

The finding from the current study that statins not reduced mortality in patients with COPD were inconsistent with most of the included studies [21,22]. However, a large multi-centre randomized clinical trial showed that statins had no effect on COPD exacerbations, which was consistent with this finding [23]. Several reasons might be accountable for this; only moderate-to-severe COPD patients were included in these studies and it is unclear whether statins were beneficial for patients with less impairment. In addition, the mean followed-up time was around 2 years, which might be a significantly different compared to the short-term effect in this study. Although the statin show a significant improvement in the both treated groups regarding the HsCRP, CAT score and FEV1 after 6 months of treatment, this improvement fail to show significant effect on CAT score when compared to placebo group i.e. the statin treatment not show symptomatic improvement measured by CAT score over placebo treatment. These findings consistent with some studies and inconsistent with others and that because it is depend on the study criteria and design as seen in Carlson et al study [24]. The statin treatment showed significant improvement of hospitalization, number of exacerbations but not mortality, again this finding is not proved yet [23,26]. The useful effect of statin was shown both in high and low dose in all primary endpoints but the side effects were higher in high dose arm as expected as the statin side effects are dose dependent [27].

In conclusion; atorvastatin use in COPD may have some beneficial effects on the disease profile mainly on the inflammatory aspects in addition to possible another useful effect on lung functions decline, frequency of exacerbations, hospitalization and even symptomatology of the patients. However, there is no clear benefits of statin in all-cause mortality outcome. The gastrointestinal and muscular adverse effects were significantly higher in the treatment groups patients.

It is recommended now to study the statin use in COPD in larger study with a longer duration and use other family drugs members and other doses to see whether it is group or drug related effects as well as using other inflammatory markers like interleukins (IL6) or tumor necrosis factor (TNF) to confirm their effect of the treatment in COPD.

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Conflict of interest

Drugs supplier and the payment for the drugs cost.

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Renal Biopsy Practice in Iraq: A Systematic Review

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Abstract

Background Renal biopsy is an indispensable tool for the accurate diagnosis and treatment of different forms of renal disease. Many advances have improved the utility and safety of renal biopsy procedure.

Objective To determine the extent to which renal biopsy used and how much it affects the Iraqi nephrology practice.

Methods A systematic review of articles published from 1990 to 2014 was carried out by searching Medline and Google scholar. We included all studies that concerned with renal biopsy as the main intervention. Eligible studies determined by predefined criteria were reviewed. Data from these studies combined and analyzed.

Results The search yielded 55 title, of which, eleven studies met the inclusion criteria. Analysis of the 11 studies (2278 patients) that underwent 2667 renal biopsies. Ten studies centered on native renal pathology and one study focused on allograft pathology.

Conclusion The Iraqi renal biopsy practice and pathology interpretation need to be rejuvenated by modern pathology services, data registry, and well-designed studies with clinical impact.

Keywords Renal biopsy, Allograft, Pathology, Iraq.

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List of abbreviation: CKD = Chronic kidney disease, IF = Immunofluorescence, EM = Electron microscopy, LM = Light microscopy, IHC = Immunohistochemistry, IP = Immune peroxidase, FSGS = Focal segmental glomerulosclerosis, MCD = Minimal change disease, MPGN = Membranoproliferative glomerulonephritis, IgAN = Immunoglobulin A nephropathy, LN = Lupus nephritis.

Introduction

Biopsy first defined by Besnier in 1895 became useful towards the end of nineteenth century [(1)]. Nils Alwall of Sweden performed the first systematic aspiration needle biopsies of the kidney in 1944 but the first published results were in 1951 by Iversen and Burn in Copenhagen. With the development of good histology, and the sequential appearance of new techniques like immunofluorescence (IF) (in 1956) and electron microscopy (EM) (in 1957), it became an integral part of clinical nephrology practice [(1,2)].

Percutaneous renal biopsy is a relatively safe procedure and a powerful tool in the diagnosis and management of renal diseases [(3)]. Refinement of biopsy technique and interpretation skills led to major development and a paradigm shift toward incorporation of modern molecular techniques. This was due to the explosion in kidney disease research in the last 20-30 years that brought new knowledge from bench to bedside and resulted in new molecular and genetic tools that enhance the diagnostic and prognostic power of the renal biopsy. These genomic technologies show a useful adjunct to the renal biopsy, and provide examples of how these may transform pathologic interpretation into molecular disease phenotypes [(4)].

Although most nephrologists recognize several clear indications for a renal biopsy, it is still underutilized [5].

Organizing the multiplicity of information available in a renal biopsy to maximize benefits to the patient, as well as to the epidemiologist and researcher, is one of the challenges that face the nephrology community. In Iraq, renal biopsy practice started at late seventies with Menghini-type needles used for liver biopsy. Then tru-cut type needles used till now with very limited use of biopsy guns. Currently all renal biopsies are performed with the help of ultrasound [6].

The aim is to review the practice of these biopsies from indications, through technique to therapeutic and prognostic implications and to provide the best available evidence about how to fully utilize renal biopsy in Iraqi nephrology practice.

Methods

It is a systematic review using the PICO (Population, Intervention, Comparison, and Outcome) model [7]. Studies populations are patients (adults and children) who had been underwent renal biopsy. Inclusion criteria included: original articles publications, whether electronically or hard copies papers which are published in English language throughout the years from 1990 to 2014 and labeled as Iraqi studies. All these studies must incorporated renal biopsy as the main intervention or part of study protocol. While, the grey literatures and unpublished data were not considered.

Searching keywords included (renal biopsy, renal pathology, glomerular, glomerulonephritis and Iraq,). Different combinations of the above keyword might also help in searching and truncation method used. Search was through full text articles and abstracts [8].

Results

The first hit of data bases was (55), then with the use of exclusion criteria (and) in combining the different keyword searches we reached to (39) search results. After removal of duplicates only (14) remained. With the use of other inclusion criteria only (11) articles left. All of them are original studies (Figure 1).

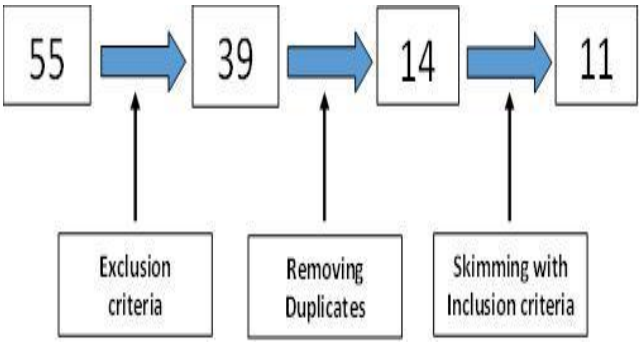

Figure 1. Data Collection

The total number of subjects included in these eleven papers is 2278. Eleven original articles have been reviewed. Ten studies involved adult patients and only one study was among pediatric age group. One study focused on allograft biopsy while all the other nine were studies of native renal pathology. A list of the eleven studies is given in table 1, representing the name of authors, year of publication, the corresponding journal, type of study, number of participant, and study objectives. The results of searched articles will be presented under the following headings: Study objectives, Practice and technique, Pathology and Therapeutic and prognostic implications.

Study Objectives

By reviewing the above studies, we found that there is more or less an agreement between almost all the studies about the main objective of the studies which is the spectrum of glomerular disease. Seven out of eleven studies conducted to have a final conclusion on glomerular diseases pattern whether by light microscopy (LM) or IF. The study by Al-Taee [9]

presents the spectrum of amyloidosis causes in Iraq. The other three studies aimed clinicopathologic correlates with therapeutic and prognostic impacts [10,11,12].

The study by Al-Omairi seems to be a study for correlation between pathology and therapeutic response in childhood lupus [13]. This in turn dictated the design of these studies. Eight of them were retrospective and cross sectional studies. The other two were prospective studies to show the effect on prognosis or to describe the effect of therapeutic intervention [11,12].

Practice and Technique

Two studies described the biopsy procedure [12,14]. Menghini-type needle used in most of biopsies before 2005. In the later years, semi-automated needle was the main tool. Studies by Ali [12] and Al-Saedi [14] described the use of different gauge needles, 16 and 18 gauge. Also, these two studies described the use of ultrasound either in pre-biopsy marking or in real time biopsy guidance. One study described the rate of complication and safety of biopsy [12,14]. There was very limited data about the rate of redo or serial biopsies.

Pathology

Light microscopy was the standard modality of nephropathology in all studies. The oldest study was by Salim and Ezzat [6] showed the full renal pathology lab potential through the study of 478 biopsies. All specimens were studied by LM and a small number, when available; had been proceeded for IF and EM. Unfortunately, neither IF, nor EM are not available for histopathology services for the last twenty years. Two studies described the practice of cutting, fixation and staining for all steps of biopsy sample examination [6,12]. One study entirely based on IF [15] and this had been conducted through collaboration with a pathology lab outside Iraq. IF findings also described in allograft biopsy by immunohistochemistry using immune peroxidase (IP) [12]. Data about adequacy of Iraqi renal biopsies recorded in two studies only [12,16]. Still focal segmental glomerulosclerosis (FSGS) is the most common primary glomerular pathology in Iraqi biopsy specimens. Together with minimal change disease (MCD) they consist about 45-50%. This was evident in four studies [11,14,17,18]. Even with IF, FSGS was the most prevalent lesion [15]. In one study by Al-Saedi, membranoproliferative glomerulonephritis was the most prevalent [16]. Tuberculosis is the most common cause of renal amyloidosis in Iraq [9]. Salim and Ezzat reported renal involvement in systemic disease in 14% of the examined biopsies [6].

Therapeutic and Prognostic Implications

Two studies described clinicopathological correlates [10,11]. One study showed the prognostic value of tubulointerstitial lesion in renal biopsies for patients with glomerular disorders [11]. One study disclosed the role of biopsy in guiding therapeutic intervention [12].

Discussion

This systematic review included 2667 renal biopsies and showed a major finding that the main objectives of Iraqi renal biopsy studies was just to describe the pattern of their findings on individual bases of their respective renal units. In addition, it's apparent that all this practice faces challenges that need to be addressed. It's well known how much renal pathology is essential for nephrology practice but in this study, we tried to have evidence how much Iraqi practice of renal biopsy influenced the care for Iraqi renal patients.

Study design: is the first challenge in renal biopsy studies. It would be dictated always by the question why we need renal pathology based studies. Do we need just shallow data or we need in depth studies that full- fill the terms of validity and usefulness? According to this systematic review still there is no consensus Iraqi nephrology practice about indications, the procedure, interpretation, clinicopathological correlates, complications, and prognostic implications.

Many developed countries have established national renal biopsy registries to document such variations and changing trends in the disease spectrum. However, such registries are largely lacking or are in primitive shape in most of the developing countries [19].

Table 1. Studies included in the review by the date of publication

Author	Year	Journal	Patients No.	Type of study	Aim and Results
Salim and Ezzat [6]	1997	J Fac Med Baghdad	369	Original	Pattern of Glomerular Ds
Shaker et al[17]	2002	SJKDT	520	Original	Pattern of Glomerular Ds
Al-Duliemi et al [10]	2005	J Fac Med Baghdad	100	Original	Clinicopathological Correlate
Mansour and Al-Shamma [18]	2005	J ABMS	136	Original	Pattern of Glomerular Ds
Al-Taee et al [9]	2005	IPMJ	1051	Original	Causes of renal Amyloidosis
Mansour and Al-Shamma [11]	2006	Med Gen Med	136	Original	Prognostic implications
Al-Saedi [16]	2009	NIJM	80	Original	Pattern of Glomerular Ds
Al-Saedi et al [14]	2011	Al-Kindy Col Med J	120	Original	Pattern of Glomerular Ds
Ali et al [12]	2013	SJKDT	47	Original	Allograft Biopsy
Al-Saegh and Assad [15]	2013	AJNT	58	Original	IF pattern of Glomerular Ds
Al-Omairi [13]	2014	AJNT	50	Original	Glomerular pattern in childhood SLE

This gap is partially filled by single or multicenter data on renal biopsies in some of these countries. Although these are not ideal, hospital or center-based biopsy studies do shed some light on the spectrum of the prevalent renal diseases in that particular location [19]. Thus, these partial results cannot conclude that the present sample of patients (2278) is actually representative of the nationwide frequency of glomerular, tubulointerstitial, or renal vascular diseases or renal grafts in Iraq.

Registry data from Saudi Arabia showed the distribution and epidemiology of primary and secondary glomerular diseases. Italian experience of national renal biopsy registry concluded how much this results meet the current challenges facing the clinical research enterprise [20-22]. The population included are children, adults, and elderly. The registry can provide separate analysis of different groups and would have been more informative and reflective of the demographics. In addition, renal allograft biopsy still not widely practiced, not well documented, and its utility is affected by many logistic issues. There are at least 500 transplants annually in Iraq and the number of graft biopsies is not concordant with these increasing number of transplants. By this we are unable to explain acute and graft dysfunction and this certainly will affect

management plan. Still there is a low threshold for graft biopsy especially early post-transplant mainly because of risk of bleeding that should be weighed against the possibility of losing a graft because of an unknown diagnosis [12,23].

The identified studies disclosed that the practice of renal biopsy as a procedure merits reevaluation. There was no adequate data about adequacy of the biopsy sample and also how much the needle gauge size affects it. Specimen adequacy assessment is the initial step during the assessment of a native renal and allograft samples. Specimen adequacy is one of the determinant of diagnostic agreement in the histopathological assessment as it should be considered in the view of clinical data. Adequacy statement in the renal biopsy report and on-site adequacy assessment should be a standard approach [24,25]. Light microscopy is the main tool for biopsy interpretation. Immunohistochemistry (IHC) but not IF is the standard in the current Iraqi renal pathology services. The sensitivity, specificity of IHC on formalin-fixed paraffin-embedded renal biopsy specimens is lower than IF with some drawbacks and therefore, not always suitable for evaluation of renal biopsies [26].

Complement antigens require careful, time-consuming antigen-retrieval procedures because of antigen masking during processing into paraffin. A false negative can be a concern. Interpretation of IHC slides is also complicated by potentially higher background in renal tissues and the difficulty of titrating the final color product to avoid over or under staining. Also, since the IP signal in IHC is a reaction product dependent on interaction of substrate and enzyme, diffusion artefacts can create problems in interpretation [26,27]. The cause behind using IHC in Iraqi studies is the unavailability of proper renal pathology lab, unavailability of IF microscopy, no proper nephropathology training, and the need for sample transport across long distance to be interpreted. In addition, EM is not available in our current practice and again this will add more challenges [27]. Most of the studies in this review didn't provide data about the complications of biopsy procedure and if there is any intervention to prevent and/or to correct. This should be in the context of improved techniques using ultrasound guidance for percutaneous renal biopsy [28,29].

A major finding of the studies is that FSGS is the commonest cause of primary nephrotic syndrome (NS). Still there is no data about classification of FSGS into histological variants. In one study, membraoproliferative glomerulonephritis was the most common cause but this needs to be critically appraised in the view of modern classification of MPGN. Another common finding in these studies is that IgA nephropathy (IgAN) is not the most prevalent, although it is considered the most common primary glomerular disease worldwide [30]. In the Middle East, FSGS is the most frequent renal disease as in the Saudi Arabian Registry, followed by MPGN. IgAN accounts for only 6.5%, while LN is the most common secondary form [20,22]. These histopathological lesions of glomerular diseases observed on renal biopsies need to be correlated with different clinical and demographic features of the patients. This in turn represents how much the indication of biopsy need to be documented and not restricted to glomerular disease diagnosis and to include other justifiable clinical indication like unexplained acute kidney injury, interstitial nephritis, or renal involvement by systemic disease. A renal biopsy doesn't only help to establish and confirm a suspected diagnosis, it may aid in understanding the mechanism of disease. It also provides useful prognostic information on the degree of glomerular, vascular and tubulointerstitial involvements and can also help in guiding treatment plan, determining the effectiveness of recommended therapy, or when treatment is futile [4,30]. Apart from few studies Iraqi renal biopsy lack these endpoints. This may be related to study design, and lack of proper follow up. This systematic review had some

limitations include the limited search strategy with the publications and sites where these articles had been published. Possibly they were non-homogenous in terms of study designs, sample size or endpoints. They were not randomized controlled trials but of acceptable quality.

In conclusion; Iraqi practice of renal biopsy needs to be extended to full spectrum to have the full benefits of renal pathology. This can be achieved through proper and modern pathology laboratory, dedicated renal pathology training and adoption of modern renal histopathology approaches. Creating an environment for a precise documentation of all clinical and pathology data in all renal units will make the bases for national renal pathology registry. The data about the spectrum of glomerular disease should be translated in to this national registry for all renal units over fair follow up period to have the exact statistics and to put the rationale for management, follow up, and future detailed analysis. Renal biopsy data should be the result of well-designed studies that hit the depth of renal pathology for better understanding of disease mechanism, induction of targeted therapy and prognostic predictions.

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None.

Author Contribution

Dr. Ali: Design, Protocol preparation and manuscript writing. Dr. Al-Saedi: data search, collection and literature review.

Conflict of Interest

Nothing to disclose.

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Human Cytomegalovirus Infection Among Neonates with Symptomatic Congenital Infections and Birth Defects

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Abstract

Background Human cytomegalovirus (HCMV) is the major viral etiology of congenital infection and birth defects, during current maternal infection the fetal transmission is high (30-40%) and the symptomatic neonates have diseases involving the neurologic, hematopoietic, respiratory and other organ systems, causing high mortality and long-term sequelae.

Objective To measure the frequency of congenital and perinatal HCMV infection among symptomatic neonates and its possible burden of disease among them.

Methods A total of one hundred ninety-eight symptomatic neonates with clinical manifestations of overt congenital infection enrolled in this study from September 2014 to March 2015. Serum samples were obtained from each subject targeted in this study. HCMV infection was defined as HCMV-IgM antibody positive by Electrochemiluminescence Immunoassay (ECLIA) techniques.

Results The prevalence of HCMV infection among symptomatic neonates with congenital infection was 25 (12.6%). The average age of HCMV detection was 9.96 (SD 6.73) days with a median of 7 days, a minimum of 3 days and a maximum of 28 days. Jaundice was the most predominant clinical finding 14 (56%), followed in order of frequency by hepatomegaly 9 (36%) and pneumonitis 7 (28%).

Conclusion The high prevalence of neonatal HCMV infection among neonates with symptomatic congenital infections could indicate a high rate of maternal HCMV primary or current infection among our population.

Keywords HCMV, congenital infection, neonates, clinical finding.

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List of abbreviation: HCMV = Human Cytomegalovirus, ECLIA = Electrochemiluminescence Immunoassay.

Introduction

Human cytomegalovirus (HCMV) is the major viral etiology of congenital infection and birth defects [(1)].

Congenital infection is the leading cause of 10% of neonatal deaths in Iraq [(2)]. HCMV transmission rates to the fetus are significantly higher than Rubella and *Toxoplasma gonidii* [(3)]. During maternal primary or current (reinfection or reactivation) infection HCMV can translocate the placental barrier and can cause infection of the developing fetus [(4)]. Perinatal HCMV infection may be acquired from the mother as a result of contact with infected genital secretions during passage through the birth canal or via ingestion of the infected breast milk (perinatal infection). This postnatally acquired infection rarely results in significant symptoms or sequelae in full-term infants [(5)], the exception being the low-birth weight and

prematurity. Infants born at preterm birth may be at higher risk of developing HCMV associated diseases [6,7].

HCMV is a common congenital infection worldwide. The prevalence of congenital HCMV is 0.64% in developed countries [8] while reliable estimate of the prevalence of congenital HCMV infection in developing countries are not available [8,9]. Since the incidence of congenital infection is directly correlated with the seroprevalence of HCMV antibodies in the population [10,11], congenital HCMV infection may indeed exert its greatest burden on developing countries with high birth rates and high seroprevalence, up to 95-100% [12].

Approximately 90% of newborns with congenital HCMV infection have no clinical symptoms of disease at birth, while about 10% of them have the signs and symptoms at birth [13,14]. Greater than 90% of symptomatic infants develop long-term neurological sequelae, such as sensorineural hearing loss, developmental disability, cerebral palsy, and impaired vision [15]. This incidence is far greater than that of the better-known chromosomal disorder Down syndrome [16]. On the other hand, among the 90% asymptomatic congenital HCMV infections, approximately 10-15% will later develop long-term neurological sequelae [15,17]. Hearing loss is the most common sequels of children with asymptomatic congenital HCMV infection who develop long-term sequelae [17,18]. Perinatal acquisition of HCMV has little significance in full-term infants and it is not associated with long-term disability. Prematurely born infants with postnatally HCMV were found to be at higher risk for HCMV associated diseases [6]. The factors that enhance the magnitude of HCMV as a health problem can be explained by the following factors: no specific antiviral therapy for HCMV infection and no licensed vaccine [19-21], a lack of awareness of congenital HCMV among health care workers and the public because most maternal and newborn infections are asymptomatic and, therefore, are not recognized at birth [22].

This study aimed to estimate the frequency rate of HCMV infection among symptomatic neonates with congenital infections and the possible burden of disease among the infected neonates.

Methods

A cross sectional study was conducted on neonates with congenital infection. A total of one hundred ninety-eight (198) neonates diagnosed with congenital infection were selected based on the inclusion criteria of the study from September 2014 to March 2015. All the selected neonates were admitted to the Neonatal Intensive Care Unit. Serum samples were obtained from the neonates, their ages ranged between one and thirty days. The neonates enrolled in this study were symptomatic neonates diagnosed with congenital infection the inclusion criteria as follows: various clinical manifestation like jaundice, petechial rash, hepatosplenomegaly, pneumonitis, congenital heart diseases (CHD), congenital malformations especially those involving the central nervous system and ophthalmological abnormalities [23]. The exclusion criteria as follows: neonates were not considered suggestive if the only finding was prematurity [24]. Clinical manifestation was determined by consultation of a pediatric specialist and verification of the information in the medical record. Neonates were admitted to the Neonatal Intensive Care Unit in Child Protection Teaching Hospital and Imamein Kadhimein Medical City in Baghdad. About 1- 2 milliliters of venous blood was obtained from each subject targeted in this study. The blood samples were allowed to clot at room temperature and then centrifuged for serum. All sera were stored at (-20 °C) until testing. HCMV infection was defined as HCMV-IgM antibody positive by ECLIA Kits (Roche, Germany) according to the manufacture's protocol.

Ethical approval to perform the study was obtained from the Research Ethical Committee at College of Medicine, Al-Nahrain University.
Statistical analysis was performed in SPSS 24 using frequency analysis to calculate rate ratios, mean, median, maximum and minimum of the study variables.

Results

HCMV infection as indicated by specific IgM measured by Electrochemiluminescence Immunoassay (ECLIA) was detected in 25 out of 198 (12.6%) symptomatic neonates as shown in figure (1).

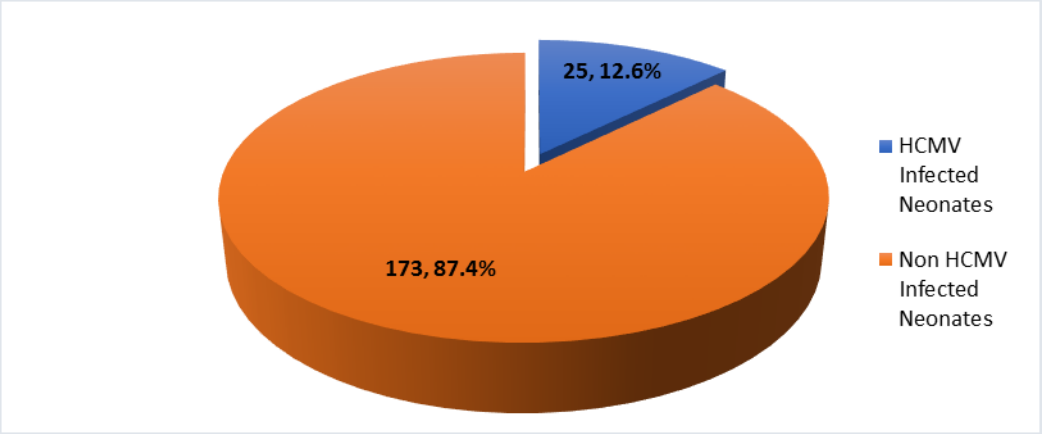

Figure 1. Positive IgM-Anti HCMV results among symptomatic neonates

Lower female rates 11 out of 25 (44%) than males 14 out of 25 (56%) were found among infected neonates. The average age of HCMV detection among symptomatic neonates was 9.96 days after birth. First week of the postpartum period was the medium age of HCMV detection. HCMV infection was detected among symptomatic neonates in a minimum age of 3 days and in a maximum age of 28 days after birth (SD 6.73) as shown in table (1).

Table 1. Characteristics of symptomatic neonates with HCMV infection

Characteristics	Number	Percentage	Mean	Median	Minimum	Maximum
Infected neonates	25	12.6				
Sex						
Female	11	44				
Male	14	56				
Age by days			9.96	7	28	3

Among the clinical manifestations of symptomatic neonates with serological evidence of congenital and perinatal HCMV infection, jaundice was the most predominant clinical finding 14 out of 25 (56%), followed in order of frequency by hepatomegaly 9 out of 25 (36%) pneumonitis 7 out of 25 (28%), prematurity 6 out of 25 (24%). Microcephaly was the most predominant neurological manifestation 6 out of 25 (24%), followed in order of frequency by hydrocephaly 5 out of 25 (20%), convulsion 2 out of 25 (8%), and vision problems 1 out of 25 (4%). Other clinical findings are shown in figure (2).

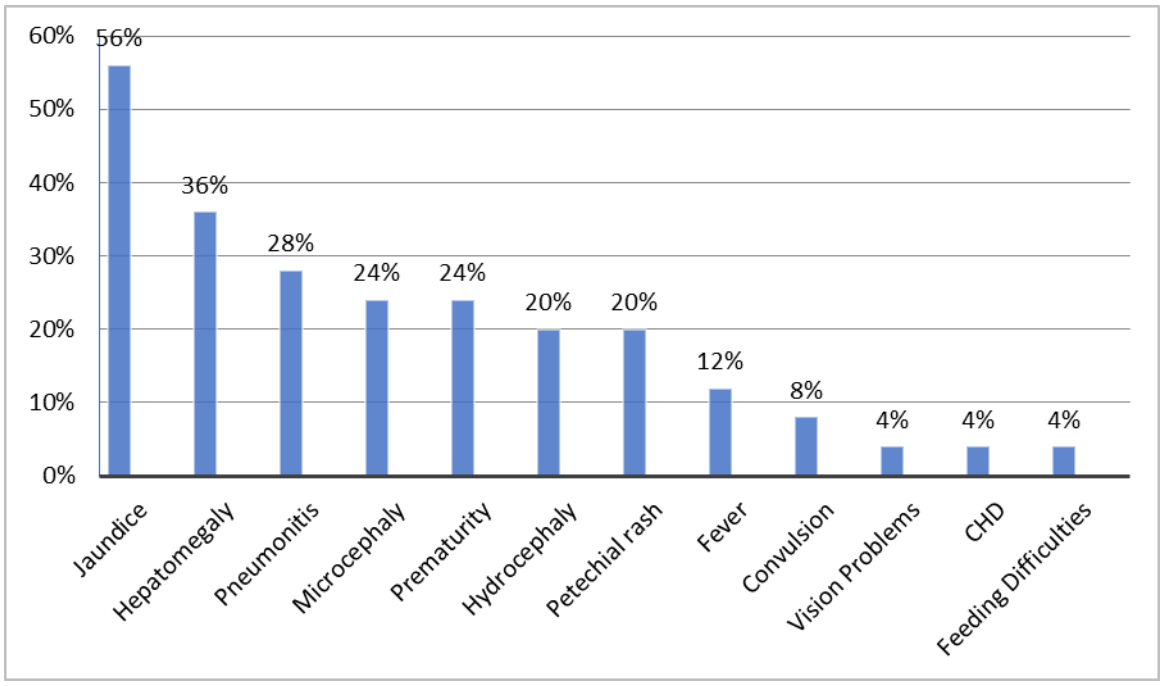

Figure 2. Clinical signs among symptomatic infected neonates, listed in order of frequency

Discussion

Among hospitalized neonates who were suggestive for congenital and perinatal HCMV infection, IgM-anti HCMV was detected in 25 (12.6%) of them. A lower result of 6% was recorded among symptomatic neonates in Iraq previously by Al-Ali and his coworkers in 1995, though they conducted their work on cord sera only, so their results reflected the congenital HCMV infection only [25]. Approximately similar result was reported by the same author among suggestive live-born infants in Mosul, Iraq by using specific IgM as a screening test [11]. A higher finding was reported 16.1% in Baghdad, Iraq by Habib et al. in 2000, by measuring specific IgM among symptomatic infants [24]. The higher results might be related to the wider range of age of suggestive infants included in that study as compared to this study. Higher results 20% of HCMV infection as indicated by specific IgM among symptomatic neonates was found in India [26], while lower results 1.6% and 2% were reported among symptomatic infants in Iran and Palestine respectively using the same test [27,28]. The variation among these results and result of this study may reflect the variation of HCMV prevalence in different countries [29]. The variation of congenital HCMV epidemiology could be related to the maternal seropositivity because women of childbearing age, who are HCMV seronegative are at major risk of giving birth to infants with symptomatic congenital infection if primary infection is acquired during pregnancy [11].

The median of HCMV detection was the first week of the postpartum period, a result that may indicate that most of the HCMV infection among symptomatic neonates was congenital infection rather than perinatal infection [30].

Jaundice was the most frequently noted presenting sign 56%, followed by hepatomegaly 36%. These results are comparable to those documented in Iraq regarding the most frequent signs and also the order of frequency among symptomatic HCMV infected infants, Habib et al. reported 65.2% for jaundice followed by 41.1% for hepatomegaly [24]. A similar finding was reported among hospitalized HCMV infected children in Palestine, but hepatomegaly was the first sing in order of frequency 60%

followed by jaundice 30% [28]. The variation in the order of frequency of jaundice and hepatomegaly between hospitalized symptomatic HCMV infected children in that study and the present study may be related to the variation in HCMV strains. There is evidence indicating that the existence of HCMV variant plays an important role in the pathogenesis of disease [31]. Jaundice was reported in 63% of symptomatic HCMV infected infants by Pass and his colleagues [32], and 70% in another study [33]. In the presented study hepatomegaly was comparable to that documented by others [34,35]. However, higher figures 74% and 70% were reported by other workers [32,33].

Pneumonitis was found in 28% of symptomatic HCMV infected neonates, a result which is found to be higher than that recorded in a study from Iraq 11.6% among infected infants [24]. In a recent study, a higher frequency of pneumonitis was detected in 47% of symptomatic infected children [36]. The variation in the results may be due to the fact that the clinical diagnosis of HCMV lung infection is challenging in children and often requires a high-index of suspicion [37].

Prematurity was found in 24% of symptomatic infected. Meier et al. have reported significant risk of HCMV transmission postnatally in preterm infants with the possibility of severe disease [43]. Immature immune system in infants, as a consequence of prematurity among neonates relates to lower production of cytokines which reduces the ability of T-cell activation and viral detection as compared to term infants [44]. In addition, premature infants are at high risk for symptomatic HCMV infection as they don't receive as many as transplacental HCMV antibodies, and the antibodies that they do receive disappear more rapidly through catabolism than in infants born near term [45,46].

Interestingly, result in the current study is higher than the results reported in a study in Iraq, which indicated 16.7% prematurity among symptomatic HCMV infected infants even though the age range in that study was wider than current study [24]. This finding could indicate that prematurity is more likely to correlate with congenital infection since HCMV impairs placental development and functions regardless of virus transmission to fetus, leading to intrauterine growth restriction that results in the baby being preterm [10]. In addition, HCMV efficiently spreads into fetal organs following fetal viremia during HCMV congenital infection. The major target organ is the fetal lungs. HCMV replication in the lungs triggers apoptosis near and within viral lesions and impairs the production of surface proteins of the lung that adversely impact lung development [47]. However, further studies need to be conducted on premature neonates with congenital and perinatal HCMV infections separately to evaluate the association between prematurity and each type of neonatal HCMV infection.

The most frequent neurological manifestations among symptomatic infected children were microcephaly and hydrocephaly, recorded at 24% and 20% respectively. A higher result was reported for microcephaly 38.9%, and an approximately similar result for hydrocephaly in Iraq among symptomatic infected infants by Habib et al. [24]. Other study in Iraq done by Al-Ali et al. described hydrocephaly in 33.3% of infected newborns and has also shown hydrocephaly as the most frequent neurological clinical finding [25]. In contrast, microcephaly and hydrocephaly were not recorded among hospitalized children with symptomatic HCMV infection in Palestine [28]. Gandhoke et al. have reported an equal frequency of microcephaly and hydrocephaly among symptomatic infected children [26]. Other neurological manifestations recorded were convulsion 8% and vision problems 4%. In two studies conducted in Iraq among symptomatic HCMV infected neonates and infants, convulsion and vision problems were recorded in lower frequency compared to this

study [24,25]. The variation in the results may be related to the wider age range of these two studies compared with the present study. A systemic review of 30 studies regarding hearing problems revealed that hearing loss was 12.65% among symptomatic HCMV infected children [38]. In the present study, hearing problems was not detected among neurological manifestations in symptomatic HCMV infected neonates. A large number of hearing problems that result from HCMV infection was detected at school age [39], which may be attributed to misdiagnosis of hearing problems among neonates in this study.

Petechial rash was described in 20% of the symptomatic HCMV infected neonates, which was found to be lower than those recorded by others: 33.3% and 32% [40]. In contrast, petechial rash was not recorded by Gandhoke [26] and Neirukh et al. [28]. This variation in the results may be due to the possibility that this clinical sign might have passed unnoticed by the physician as it may be transient, disappearing with 48 hours [41] and requires very careful clinical examination.

Fever for unknown reasons was shown in 12% of symptomatic HCMV infected neonates. Fever for unknown reasons may indicate the prenatal HCMV infection among neonates in this study since it presents the usual clinical picture of HCMV mononucleosis among immunocompetent children [42]. Heart diseases and feeding difficulties were last regarding the order of frequency. The variations in the results of clinical findings that were found among different studies may reflect the variations in the severity of disease and the degree of organ involvement. Clinical findings associated with HCMV infection are broad and non-specific [13]. This variation may be related to the type of HCMV genotype and tissue tropism associated with the virus strain. From a prognostic viewpoint, the prompt identification of infected infants would help define a population at risk for developmental abnormalities so the establishment of diagnosis early in life is important for the infants and for the families.

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Author Contribution

Alwan: collection of the sample, conduction the experimental aspects of the study, and writing the manuscript. Kadhim: concepts of the study, revision and approval the final version of the manuscript. Al-Saffar: the study design, implementation of the statistical aspects, revision of the final version of the manuscript. Arif: clinical consultation. Wickes: general consultation. Fu: experimental consultation.

Conflict of Interest

Authors declare no conflict of interest.

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