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3. Chapter in book: Phillips SJ, Whisnant JP. Hypertension and strock. In: Laragh JH, Brenner BM. editors. Hypertension: Pathophysiology, diagnosis, and management. 2nd ed. NewYork: Raven Press; 1995. p. 465-78.

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Delivering Quality Care and Patient Safety – A New Paradigm

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Abstract

Quality in healthcare is imperative and not optional. To deliver continuous process improvement, the focus must be on the patient from start to finish, as often the patient's experience of receiving care is the product of the process as in doctor consultation setting. Value healthcare can be formulated as an equation: quality divided by cost. Sometimes to do less is to achieve more, as beyond the point of optimal healthcare, adverse effects will outweigh perceived benefits. Patient safety is proportional to process quality; hence the overarching importance of focusing on quality. However, costs must always be harnessed to ensure we achieve optimality of healthcare delivery.

Keywords Change management, errors, events reporting, frontline staff, harm events, healthcare delivery, information flow, leadership, near misses, patient safety, quality care, risk management, process improvement, system data.

Citation Alani A. Delivering Quality Care and Patient Safety – A New Paradigm. Iraqi JMS. 2017; Vol. 15(3): 214-219. doi: 10.22578/IJMS.15.3.1

List of abbreviation: PSO = Patient safety organizations

Hippocrates got it right more than two thousand years ago when he said: first do no harm (see Bibliography below). He has, and quite rightly so, put patient safety at the heart of all priorities for physicians. However, fast forward to today, we can easily see that healthcare has developed huge inefficiencies which in turn compromised patient safety.

Healthcare planners have recognized that the delivery of quality care is a function of organization design and not down to individual effort. No longer weaving the whole system of healthcare around surgeons and physicians; rather we are truly moving towards patient (or person) centric model. Health is no longer defined as the absence of illness; healthcare today focuses as much on wellbeing as it did on illnesses and disorders.

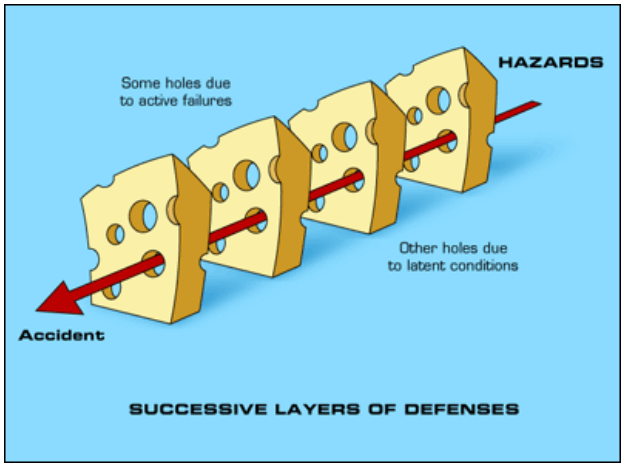
In 2006, I wrote in the BMJ about simple change, big gain: a call to focus on low-hanging fruit to implement changes that would really bring about improvement. All healthcare processes start and end with the patient; and patient's experience is as important as the outcome of the process. Hence using techniques like value stream mapping provides the ability to implement system-thinking to identify bottlenecks (both capacity constraints and time-traps as examples of drivers of process efficiency), rather than shifting issues from one department to another.

The Five Bad Habits of Healthcare: More vs Better

Drawing on the work of the 2010-2011 World Economic Forum Healthcare Industry Global Agenda Council, the team identified five important and pervasive "bad habits", which contribute to this spending problem:

1. Favoring current practice over the best available evidence.
2. Following what others are doing even when it is wrong.
3. Behaving as if more healthcare is identical to better healthcare.
4. Focusing on illness at the expense of prevention.
5. Failing to present information or choices effectively.

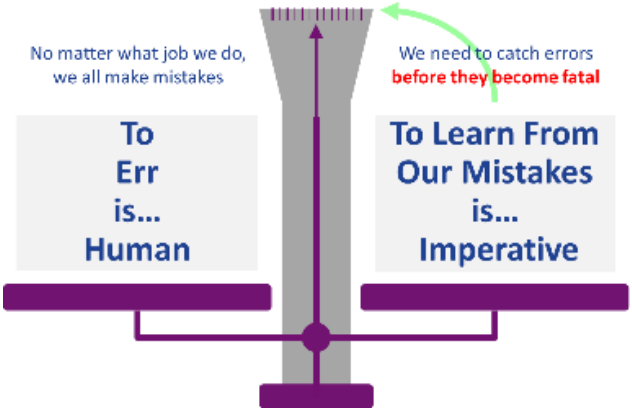
It is easy to miss a step or omit a check as an oversight, an issue that can be addressed by something as simple as a "checklist manifesto." Having said that, you will rarely find checklists indoctrinated into healthcare delivery even in teaching hospitals because they are trapped in the "five bad habits." Even when you have in place more than one layer of checks, there is still the typical example of what's known as Swiss cheese model. Every step in a process has the potential for failure, to varying degrees. The ideal system is analogous to a stack of slices of Swiss cheese. Consider the holes to be opportunities for a process to fail, and each of the slices as "defensive layers" in the process. An error may allow a problem to pass through a hole in one layer, but in the next layer the holes are in different places, and the problem should be caught. Each layer is a defense mechanism against potential error impacting the outcome.



Patients don't care how much you know until they know how much you care

While patients are sometimes forgiving and always understanding, efforts to improve quality of care and patient safety still have a long way to go. "I know you didn't mean to hurt me" said Elizabeth, an eight-years old, paralyzed from the waist down after a recurrence of cancer was missed (excerpted from R. Gibson, "Wall of Science"). Betsy Lehman, Boston Globe health reporter, died from an overdose during chemotherapy. Willie King had the wrong leg amputated. Ben Kolb was eight years old when he died during "minor" surgery due to a drug mix-up. Globally, improving health care safety is still plagued by fear, gripped by reluctance, crippled by punishment. According to OpenSafety.org, only 44% of hospital workers are sure that they will not face punishment for mistakes.

The root cause for the many healthcare delivery errors cited above is the fear of admitting and owning up to incidents. Punishment is a huge hindrance and must be overcome if progress is to be made in patient safety and quality care. We need to always remind ourselves that non-harm events herald harm ones. If we can encourage staff to be candid and report back all events as and when they occur, we will significantly improve safety and quality.



Admitting mistakes and taking ownership can be achieved through self-leadership and staff empowerment: to take the initiative to fix and prevent errors, and educate others not to fall

in the same trap. Self-leadership is just the starting point: by acting as a role model for others and through institutionalizing this behavior, the change in culture will be the foundation for future improvements in patient safety.

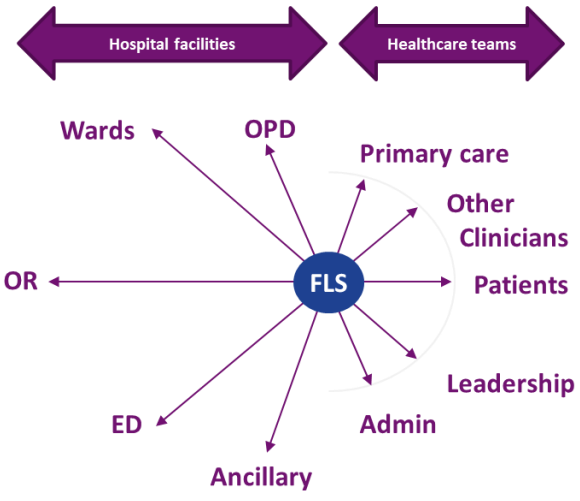
Patient Safety in Numbers

$4.5 billion Adverse drug events and patient falls cost US hospitals annually. 780,000 surgical site infections occur in the US each year, up to 60 percent of them preventable.

250,000 Ventilator-associated pneumonia (VAP) accounts for up to 18% of all hospital-acquired infections, causing 1.75 million excess hospital days (US).

Frontline Staff – A Pivotal Role in Patient Safety

Frontline staff (FLS), those in direct contract with patients, are the focal point of healthcare delivery channels and information flow. They are the single-most important area to work with when it comes to improving patient safety. Among other clinicians, front-line healthcare givers are the ones who spend the most time interacting and care for patient. This group includes all non-manager workforce who are out there day in day out working with patients at the bedside, on the ward, in labs, pharmacies and ancillary services.

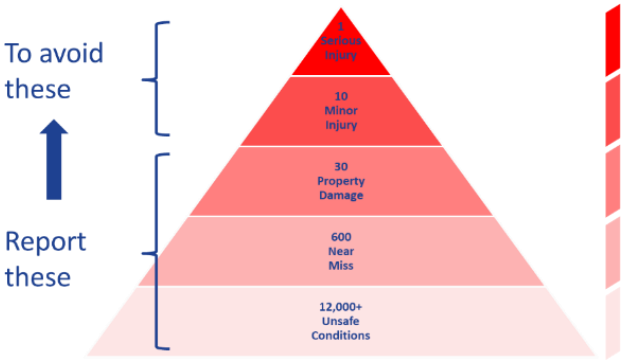


The Events Pyramid

By reporting near misses and unsafe conditions we can take corrective action and avoid serious injuries. For every serious injury event, more than 12,000 unsafe conditions occur.

The aim is to move from considering error reporting a task to making it recognized as a duty. That's the cultural change we are trying to achieve.

Owning up to mistakes takes a bottom-up approach (vs top-down). It embodies self-leadership – especially taking the initiative and demonstrates leading by example – role model for others. It educates staff by bridging the gap between knowledge and know-how and empowers them to voice concerns. It's not "Project of the Month" … this is change for good… "Change is the new culture"



Delivering Quality Care – It's a State of Mind

Changing mindsets is not as hard as it sounds. It can be achieved by combining the four elements together: purpose, feedback, skills and role-models. Start by developing a story describing the end-game and assign "deliverables" to various teams to write their own part of the story. Review the "story" regularly to ensure it remains meaningful to all involved. Ensure information flow to be both downstream as well as upstream. Ensure buy-in by making the story tell how life will be better for all.

Transpersonal psychology suggests that the innate desire to develop and grow infuses human beings with energy. The overarching aim is to achieve higher performance: employees will not put sustained effort into a

new kind of behavior if they have only a rational understanding of why it matters to the organization; it must mean something much deeper to them, something that they know will influence their personal growth; giving change a personal meaning for participants. Saving lives and preventing errors is a powerful purpose that provides deep meaning to frontline staff.

Once success is realized and results are at hand, provide transformational workshops for a small group over a brief period, the graduates of such workshops will create a critical mass who willingly embrace the new behavior and culture to make it sustainable by taking it on a roadshow throughout your organization.

Safety and Management – The Paradox

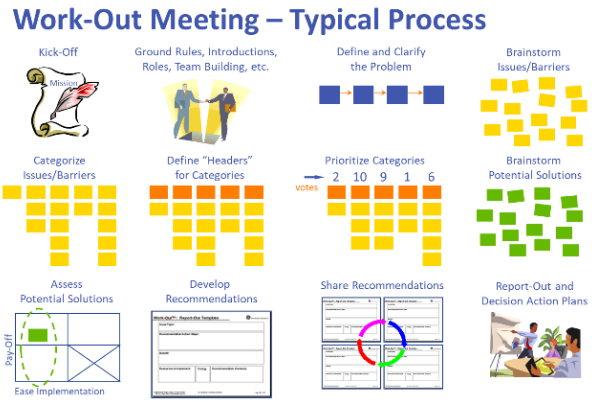
It sometimes appears that the aim of improving safety goes against management theory concepts. Our priority is safety: first and foremost. However, there are at least two aspects as viewed through patient safety lens when compared to management:

SAFETY CULTURE	MANAGEMENT THEORY
Make it hard for operators to err	Simplify process
Make "alerting and event management" systmens sophisticated to capture as much information as possible when errors occur	Streamline systems
Prepare for the unanticipated	Implement standard operating procedures (the anticipated)
Stop on errors to investigate and do root cause analysis	Do not first, fix it later
Achieve higher quality first and foremost	Meet targets
Document errors with as much details as possible – capture lessons learned and share knowledge	Document best practices and build organization-wide "gold standard" knowledge base
One event is enough to invoke analysis and learn lessons to prevent future ones	Require large sample to run statistical analysis and define average and then make decisions

Change Won't Happen by Itself

Changes that have been identified as improvementsrequire strong leadership and won't happen by themselves. We need to introduce methods, tools and even sometimes new technology to embed change. To identify the way forward and try new ideas with a cross-functional multi-disciplinary team, we will need to expedite change implementation (avoid going into analysis paralysis). We can start with a pilot hospital and a create a blueprint to take it forward to other areas/teams/departments. To ensure governance, we may need to deploy a system to capture and report out on medical events.

One method is known as kaizen (Japanese for change for better), sometimes referred to as action work-out: a process of concentrated team-based decision-making and empowerment used to resolve issues and improve processes. A team of experienced, knowledgeable people with a stake in an issue is chartered to develop solutions and action plans. The team is empowered by their sponsor to proceed with implementation and is accountable for implementing their action plans. The sponsor removes barriers and provides support and follow-up. Kaizen provide a process to drive improvement; an opportunity to empower people; and are results-based to inform decisions, ensure accountability with timely follow-ups.



How Not to be Trapped in the Blame Game?

No-Blame System enables a culture of candor. The system is based on "High Reliability Organizations" HRO's (like aviation industry) which encourages the reporting of errors and near misses, exploiting these incidents to improve their operative processes. No-Blame System consists of three main components:

1. Reporting: to create a "Book of Learning" which is usually referred to as "Book of Errors": we want here to emphasize not only logging events but the fact that learning and not blaming is the aim
2. Debriefing: once events are documented (Book of Learning), teams can review and group events
3. Narrative: this is all about "story-telling" to help others avoid running through the same conditions that caused the errors logged and analyzed (steps 1 and 2).

Healthcare is Too Complex

"Our systems are too complex to expect merely extraordinary people to perform perfectly 100 percent of the time. We as leaders have a responsibility to put in place systems to support safe practice."- James Conway, IHI Senior Fellow; former Executive Vice President and Chief Operating Officer, Dana-Farber Cancer Institute. To address the inherent complexity in healthcare delivery, patient safety organizations (PSO) provide its members a single common medical event reporting platform with a comprehensive set of data analytics and an advisory support to identify the root causes of risk. PSO's help hospitals make lasting safety improvements. In the US, PSO's are certified by the US Secretary of Health and Human Services (HHS) as part of the Agency for Healthcare Research and Quality (AHRQ). PSO's serve as independent, external entities that collect, analyze and aggregate information on patient safety events. They give confidential advice to health care providers seeking to understand and minimize the risks in delivering patient care. Importantly, PSO's are evaluated by the quality of the data collected and the insights provided.

PSO's distinguish themselves itself by the way they capture data and analyze it, by their ability to pinpoint the risks of patient harm. PSO help develop new knowledge by first providing every member with a common event reporting system that captures data in the richest possible manner. Then, database is organized so that we run analytics tools and by using the expertise of PSO members to find those critical insights. The risks of harm are systemic, myriad, and complex.

Patient Safety is at the Heart of Quality Care

Delivering improved healthcare is all about providing quality care with widest access from a reasonable cost base. This can be realized by deploying event or incident management where data is captured through an event reporting system that is used by all the PSO members. The system enables members to not only capture more event reports and near misses, but to also capture more data in each report. The result is a powerful dataset that is aggregated and protected in the PSO.

The power of analytics: to make use of the information captured, we need to create the infrastructure necessary to anonymize and analyze the data. Analytical tools in data integration will enable the PSO to identify and share insights within the membership.

Benefit Realization: PSO's will provide insights to hospital administrators, providers, and policymakers to assist them in identifying and isolating factors that contribute to medical errors. In particular, the PSO will focus on the complex root causes of risk. For example, what environmental factors should be monitored to reduce risk for a certain type of procedure? How have some members been successful at reducing rates of ventilator associated pneumonia? Why have others been less successful?

The Shape of Success

So, what is it we are trying to accomplish? The aim is to deliver empowered, up-skilled and toolkit-equipped front-line staff ready to voice their concerns; to setup or join a PSO with the benefit of having access to world-class knowledge-base and research on patient safety; to have an in-house core team who can spread the word, disseminate acquired knowledge, skills and realized benefits to other teams; establish data collection mechanism to show (prove) improvement in patient safety: "Book of Learning" to capture all events related to patient safety (number of harm events reported, number of non-harm events reported and number of near misses reported). Eventually we will have an open culture and conducive environment to make best use of the above and will be a model for others to follow.

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The Effect of Tumor Necrotic Factor Alpha Polymorphism on Response to Biological Treatment for Rheumatoid Arthritis Patients

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Abstract

Background The tumor necrotic factor alpha (TNF-α) was associated with rheumatoid arthritis (RA) pathogenesis and inflammation of joint, for this consider important target for drugs (TNF inhibitors) as for example infliximab.

Objective To investigate the role of TNF-α-308 polymorphism G>A in unresponsiveness to biological treatment (infliximab) in RA patients.

Methods This study included 29 RA patients treated with infliximab attending Department of Rheumatology in Baghdad Teaching Hospital and diagnosed according to American College of Rheumatology (ACR) with 30 persons as healthy controls. The age range was 20 to 68 years. The DAS28 was calculated for each RA patients. Blood samples were taken from them during the period from May 2014 to January 2015. The blood samples were tested by polymerase chain reaction (PCR) for TNF-α-308 G>A then by restriction fragment length polymorphism (RFLP) for A allele and G allele. HLA-DR genotyping by PCR-SSO.

Results The frequency of A allele was 23 (39.7%) in RA patients while G allele was 35 (60.3%) in RA patients, whereas in in controls A and G alleles frequencies were 5 (8.3%) and 55 (91.7%) respectively. The A allele was associated with high DAS28 19 (82.6% while for G allele was 1 (2.9%) and DAS28 showing significant association with TNF-α-308 polymorphism G>A. The distribution of A allele in female RA patients was 16 (36%) whereas G allele was 28(64%), while in male RA patients, A and G allele were 7 (50%) for each of them. The HLA-DRB1 not showing significant association with TNF-α-308 polymorphism G>A.

Conclusion The TNF-α-308 polymorphism G>A has effect on response to biological treatment (infliximab). The A allele of TNF-α was associated with unresponsiveness to infliximab and RA patients who carry this allele have high DAS28 score. The HLA-DRB1 not showing association with TNF-α-308 polymorphism G>A.

Keywords RA, TNF-α polymorphism, infliximab, HLA-DR.

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List of abbreviations: ACR = American college of rheumatology, DAS = Disease activity score, HLA = Human leukocyte antigen, RA = Rheumatoid arthritis, TNF = Tumor necrosis factor.

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease affecting 0.2-1% of the population worldwide and is associated with progressive destruction of the joints causes early mortality, disability and

compromised excellence of life in the industrialized and developing world. Assessment of disease activity and treatment responses is based on measurement of disease activity score (DAS) [1].

Tumor necrotic factor (TNF), which is produce by monocytes, macrophage, stimulated T-cell, NK cell, neutrophil and fibroblast play important role in pathogenesis of rheumatoid arthritis, which act by activating leukocytes, endothelial cells, and synovial fibroblasts, inducing production of cytokines, chemokines, adhesion molecules, and matrix enzymes; suppression of regulatory T-cell function; activation of osteoclasts; and resorption of cartilage and bone; mediates metabolic and cognitive dysfunction. Consequently, TNF was target for drugs to treatment RA including infliximab which act as TNF inhibitor [2].

Tumor necrotic factor alpha is considered one of important mediator in pathogenesis of RA, polymorphism in TNF-α have been associated with severity and susceptibility of RA to biological treatment. Promoter polymorphisms at TNF have been associated with disease susceptibility, or severity of joint damage and autoantibody production in RA indifferent populations [3].

Individuals with HLA DRB1 alleles *0101, *0401, *0404 and *0405 have a much greater relative risk developing RA. Genes – including those risk alleles within HLA-DR4 – have been implicated but are insufficient to explain the vast majority of cases [4].

This study aimed to investigate the role of TNF-α-308 polymorphism G>A in unresponsiveness to biological treatment (infliximab) in RA patients.

Methods

This study included 29 RA patients on biological treatment with 30 individuals of healthy controls. The RA patients attended Rheumatology Department in Baghdad Teaching Hospital was examined by rheumatologist and DAS28 was calculated for each patient according to American college of rheumatology (ACR).

Five ml of blood was taken from each patient and healthy control by venipuncture, then 3 ml was added in to EDTA tubes for TNF-α-308 polymorphism and HLA-DR genotyping while 1.6 ml was added to 0.4 of sodium citrate for ESR.

DNA extraction from blood samples

Reliaprep blood gDNA (Miniprep system) provided by promeqa/USA and extraction of DNA was done according to manufacture instructions manual.

PCR for TNF-α-308 G>A

Tumor necrosis factor alpha was done as proposed by Dalziel et al [5].

Amplification primer for 308 tumor necrosis factor polymorphism.

Forward primer: AGGCAATAGGTTTTGAGGGCCAT

Reverse ward primer: ACACTCCCCATCCTCCCTG CT

Restriction fragment length polymorphism was done on gel by addition NCOI enzymes to PCR product [5].

HLA-DR genotyping was done PCR-SSO according to procedure by Innolipacompany (Beljum).

The study was approved by the Ethical Committee of the College of Medicine, Al-Nahrain University.

Statistical analysis

Distribution of genotyping and frequency of alleles were compared by chi-sequare test. Significance was attributed to probability values P≤ 0.05. Computer SPSS and Microsoft excel program were used for determination of probability values.

Results

This study was showed mean of age for RA patients on biological treatment (infliximab) was 42.72 years while healthy controls mean age was 43.6 years. The age range was 26 to 68 years in RA patients on other hand the age range in controls were 18 to 65 years with non-significant P value (>0.05) as in table (1).

Table 1. Mean of age in RA patients on treatment with infliximab and control

Age (Years)	Study groups	
	Control	RA patients
Mean	43.6	42.72
Standard deviation	11.28	11.24
Median	43	40
Minimum	18	26
Maximum	65	68
P value	>0.05	

The current results showed that the GG genotype of TNF-α-308 polymorphism G>A was detected in 16 (55.20%) out of 29 patients on treatment with infliximab while in control was in 27 (90.0%) out of 30 persons. In addition, the frequency of AA was 10 (34.50%) out of 29 patients on treatment with infliximab while in controls was 2 (6.70%) as well as the frequency G/A genotypes was detected in 3 (10.30%) of patients while in controls were only 1 (3.30%) and shown significant difference (P value <0.001) (Table 2 and Figure 1).

Percentage positivity of A alleles in RA patients on treatment was 23(39.70%), while in controls was 5 (8.30%). The frequency of G allele was 35 (60.3%) while in controls was 55 (91.7%) as shown in table (3).

Table (4) showed that A allele of TNF-α has high DAS score frequencies (82.6%) with no good response to biological treatment (infliximab), on other hand the G allele showing high frequencies of the remission cases with good response to biological treatment(infliximab) with significant association between DAS and TNF-α (P value <0.001).

The frequency of G allele was 28 (64%) in RA females' patients while A allele was 16 (36%), however, A and G allele in RA males' patients was 50% for each allele but the association between TNF-α allele and gender not showing significant association (P value=0.532) as shown in table (5).

Table 2. The TNF-α-308 polymorphism genotyping in controls and treated RA patients

TNF-α Genotypes		Studied Groups		
		Control	RA patients on treatment	Total
GG	Count	27	16	43
	%	90.0%	55.2%	47.8%
G/A	Count	1	3	4
	%	3.3%	10.3%	4.4%
AA	Count	2	10	12
	%	6.7%	34.5%	13.3%
Total	Count	30	29	59
	%	100%	100%	100%
P Value		0.001		

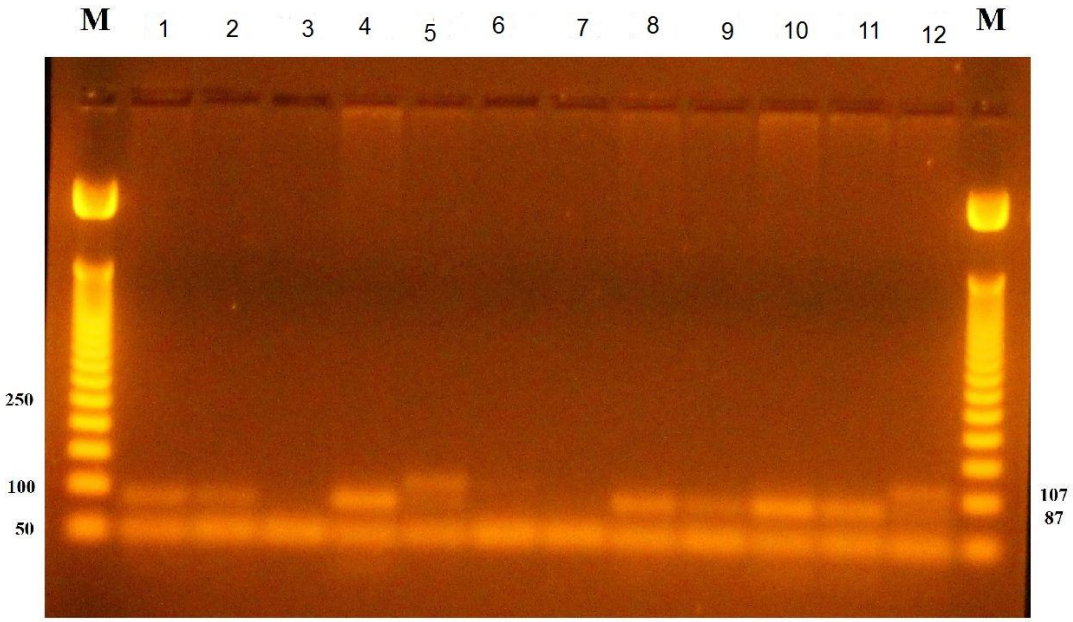

Figure 1. Gel electrophoresis for PCR product of TNF-α-308 G>A after digestion with restriction enzyme NCOI (107 bp was AA, 87 and 107 bp GA, 107 bp GG), M:1000 bp marker, (90 mint., 100 volt)

Table 3. The frequency of A and G alleles in RA patients on treatment

TNF-α-308 G>A		Studied Groups		
		Control	RA patients on treatment	Total
Allele A	Count	5	23	28
	%	8.3%	39.7%	15.6%
Allele G	Count	55	35	90
	%	91.7%	60.3%	85.4%
Total	Count	60	58	118
	%	100%	100%	100%

Table 4. Association between DAS score and TNF-α-308 G>A polymorphism in rheumatoid arthritis patients

DAS score	TNF-α-308 G>A			
	A	%	G	%
Remission	0	0.0%	12	34.3%
Mild	2	8.7%	10	28.6%
Moderate	2	8.7%	12	34.3%
High	19	82.6%	1	2.9%
Total	23	100%	35	100%
P value	<0.001			

Table 5. Association between TNF-α-308 G>A alleles and gender

Gender type	A	G	Total
Female	16	28	44
%	36%	64%	100%
Male	7	7	14
%	50%	50%	100%
Total	23	35	58
P value	0.532		
Odds ratio	0.57		
95% confidence interval	0.17 - 1.93		

Discussion

This study included 29 patients on treatment with infliximab, the wild genotyping GG was (55.20%) of RA patients on treatment while in control was (90%), this genotyping showing good response to biological treatment, controversy to genotyping AA and GA, Mugnier et al. (2003), mentioned that the GG genotyping represent wild genotyping and more common while AA and GA represent mutant genotyping [(6)]. Translation and posttranslation of TNF-α may causes 308 mutation in TNF-α, Wilson et al. (1995), mentioned that the A dimorphism with possible functional consequence (G to A transition at position -308 in the promoter/enhancer region) has been described for the TNF locus. TNF-α A allele associated with high TNF-α production in addition to increase susceptibility to infection and autoimmune diseases especially RA [(7)]. In some studies, 308 mutations have been attributed to geographical variation and cultural constraints resulting in higher endogamy therefore, the effect of inbreeding in Jammu and Kashmir population may lead to increase minor allele frequency in residents [(8)].

The frequency of A allele was high in RA patients on treatment, which showing weak responses to infliximab. The A allele associated with bad outcome of RA disease as well as high TNF-α production this result agrees with Maxwell et al. (2008) who revealed that the A allele frequencies were more than G allele in RA patients receiving biological treatment with poor response [(9)]. Marotte et al. (2006) who used in their study large size of samples from RA patients on biological treatment showed that the A alleles were more frequency in RA patients with inadequate response to drugs [(10)]. This study found that the G allele was more frequent in control than RA patients, this mean G may consider low risk allele for RA while A allele risk for RA as explained by Lee et al. (2007) who mentioned that the A allele consider risk for RA in Latin American [(11)].

Current study found that the patients who have A allele have high DAS score and showing bad response to infliximab as when compared with G allele this result also mentioned by Hajeer et al. (2003) who observed that RA patients with TNF-308 genotypes A/A or A/G show a poor response to infliximab treatment at 22 weeks [(12)]. They therefore, believe that TNF-genotyping in RA patients may be a useful tool for predicting efficacy of treatment with infliximab, however this finding not agree with Lee et al. (2010), who showed that the polymorphism of TNF-α have no effect on responses to infliximab in patients with RA [(13)]. The infliximab work better with normal G allele in tumor necrosis factor alpha while polymorphism with homozygote AA and heterozygote GA lead to less inhibition with tumor necrosis factor inhibitors and result in low outcome of biological treatment which is very costly as well as consider last choice for treatment of RA patients and patients

disabilities of RA disease development [14]. In the present study no significant association between HLA genotyping and A, G allele of TNF alpha in RA patients on treatment and this result agree with Lacki et al. (2000), who mentioned that the TNF-α-308 polymorphism not showing association with HLA-DR [15], but this result not consistent with Jimena Cuenca et al. (2001), who mentioned alteration in HLA allele may result in variation in TNF gene for this increasing susceptibility but depending on ethnicity [16]. In study by Braun et al. (1996), mentioned that the TNF-α A allele associated with high TNF-α production [17]. However, in this study the detection was made to association between TNF-α-308 polymorphism and HLA-DRB1 whereas in other study by Jawaheer et al. (2002), who mentioned that the association between TNF-α-308 polymorphism and HLA-DRB3 [18]. Finally, HLA-DRB1 may associated with high TNF-α production not with polymorphism (whether TNF-α was carried A or G allele), this agree with Padyukov et al. (2004) who hypothesis that the HLA-DRB1 associated with high TNF production [19].

This study concluded that TNF-α-308 polymorphism A associated with bad responses to infliximab while G allele associated with good response. HLA-DRB alleles not associated with TNF-α-308 polymorphism.

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Author contribution

Hachim: conducted the sampling, processing and working. Dr. Abbas: design of the work, data interpretation, drafting and critical revision of the article. Dr. Alosami: samples collection.

Conflict of interest

The authors declare no conflict of interest.

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Value of Plain Abdominal Radiograph, Ultrasound and Computerized Tomography in the Diagnosis of Intestinal Obstruction

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Abstract

Background The diagnosis of mechanical intestinal obstruction is sometimes very challenging especially in the absence of previous abdominal surgery or obstructed hernia and there are limited studies comparing or evaluating the usefulness of plain abdominal x-ray, ultrasonography, and computerized tomography (CT) in intestinal obstruction.

Objective To compare the efficacy of plain abdominal x-ray, ultrasound and CT in the diagnosis of intestinal obstruction. Also, to assess ultrasound value and usefulness in pregnant women and critically bed ridden patients with intestinal obstruction.

Methods In this prospective study with signs and symptoms of mechanical intestinal obstruction, a total of sixty two patients were investigated by plain x-ray, ultrasound, CT scan and the findings were compared with reference to the presence or absence of obstruction, the level, site, cause and strangulation of bowel for a period of eight years (2009-2016). The final diagnosis was obtained by surgery and only those with proved intestinal obstruction per-operative were included in the study.

Results Out of the sixty two patients (41 males and 21 females) included with an age incidence (22-65 yr), 58 had mechanical intestinal obstruction (50 had small bowel obstruction and 8 had large bowel obstruction), of the remaining 4 patients; 2 had mesenteric vascular occlusion and 2 had pseudo-obstruction. The level and site of obstruction was correctly predicted in 91.9% on CT, in 82% on ultrasound and 90.3% on plain film. CT was the best 85.4% to both, ultrasound 24% and plain film 8% in determining the cause of obstruction. Regarding strangulation of bowel, CT was superior while plain film was the least informative with the ultrasound in between.

Conclusion CT is the best tool for the diagnosis, strangulation of the bowel and detecting cause of intestinal obstruction and recommended to be the investigation of choice in equivocal cases. Ultrasound is the best and sole investigation in pregnant women and critically bed ridden patients especially in early cases.

Keywords Intestinal obstruction, plain abdominal x-ray, ultrasound, CT scan.

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List of abbreviations: none.

Introduction

Abdominal radiography can be entirely normal in patients with complete, closed loop, or strangulating obstruction [1].

Therefore; if the patient clinical profile and the results of physical examination are highly consistent with intestinal obstruction or in cases where plain x-rays films are inconvenient

as in pregnancy or critically ill bed ridden patients or being normal or equivocal and little informative or more details are needed, in this case more sophisticated investigations are needed greatly and these would be namely a more advanced imaging studies, these would be ultrasound and computerized tomography (CT) scan [1-8].

This need requires to prove that these imaging modalities are sensitive, specific and accurate in intestinal obstruction when ordered, performed properly and interpreted by expert radiologist. This is supported by two prospective clinical trials [9,10].

Sonography criteria have been established for small bowel, colonic obstruction and even ileus through: simultaneous observation of distended and collapsed bowel segment, free peritoneal fluid, inspissated intestinal contents, paradoxical peristalsis, highly reflective fluid within the bowel lumen, bowel wall edema between serosa and mucosa, a fixed mass of a peristaltic, fluid filled, dilated intestinal loops.

Ultrasound is well suited to pregnant women and critically ill patients because it is safe and can be performed at the bed side, the risk associated with transport to the radiology suite is avoided. Given that ultrasound is relatively inexpensive, is easy and quick to perform, and often can provide a great deal of information about the location, nature, severity of the obstruction and possibility of strangulation, it should be employed early in the evaluation of all patients with intestinal obstruction [11-13].

CT scan has several advantages over the plain film, ultrasound especially with contrast; it can ascertain the level of obstruction, it can assess the severity of the obstruction and determine its cause, it can detect closed loop obstruction and early strangulation. The CT scan findings, that every surgeon should be aware of which, can help in assessing precisely cases of intestinal obstruction with or without strangulation are; it can show thick adhesive bands, collapsed distal bowel loops and absence of air in the large bowel, distended fluid filled loops with air fluid levels, hyperemia, bowel wall thickening in cases of strangulation and ischemia, free fluid in the peritoneum. It may show the site of obstruction and the cause, it also can detect inflammatory or neoplastic processes both outside and inside the peritoneal cavity, it can visualize small amounts of intraperitoneal air or pnematosis cystoids intestinalis not seen in conventional films [14,15].

The aims of this study were to evaluate the efficacy of plain abdominal x-ray film, ultrasound, and CT scan in the diagnosis of intestinal obstruction. In addition to assess the value of plain films, ultrasound and CT scan in determining the level, site, cause and the presence or absence of strangulation. Also, to evaluate the importance of ultrasound in the assessment of intestinal obstruction in pregnant women, and bed ridden, critically ill patients with signs and symptoms of intestinal obstruction.

Methods

Patients

A prospective study with a total number of 62 patients presented to Al-Imamein Al-kadhimein Medical City from October 2009 to October 2016 with the diagnosis of intestinal obstruction.

Exclusion criteria

a. Previous abdominal surgery.
b. Obstructed hernia.
c. Paralytic ileus.

After workup on those selected patients through history taking, clinical examination and the proper investigations, only those with high suspicion of intestinal obstruction were included and this group is even downsized to those who required surgery and proved of have one type of intestinal obstruction.

Methods

All the necessary detailed data about each patient clinical presentation were covered.

The 62 patients elected for the study underwent the following investigations in addition to the regular investigations.

1. Plain abdominal x-ray film both in erect and supine position for the diagnosis and to help figure out the level and, the involved part and the cause of obstruction. For the features of strangulation; thickened small bowel loops, mucosal thumb printing, pnematosis cystoids intestinalis, or free peritoneal air.
2. Ultrasound done looking for:
   A. The line of demarcation between collapsed and distended loop.
   B. Bowel wall edema between serosa and mucosa for possibility of ischemia.
   C. A fixed mass of a peristaltic fluid filled dilated intestinal loops.
   D. Presence of free peritoneal fluid.
   E. Inspissated intestinal contents.
   F. Paradoxical peristalsis.
   G. High reflective fluid within the bowel lumen.

3. Computerized Tomography, carried out for each patient included looking for;
   A. It can ascertain the level of obstruction.
   B. It can assess the severity of the obstruction and determine its cause.
   C. It can detect closed loop obstruction and early strangulation.

The CT scan findings that every surgeon should be aware of which can help in assessing precisely cases of intestinal obstruction with or without strangulation are;

   A. It can show thick adhesive bands.
   B. Collapsed distal bowel loops and absence of air in the large bowel.
   C. Distended fluid filled loops with air fluid levels, hyperemia, bowel wall thickening in cases of strangulation and ischemia.
   D. Free fluid in the peritoneum.
   E. It may show the site of obstruction and the cause.
   F. It also can detect inflammatory or neoplastic processes both outside and inside the peritoneal cavity.
   G. It can visualize small amounts of intraperitoneal air or pnematosis cystoids intestinalis not seen in conventional films.

Those 62 patients were compared and proved with reference to per-operative findings regarding the presence or absence of intestinal obstruction, the level of obstruction and the cause and as mentioned earlier the final diagnosis was obtained by surgery and only those with intestinal obstruction were included.

Results

Sixty-two patients were included in this study. Forty-one of them were male patients while twenty-one of them were female patients only.

Fifty-eight (58) out of 62 (93.5%) patients had mechanical intestinal obstruction. Of those 58 patients, 50 patients (86.2%) had small intestinal obstruction and the rest 8 patients (13.9%)had large bowel obstruction.

Regarding the remaining four patients; two of them has mesenteric vascular occlusion and the other 2 patients had pseudo-obstruction (Oglivie's syndrome) (Table 1).

Regarding diagnosis and level of obstruction, the accuracy of x-ray was 96.55%, ultrasound 87.93%, CT scan 98.27% (Table 2).

Table 1. The cause of intestinal obstruction in the 62 patients included in this study

Cause of Obstruction	Number of Patients (total 62)
Mechanical intestinal obstruction	58 (93.54%)
Small bowel mechanical	50 (80.64%)
Large bowel mechanical	8 (12.90%)
Mesenteric vascular ischemia	2 (3.22%)
Pseudo-obstruction	2 (3.22%)

Table 2. The Accuracy of each investigation for the level and site of obstruction

Investigation	Number of patients	Diagnostic accuracy
x-ray	56	96.55%
Ultrasound	51	87.93%
CT Scan	57	98.27%

For identifying the cause, the CT scan was superior 85.4%, followed by Ultrasound 24%, and the least was x-ray 8% (Table 3).
While the results about the possibility of strangulation was best seen on CT scan 75.8%, followed by ultrasound 17.7% in early cases and the least informative and difficult to interpret by the surgical team and its result was 0% (Table 4).

Table 3. The Accuracy of investigations modalities for the cause of obstruction

Investigation	Number of patients	Accuracy
X-ray	5	8.62%
Ultrasound	15	25.86%
CT scan	53	91.37%

Table 4. The Accuracy of the investigation modalities in strangulation obstruction

Investigation	Number of patients	Accuracy
X-ray	0	0%
Ultrasound	11	18.96%
CT scan	47	81.04%

Discussion

For a long time, abdominal x-ray films, because of its low cost and availability, has long been considered the first choice of investigations in patients with intestinal obstruction [(16)], but the organizational structure of the abdomen is responsible for overlapping images, low resolution, difficult to show clear signs, site of obstruction, cause of obstruction assessment of blood circulation and strangulation which make it difficult on the surgeon to interpret [(7)].
This group of abdominal x-ray films of the site of obstruction, causes, strangulation and their accuracy rates were 90.3%, 8%, and 0% respectively. The opinion about strangulation on plain film is very difficult to be seen.
Mechanical intestinal obstruction is one of the common surgical acute abdomen and if not handled properly the transition to intestinal strangulation the mortality can be as high as 20-30 %; therefore, early diagnosis and surgical treatment can significantly reduce the mortality [(17)].
Clinically in intestinal obstruction one do not need only to know whether obstruction is there but more needed to determine the exact location of obstruction, cause and whether strangulation is present.
For the site and diagnosis, plain film was 96.55%, ultrasound 87.93% and CT 98.27% correct, which make it superior in this regard.
It is worth mentioning that ultrasound diagnosis of intestinal obstruction when fluid is still in the bowel is relatively good in comparison plain film when air accumulates in the bowel and becomes distended with air.
When the bowel loops are distended with fluid it makes a good acoustic window and in this situation the intestinal structure, mucosal folds, the ileocecal valve, mucosal thickening,

the cause of obstruction and intestinal contents can be clearly shown in addition to closed loop syndrome [2,7,8,11-15].

In the early phase of intestinal obstruction when the loops at this time show no obvious expansion of intestinal gas and filled with fluid and in comparison, to x-ray can be superior to plain film in finding changes in intestinal expansion and bowel movement and blood supply which can clearly suggest intestinal obstruction [18].

For all the advantages and usefulness of ultrasound in diagnosis, working on the cause, finding the type of obstruction (mechanical or adynamic), ileus follow-up, blood supply and strangulation, the ultrasound is the superior tool of investigation in a case of suspected intestinal obstruction during pregnancy and critically ill patients [19,20].

With the wide application of CT scan the current diagnosis of intestinal obstruction has developed into a fast and easy proven method [21]. It clearly shows the obstructed bowel and its adjacent mesentery, peritoneal cavity anatomy structure, thus contributing to obstruction site [22]. CT scan can also observe the lesion, revascularization situation, see if the wall thickening of 5 mm scan cuts, diffuse or localized adjacent mesenteric swelling, vague massive ascites and the prompt diagnosis strangulation [23].

Several authors have recommended that patients with suspected small bowel obstruction and equivocal plain film should undergo CT scan before a small bowel contrast series ordered [3,6].

Prospective studies comparing the value of plain film, ultrasound and CT scan have demonstrated that the accuracy rates in the diagnosis of bowel obstruction regarding all the data about the diagnosis, site, level and strangulation were very close to the study results apart from the diagnosis of strangulation on plain films; see (Tables 5, 6, and 7) [14,15,24].

Table 5. Comparison of accuracy of the investigation modalities in determination of site and level between this study and other studies

Investigation	Current study	Megibow et al [14]	Balthazar et al [١٥]	Markogiannakis [24]
CT scan	96.55%	94.0%	93.0%	90.2%
Ultrasound	87.93%	83.0%	70.0%	69.1%
X-ray	98.27%	91.0%	60.0%	83.7%

Table 6. Comparison of accuracy of the investigation modalities in determination of cause of obstruction between this study and other studies

Investigation	Current study	Megibow et al [14]	Balthazar et al [١٥]	Markogiannakis [24]
CT scan	85.4%	85.3%	87.0%	86.3%
Ultrasound	24%	36.0 %	23.0%	27.7%
X-ray	8%	32.0%	7.0%	7.5%

Table 7. Comparison of accuracy of the investigation modalities in determination of strangulation and ischemia between this study and other studies

Investigation	Current study	Megibow et al [14]	Balthazar et al [١٥]	Markogiannakis [24]
CT scan	81.04 %	79.0%	84.0%	76.9%
Ultrasound	18.96%	75.0%	11.7%	10.5%
X-ray	0%	15.0%	5.3%	8.1%

This study concluded that CT scan is highly accurate method in the evaluation of intestinal obstruction especially for determining the level, cause and possibility of strangulation and should be the investigation of choice when the clinical, plain film, and ultrasound findings are equivocal.

Ultrasound is the investigation of choice in cases of intestinal obstruction in pregnant women and at the bedside when the patient is critically ill.

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Conflict of interest

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Comparison of Antimullerian Hormone Level Between Women with Polycystic Ovary Syndrome and Normal Ovulatory Infertile Women of Reproductive Age

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Abstract

Background Polycystic ovary syndrome (PCOS) is the most common cause of chronic anovulation and hyperandrogenism in young women. This syndrome is characterized by an increase in the number of small antral follicles that are between 5-8 mm in size. Antemüllerian hormone (AMH) is secreted mostly by this type of follicle and when it is much too high, the production of a healthy egg every cycle can be halted as it works by reducing the receptors of the ovary to follicle stimulating hormone (FSH).

Objective To compare the AMH level between women presented with PCOS with its level in normal ovulatory infertile women and to determine its correlation with the clinical, hormonal and ultrasonographic parameters in both groups.

Methods This is a prospective cross-sectional study done at Um-Albaneen Infertility Center in Al-Imamein Al-Kadhimein Medical City from march 2015 to January 2016. One hundred infertile women were recruited in this study, 50 women with PCOS and 50 women have other factors of infertility apart from PCOS and ovulatory dysfunction. Sera were taken from all the participants at day (2-3) of menstrual cycle and were investigated for AMH, FSH, luteinizing hormone (LH), androstenedione, testosterone and estradiol (E2) levels. The number of early antral follicles (2-9 mm in diameter) was estimated by transvaginal ultrasound scanning.

Results Level was significantly higher in PCOS women (42.6±23.8) compared to the normal ovulatory infertile women (16±7.5), P-value <0.001. There was positive correlation between AMH and LH, testosterone, androstenedione, number of antral follicles (antral follicle count) and ovarian volume. However, the correlation was negative with age, body mass index, estradiol, and FSH.

Conclusion AMH strongly correlated with testosterone level and the number of small antral follicles in PCOS women, so it can be considered as a good diagnostic marker for PCOS.

Keywords Antimullerian hormone, PCOS.

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List of abbreviation: AML = Antemüllerian hormone, BMI = Body mass index, E2 = estradiol, FSH = Follicle stimulating hormone, LH = Luteinizing hormone, PCOS = Polycystic ovary syndrome,

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders among females. PCOS is a complex, heterogeneous disorder of uncertain etiology, but there is strong evidence that it

can, to a large degree, be classified as a genetic disease [1]. The condition was first described in 1935 by American gynecologists Irving F. Stein, Sr. and Michael L. Leventhal, from whom its original name of Stein-Leventhal syndrome is taken [2]. PCOS produces symptoms in approximately 5-8% of women of reproductive age group. It is thought to be one of the leading causes of female subfertility and the most frequent endocrine problem in women of reproductive age [3].

Diagnostic Criteria of PCOS

•Rotterdam

In 2003, a consensus workshop sponsored by the European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) in Rotterdam indicated that PCOS to be present if any 2 out of 3 criteria are met [4]:

- Oligoovulation and/or anovulation.
- Excess androgen activity (clinical or biochemical).
- Polycystic ovaries (by gynecologic ultrasound) ≥12 follicles of 2-9 mm and/or enlarged ovarian volume of ≥10 mL in one or both ovaries.

•Androgen Excess PCOS Society.

In 2006, the Androgen Excess PCOS Society suggested a tightening of the diagnostic criteria to all of [5]:

- Excess androgen activity.
- Oligoovulation/anovulation and/or polycystic ovaries.
- Other entities are excluded that would cause excess androgen activity.

Ovarian Dysfunction in PCOS

The ovulatory dysfunction in PCOS can be ascribed to disturbed follicular development with excessive early follicular growth and abnormal later stages of arrested follicle growth well before expected maturation [6]. This pattern of follicular growth with failure in the selection of a dominant follicle for ovulation results in one of the hallmarks of PCO morphology. Infertility affects 40% of women with PCOS, which is the most common cause of anovulatory infertility. Approximately 90-95% of anovulatory women presenting to infertility clinics have PCOS [7].

Antimullarian Hormone (AMH)

It is produced by the Sertoli cells of the fetal testis, induces the regression of the Müllerian ducts. However, after birth, this sex-dimorphic expression pattern is lost and AMH is also expressed in granulosa cells of growing follicles in the ovary. AMH is a glygoprotein hormone structurally related to inhibin and activin, and a member of the transforming growth factor-β (TGF-β) family [8]. In humans, the gene for AMH is on chromosome 19 [9].

In healthy females, AMH is either just detectable or undetectable in cord blood at birth and demonstrates a marked rise by three months of age; while still detectable it falls until four years of age before rising linearly until eight years of age remaining fairly constant from mid-childhood to early adulthood; it does not change significantly during puberty; from 25 years of age AMH declines to undetectable levels at menopause [10]. AMH continues to be expressed in the growing follicles in the ovary until they have reached the size and differentiation state at which, they are to be selected for dominance by the action of pituitary follicle stimulating hormone (FSH) [11]. AMH is not expressed in atretic follicles and theca cells [12].

AMH expression is strongest in preantral and small antral follicles (≤ 4 mm) [11]. AMH expression disappears in follicles of increasing size and is almost lost in follicles larger than 8 mm. This expression pattern suggests that, AMH may play a role in initial recruitment and in the selection of the dominant follicle [11]. So, there are local selectors for follicle recruitment and growth within the ovary that might contribute to the impaired follicle development in PCOS. AMH reflects the size and activity of the follicular pool [13,14]. There is also evidence of AMH involvement in the regulation of

recruitment of primordial follicles into the growing pool, presumably by decreasing the granulosa cell sensitivity to FSH [15]. In the small primordial and transitional follicles of anovulatory PCOS, AMH protein expression is reported to be reduced. This may contribute to the inappropriate recruitment of growing follicles. Additionally, in both circulation and antral follicular fluid of PCOS women, AMH levels are increased, and these are associated with poor reproductive responsiveness to treatment [16-18]. These high circulating levels may be a reflection of the increased pool of granulosa cells instead of an increased expression. Since high levels of AMH (normal value 7-70 pmol/l in young age female) are associated with lower levels of FSH, it has been suggested that the AMH excess is involved in the lack of FSH-induced aromatase activity that is characteristic of follicular arrest in PCOS [17]. In addition, testosterone exposure upregulates AMH expression in granulosa cells of small bovine follicles in culture and could possibly represent a mechanistic origin of PCOS [19].

The objectives of this study were to compare the serum level of AMH in women with PCOS with its level in normal ovulatory infertile women, and to assess if it could be used as diagnostic marker for PCOS.

Methods

This prospective study was conducted on 100 infertile women who were recruited from the outpatient clinic of the Department of Gynecology and Obstetrics at Um-Albaneen infertility Centre in Al-Imamein Al-Kadhimein Medical City during the period from march 2015 to January 2016. After verbally informed consents were obtained from the patients, they were divided into two groups,

The study group: includes 50 infertile women diagnosed to have polycystic ovary syndrome according to presence of any 2 out of 3 Rotterdam criteria (mentioned in introduction). Exclusion criteria include any woman with unexplained infertility (when no abnormality was found as a cause of her infertility) or endometriosis (laparoscopically diagnosed after confirmation by histopathological study), hypothalamic amenorrhea, thyroid diseases, hyperprolactinemia, hyperandrogenism from another cause e.g. adrenal or androgen secreting ovarian tumors were excluded. Furthermore, any patient with at least one follicle with a diameter >9 mm at a transvaginal ultrasound (U/S) done at a midcycle, or a serum estradiol (E2) level above 80 pg/ml and those with a history of tubal surgery, salpingectomy or ovarian cyst were excluded as well. All of the above conditions were excluded because they may bias the results of our study as they may have an effect on AMH level, as well to determine the pure correlation between infertile women with PCOS and the level of this hormone.

The control group includes 50 infertile women with other causes of infertility other than PCOS, such as male factors, tubal causes and with normal ovulatory cycle (25-35 days) and having no endocrine abnormalities (normal prolactin, basal FSH and E2, and no hyperandrogenism) and a normal ultrasonic ovarian morphology.

The control group was matched with study group for age (±2 years) and body mass index (BMI) (±10%). The control group did not receive any hormonal therapy. A complete history was taken from all the participants in both groups, as well clinical and physical exam was performed. The BMI was determined by measuring the weight and the height of the patient, BMI = weight kg / height m^2. Early-morning blood sample (5 ml) was obtained during the follicular phase for both control and study groups, at (Days 2-3 of the cycle) for the measurement of luteinizing hormone (LH), FSH, E2, Testosterone and AMH. Serum was separated from all blood sample and frozen at -2 °C until used for analysis. Serum AMH levels were determined using enzyme linked immunosorbent assay (ELISA). Serum FSH, LH and E2 levels were determined by using VIDAS method which is automated quantitative test, using ELFA technique (Enzyme Linked

Flourecent Assay). Transvaginal U/S was done at day 13 of the cycle to all participate to assess the number of small follicles (2-9 mm) and calculate the ovarian volume. Ovarian ultrasound scanning was performed using 4.5-7.2 MHz transvaginal probe, done by the same operator.

Statistical analysis

Statistical analyses were performed using the SPSS Statistics (Statistical Package for the Social Sciences) version 17. Descriptive analysis was used to show the mean and SD for age, BMI, Serum FSH, LH, E2, Testosterone and AMH. Comparisons of two independent groups were made using the Student t test. The correlation between AMH and the various parameters were evaluated, multiple regression analysis was used to evaluate the preferential effect of different studied variables on AMH level. A P-value ≤ 0.05 was considered significant and ≤ 0.001 highly significant.

Results

For both groups (the PCOS and the control groups), the age range was (18-35) years old and the BMI range was (18-39) kg/m^2. Comparison of the demographic characteristic, clinical, hormonal and ultrasound data for PCOS with that of the control group revealed the following results (Table 1). The mean FSH and E2 were not significantly different between the two groups. The mean LH, testosterone, androstenedione, AMH, number of antral follicular (2-9 mm) and ovarian volume were significantly higher in PCOS group.

Table 1. The demographic characteristics with clinical, hormonal and ultrasonographic data of study and control groups

Variables	PCOS n=50 Mean±SD	Control n=50 Mean±SD	P-value
Age (year)	27.5±4.1	28.6±4.6	0.295
BMI (kg/m^2)	26.1±4.9	25.9±3.7	0.848
AMH (pmol/L)	42.6±23.8	16.1±7.5	0.001*
FSH (IU/L)	5.6±1.7	6.1±1.8	0.236
LH (IU/L)	8.9±4.4	4.7±2.3	0.001*
Testosterone (nmol/L)	2.3±0.8	1.5±0.6	0.001*
E2 (pmol/L)	110.1±56.6	118.4±58.1	0.547
No. of antral follicles	21.3±7.3	7.5±3.4	0.001*
Ovarian volume (cm^3)	28.7±6.7	7.8±1.6	0.001*
Androstenedione (nmol/L)	9.2±4.3	6.6±1.8	0.002*

P value < 0.05 is significant, P value <0.001 is highly significant

Using the Pearson correlation (r) between AMH and other parameters in all group of patients, we found that there were negative statistical correlations between AMH and age, BMI, FSH and E2. There were positive correlations between AMH and LH, testosterone, androstenedione, number of antral follicle and ovarian volume.

From tables 2 and 3, there was positive correlation between AMH and LH, testosterone, androstendione, number of antral follicles and ovarian volume. Testosterone and androstendione strongly correlated with AMH exclusively in PCOS group (r=0.557; p=0.001), (r=0.451; p=0.007) respectively, also the number of small antral follicles. Multiple regression analysis was performered in the PCOS groupincuding AMH as dependent variable, and LH, FSH, testosterone, androstendione, E2 and ovarian

volume as independent variables. Testosterone was the only determinant for AMH level (r= 0.557; p<0.001), whereas other parameters were no longer Significantly related.

Table 2. Correlation between AMH and clinical, hormonal and ultrasounographic parameters (n = 100)

Variables	AMH	
	r	p
Age	- 0.205	0.089
BMI	-0.130	0.283
FSH	-0.358	0.002
LH	0.281	0.018
Testosterone	0.472	0.001
Androstenedione	0.371	0.002
E2	-0.095	0.434
AFC <10 mm	0.627	0.001
Ovarian volume	0.478	0.001

Table 3. Correlation between AMH and clinical, hormonal and ultrasonographic parameters in the study and control groups

Variables	PCOS		Control	
	r	p	r	p
age	-0.137	0.433	-0.098	0.575
BMI	-0.086	0.623	-0.195	0.262
FSH	-0.347	0.041	-0.356	0.036
LH	0.336	0.048	0.341	0.045
Testosterone	0.557	0.001	0.199	0.504
Androstendione	0.451	0.007	0.227	0.379
Estradiol	-0.085	0.627	-0.074	0.673
AFC <10	0.625	0.001	0.475	0.008
Ovarian volume	0.436	0.009	0.369	0.029

Discussion

The results of the present study revealed no significant correlations between AMH with age and BMI in both groups. As the mean age of the control group (28.6±4.6) and (27.5±4.1) for PCOS group, p-value 0.295, and BMI means (26.1±4.9) and (25.9±3.7) for PCOS and control groups respectively, p-value 0.848. This is in agreement with Pigny et al. [18]. However, Nardo et al. [20] indicated that AMH is generally decreased with chronological age and Chen et al. [21] found that AMH had a significant negative association with BMI and age. In the current study, there were negative statistical correlations between AMH with age and BMI, however, this does not reach the level of significance, probably because of the small sample size.

The results of the present study have shown higher serum AMH levels in the study group than that in the control group, as well the mean number of antral follicle count was significantly higher in the study group (21.3±7.3) compared to the control group (7.5±3.4) with a P- value of 0.001, and a significant positive correlation between AMH and number of follicles <10 mm in the whole group of patients (r=0.627) and in each group

separately (PCOS group r=0.625 and control group r=0.475) was found; these results are in line with the fact that serum AMH levels reflect the number of small antral follicles because the highest expression of AMH has been demonstrated in the stage of pre-antral and small antral follicle size (4-6 mm) and disappears in follicle size larger than 9 mm, this was demonstrated in several studies [18,20,22-26].

The current study findings regarding FSH are comparable with the results of previous studies [27,28]. However, Pigny et al. [18] found no relationship between AMH and FSH in PCOS and control groups. Current results revealed that there is negative correlation between AMH and FSH, the mean of FSH in PCOS group was (5.6±1.7) and in the control group was (6.1±1.8) with p-value 0.236, which is not significant. So with increasing age there will be an increase in the level of FSH and a decrease in AMH level, so AMH could be used as a marker of ovarian reserve [24]. Also it was found that there was positive correlation between AMH and LH as the mean of LH in PCOS was (8.9±4.4) and in the control group was (4.7±2.3) with p-value <0.001, which is significant, r=0.281 that's mean positive correlation.

In the present study, significant positive correlation was found between AMH and serum testosterone in the PCOS group exclusively. This finding is in accordance with the results of previous studies [18,21,27-29], and add to the existing evidence for the role of small ovarian follicles in the production of both AMH and androgens. However, Nardo et al. [20] indicated that AMH is similarly related to testosterone in women with and without PCOS. In the current study, the mean testosterone in PCOS group was (2.3±0.8) and in the control group (1.5±0.6), p-value <0.001, which is significant, r=0.472 indicates positive correlation between AMH and testosterone.

Pigny et al. [18] suggested that the increase in AMH serum levels in PCOS is a consequence of androgen-induced excess in small antral follicles and that each follicle produces normal amount of AMH.

However, Pellatt et al. [30] found that raised serum AMH in PCOS is a reflection of both an increase in production per cell and the increase in follicle number since they used cells from size-matched follicles in patients and controls plated at the same density.

It could be also speculated that since AMH inhibits FSH-induced aromatase activity in cultured mouse [31] and human granulose cells [21], it may also be responsible for the reduced aromatase activity in PCO granulosa cells [21] and contributes to the elevated androgen levels. Moreover, Crisosto et al. [32] proposed that AMH expression is modulated by androgens in bovine granulosa cells from small follicles; suggesting that androgens, by inhibiting AMH expression, may promote follicle recruitment, increasing the early growing follicular pool. Multiple regression analysis demonstrated that testosterone was the only determinant for AMH level in the PCOS group (r=0.557 ; P<0.001). This is in contrast with Pigny et al. [18] who found that only the number of 2-5 mm follicles, was significantly related to AMH. However, Eldar-Geva et al. [28] revealed that the number of small follicles and serum androgens were correlated to AMH.

This study concluded that there was strong correlation between AMH, testosterone and the number of small antral follicle, which were increased in PCOS patients so AMH can be considered as a good diagnostic marker for PCOS .

The authors of this study recommend that AMH can be used as diagnostic marker for PCOS and also may be used as prognostic marker for the extent of ovarian dysfunction in PCOS patients and whether it can predict response to ovulation induction and monitoring infertility treatment. Also, AMH can be used for the assessment of ovarian reserve and provide insight into the number of fertile years women has left .

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Author contributions:

Dr. Jumaa: cases collection, obtaining the results of the hormonal study and the findings of transvaginal ultrasound scan. Dr. Almoayad and Dr. Abdulrasul supervised the study and wrote the article and revised it.

Conflict of interest

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Indirect Immunofluorescence Expression of ZO-1 and Pan-cadherin during Maturation of Hepatocytes and Biliary Network Formation in Mice

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Abstract

Background The tight junctional complexes had been demonstrated in hepatocytes and in both epithelium and endothelium. In multicellular organisms, cell-cell adhesion is critical for development and morphogenesis.

Objective To investigate the spatiotemporal organization of the immunohistochemical markers anti ZO-1 and anti Pan-cadherin during the prenatal development of the mice liver and to correlate the expression of these markers with histogenesis during hepatocyte maturation and biliary network formation.

Methods Forty eight (48) pregnant mice were scarified at subsequent gestational days from day 14 till the first day of postnatal life. Paraffin blocks and sections of the liver extracted from the embryos obtained from these pregnant mice and from neonates at the first postnatal day of life were prepared. The sections were stained using the anti ZO-1 and anti Pan-cadherin immunohistochemical markers.

Results The sequential steps of anti ZO-1 indirect immunofluorescence reactivity in the developing liver tissue showed chronological variability, during days 14, 15, and 16; positive cell surface labeling was weaker adjacent to the blood vessels. The next step includes anti ZO-1 reactivity in late prenatal and postnatal liver tissue showing marked uniform reactivity. The sequential steps were also demonstrated in the anti Pan-cadherin immunohistochemical reactivity. The reactivity of the liver tissue during days 14, 15, and 16 showed disregarded cell surface labeling that increased markedly in later prenatal and postnatal periods.

Conclusion The initial stages in the development include the undifferentiated specification the hepatic lineage characterized by regional establishment of zonula occludens in the developing liver cells and paucity in the development of the zona adherens complexes. The later stages showed histological and functional maturation reflected by anti ZO-1 and anti Pan-cadherin reactivity that represent a requirement for the physiological functions in the entire liver tissue.

Keywords Embryo, mice, Zo-1, Pan-cadherin, immunhistochemistry.

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List of abbreviation: FITC= Fluorescein isothiocyanate, IgG=Immunoglobin G, IHC= Immunohistochemistry, TR= Texas Red, ZO-1=zonulaoccludans.

Introduction

Several studies used mice for research on liver structures and functions assumed that there is similarities between the humans, mouse and rat liver [1]. In mice, the development of the liver is the same that in

human by formation of hepatocytes and biliary epithelial cells that are derived from the endodermal germ layer, while the stromal, stellate, kuppfer cells and blood vessels all are of mesodermal germ layer [2]. A mouse liver extent the whole sub-diaphragmatic space and divided into four lobes (right, medial, left, and caudate lobes) [3].

The junctional complexes are intercellular connection that are of three major types; vertebrates, tight junctions, adherens junctions (including zonulaadherens and macula adherens) and gap junctions [4]. The bile canaliculi between liver cells are split from the intercellular space by the tight junctions, which forming a barrier to large molecule and substance exchanging between the biliary section and the blood section [5]. The zonula occludans-1 (ZO-1) has been verified in hepatocytes of liver in both epithelium and endothelium [6]. The procedure for cell-to-cell adherens junctions has been illustrated in rat liver [7]. The gap junctions occur between liver cells at patchy distances from the bile canaliculi [8].

Pan-cadherin is a termed of antibodies that detect cadherin proteins in a variety of tissue organs. The Cadherin is a polypeptides (about 720–750 amino acids long) that part of super family of trans-membrane glycoproteins [9].

This study was designed to investigate the spatiotemporal organization of the immunohistochemical markers (Anti ZO-1 and Pan-cadherin) during the prenatal development of the mice liver till the 1st day of parturition.

The expression of these markers would be correlated with histogenesis during hepatocyte maturation and biliary network formation.

Methods

Forty-eight (48) adult femalespregnant mice aged about 10-12 weeks, weighing between 25-30 g, apparently active and healthy had been used in this study. These mice were divided according to the days of pregnancy to (day 14,15,16,17,18,19,20 and postnatal "1st day after parturition"), and select six (6) mice for each of these days. The number of embryos (or newborns) obtained ranged from (6-8) from each mouse. Two (2) of these embryos (or newborns) were taken randomly from each mouse. The total number of embryos was (12) for each day. The liver embryos were fixed in 10% formalin for 48 hours and paraffin blocks were prepared [10]. Sections of 5 µm thickness were cut using the electrical microtome (Richert - Jung, 2030 MOT Biocut).

Two immunohistochemistry (IHC) markers had been used in this study from (SANTCRUZ BIOTECHNOLOGY and US BIOLOGICAL):

1- Monoclonal, Anti ZO-1 (R40.76) (primary).
   - Goat anti-rat IgG-FITC: sc2011 (secondary).
2- Monoclonal, Anti Pan-Cadherin, (primary).
   - Goat anti-mouse IgG-TR: sc-2781 (secondary).

Images of Anti ZO1 and Anti Pan-cadherin were captured by Zed Axis camera (5 mega pixels) placed directly over the head of the fluorescent microscope linked with a desktop computer to saved images.

Results

Indirect immunofluorescence reactivity of ZO-1

Section of the adult rat kidney was used as a positive control of the anti ZO-1 IHC reactivity. Sections of embryos in each day were used as a negative control by stained only the secondary of ZO-1.

During 14, 15, 16 days

The paraffin sections of the developing hepatic parenchyma during the 14th, 15th and 16th days of gestation showed positive IHC reactivity to anti ZO-1 antibody. The endothelial cells of the hepatic sinusoids showed very weak reactivity at this developmental stage. The fluorescent IHC reactivity appeared to be fainter at the peri-sinosoidal hepatic parenchymal tissue. The variable cellular elements of the liver tissues could not been distinguished as the histological criteria of the tissue are not demonstrated using the IHC labeling. The cell surface

immunoreactivity verifies variability of the cell-size at high power field. The size variability ranged from small, intermediate, to large cell size. The small size immunoreactive cells are arranged in a linear-chain like architecture all over the field. The cellular reactivity showing the intermediate and large size of cells is less numerous in comparisons to the small size cells. The cell surface IHC fluorescence reactivity appears more intense around the larger cell (Figure 1).

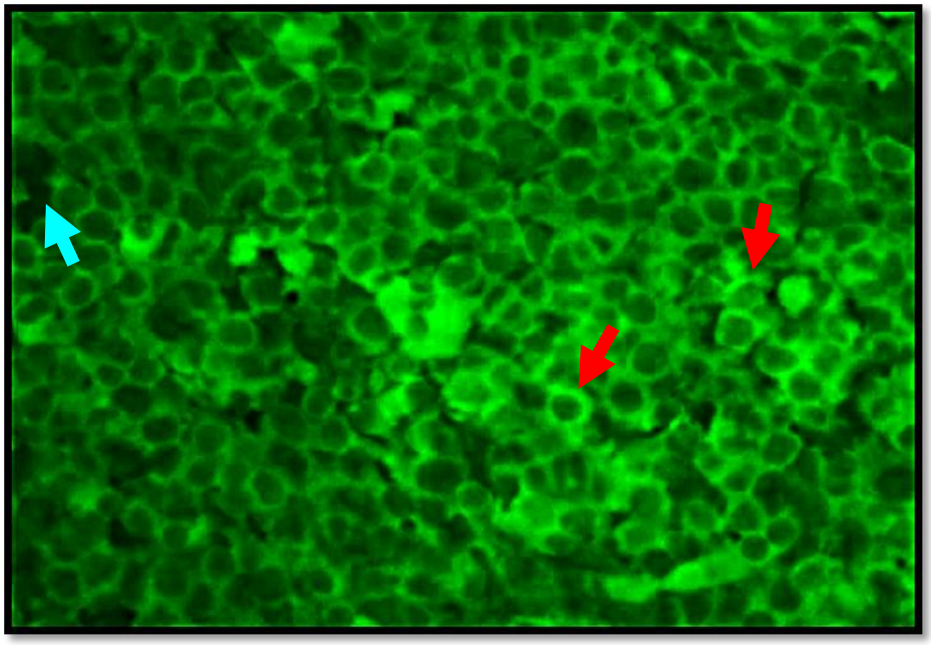

Figure 1. The section of mouse liver embryo at the day 15 showed positive reactivity to anti ZO-1 antibody in the cell surface by using indirect immunoflourescence (Red arrow), hepatic vessel (Blue arrow). 100X

During 17th and 18th days

The vascular endothelial cell showed weak reactivity, the vascular lumen contained many strongly fluorescent red blood cells. The IHC reactivity showed uniform intensity in all parts of the liver parenchyma. The immunoreactivity on the cell surface showed mostly large and intermediate size cells (Figure 2).

During days 19 - to - postnatal (1st day after parturition)

The fluorescence reactivity all over the section showed distinguished cell surface binding demarcating the cellular boundaries. Other cellular element showed the same pattern of reactivity seen in the previous stages (Figure 3).

Indirect immunofluorescence reactivity of Pan-Cadherin

Section of the adult rat kidney was used as a positive control of the anti Pan-cadherin IHC reactivity. Sections of embryos in each day were used as a negative control by stained only the secondary of Pan-Cadherin.

During days 14, 15 and 16:

The Pan-cadherin IHC reactivity was very weak. No regional variability was seen in different parts of the sections. The intravascular red blood cells showed strong fluorescent reactivity. The ghost-like fluorescence reactivity does not allow clear examination of the cellular outlines (Figure 4).

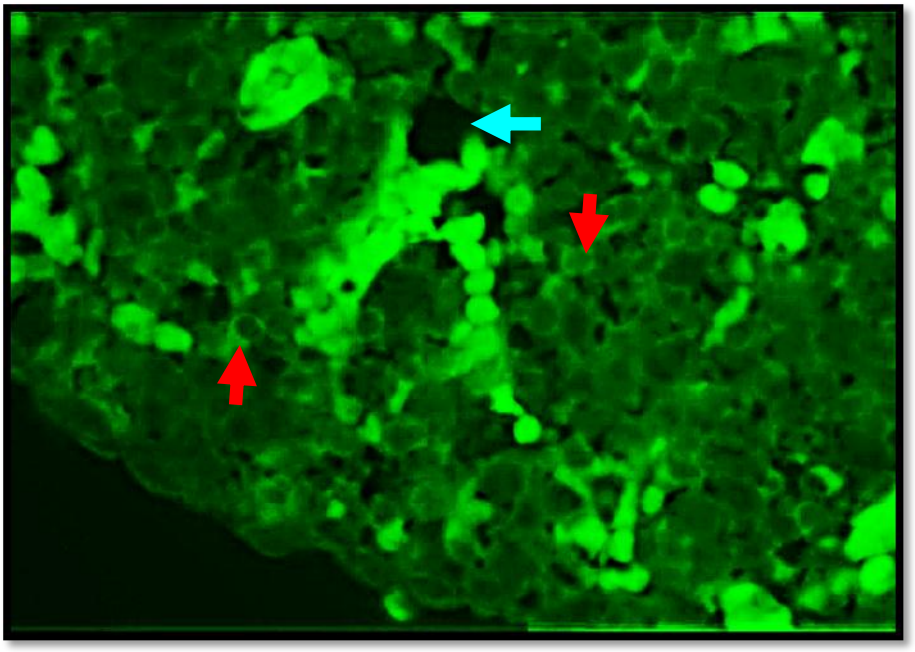

Figure 2. The section of mouse liver embryo at the day 18 showed positive reactivity to anti ZO-1 antibody in the cell surface by using indirect immunoflourescence (Red arrow), hepatic vessel (Blue arrow). 100X

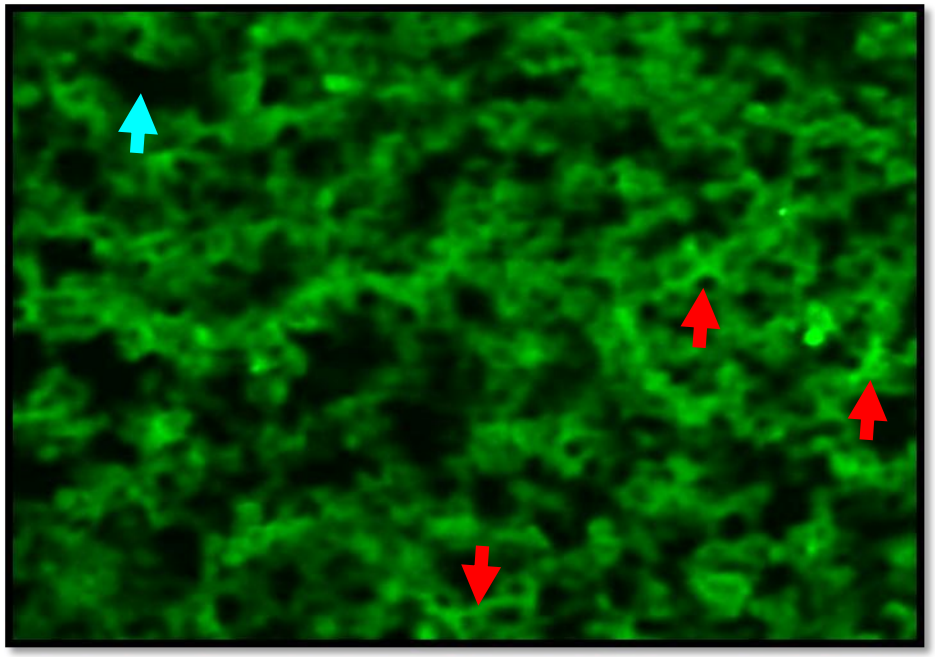

Figure 3. The section of mouse liver embryo at theday 20 showed positive reactivity to anti ZO-1 antibody in the cell surface by using indirect immunoflourescence (Red arrow), sinusoid (Blue arrow). 100X

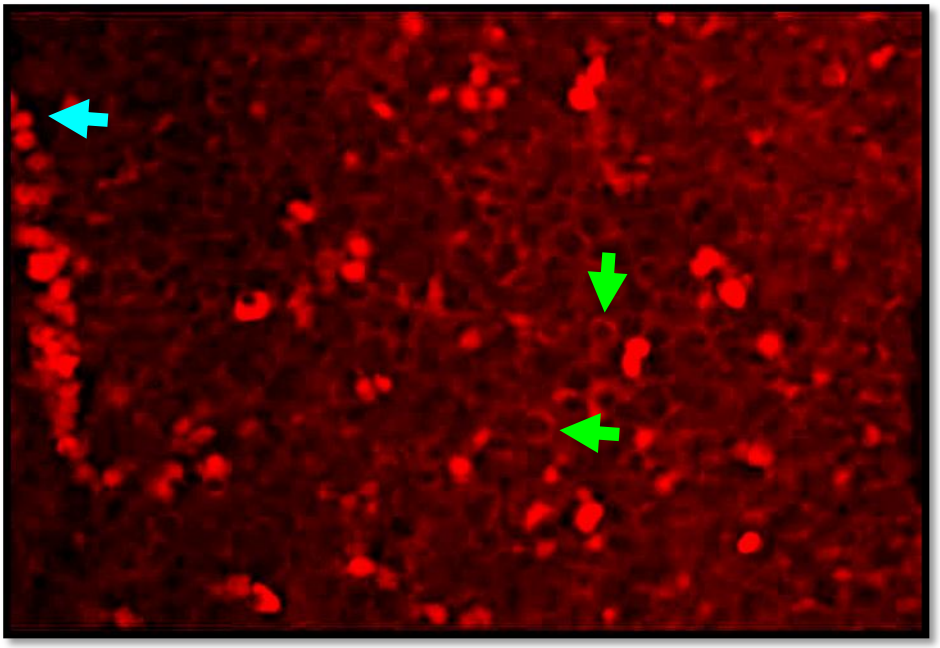

Figure 4. The section of mouse liver embryo at the day 15 showed positive reactivity to anti Pan-cadherin antibody in the cell surface by using indirect immunoflourescence (Green arrow), hepatic vessel (Blue arrow). 100X

During days 17- to - postnatal (1st day after parturition)

The IHC reactivity of the pancadherin in the paraffin sections showed obvious fluorescence in all part of the sections. The outline of the cellular architecture showed large and

The liver develops in a sequential range of steps that are regulated by (intrinsic mechanisms) and (extracelluar signals) leading in differentiation of liver paranchyme (11).

The tight junctional complexes had been demonstrated in the liver cells, and the cell-cell adhesion was confirmed to have a critical role during morphogenesis (12).

These actualities were supported by the results of the IHC descriptions of this study, which suggest the formation of junctional complexes as an important cellular apparatus involved in spread of signaling regulating the maturation of the hepatic tissues. The developmental peroid involved in this study covers the duration of hepatocyte maturation and billiary network formation extending between gestational day 14 till the 1st day of postnatal life.

intermediate cell size, simulating the cellular pattern of immunohistochemical reactivity observed in the similar stages labeled with anti ZO-1 antibody (Figure 5).

Discussion

The sequential steps of anti ZO-1 IHC reactivity in the developing liver tissue showed chronological variability, during days 14, 15, and 16 positive cell surface labeling was seen, which was weaker adjacent of the blood vessels. The outline of cell surface reactivity distinguished variable cell size ranging from small to large cells. The next step includes anti ZO-1 reactivity in late prenatal and postnatal liver tissue showing marked uniform reactivity.

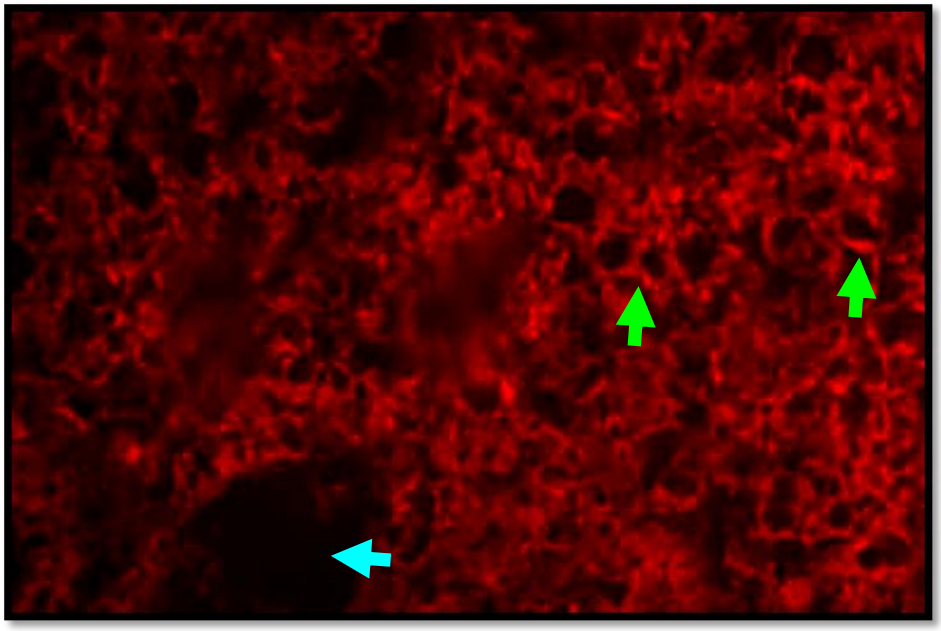

Figure 5. The section of mouse liver embryo at the day 20 showed positive reactivity to anti Pan-cadherin antibody in the cell surface by using indirect immunoflourescence (Green arrow), hepatic vessel (Blue arrow). 100X

The IHC reactivity of ZO-1 during day 14, 15, 16 days described in this study showed very weak reactivity in the endothelial cell of the hepatic sinusoids at these early developmental stages. This finding supported by the finding of (Sheridan, 1966) suggesting that minute tight junctions observed within the primitive tissues of the young chick embryo represent the sites of lowered cell to cell electrical resistance, which have been reported by Sheridan [13].

Potter et al. [14] had discovered that electrical coupling between developing cells occurs prior to cell differentiation in embryonic development. Also, preliminary report done by Sheridan [13] demonstrated low electrical resistance between cells in chick embryos and other developing vertebrate. These reported are in agreement with conclusion of the ZO-1 IHC reactivity investigated in this study.

The sequential steps were also demonstrated in the anti Pan-cadherin IHC reactivity, disregarded cell surface labeling of the liver tissue was seen during days 14, 15, and 16 that will have increased markedly in late prenatal and postnatal periods.

Accordingly, the development of junctional complexes during histogenesis of the liver tissue can be divided into distinct stages based on the IHC reactivities of markers involved in this study.

The initial stages in the development include the undifferentiated specification the hepatic lineage characterized by regional establishment of zonula occludens in the developing liver cells and paucity in the development of the zona adherens complexes.

From the results of this study, it was concluded that later stages showed histological and functional maturation required for the physiological value of these junctional complexes in the entire liver tissue.

This conclusion is in agreement with descriptions of Zaret [15] that reported detection of liver-specific markers in mouse since days 8-9, the hepatic paranchyma at this stage possess the potential to differentiate into both hepatocytes and bile duct epithelial cells [16].

The zona occludens and zona adherens are recommended for intercellular communications [5]. The signaling control of the

liver development includes many of the fibroblast growth factors, which displaying hepatogenic properties in many embryonic species [17]. The dynamics of signaling controlling hepatic specification in mouse embryo was also established by [18]. The surfacing of the junctional complexes in association with maturation of the liver cells could play role in transmitting intercellular signaling exhibiting the hepatogenic properties. It was stated that hepatic cells maturated when they switch from a hematopoietic microenvironment to a metabolic organ [19], then after the growth of liver cells arrested during the postnatal development [20]. The postnatal differentiation of the hepatocytes and other non-parenchymal cells to creates the organized liver lobules that are the basic units of the liver tissue. This report could be implicated for the rationality of choosing the limitation of the first postnatal day as the latest stage involved this study.

The endodermal cells committed to the hepatic cells lineage undergo maturation programs to acquire various specific metablolic functions and differentiate into either hepatocytes or bile duct cells. The previous suggestions reported that the embryonic hepatic cells should not simply be considered as immature nonfunctional cells [21].

This suggestion could interpret the pattern of IHC reactivity in this study that show equivalent binding in both late prenatal and postnatal stages and establishing evidences that the late prenatal and the postnatal hepatic paranchyma achieve the property of specific maturation.

This study concluded:

1. The immunehistochemical results represent a step to elaborate the mechanism underlying the fate of hepatoblast differentiating into hepatocytes or biliary epithelial cells.
2. The histology of the developing liver provides a framework for interpretation the immunohistochemical investigations of liver tissue organization.
3. The histology of developing liver supported reports that the liver tissue from day 19 till the early postnatal days showed no marked morphological changed.
4. The Anti Pan-Cadherin reactivity showing disregarded labeling of the liver tissue in younger embryo.
5. The junctional complexes were divided into distinct stages. The initial stage charactrized by regional establishment of zonula occludens and paucity of the zona adherens. The later stages showed histological and functional maturation that require the physiological value of junctional complexes in the entire liver tissue.
6. Equivalent immunhistochemical reactivity in both late prenatal and postnatal stages indicating specific maturation.
7. The mosaic Anti ZO-1 reactivity gives insights on the maturation pattern of hepatocytes which depend on cell-cell and cell-matrix interactions controlled by adhesion molecules and intercellular junctions.
8. Uniform Anti Pan-Cadherin reactivity at late prenatal and postnatal days indicate normal tissue integrity as cadherins regulates morphogenesis in the liver during development, this functional criterion was associated with maturation of the liver tissue

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Author contributions:

Dr. Ali: the M.Sc. candidate performing the laboratory research work and performing production of the results. Dr. Mubarak: the supervisor of the MSc research performing the interpretation of the results.

Conflict of interest

The authors disclose no any financial and personal relationships with other people or organizations that inappropriately influence (bias) this work.

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Evaluation of Intussusception in Children: Our Experience in 47 Cases

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Abstract

Background Intussusception is the most common cause of intestinal obstruction in infants and young children.

Objective To review the clinical, epidemiological, management pattern and outcome of intussusception.

Methods A retrospective descriptive review of 47 patients who were diagnosed and managed for intussusception during the period from January 2012 to October 2014 in Al-Ramadi Teaching Hospital for Maternity and Children. Data were collected from the Pediatric Surgery Unit records, which include demographic characteristics of the patients, history of preceding gastroenteritis or respiratory infection, clinical signs and symptoms, imaging studies, type of management, operative finding, outcome and mortality. Data were analyzed by Epi Info7TM, using chi-square to obtain p value which regarded significant when it was < 0.05.

Results Records of 47 patients were reviewed; (68.4%) were under one year of age. Peak incidence between 6-12 months of age (55.3%). Male: female ratio was 2.35:1. 27 patients (57.4%) were from rural area, peak seasonal incidence occurred in summer and winter, 41 patients (87.2%) presented with abdominal pain, a palpable abdominal mass was detected in 24 patients (51%), red currant jelly stool reported in (74.5%). Ultrasonography were done for 39 patients and showed an abdominal mass suggestive intussusception (as a target sign or pseudo kidney mass) in 34 patients (87.1%). (95.7%) of patients treated by surgical reduction, while only two patient reduced by hydrostatic enema, Meckle's diverticulum were the commonest pathological lead point (44.4%). No intussusception- associated death was recorded.

Conclusion Colicky abdominal pain was the most presenting symptoms and red currant stool was a significant sign especially in children ≤ 12 months. Majority of cases presented after 24 hours were from rural area, seasonal peaks occurred in summer and winter months and surgery remain the mode of management.

Keywords Intussusception, clinical, epidemiological, management pattern and outcome.

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List of abbreviation: No abbreviations

Introduction

Intussusception is the commonest cause of intestinal obstruction in infant and young children [1,2]. It occurs when proximal segment of bowel invaginates into another just distal to it, resulting in venous congestion, bowel wall edema leading to an out pouring of mucous and blood from the engorged intussusceptum, and later leading to an obstruction, if left untreated is a potentially lethal condition [1-3].

More than 60% of children are younger than one-year old and accurate estimates of the incidence are not available for most of the developing countries, intussusception rates reported from various parts of the world ranged from 24 to 230 cases per 100,000 children annually and the male to female ratio range from1.4:1 to 4:1 [2]. The peak incidence is between 5 and 7 months of age [4], with no distinct seasonality were observed [4,5].

In children, the cause of intussusception is idiopathic in majority of cases [1,2,4]. However, a strong association with viral (adenovirus) and bacterial infection has been observed [6-8]. Susceptible individuals may have an altered anatomic or immune status that, when they are infected with adenovirus, predisposes them to intussusception [9].

In 2-8%, Intussusception is secondary to a pathological lead points especially in children >2 years of age [1], such as Meckel's diverticulum, polyps and small bowel lymphomas, etc. [10,11]. Intussusception has been also reported to occur post operatively [12] and after abdominal trauma [13].

Intussusception may be ileo-colic (80%), ileoileal, cecocolic, colocolic, ileoileocolic and jejunojejunal in type [1,2,14].

Classically, colicky abdominal pain and vomiting (milk then bile) will be the presenting symptoms in a previously healthy infant. Between episodes child initially appears well. Later on, they may pass a red currant jelly stool [1,2].

Lethargy or alterations of consciousness can be the sole presenting symptom of intussusception, which makes the condition's diagnosis challenging [15].

Clinical signs include dehydration, abdominal distension and a palpable sausage shaped mass in the right upper quadrant. Rectal examination may reveal blood or rarely the apex of an intussusception. Prolapse of the intussusceptum through the anus is a grave sign, particularly when the intussusceptum is ischemic [16]. The classic triad of abdominal pain, palpable abdominal mass and red currant jelly stool occurs only in one third of cases [17].

Diagnosis of intussusception can be confirmed by X-ray of the abdomen, which may show signs of intestinal obstruction and in 25-60%, abdominal plain films demonstrate a right upper quadrant soft tissue density that displaces air-filled loops of bowel. Diagnosis can also be confirmed by an ultrasound scan (noninvasive, sensitive and specific method for the diagnosis). Intussusception has a characteristic sonographic appearance, this is described as an abdominal mass with a target sign on transverse section and a pseudo kidney or sandwich sign on longitudinal section [2,16].

Contrast enema, it's both diagnostic and therapeutic and the classic signs of intussusception on contrast enema are the meniscus sign where the apex of the intussusception project into the contrast material and the coiled spring sign, which produced when small amount of contrast accumulate between the intussusceptum and intussuscepients.

After resuscitation with intravenous fluids, broad spectrum antibiotics and nasogastric drainage, non-operative reduction of the intussusception can be attempted in early and uncomplicated cases [2,16].

Non-operative reduction techniques using enemas may be hydrostatic (using barium, water soluble contrast) [1,2,4,16,18], or pneumatic (using either air or carbon dioxide) [18-20]. Both of these procedures can be performed under fluoroscopic or ultrasound guidance [18].

Operative reduction indicated when there are signs of peritonitis, a pathological lead point or in the presence of profound shock and when facilities for non-operative reduction are limited [2,4,16].

In general, the longer the duration of symptoms (particularly if >24 hours) the lower the likelihood of successful non-operative reduction [18].

The aim of this study was to evaluate the management and outcome of intussusception.

Methods

Study design

A retrospective descriptive study was conducted at Al-Ramadi Teaching Hospital for Maternity and Children over a period from January 2012 to October 2014.

Study sample

Records of forty-seven patients who were diagnosed on the basis of history and clinical examination, radiological investigations and further confirmed on surgical exploration and managed for intussusception were reviewed.

Data collection

Data were collected from the Pediatric Surgery Unit records and the patient case files.

These data include demographic characteristics of the patients (age, gender, residence). History of preceding medical illnesses, time of presentation (season) and duration of symptoms before presentation (when the time interval between the onset of first symptom and presentation of patient is more than 24 hours it is considered as delayed presentation and time period less than 24 hours is called as early presentation.

Clinical signs and symptoms, which include abdominal pain (screaming attacks), vomiting, presence of bloody stool, fever, lethargy, abdominal tenderness, distension, mass, finding on per rectal examination and presence of prolapsing mass or not. Imaging studies (erect abdominal x-ray and contrast study) and ultrasonography. Type of management (barium reduction or surgical reduction) as well as operative finding (type of intussusception and presence of pathological point and its type), and outcome among the sample study.

Statistical analysis

These data were analyzed by Epi Info7TM using chi-square to obtain the difference between variables (p value) and p value was regarded significant when it was <0.05. Percentages were calculated Manually.

Results

Records of forty-seven patients who were diagnosed and managed for intussusception were retrospectively reviewed. In this study, age of patients ranged from 4months to 5 years with a mean age of(13 months ± 10.9 STD).

Thirty-two patients (68.08%) were under one year of age. Twenty-six of them (55.3%) were between 6-12 months of age. And fifteen patients (31.92%) were older than one year.

Thirty three patients (70.2%) were males and fourteen patients (29.8%) were females with a male:female ratio of 2.35:1 as shown in figure (1).

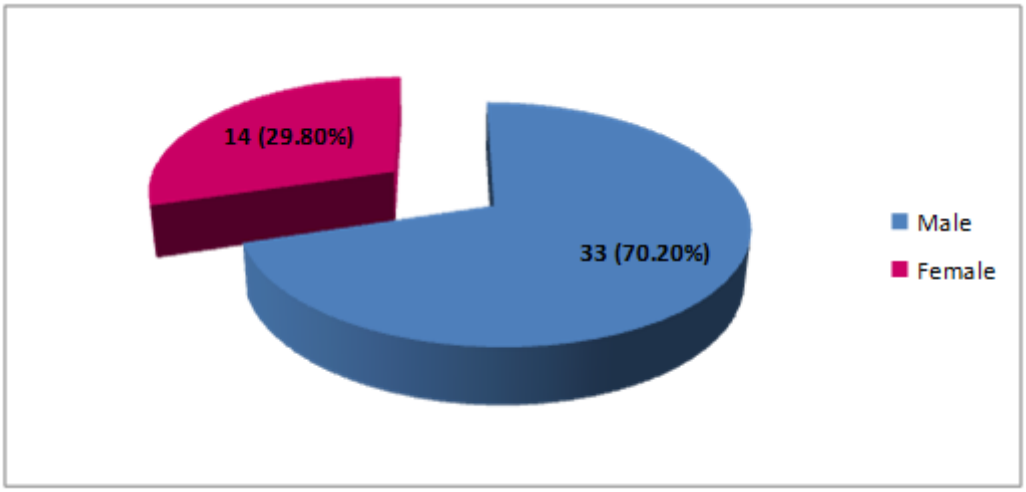

Figure 1. Frequency distribution of intussusception by gender

Twenty-seven of patients (57.4%) came from rural area while twenty patients (42.6%) were from urban society. Thirty-one patients were presented after 24 hours of the onset of symptoms while sixteen patients were presented within first 24 hours, and there was statistical difference between rural and urban residence in relation to duration of symptoms (whether ≤ 24 hours or > 24 hours) at time of presentation (p. value was highly significant< 0.001) as shown in table (1).

Peak incidence occurred in summer and winter months as shown in figure (2).

Table 2. Relation between residence of children with intussusception and duration of symptoms on presentation

Residence	Duration of symptoms in hours				Total	p. value
	≤ 24 hours		> 24 hours			
	No.	%	No.	%		
Rural	5	18.5%	22	81.5%	27	< 0.001
Urban	11	55.0%	9	45.0%	20	
Total	16	34.04%	31	65.96%	47	

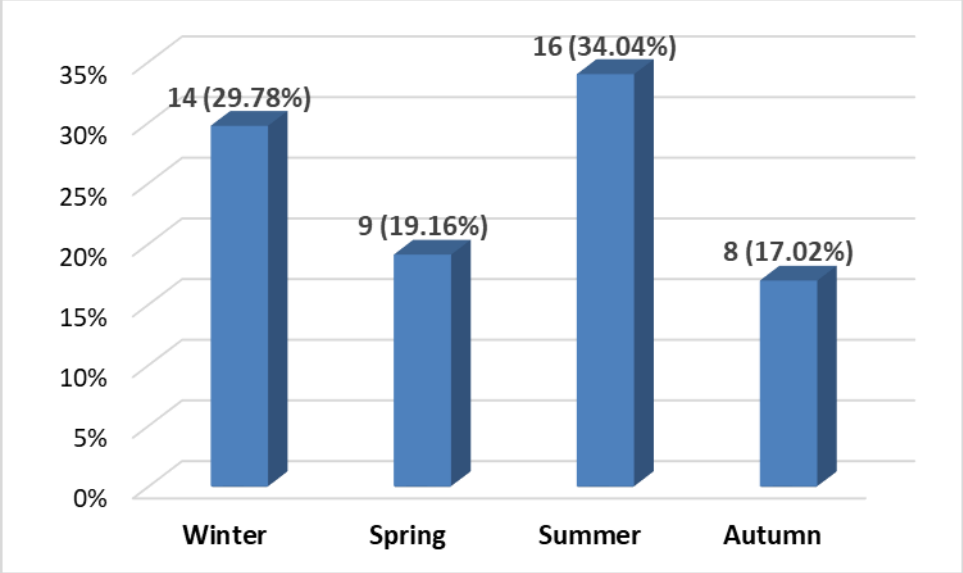

Figure 2. Seasonal distribution of children with intussusception

History of preceding medical condition was reported in about 75% of children, (34 %)of them with respiratory tract infection, eleven patients (23.40%) with gastroenteritis, five patients (10.64%) with tonsillitis and three patients (6.39%) with urinary tract infection, however, in twelve patients (25.53% of cases) there was no preceding illnesses in as shown in figure (3).

In the underlying table, we compared between the clinical features of two age groups (below and above one year) and the finding showed the that patients whom age was less than 1 year were presented more frequently with abdominal pain and bleeding per rectum, while vomiting and lethargy were approximately presented equally in both groups. Regarding clinical signs, red currant jelly stool on per

rectal examination were reported more frequently in children whom age less than 1 year (significant P value 0.024), while abdominal mass and prolapsing mass were reported more in children older than 1 year and abdominal tenderness and distension were reported equally in both groups as shown in table 2.

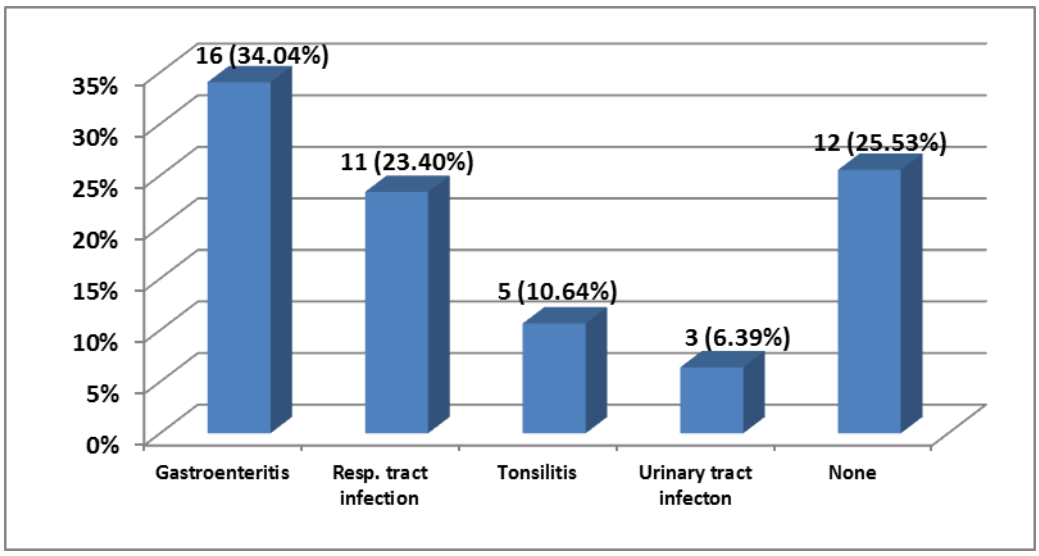

Figure 3. The preceding medical conditions

Table 2. The clinical features in relation to age of patients

Clinical features		Total no.	Age in months				X^2	P. value
			≤12 months		>12 months			
			NO.	%	NO.	%		
Abdominal pain	Present	41	29	90.6%	12	80.0%	1.013	0.314
	Not	6	3	9.4%	3	20.0%		
Vomiting	Present	30	21	65.6%	9	60.0%	0.137	0.711
	Not	17	11	34.4%	6	40.0%		
Bleeding per rectum	Present	30	23	71.8%	7	46.6%	2.751	0.097
	Not	17	9	24.2%	8	53.4%		
Fever	Present	16	13	40.6%	3	20.0%	1.894	0.168
	Not	31	19	59.4%	12	80.0%		
Lethargy	Present	23	16	50.0%	7	46.6%	0.044	0.833
	Not	24	16	50.0%	8	53.4%		
Abdominal tenderness	Present	38	26	81.2%	12	80.0%	0.010	0.920
	Not	9	6	18.8%	3	20.0%		
Abdominal distension	Present	15	10	31.2%	5	33.3%	0.020	0.888
	Not	32	22	68.8%	10	66.7%		
Abdominal mass	Present	24	14	43.7%	10	66.7%	2.107	0.147
	Not	23	18	56.3%	5	33.3%		
Red currant jelly stool	Present	35	27	84.38%	8	53.3%	5.066	0.024
	Not	12	5	15.63%	7	46.7%		
Prolapsing mass	Present	1	----	0.0%	1	6.6%	2.133	0.144
	Not	46	32	100%	14	93.4%		

Erect abdominal x-rays were done in in forty-two patients and which were conclusive in nineteen (45.2%) patients only, showing multiple fluid level, distended bowel loops or soft tissue density mass in the upper abdominal quadrant.

Ultrasonography were done for thirty-nine patients and showed an abdominal mass suggestive intussusception (as a target sign or pseudo kidney mass) in thirty-four patients (87.1%). Diagnostic barium enema done for seven patients (14.8%) only and were diagnostic in five patients (71.5%) as shown in table (3).

Table 3. Frequency of results by the diagnostic modalities

Diagnostic modality	Finding	No. of patients	Percentage
Erect x-ray of abdomen	Conclusive	19	45.2%
	inconclusive	23	54.8%
Ultrasound of abdomen	Diagnostic	34	87.0%
	Not diagnostic	4	13.0%
Barium study	Diagnostic	5	71.5%
	Not diagnostic	2	28.5%

Two patients (4.25%) achieved reduction during diagnostic enema and forty-five of patients underwent surgical treatment (95.75%). In thirty-two patients, the intussusception reduced manually (71.2%) while surgical resection with end to end anastomosis performed in thirteen patients (28.8%) because of ischemic bowel or failure of manual reduction and presence of pathological lead points and all of them were presented after 24 hours as shown in figure (4).

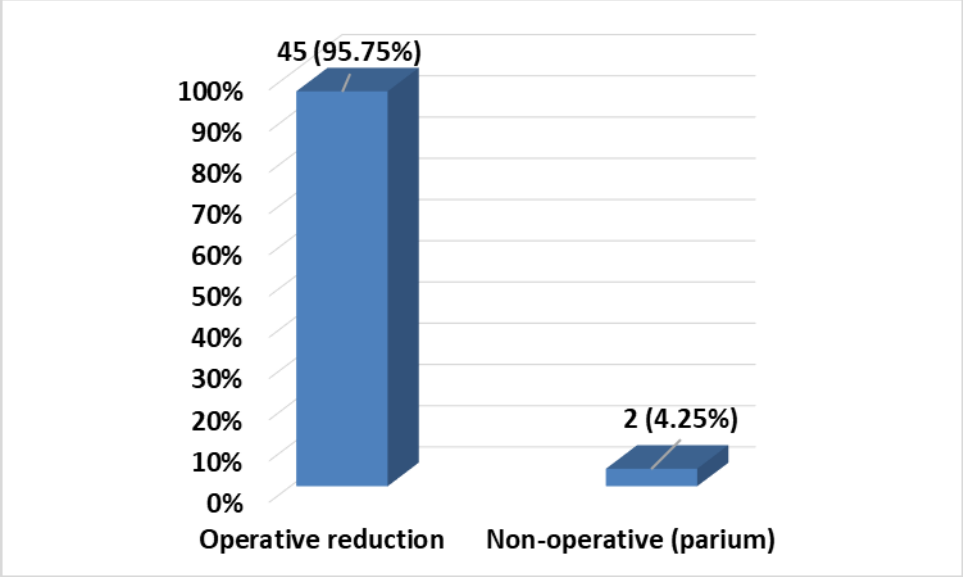

Figure 4. The mode of management

The most common type of intussusception was ileocolic type in thirty-five patients (77.8%) followed by ileoileocolic in seven patients (15.5%) then ileoilial and jejunojejunal types with (4.5%) and (2.2%) respectively as shown in figure (5).

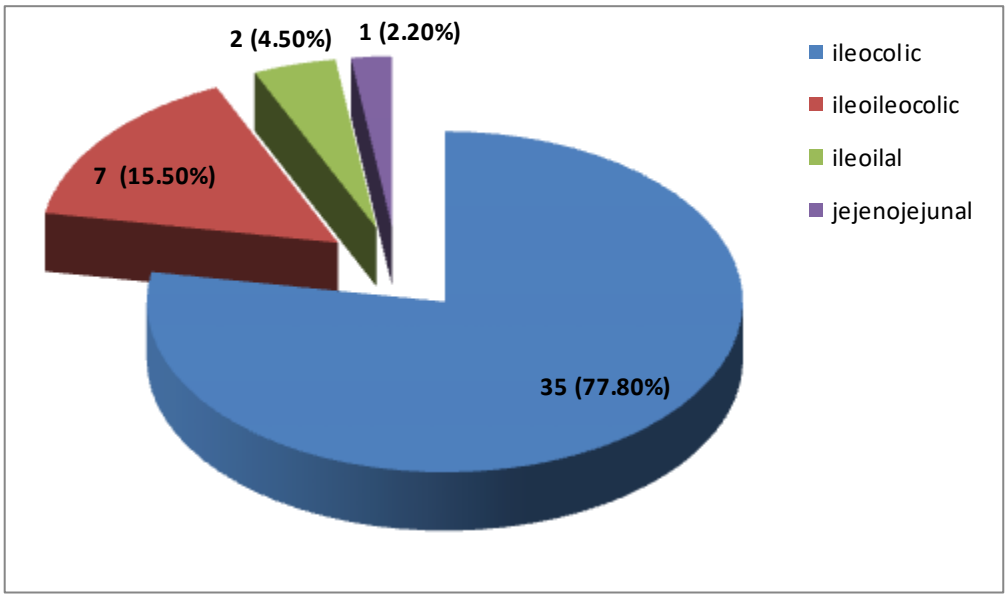

Figure 5. Types of intussusception.

Of the forty-five patients who underwent operative reduction, idiopathic (primary) intussusception were reported in thirty-six patients (80%), and pathological lead points (secondary) were identified in nine patients (20%) as shown in figure (6).

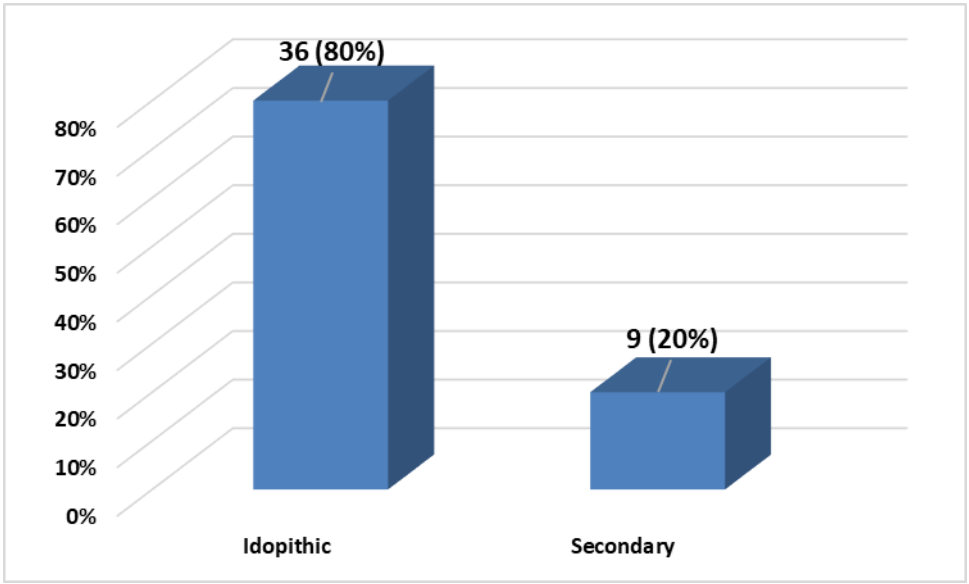

Figure 6. The underlying pathology for intussusception in patients underwent operative reduction

Regarding the pathological lead points, meckle's diverticulum were the commonest lead point, identified in four patients (44.4%). Three patients have lymphomas (33.3%), one patient had polyps (11.1%) and one patient has hemangioma (11.1%), as shown in table (4).

Recurrent intussusception was reported in one patient (2.1%) after four days of primary surgery and only four patients (8.4%)

developed complications, one patient had superficial wound infection and two had pneumonia and one patient had skin excoriation, length of hospitalization ranges from 3-5 days for patient managed by manual reduction to 5-7 days for those managed by surgical resection, no surgical complications were recorded.

All patients improved and discharged and no intussusception associated mortality were reported in this study as shown in table 5.

Table 4. Distribution of the pathological lead points

	Underlying pathology	No. of patients	% of patients
Secondary intussusception (pathological lead points)	Meckel's diverticulum	4	44.4%
	lymphomas	3	33.3%
	polyps	1	11.1%
	hemangioma	1	11.1%

Table 5. The duration of symptoms, complications, length of hospitalization and outcome

Duration of symptoms	No. of patients	% of patients	Complication	No.	Length of hospitalization and outcome
≤ 24 hours	16	34.04%	Pneumonia	1	3 -5 days postoperatively
> 24 hours	31	65.96%	Wound infection	1	5-7days postoperatively
			Pneumonia	1	
			Skin excoriation	1	
Total	47	100%	4		All survived (No death)

Discussion

This descriptive retrospective study showed the majority of cases were under one year (68.4%) and the commonest affected age group was between 6-12 months (57.4%), similar result was approximately encountered by Hanoudi and Hameed [23] (63%) and Khalaf [24] (61%). The possible explanation that this is the time of weaning and introduction of new food protein (solid food), which may result in change in normal flora and in swollen Payer Patches in mesentery of the terminal ileum that act as a lead point for intussusception [1].

In this study, a predominance of male was found with a male-female ratio of (2.35:1), which comparable to finding of Mohammed [25] (2.3:1) and Kella et al. [26] (2.5:1), but higher than that were found by Al-Sawafet al. [27] (1.5:1).

Twenty-seven patients (57.4%) were from rural area and twenty patients (42.6%) from urban society. In contrast, Khalaf [24] and Al-Sawaf et al. [27] found majority of their patients were from urban area. Regarding residence, the higher percentage of those from rural area might be explained by limited accessibility to health care facilities and awareness of the disease in the rural society. Bines et al. [28] suggested possible environmental and cultural exposures including exposure to enteric pathogens and child nutritional practices might had a role.

In this study, sixteen patients (34.04%) were presented within the first 24 hours from the onset of symptoms. Furthermore, most of patients (65.96%) were presented after 24 hours. Similar findings were encountered by Khaleel and AL-Alwan [29] and Hashim [30], in

contrast, Crankson et al. [21] and Al-Sawaf et al. [27] were found the majority of their patients presented within the first 24 hours. This delay in presentation may be due to lack of awareness or misdiagnosis of the condition by first treating health professionals or delay in arrival to hospital due to socio-economic problems including security situation in our governorate (Al-Anbar). In this study, there was a high statistical difference between rural and urban areas (p. value <0.001) in regard to duration of presentation of intussusception, (Table 1), a result that might be explained by what mentioned earlier regarding our society.

Regarding seasonal incidence, two peaks identified, first one occurred in summer months and the second occurred in winter months. Similar finding encountered by Đorđević et al. [31]. This finding differs from that of Khaleel and AL-Alwan [29] who found peak seasonal occurrence in spring. In other studies [32-34], no distinct seasonality of intussusception was detected. The seasonal distribution in this study might be explained by increased occurrence of gastroenteritis and upper respiratory tract infections during these seasons.

In this study, preceding gastroenteritis and respiratory tract infection was reported in twenty patients (34.4%) and thirteen patients (23.4%) respectively, approximately similar results were reported by Alamdaran et al. [35].

The most common clinical presenting symptoms in this study was abdominal pain, which reported in 87.2% followed by vomiting and bleeding per rectum 63.8% for both, comparable results were found by Mohsen and Hashim [36] (89%, 62%, 63.3%), other studies [21,37] found that the vomiting, rectal bleeding were most common presenting features.

Lethargy were found in 23 patients (48.9%), this is lower than the result (66%) encountered by Hashim [30] and Dominques-Carral et al. [38], this can be explained by dehydration and electrolyte imbalance as a result of vomiting and bowel obstruction. The absorption of toxic metabolic product from a strangulated bowel and the releases of neuropeptide were suggested by Kaiser et al. [22].

The red currant jelly stool on per rectal digital examination recorded in 35 patients (74.5%), the majority of them 27 (3/4) were under 1 year when compared with older children this indicate that with the younger patient, it is more likely to find red currant jelly stool as our study showed significant relation with age, on the other hand, Tareen et al. [39] found 36% of his cases presented with red stool and this might be due to a lot of his cases presented early within 24 hours (63% of cases), while in this study, approximately 66% of cases presented after 24 hours.

In this study, a palpable abdominal mass had been detected in 24 patients (51%), William [18] reported palpable mass in 69% and 84% of cases respectively; this might be due to the majority of our cases presented after 24 hours with the presence of abdominal distention which make the abdominal muscles more rigid and in turn make the palpation of the mass difficult.

Prolapsing mass was reported in 1 patient only (2.1%) who presented after 48 hours of the onset. Keïta et al. [40] and Nasar [41] reported 4 cases (10.8%) and 10 cases (20.08%) with anal prolapse respectively, this might be explained by the longer duration of symptoms before presentation the more likely the prolapse occur.

In this study, the classic triad of abdominal pain, current-jelly stool and palpable mass were documented in 17 patients (36.1%), which is comparable to results encountered by Huppertz et al. [17].

Generally, none of the clinical features mentioned above was of statistical difference in relation to age of the child (whether ≤ 12 months or > 12 months) except for red currant jelly stool, which was statistically significant in children ≤ 12 months (p. value 0.024) (Table 3).

Ultrasonography were done for 39 patients of total and showed an abdominal mass suggesting an intussusception as a target sign or pseudo kidney mass in 34 patients (87.1%),

same results gained by Mohsen and Hashim [36]. However, Stanley et al. [42] and Naseem et al. [43] found that the ultrasound was highly sensitive in 95-100% of cases.

Regarding missed cases to be diagnosed by abdominal x-ray or ultrasound, it might be due to technical or personal causes as both depend mainly on the person interpreting them in addition to the technique and the type and model of device used. Furthermore, Barium enema done for 7 patients only (14.8%) and it was diagnostic in six patients showed a coil spring sign, this finding was comparable to results of Khalaf [24].

Regarding management, 2 patients (4.2%) achieved reduction during diagnostic enema and the 45 patients underwent laparotomy, out of them 32 patients (71.2%), the intussusception reduced manually while surgical resection with end to end anastomosis performed in 13 patients (28.8%), because of ischemic bowel in 4 cases (8.8%) or due to presence of pathological lead point in 9 cases (20%). These findings were comparable to what reported by Khaleel and AL-Alwan [29] and Abdur-Rahman et al. [44]. Other studies like Al-Sawaf et al. [27] reported 25% of his patients treated by air enema. Moreover, Takeuchi et al. [45] reported the 93% of patients treated by an enema and only few cases managed by surgery. In this center, operative reduction was still the main mode of management because lack of facilities and trained radiologist who is familiar with the non-operative reduction by using hydrostatic or pneumatic reduction especially under ultrasonic or fluoroscopic guidance, additionally, delayed presentation might be a reason.

The most common type of intussusception was ileo-colic type in thirty-five patients (77.8%), Similar results approximately encountered by Crankson et al. [21] and Hanoudi [23].

Intussusception was idiopathic in 36 patients (80%), the pathological lead points were identified in 9 patients (20%) of total Meckel's diverticulum was the commonest pathological lead point, identified in 4 patients (44.4%) and lymphomas in 3 patients (33.3%) a result Which is comparable to what was found by Zain [10].

Recurrent intussusception was reported in one patient (2.1%) after four days of primary surgery, which closer to what shown by Hanoudi [23]. Best explanation for this finding was the surgical management done for vast majority of the cases.

Mortality rate was zero in this study similar to what reported by Al-Maliki [46] and Jehangir et al. [47], in contrast, Al-Sawaf [27] and Talabi et al. [48] reported 3.1%, 15.4% mortality in their study respectively.

In conclusion, colicky abdominal pain was the most presenting symptoms and red currant stool jelly was a significant sign especially in children ≤ 12 months while palpable abdominal mass occurred with a higher percentage in children > 12 months than those ≤ 12 months, majority of patients who presented after 24 hours were from rural area, a significant percentage of patients were presented with coexisting medical illnesses, seasonal peaks occurred in summer and winter months, pathological lead points were identified in about fifth of the patients and in this group the management was more radical (resection), delayed presentation(> 24 hours) was the main factor of morbidity and no mortality were reported.

The authors recommend that the varying age of children with intussusception and its variable presenting features should be kept in mind of the first treating health professionals to insure early diagnosis and referral to a specialized center for timely definitive treatment to decrease morbidity and mortality, despite the advance in the management of intussusception by non-operative techniques, surgery remained the main stay of treatment in authors' center because of limited facilities for the non-operative reduction in this center, the authors recommend that the facilities for non-operative reduction like ultrasonic guided barium or hydrostatic reduction and pneumatic reduction should be available and used especially for those presenting early to reduce

the risk of surgery and its impact on the patient and the hospital.

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Author contributions:

Dr. Maklef collected the patient data, Dr. Al-Ani and Dr. Ghani performed the statistical analysis and all together shared in writing the article.

Conflict of interest

None.

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The Effectiveness of Diode Laser 810 nm in the Removal of Oral Pyogenic Granuloma in Repetitive Pulsed Mode

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Abstract

Background A variety of benign soft tissue swellings can be found arising from oral mucosa, most of which are inflammatory hyperplasia and granuloma. Surgical diode lasers have been used in oral surgical procedures with beneficial and undoubted operative advantages and a better quality of the outpatient operation compared with electro- and coldblade techniques.

Objective To assess the effectiveness of diode laser 810 nm in the removal of pyogenic granuloma of oral cavity in repetitive pulsed mode.

Methods This study was conducted at the consultation clinic of the Maxillofacial Surgery Department in the Al-Imamein Al-Kadhiemein Medical City on 35 patients who suffered from oral pyogenic granuloma. The period of the study was from January 2013 to January 2015. The laser used in this study was diode laser 810 nm in repetitive pulsed mode. The maximum power was 5-8 watts. The pulse duration was 0.2-0.4 second and 0.2-0.4 second pulse interval. Excisional biopsies were sent for Histopathological study. Clinical examination was done and had included presence of swelling, infection, disturbance of function, pain and bleeding.

Results Patients with pyogenic granuloma were treated efficiently with repetitive mode of diode laser 810 nm. Minimal to moderate swelling, no disturbance of function, no infection and mild pain were observed postoperatively, no bleeding was seen neither intraoperatively nor postoperatively.

Conclusion Repetitive pulsed mode of diode laser 810 nm is efficient and safe in removal of pyogenic granuloma of oral cavity.

Keywords Diode laser, oral pyogenic granuloma

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List of abbreviation: cw = Continuous wave, GaAlAs = Gallium Aluminum Arsenide

Introduction

A variety of benign soft tissue swellings can be found arising from oral mucosa, most of which are inflammatory hyperplasia and granuloma. These lesions can be divided into those which arise from the mucosa covering the alveolar processes and those which arise elsewhere in oral cavity. The soft tissue masses which are excised should be sent for histological examination [(1)]. Lasers are rapidly becoming the standard of care for many procedures performed by oral and maxillofacial surgeons. The reason for this transition is due to the fact that many procedures can be executed more efficiently and with less morbidity using lasers as compared to a scalpel, electrocautery or high frequency devices [(2)]. The laser-tissue effects and interactions depend on the interplay of irradiation parameters such as wavelength of

laser source, physical properties of the irradiated tissue, laser pulse energy, continuous wave (cw) or pulsed irradiation, laser beam size on the tissue, laser pulse duration and repetition rate [3]. Laser excision of benign soft tissue lesions, such as fibroma, papilloma, mucocele, gingival lesions, benign salivary glands lesions, salivary stones, epulis fissurata, tongue lesions and hyperplastic tissue excisions is minimally invasive and can make the surgery less extensive, and may reduce the need for general anesthesia or in-patient hospital care, resulting in the lowered overall costs [4,5].

There are many advantages to the use of lasers in maxillofacial surgery. These advantages include: hemostasis and excellent field visibility, reduced postoperative pain and edema, precision, enhanced infection control, lack of mechanical tissue trauma, reduced scarring and tissue shrinkage, less instruments at the site of operation and prevention of tumor seeding [6].

The aim of this study is to assess the effectiveness of repetitive pulse mode of diode laser 810 nm in removal of pyogenic granuloma of oral cavity.

Methods

A total of 35 patients who had oral pyogenic granuloma were treated at the Department of the Maxillofacial Surgery, Al-Imamein Al-Kadhiemein Medical City during the period from January 2013 to January 2015. Photographs had been taken for all lesions pre- and postoperative period of treatment. Patients with any systemic diseases were excluded from the study and all operations were performed by the same surgical team throughout the procedures. The patients, the surgeon, and the operative staff wore safety glasses. The diagnosis of all lesions was confirmed by histopathological examination. All treatments were performed under local anesthesia.

The type of laser, which was used in this study is diode laser (Diomed 15 laser). It is an integrated 810 nm wavelength, Gallium Aluminum Arsenide (GaAlAs) semiconductor laser. Its maximum output power is 15 W, 0.1-1.0 second is the pulse duration, 0.1-1.0 second is the pulse interval and it works in continuous, single, and repeated pulsed modes.

The laser surgical operations had been done at repetitive pulsed mode for 5-8 W maximum power, 0.2-0.4 seconds pulse duration and 0.2-0.4 second pulse interval. All patients were told about laser surgical procedures, its complications before the surgery and obtained patient's agreement (Medical Ethics).

In all the patients treated, the treated tissue and its surrounded areas were cooled by gauze pack soaked in normal saline after the laser surgical procedure. Some of the patients were given suitable postoperative care such as 0.12% chlorhexidine mouthwash, Amoxicillin capsules 500 mg as antibiotics and Ponstan tablets 500 mg as analgesics.

All patients were seen on a regular basis for follow‐up: At one and two weeks after treatment.

Each patient was evaluated clinically for swelling, infection, disturbance of function, pain and bleeding (Figures 1 and 2).

Results

This study has enrolled 35 patients with oral pyogenic granuloma. The results of this study were evaluated clinically depend on swelling, infection, disturbance of function, pain and bleeding.

In all cases, there was no incidence of infection in the days following the procedure. Postoperative swelling was minimal to moderate. No sutures were required. No bleeding was seen neither intraoperative nor postoperative period. Postoperative pain was mild in few patients. No disturbance of function was observed.

The patients were satisfied with the treatment protocol and the results obtained. They were comfortable either intraoperatively or postoperatively (Figures 3, 4, 5 and 6).

Figure 1. Pyogenic granuloma of left side of palatal mucosa

Figure 2. Pyogenic granuloma of right side of maxillary alveolar mucosa

Figure 3. Complete excision of granuloma by 5 W pulsed mode diode laser

Figure 4. One week later

Figure 5. Complete excision of granuloma by 5W pulsed mode diode laser

Figure 6. Excisional biopsy of lesion sent for histopathological examination

Discussion

Laser excision is most desirable for any solid, exophytic-type lesion because of the improved visibility and precise control of tissue removal [(6)].

Currently, laser soft tissue applications have constituted the primary area for the clinical use of lasers in dentistry [(7)]. For therapeutic purposes, the laser-tissue interaction mechanisms are mainly determined by two parameters, namely the laser exposure time on the tissue and the effective power density taking into account the tissue-specific absorption [(5)].

Many reports have proved the benefits of laser soft tissue surgery include: minimal swelling, dry surgical field, sterilization of the surgical site, reduced postoperative pain (laser energy can inhibit pain receptors at the site of operation), a relatively bloodless surgical and postsurgical course and no-touch technique.

In this clinical work, mild postoperative pain was detected in few cases; the postoperative swelling was greatly reduced (laser energy can cause sealing of lymphatic vessels). These results are in agreement with results of reports which proved the excellent postoperative conditions, minimal pain and swelling [(8,9)].

Diode laser have high penetration depth in the tissue and it has been highly absorbed by hemoglobin and cause good hemostasis.

Clinically, the intraoperative bleeding was minimal or not observed and no postoperative bleeding has occurred. Postoperative infection has not been encountered. These results are in agreement with reports which proved the homeostasis was optimum immediately after the removal of the benign exophytic lesions with diode laser [10] and after removal of hemangioma [11].

The advantages of laser application are relatively bloodless surgical and post-surgical courses with minimal swelling and scarring. Diode laser has been used for excisional biopsy of pyogenic granuloma and gingival pigmentation [12].

The safety and efficacy of laser systems, especially the diode laser, are already evaluated for the treatment of oral surgery, for example upper and lower frenulectomy, fibroma and excision of epulis fissuratum and gingival hyperplasia.

High degree of patient acceptance and satisfaction, without compromising health and function, has been found in this clinical work. This result is in agreement with reports, which have concluded that the use of the 810-nm diode laser may indeed be the best choice in oral soft tissue surgery [13,14].

From this clinical study, it is concluded that surgical diode laser in repetitive pulse mode is an efficient, precise and modern method in removal of pyogenic granuloma of oral cavity. This treatment modality provides satisfactory results and well patient's acceptance without compromising their health and functionality.

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Conflict of interest

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The Neonatal Outcome in Infant Born to Gestational and Pre-Gestational Diabetic Mothers

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Abstract

Background Diabetes is the 2nd commonest medical complication of pregnancy after hypertension. Infants of diabetic mothers are those infants born to a mother who suffers from diabetes mellitus, but the term refers especially to infants born to a mother who has persistently elevated blood sugar during pregnancy.

Objective To study the complications in infants of diabetic mothers and the difference in the complications between infants of gestational and pre-gestational diabetic mothers.

Methods A cross sectional observational study carried out on (100) neonates of diabetic mothers that delivered in Al-Imamein Al-Kadhimein Medical City; the data was collected between 1st of September 2015 to 1st of December 2015. Maternal history was taken about type of diabetes and babies were divided into 2 groups; 1st group was infants of pregestational diabetic mothers and 2nd group was infants of gestational diabetic mothers. Thorough physical examination of these infants at birth at the neonatal care unit was done. Laboratory investigations included blood sugar, serum calcium, hematocrit, total serum bilirubin and echocardiograph was done in all babies but chest x-ray, abdominal ultrasound, brain ultrasound and electrocardiogram were done whenever indicated.

Results The 1st group (36 cases) 36% of infants was product of mothers having pregestational diabetes and the 2nd group (64 cases) 64% infants of gestational diabetic mothers. Mothers were delivered by cesarean section in 69%. Mothers were primigravida in 31%. Female 59% to male ratio 1.4:1. Hypoglycemia at birth was documented in 39% of cases, was nearly equal in both groups (20%, 19%) respectively. Hypocalcemia in 18%, was more in infants of pregestational diabetic mothers (11% and 7%) respectively. Respiratory complications were the most prominent complications 41 % nearly equal in both groups (22% and 19%) respectively. Macrosomia seen in 23%, more in infants of gestational diabetic mothers (7% and 16%) respectively. Forty % of infants of both group have been discharged in the first 24 hours.

Conclusion The respiratory complications were the most prominent followed by hypoglycemia that occurred mainly to infants of pregestational diabetic mothers. There are statistical differences in infants of pregestational diabetic mothers regarding hypoglycemia, hypocalcemia and respiratory complications. Macrosomia is more in infants of gestational diabetic mothers.

Keywords Neonate, infants, gestational, pregestational diabetes.

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List of abbreviation: CNS: Central nervous system, GDM = Gestational diabetic mothers, GIT = Gastro intestinal tract, IGD = Infants of gestational diabetes IGD, IPGD = Infants of pregestational diabetes, PGDM = Pregestational diabetic mothers

Introduction

Infants of diabetic mothers are those infants born to a mother who suffer from diabetes mellitus. But the term refers especially to

infants born to a mother who has persistently elevated blood sugar during pregnancy [1].

Diabetes is the second commonest medical complication of pregnancy after hypertension [2]. Fetal and neonatal mortality rates were as high as 65% before the development of specialized maternal, fetal, and neonatal care. Since the discovery of insulin, infants of diabetic mothers have experienced an almost 30-fold decrease in mortality and morbidity [3].

There are two types of diabetes that occur in pregnancy: Gestational diabetes refers to a mother who does not have diabetes before becoming pregnant but develops a resistance to insulin because of the hormones of pregnancy. Pregestational diabetes describes women who already have diabetes before they become pregnant [4].

This study aimed to study the complications in infants of diabetic mothers and the difference in the complications between infants of gestational and pre-gestational diabetic mothers.

Methods

A cross sectional observational study was carried out on (100) neonates of diabetic mothers that delivered in Al-Imamein Al-Kadhimein Medical City; the data was collected between 1st of September 2015 to 1st December 2015.

Maternal history was taken about types of diabetes either gestational or pregestational as well as type of treatment used to control blood sugar, parity and mode of delivery. According to maternal history of diabetes, the neonates were divided into two groups; 1st group was infants of pregestational diabetic mothers and the 2nd group was infants of gestational diabetic mothers.

Thorough physical examination of these babies was done including: assessment of gestational age by Ballard Maturational Assessment then classified into term (gestational age 37-40 weeks), preterm (gestational age less than 37 weeks) [5], gender, weight, length, head circumference, systemic examination looking for congenital anomalies and assessment of respiratory distress. Babies with weight of $\geq$ 4000 gm were labeled as macrocosmic and with birth weight < 2500 gm as low birth weight (LBW) [6]. The head circumference from 33 to 36 cm were considered normal range [7].

The laboratory investigations included serial blood sugars initially at birth and half hourly in hypoglycemic neonates; done by a heel prick after sterilization with alcohol swab and measuring random blood sugar by glucometer (Accu-Check Go (ROCHE) [8]. Hypoglycemia was labeled when the blood sugar was less than 25 mg/dl in the 1st 24 hours [9]. Serum calcium was done within the 1st 24 hours of birth, which was done by non-tourniquet venous sample. Hypocalcemia was considered when total ionized serum calcium was less than 8 mg/dl (2 mmol/dl) [10]. The hematocrit was done by using heparinized micro capillary tube (0.75 ml) centrifuged at speed of (10,000-15,000) round per minute, plasma separate packed cell volume was measured to give the hematocrit level, polycythemia considered when hematocrit more than 65% [11]. Total serum bilirubin was done by spectrophotometer; physiological jaundice was considered when the total serum bilirubin was 1-3 mg/dl and the rate of rise was less than 5 mg/dl in 24 hr [12]. Echocardiography was done to all neonates. Chest X-ray, abdominal ultrasound, brain ultrasound and CT scan of brain as needed.

Simple statistical analysis was done including percentage estimations and tabulation using Microsoft excel program. Chi-square (X2) test was done using SPSS version 21. P value was calculated, statistically significant if its <0.05 and highly significant if < 0.001 [13].

Results

Of one hundred neonates, there were 64% with gestational diabetes mellitus (GDMs) and 36% with pre-gestational diabetes mellitus (PGDMs). The mean of maternal age for both groups was (28±5) years. Types of treatment for PGDMs and GDMs were on diet, insulin and without treatment. The results are highly significant as P. value < 0.001 as shown in table 1.

Table 1. Distribution of mothers according to type of treatment (pregestational or gestational)

Type of treatment	PGDM No. = 36 No. (%)	GDM No. = 64 No. (%)	Total (%)	P .value
Diet	19 (52.78)	22 (34.38)	41	
Insulin	15 (41.67)	15 (23.43)	30	0.0005*
No treatment	2 (5.55)	27 (42.19)	29	

*Statistically highly significant

There were 31% primigravida {10 (27.78%) with PGDM and 21 (32.81%) with GDM}, while 69% were multigravida {26 (72.22%) with PGDM and 43 (67.19%) with GDM} as shown in table 2.

The mode of delivery was by Cesarean section (C/S) in 69% and 31% by normal vaginal delivery (NVD). Nine cases (25.0%) of pregestational diabetic mothers (PGDMs) delivered by NVD, 22 (34.37%) of gestational diabetic mothers (GDMs) delivered by NVD, 27 (75.0%) of PGDMs delivered by C/S and 42 (65.63%) of GDMs delivered by C/S, none with assisted delivery as shown in table 2.

Table 2. Distribution of mothers according to parity and mode of delivery (pregestational or gestational)

Parameter		PGDM No.=36 No. (%)	GDM No.=64 No. (%)	Total %	P. value
Parity	Primigravida	10 (27.78)	21 (32.81)	31	0.6577
	Multigravida	26 (72.22)	43 (67.19)	69	
Mode of delivery	NVD	9 (25.0)	22 (34.37)	31	0.3747
	C/S	27 (75.0)	42 (65.63)	69	
	Assisted delivery	0	0	0	

There were 59% females and 41% males with female to male ratio 1:4:1 as shown in table 3. Preterm babies were 51% and 49% term babies as shown in table 3. The mean weight was (3.2±0.75) Kg with a range of (1.2-4.8) Kg, low birth weight found in 24% and macrosomia found in 23% as shown in table 3.

Table 3. Infants variables

Infants variables		IPGD No. = 36 No. (%)	IGD No. = 64 No. (%)	Total %	P. value
Gender	Female	21 (58.33)	38 (59.38)	59	1.000
	Male	15 (41.67)	26 (40.62)	41	
Gestational age	Preterm	23 (63.89)	28 (43.75)	51	0.0629
	Term	13 (36.11)	36 (56.25)	49	
Birth weight (kg)	< 2.5	7 (19.44)	17 (26.56)	24	0.4738
	2.5-3.9	22 (61.11)	31(48.44)	53	
	≥ 4.0	7 (19.44)	16 (25.0)	23	

IPGD = Infants of pre-gestational diabetes, IGD = Infants of gestational diabetes

Hypoglycemia was found in 39%, as it was observed in (14 IPGDMs and 13 IGDMs); 70% as asymptomatic neonates and 30% presented with (jitteriness, seizure, tachypnea and irritability). The hypoglycemia improves from the 1st day to the 2nd day for all babies. Hypocalcaemia presented as asymptomatic in 50% and the other with symptoms just like hypoglycemia. Polycythemia presented in 7%; only 3 patients need partial blood exchange transfusion whom IPGDMs. The results for hypoglycemia and hypocalcaemia are statistically significant as P. value < 0.05. As shown in table 4.

Table 4. Distribution of metabolic abnormalities in infants (pregestational or gestational)

Metabolic abnormalities		IPGD No. = 36 No. (%)	IGD No. = 64 No. (%)	Total %	P. value
Hypoglycemia	Positive	20 (55.56)	19 (29.69)	39	0.0182**
	Negative	16 (44.44)	45 (70.31)	61	
Hypocalcemia	Positive	11 (30.56)	7 (10.94)	18	0.0277**
	Negative	25 (69.44)	57 (89.06)	82	
Polycythemia	Positive	4 (11.11)	3 (4.69)	7	0.2479
	Negative	32 (88.89)	61 (95.31)	93	

** Statistically significant

The respiratory complications occurred in 41% of infants diagnosed by clinical feature and radiological findings in form of hyaline membrane disease (RDS) and transient tachypnea of the newborn (TTN). In relation to cardiac complications, 6% of infants diagnosed as congenital heart disease which was documented by echocardiograph as 1% atrial septal defect (ASD), 3% ventricular septal defect (VSD), 2% patent ductus arteriosus (PDA). One newborn (IPGDM) had diaphragmatic hernia. All babies had occipitofrontal circumference (OFC) within normal ranges except for two infants with OFC more than 40 cm diagnosed to have hydrocephalus by brain ultrasound. The differences in respiratory complications between the 2 groups were highly significant with a P-value < 0.001 as shown in table 5.

Concerning discharge from the hospital, 10 cases (27.77%), 15 cases (41.67%), 11 cases (30.56%) discharged in the 1st 24 hours, 2nd-5th day, more than 1 week respectively for IPGDMs while 30 cases (46.88%) of infants of gestational diabetes were discharged in the first 24 hr as shown in table 6.

Discussion

The study included 100 mothers, 36% of whom had PGDM and 64% GDM, which is close to the study in Iran by Tabib et al. in 2013, which found that 40% PGDM and 60% GDM [14].

Type of treatment of diabetes mellitus was diet control in 41% while insulin used in 30%, 29% without treatment and no patients on oral hypoglycemic agent; this differ from a study done in Switzerland by Ullmo et al. in 2007, which showed that 81% of mothers on insulin treatment while 19% were on diet control [15]; also differs from a study done in Islamabad by Alam et al. in 2006 who found that 47.5% on insulin 12.5% on diet and 40% without any treatment [16]. High percentage of mothers in the current study were on no treatment this reflects poor compliance of therapy in our patients.

Table 5. Distribution of systemic complications of infants (pregestational or gestational)

Systemic complications		IPGD No. = 36 No. (%)	IGD No. = 64 No. (%)	Total %	P .value
Respiratory complications	RDS	22 (61.11)	13 (20.31)	35	0.0001*
	TTN	0 (0)	6 (9.38)	6	
	None	14 (38.89)	45 (70.31)	59	
Cardiac complications	ASD	1 (2.78)	0 (0)	1	0.0537
	PDA	1 (2.78)	1 (1.56)	2	
	VSD	3 (8.33)	0 (0)	3	
	None	31 (86.11)	63 (98.44)	94	
GIT complications	Diaphragmatic hernia	1 (2.78)	0 (0)	1	0.3600
	None	35 (97.22)	64 (100)	99	
CNS complications	Hydrocephalus	2 (5.56)	0(0)	2	0.0640
	None	34 (94.44)	64(100)	98	
Birth injury	None	0 (0)	0 (0)	0	0
Musculoskeletal anomalies	Sacral agenesis	0 (0)	0 (0)	0	0

* Statistically significant

Table 6. Distribution of day of discharge in infants (pregestational or gestational)

Day of discharge	IPGD No. = 36 No. (%)	IGD No. = 64 No. (%)	P. value
1st	10 (27.77)	30 (46.88)	0.1242
2nd–5th	15 (41.67)	23 (35.94)	
More than 1 week	11 (30.56)	11 (17.18)	

Female to male ratio was 1.4:1, which differs from a study done in Pakistan by Shirazi et al. in 2010 in which, female to male 1:1.3 (17). This is due to small sample and short period of time in the current study.

The mode of delivery was by C/S in 69, which is close to a study from Switzerland done by Ullmo et al. in 2007, which found that 61% of mothers were delivered by C/S (15). This high rate of C/S deliveries in this study is due to bad obstetrical history, failure of induction, macrosomia and miscalculation. Both groups with the same risk for having C/S delivery.

There were 51% preterm babies and 49% term infants. This is higher than a study done by Nwankwo et al. in Nigeria in 2013, which showed that 41% preterm labor [18], also higher than a study from Pakistan done by Shirazi et al. in 2010, preterm in 19% while full term 81% [17]. This high rate of preterm babies is due to the fact that diabetes in pregnancy increases the risk of preterm labor because of polyhydramnios, which cause sudden rapture of membrane [19]. High percentage of preterm in our study reflects poor glycemic control in the mothers.

The mean of birth weight was (3.2±0.75) Kg, which is less than the study in Nigeria by Opara et al. in 2010, which found that the mean weight was (4.14 ±0.838) Kg [20]. This difference is due to the fact that we have more preterm

labor, small mass of the study group and poor compliance of the mothers.

Macrosomia seen in 23%, which is less than the study in Saudi Arabia by Yaseen et al. in 1999 in which, 30% were macrosomic [21], but higher than the result in a study done in Pakistan by Shirazi et al. in 2010 as macrosomia was 15% [17]. Also, higher than a study done by Nwankwo et al. in Nigeria in 2013, (5.1%) [18]. Macrosomia is more in IGDMs, which is the same as a study done in Ohio by Cordero et al in 2015 [22].

Multigravida mother were 69%, this is less than the study done by Nwankwo et al. in Nigeria in 2013 in which, multigravida account for 74% [18]. This is due to increased risk of gestational diabetes with increase maternal age.

Hypoglycemia 39%, which is close to a study done in Islamabad by Alam et al. in 2006 (35%) [16] and close to a study done in Ohio by Cordero et al. in 2015 (36%) was more in IPGDMs [22].

Hypocalcemia 18%, which is close to a study done in Islamabad by Alam et al. in 2006 15% [16], more common in IPGDMs in a study done by Shirazi et al. in 2010 [17].

Polycythemia 7%; is close to the study of Shirazi et al. in 2010 (8%) [17].

Cardiac anomalies 6%, this result is close to a study in Iran by Tabib et al. in 2013 as (8.8%) of infants with congenital heart disease being more in PGD [14]. Also, it is close to the result of a study by Ullmo et al in 2007 (5%) [15].

Respiratory complications 41% and more common in IPGDMs, the results are more adherent to by Ullmo et al. study in 2007 (36%) [15]. Also, is close to a study in Nigeria by Opara et al. in 2010 in which, respiratory complications were noted in 34% [20].

For congenital anomalies; anomalies related to gastrointestinal system, only one case 1%, which is similar to Shirazi et al. study in Pakistan in 2010, which is also 1% of duodenal atresia and 1% of anorectal atresia [17].

Central nervous system complications 2%, which was near the result in Shirazi et al. study done in Pakistan in 2010, which was 1% [17].

The mean of infants stay in hospital was (4.61 ±3.35) days, which is less than in Opara et al. study in Nigeria in 2010 in which, the mean of duration of admission was (6.97±2.63 days) [20]. The time that needed to stay in hospital is less due to the fact that we have more deliveries at the same period, as we have 468 mothers were delivered at the same time and more preterm babies who need more time to recover from complications as hypoglycemia and polycythemia. Infants of diabetic mothers are at risk of deaths.

This study concluded that the respiratory complications are the most prominent that we faced in the neonatal care unit and followed by hypoglycemia that occur mainly in infants of pregestational diabetic mothers. There are statistical differences in infants of pregestational diabetic mothers regarding hypoglycemia, hypocalcemia and respiratory complications. Macrosomia is more in infants of gestational diabetic mothers.

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Effect of Helium-Neon Laser on the Lymphocyte Cells and their DNA

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Abstract

Background Laser light is widely used for a wide range of medical applications. He-Ne laser application in medicine as in any type of laser is based on the interaction of laser light with the biological system.

Objective To show the effect of helium-neon (He-Ne) laser (632.8 nm) irradiation on human lymphocyte blood cells and their DNA.

Methods This study involved 72 blood samples, taken from apparently healthy volunteers. The samples were divided into two groups; the 1st group consisted of 27 samples were processed only for lymphocyte blood cells separation, while the 2nd group, which consisted of 45 samples were employed to evaluate the influence of He-Ne laser irradiation on the extracted DNA from the lymphocyte blood cells.

Results At the used doses of He-Ne laser (18, 35, 52.5, and 69 J/m²), a significant difference was found ($P < 0.05$) in survival percentage of lymphocyte cells (99.8, 99.74, 99.68, and 99.59) in comparison with those cells untreated with He-Ne laser irradiation. Immediately after He-Ne laser irradiation alone, the following doses (18, 35, and 69 J/m²) were applied on the extracted DNA, the DNA demonstrated a significant damaging where the fraction of DNA survival percentage was (88.6, 87.7, 86.1) respectively, with significant difference ($P < 0.05$) between the DNA survival before and after He-Ne laser irradiation.

Conclusion The percentage of lymphocytes survival is decreasing with increasing dose of He-Ne laser and longer exposure time where time exposure (2.5, 5, 7.5, and 10 s). He-Ne laser irradiation causes a significant degree of DNA damaging independent on the irradiation doses.

Keywords Lymphocyte cells, He-Ne laser irradiation, DNA.

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List of abbreviation: Helium-neon laser = He-Ne laser, phr = Photoreactivation gene, RecA = Recombination gene, UV = Ultraviolet radiation

Introduction

Laser light is extensively used for a wide range of applications in medicine. However, the effect of laser light irradiation is debatable and the mechanisms of its exact action are still unclear.

Laser in experimental medicine requires detailed information on the mechanism of their biological effects [1]. Since laser light has the unique properties of polarized coherent, monochromaticity (single wavelength), and directionality, which may enhance laser absorption by different tissues [2]. Tissue bio-stimulation is only possible if irradiated cells possess molecular photoacceptors or photosensitive capacity that absorb the light and enter into state of excitation, that trigger intracellular cascade of signals leading to measurable biological effect [3]. The bio-

stimulatory effect of laser irradiation is determined by the magnitude of the absorbed light energy, which depends on many factors; wavelength of laser source, power, exposure time, and characteristics of absorption and scattering of tissue [4].

Helium-neon (He-Ne) laser application in medicine as in any type of laser is based on the interaction of laser light with biological system [5]. He-Ne laser (632.8 nm wavelength) has low photon energy and output power that produces minimum biomolecular damage [6]. Because it produces a temperature elevation of less than 0.5 °C in the irradiated cells [7,8], the irradiation of He-Ne laser causes photochemical interaction with the cells rather than thermal effect.

He-Ne laser (632.8 nm) irradiation of the lymphocytes may cause such photochemical interaction that may be useful in medicine [9]. Laser irradiation, in this red spectral area, influences the proliferative activity of peripheral blood lymphocytes [10], promotes tissue repair [11], and has a protective effect on lymphocyte cells by stimulation of cytokines production [12]. Moreover, He-Ne laser irradiation (632.8 nm) of cells has been reported to result in a variety of effects on cell structure and function [13], such as remodeling of the cytoskeletal network [14]. Cellular proliferation could be triggered by the interaction of a He-Ne laser with the mitochondrial photoacceptor-cytochrome oxidase, which is the enzyme that catalyzes the final step in the mitochondrial respiratory chain for transfer of electron from cytochrome c to molecular oxygen [15]. Another effect is an increased content of ATP (adenosine triphosphate), growth of the electric potential across inner membranes [16], and formation of giant mitochondria [17]. Low energy lasers (low-level laser) light in the red and far red regions of electromagnetic radiation spectrum are considered to have positive effects in wound healing [18,19]. Since ionizing energy such as X and γ- rays, UV light and α- particles cause cell and tissue damaging, therefore a lot of works have been carried on showing that the low-level laser irradiations modify the response of cells to ionization [18,20].

The present study was done to show the effect of He-Ne laser (632.8 nm) irradiation on human lymphocyte blood cells and their DNA.

Methods

This study was carried out at the Department of Physiology and Medical Physics, College of Medicine, Al-Nahrain University, during the period from November 2010 to June 2011.

In order to assess the effect of He-Ne laser on DNA of human blood lymphocyte cells, 72 blood samples were taken from healthy volunteers (47 females and 25 males), with age ranged from 19-45 year, with mean age of 32.4±7.68. The 72 blood samples were divided into two groups. The 1st group, consisted of 27 samples, was used to estimate the effect of He-Ne laser on lymphocyte cells; therefore, these samples were processed only for peripheral blood lymphocytes cell separation (PBL) using Boyum method [21]. The 2nd group, consisted of 45 blood samples, were processed for DNA extraction to evaluate the influence of He-Ne pre-irradiation on the extracted DNA from the lymphocyte cells. The degree of damage by He-Ne laser on lymphocyte cells number, and DNA concentration measured by the haemocytometer, and the spectrophotometer respectively.

A continuous He-Ne laser beam, of 1 mm diameter and 632.8 nm wavelength was employed (Griffin and George, Britain). The laser maximum output power was 1 mW. To ensure uniform illumination on the sample, the He-Ne laser beam diameter was expanded to a spot of 1.3 cm (using a converging lens) which corresponded to the sample tube diameter of 1.3 cm. Irradiation of laser was done employing different exposure times (2.5, 5, 7.5, and 10 s), which equal to energy doses of (18.8, 37.6, 56.4, and 75 J/m^2) respectively. Since at each lens surface about 4 % from the intensity reflected back, so about 8% will be lost at the

two lens surfaces, therefore, the final energy doses became (18, 35, 52.5, 69 J/m^2).

Twenty-seven blood samples, each one undergoes lymphocyte cells isolation. Each lymphocyte cells suspension sample was divided into approximately five equal parts, one of them (untreated) was used as a standard. The trypan blue exclusion test was employed to assess lymphocyte viable cells number for the untreated sample part by a haemocytometer. The counts expressed as number of viable cells/ mm3 and the other four parts of the sample, each one was exposed to He-Ne laser beam for only one of the following doses (18, 35, 52.5, and 69 J/m^2). After each irradiation, the fractional of cell survival % was evaluated relative to untreated (standard) cells viability.

Forty-five blood samples were used to study the effect of He-Ne laser irradiation on the DNA. The DNA was extracted from human blood lymphocyte cells using phenol-chloroform method [22]. A part from each extracted DNA sample was employed to evaluate the DNA purity. The optical density (OD) of DNA, which is measured by spectrophotometer at UV wavelength of 260 nm for the untreated sample part was used as a standard. The second part, firstly irradiated with He-Ne laser beam (632.8 nm), and then incubated for 45 min at room temperature. After each irradiation, the OD of DNA was measured. Three different He-Ne laser exposure time periods were used (2.5, 5, and 10 s). Therefore, the 45 samples in this group divided into three sub-groups. Each sub-group contains 15 samples, and each sub-group irradiated with He-Ne laser beam for one of the used exposure time 2.5, 5, or 10 s (doses 18, 35, 69 J/m^2).

Statistical analysis

The mean and the standard deviation for each group parts data were estimated employing Microsoft Excel program. A paired sample t-test was used comparing the data for pre-laser irradiation and then after UV-light irradiation. The difference was considered statistically significant, when the P value was less than 0.05 [23].

Results

Lymphocyte cells results

The average percentage of lymphocytes viability of untreated part samples (27 blood samples; standard) was 99.9±0.06.

Table (1) shows the effect of different laser times of exposure (2.5, 5.0, 7.5, and 10 s), which correspond to the following doses of energy (18, 35, 52.5, and 69 J/m^2) respectively, on lymphocytes viability percentage. There is a small difference in the cells viability after He-Ne laser irradiation in comparison to that before cells irradiation (untreated) ($P < 0.0001$).

It is clear from the results that the viability of lymphocyte cells is higher at the smallest time of exposure (2.5 s), as shown in figure 1.

Table 1. The mean of the lymphocyte blood cells viability % after different time exposure and He-Ne laser doses irradiation

Time exposure (s)	Dose (J/m^2)	Mean±SD	*P* value
0	0	*99.9±0.06	
2.5	18	99.8±0.14	< 0.0001
5.0	35	99.74±0.15	< 0.0001
7.5	52.5	99.68±0.17	< 0.0001
10	69	99.59±0.23	< 0.0001

*The comparison is done with lymphocyte viability before treatment

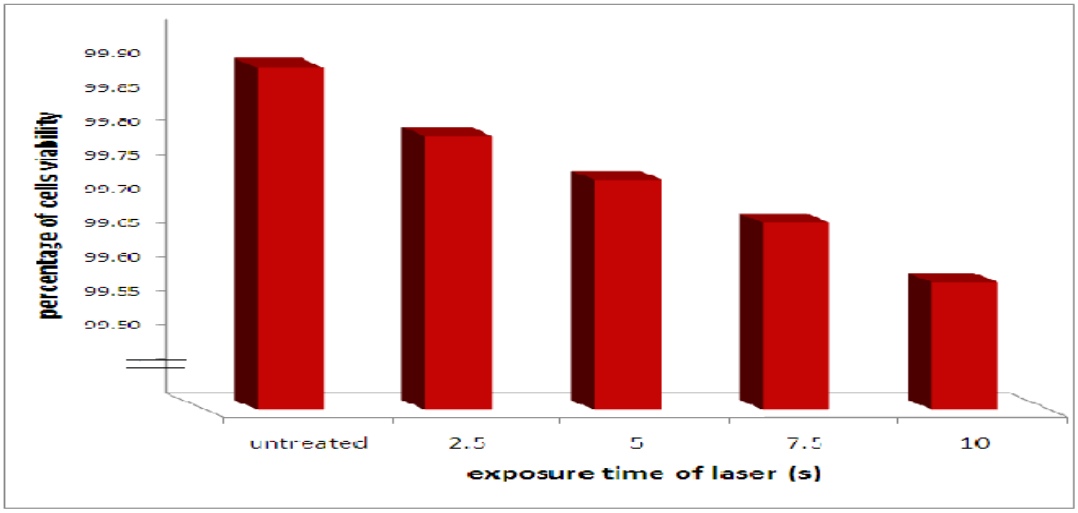

Figure 1. Histogram of the average percentage of lymphocyte cells viability for different He-Ne laser exposure times (doses)

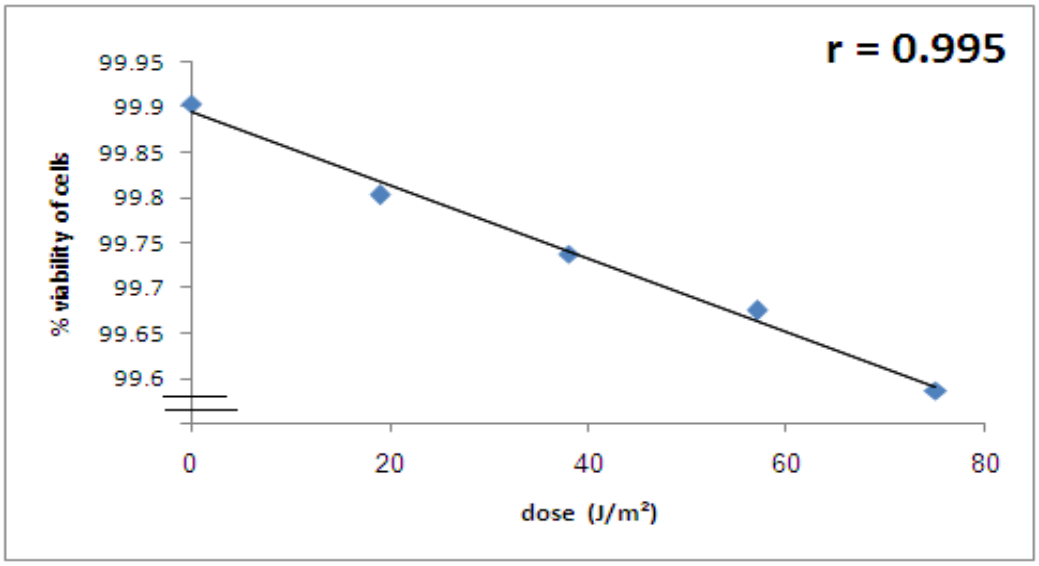

Figure 2. The relation between % viability of lymphocyte cells and the laser light energy doses

Table (2) represents the absorption optical density (OD) of DNA before and after He-Ne laser irradiation. These data showing that He-Ne (632.8 nm) irradiation alone employing the following three doses (18, 35, and 69 J/m2) causes a reduction in the DNA absorption (OD), which mean a reduction in DNA concentration survival compared with standard DNA (OD) results (untreated sample). The percentage of DNA survival after the three laser-irradiation doses are (88.7%, 87.4%, and 87.6%). These results demonstrate a significant DNA damage immediately after laser irradiation, and it is independent on the He-Ne laser doses.

Discussion

The photobiological reaction in the cells to light in general and to He-Ne laser specifically is mainly related to the magnitude of the absorbed dose. He-Ne laser light (632.8 nm) induces many effects as result of a photobiological response, including increased

temperature as well as an electronical excitation of the photoacceptor molecules [24].
The effect of the low dose used in this work of He-Ne laser is mainly due to electro-excited state rather than due to increase in the temperature of the phohotoacceptor molecules, since the temperature elevation in the irradiated tissue is limited to less than 0.1→0.5 °C [25,26].

Table 2. The optical density (OD) of DNA by spectrophotometer for before and after He-Ne laser irradiation, and the DNA survival % after laser irradiation

dose (J/m²)	OD before laser	OD after laser	*P* value	DNA survival %
18	3.35±0.51	2.98±0.55	< 0.0001	88.6±6.8
35	3.41±0.38	2.98±0.44	0.0001	87.7±9.8
69	3.54±0.18	3.1±0.29	< 0.0001	86.1±7

In this work, the effect of low energy He-Ne laser (632.8 nm) irradiation alone immediately was examined on lymphocytes using different times of exposure (2.5, 5, 7.5, and 10 s) that give an energy values of (18, 35, 52.5, and 69 J/m^2), respectively. These different energies or doses of He-Ne laser lead to a little degree of cell death or sub-lethal damage, since the mean viability percentage of lymphocyte cells are (99.8%, 99.74%, 99.68%, and 99.59%) respectively (Table 1) while figure (2) showed that the lymphocyte mean viability with doses of laser irradiated is highly correlated (r= 0.99). No cells protection was observed, because no incubation time was given after the irradiation. These results are in agreement with El-Batanouny and coworkers [27]; they reported that low dose of He-Ne laser causes the lowest percentage in cells damage and promote the cell cycle of lymphocyte cells.
While the degree of cell damaging after He-Ne laser may be explained by other worker results, such as Stadtman 1992 who observed a significant decrease in lipid peroxidation and proteins damage post He-Ne laser irradiation, which cause a decrease in oxidative stress, this may become a threat to cells survival [28].
Other authors showed that the irradiation of human lymphocyte cells with a He-Ne laser can activate some short-term reactions in these cells, increase in chromatin template activity, lead to increase activation of mitochondrial function concurrently with the formation of giant mitochondria [29].
Gulosoy et al. in 2006 [30] reported that He-Ne laser caused increasing proliferation of blood mononuclear cells after 7 days of laser irradiation and suggested that the optimum He-Ne dose of 2.5 J/cm^2. But Dube et al. in 2001 [14] showed no significant effect of He-Ne laser of 1.5 kJ/m^2 doses on human B-lymphocyte line NC37 cells survival indicating that He-Ne laser has no cytoxic effect on these cells.
Hu et al. in 2007 reported immediate rises in the growth factors such as cytochrome C oxidase enzyme, Adenosine Triphosphate (ATP) content, and JNK phosphorylation in melanoma cell line A2058 after He-Ne irradiation of dose 1.0 J/cm^2, which leads to significant cell proliferation after 3 days of irradiation [31].
Gao and Xing in 2009 [32] reported that low level laser (red and near infra-red light) is absorbed by mitochondrial respiratory chain resulting an increased reactive oxygen species and ATP/cAMP which initiates signaling cascade promoting cellular proliferation and cytoprotection.
In general, He-Ne laser can stimulate the intracellular or extracellular effects, which pass in the initial commitment phase since the cell responds to signal that commit the cell to undergo self-destruction [30,33] showing that the irradiation of mononuclear cells with He-Ne laser can stimulate short term reactions and

irradiated cells did not enter S phase of the cell cycle. The intracellular effect such as the generation of singlet oxygen in the different cell type, which can stimulate a redox control over the parameters cellular homeostasis [19,34-36]. The extracellular signals include receptor ligand, proteins, and activate calcium channels [37].

In the current study, the results of the fraction DNA survival %, which measured immediately after the He-Ne laser irradiation alone, showed a significant degree of DNA damaging independent on the irradiation doses, (Table 2), no such DNA damaging was reported previously. This may be attributed, that most of the workers studied the effect of He-Ne laser irradiation on DNA within the cells and not extracted one [14,38]. So, a further work required to investigate this situation.

Different mechanism may be involved in the protection phenomena. Since Manteifel et al. in 1999 [39] demonstrated that the action of He-Ne laser irradiation excludes direct ruptures of covalent bond of DNA. Because the DNA do not have absorption bands in the visible spectral region, therefore, it is believed that the products of expressed genes are involved in the repair of DNA damage caused by the ionizing radiation [40]. This hypothesis further supported by Kohli et al. in 2001 [41], they observed that He-Ne laser pre- irradiation on E. coli strain KY706.pPL-1 leads to the induction of photolysis gene "phr". Ihara et al. in 1987 [42] suggested the role of singlet oxygen in induction of phr gene, the magnitude of the gene induction depend on the laser fluence, the photon energy of He-Ne laser irradiation may induce singlet oxygen which leads to sub-lethal damage of DNA. The singlet oxygen species response to the transcription of UVrA, UVrB, recA, and UmuDC genes, which trigger the DNA repair processes [43,44].

In conclusion, the percentage of lymphocytes survival is decreasing with increasing dose of He-Ne laser and longer exposure time. He-Ne laser irradiation causes a significant degree of DNA damaging independent on the irradiation doses.

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Author contributions:

Dr. Abdullah and Taha did the sample collection, procedure and writing of the manuscript, Dr. Ahmed participated in medical consultation and final revision of the manuscript.

Conflict of interest

Authors declare no conflict of interest.

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Snodgrass Urethroplasty for Mid and Distal Penile Hypospadias

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Abstract

Background Hypospadias describes a urethral opening proximal to the normal position at the glandular tip (anywhere on the ventral surface) and it is considered the most common penile anomaly. There are many surgical modalities for reconstruction but with different success. The goal of modern hypospadias repair is to achieve functionally as well as cosmetically normal looking glans, meatus and phallus. Snodgrass urethroplasty was introduced in 1974 with more reliable creation of a normal appearing meatus.

Objective To see the short-term results of tubularized incised plate urethroplasty as described by Snodgrass in mid and distal penile hypospadias.

Methods This is a prospective study for evaluation of fifty male children with age ranged from one to ten years with penile hypospadias (distal and mid shaft) were included in this study over a period from January 2008 to January 2016 in our pediatric surgical center.

Results The majority were between 2-4 years. Meatal stenosis (12%) and urethrocutaneous fistula (6%) were the most common complications encountered in this study. Inguinal hernia (8%) and undescended testis (10%) were the most common associated congenital anomalies with hypospadias.

Conclusion Snodgrass urethroplasty is simple, single stage operation in the management of mid and distal penile hypospadias with good cosmetic and functional results.

Keywords Hypospadias, Snodgrass technique, meatal stenosis, urethrocutaneous fistula.

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List of abbreviation: TIP = tubularized incised plate urethroplasty

Introduction

The term hypospadias is derived from the Greek word (hypos) meaning under and (spadon) meaning rent or fissure [1]. It's one of the most common anomalies of male external genetilia in which, the penis is similar to the normal one except on the ventral aspect where the foreskin, the urethra, and urethral spongiosum are deficient [2].

The ectopic external urethral meatus exists ventrally anywhere from the glans to the perineum and according to this abnormal meatal location, hypospadias classified into: anterior (50%) involving glanular, coronal and subcoronal regions. Middle (30%) involving the distal penile, mid-shaft and proximal penile regions. Posterior (20%) involving the penoscrotal, scrotal and perineal region [3].

Surgical repair of hypospadias has one of the most taxing problems for reconstructive surgeons, urologist and paediatric surgeons alike because of high complications rate [4]. The very fact that there are about 300 different operations to manage this tricky problem itself is testimony that no single operation is favored

by all surgeons and no single technique provides uniformly good results. One stage repair is naturally favored as it decreases number of hospitalizations and thus is economical [5,6].

The aim of hypospadias surgical treatment with or without curvature of penis: 1- trying to bring the urethral opening at the tip of the glans, 2- achieving a regular and straight penis, 3- creating favorable conditions for a successful psychosexual life [7]. There are two windows of opportunity to perform surgery based on the following factors, namely the environment in which, the patient will be managed, the anesthetic risk and the psychological effect of genital surgery. There is an increasing trend to perform surgical correction between six months to 18 months of age. The second option is between three to four years of age i.e., before the boy start s school [8].

Urethrocutaneous fistula formation is the commonest complication of hypospadias repair, with reported incidence of 4-25%. The successful repair of this lesion depends on several basic principles. Various techniques have been described for fistula repair but with loupe magnification [5-7].

This study aimed to see the short-term results of tubularized incised plate urethroplasty as described by Snodgrass in mid and distal penile hypospadias.

Methods

A total of fifty boys with age ranged from one to ten years with penile hypospadias (distal and mid shaft) were included in this study over a period from January 2008 to January 2016 in our pediatric surgical center at Al-Imamein Al-Al-kadhimein Medical City. All cases with penoscrotal hypospadias and those with severe chordee were excluded from the study. All patients underwent the same surgical procedure, which is Snodgrass operation (TIP: tubularized incised plate urethroplasty). Total blood investigation including complete blood count, blood group, bleeding time, prothrombin time, urea, sugar and creatinine in addition to abdominal ultrasound to exclude other associated congenital genitourinary tract anomalies were done prior to surgery. We exclude all boys with proximal hypospadias from this study.

Snodgrass operation was done under general anesthesia in a classic way with insertion of Foley's catheter of eight to ten French on the basis of patient age and size of the penis. All cases had dressing opened on the 7th day postoperatively and keeping the patient on oral antibiotics and local fucidin ointment for at least 10 days after surgery while Foley's was taken out on the 8th day and patient discharged from hospital. Patients were informed for follow up after one week of discharge to evaluate the state of wound and to see the urinary stream or any postoperative complications especially urethrocutaneous fistula and meatal stenosis.

Results

The Snodgrass (TIP) urethroplasty was performed in fifty boys with mid and distal penile hypospadias. The age of patients ranged from 1 to 10 years. There were 7 (14%) coronal, 19(38%) subcoronal, 16(32%) distal shaft and 8(16%) midshaft hypospadias. The most common type of hypospadias in this study was subcoronal type and occurred in 19 patients (38%) followed by distal shaft, which occurred in 16 patients (32%) as shown in table (1).

The most common age for operation was at age between two to four years accounting for 54% of total patients as shown in table (2).

There were also associated congenital anomalies with those patients with hypospadias, five (10%) of them were associated with undscended testis and four patients (8%) had inguinal hernia as shown in table (3).

Table 1. Types of hypospadias

Types	No. of patients	Percentage
Coronal	7	14%
Subcoronal	19	38%
Distal shaft	16	32%
Mid shaft	8	16%
Total	50	100%

Table 2. Age distribution of children with Hypospadias underwent Snodgrass operation

Age	No. of patients	Percentage
1-2 year	6	12%
2-4 year	27	54%
4-6 year	12	24%
6-8 year	5	10%
Total	50	100%

Table 3: Associated congenital anomalies

Associated anomalies	No. of patients	Percentage
Undescended testes	5	10%
Inguinal hernia	4	8%
Congenital heart disease	1	2%
Omphalocele	1	2%
Scoliosis	1	2%

Early complications were seen in two patients in whom complete dehiscence did occur. The first child aged 2 years and the second aged three years. The repair was tried after six months with better results. Meatal stenosis was the most frequent complication of Snodgrass operation in our series and happened in 6(12%) patients followed by Urethrocutaneous fistula (6%). As shown in table (4).

Table 4. Complications after Snodgrass operation according to type of Hypospadias

Types of Hypospadias	No. of patients	Wound dehiscence	Meatal stenosis	Urethrocutaneous fistula	Good cosmetic appearance
Coronal	7	0	1	0	6
Subcoronal	19	1	3	1	17
Distal shaft	16	1	1	1	15
Mid shaft	8	0	1	1	7
Total	50	2(4%)	6(12%)	3(6%)	45(90%)

Discussion

Snodgrass technique for mid and distal penile hypospadias described has several advantages over other techniques for hyposadias. Mathew, onlay island pedicle flap procedure has widely used with minimal complications but the meatus appearing rounded like fish mouth in contrast to slit like of a normal meatus was their disadvantage. Meatal advancement and glanulopasty was developed to correct meatus which had urinary stream going downward as its demerits.

The hypospadias does not present a major concern for the pediatric surgeon only because of its high incidence (1:300), but also because of its numerous variations, from the mildest forms to the most complex ones, where it is difficult even to distinguish the sex of patient on the basis of its external appearance at first sight. In this study, the meatus was vertically oriented and extended to the apex with straight voiding in 90% of them. This is in agreement with result of Acimi [9], and that of O'Connor and Kiely [10]. Meatal stenosis was the most frequent complication in this study and it was seen in 12%, which is higher than that of Din et al. [11], while it is lower than Qassim study who observed 20% incidence of meatal stenosis in his series [12]. In 2002, Lorenzo and Snodgrass concluded that failure to deeply incise the urethral plate too far distally can play an important role in developing stenosis [13]. It can be stated that meatal stenosis and its associated poor urinary stream is mainly due to technical error. To minimize this, urethral plate must be deeply incised and avoiding too far distal tabularization (not exceeding the mid-glanular point). Along term daily urethral dilatation, which started after removing the silastic urinary catheter after 10-14 days and continue for three months, this dilatation prevents or at least minimizes epithelial apposition enhancing the secondary rather than primary wound healing and these procedures will reduce incidence of meatal stenosis.

The other complications encountered by Snodgrass operation were urethrocutaneous fistula in (6%) and this complication was observed only in those patients with meatal stenosis suggesting that the impaired normal flow of urine due to stenosis produced back pressure which adversely affect the suture line that considered as a weak point enhancing the development of fistula. It is thought that preventing meatal stenosis in Snodgrass procedure significantly minimizes this complication. This complication was lower than the study of Thapa and Pun [14] who observed (10%) fistula and study of Uzair et al. [15] who observed (9.6%) urethral fistula, but it is higher than the result of Din et al. [11] who observed only 2% urethral fistula. The higher incidence of fistula in present study in comparison to other western studies like Cheng et al. [16] in USA may be due to development of sub-specialties like pediatric urology, pediatric plastic surgery and also due to strict sterile theatre environment, proper suture materials and proper instruments.

This study concluded that Snodgrass technique is simple, single stage operation in the management of distal and mid penile hypospadias and gives good functional and cosmetic result with low complications rate.

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Conflict of interest

The author declares no any interest in publishing this article or competitive intention.

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The Identification of Risk Factors that Predict Occult Cystobiliary Communication in Liver Hydatid Cysts

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Abstract

Background Post-operative biliary leakage in patients with liver hydatid diseases is still a major problem especially after conservative surgery. Radiologic and intraoperative findings may not be helpful to detect occult biliary communications in asymptomatic patients.

Objective To identify the risk factors to predict occult cystobiliary communications (CBC) preoperatively to avoid development of biliary leakage after surgery.

Methods This prospective study conducted at the Gastroenterlogy and Hepatology Teaching Hospital in Medical City, Baghdad from the 1st of December 2013 to the 29th of March 2016. Clinical assessment, laboratory tests and imaging studies were under taken for 85 patients with uncomplicated liver hydatid cysts. Endocystectomy and or partial pericystectomy were undertaken. Post-operative follow up and management of biliary leakage for those with cysto-biliary communication not detected intra operatively. Data were analyzed to predict risk factors for occult CBC.

Results Of the 85 patients, 64 patients had no evidence of CBC neither intra nor postoperative, while the remaining 24 patients had an occult CBC, that have been discovered during operation or evident in the following days. Significant clinical predictors of communication were cyst size ≥ 10 cm, elevated total serum bilirubin (TSB) and gamma glutamyl transferase (GGT) ($P < 0.001$). Other findings were associated more with patients having CBC than those without, including high white blood cell count (WBC), alkaline phospatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT) levels. Nine of 13 patients who developed post-operative bile leak stopped spontaneously, the other 4 patients mandate endoscopic retrograde cholangiopancreatgraphy (ERCP) to close their fistulas.

Conclusion Awareness about the risk factors for CBC can predict preoperative diagnosis of occult CBC. Endoscopic biliary interventions should be considered as a part of complementary treatment of those cases with refractory CBC and to be discussed in patients's consent preoperatively.

Keywords Liver hydatid cyst, cystobiliary communication, biliary leakage.

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List of abbreviation: ALT = Alanine Aminotransferase, ALP = Alkaline Phosphatase, AST = Aspartate Aminotransferase, ERCP = Endoscopic retrograde cholangiopancreatography, GGT = Gama Glutamyl Transferase, TSB = Total Serum Bilirubin, WBC = White blood cells count

Introduction

Hippocrates describes hydatid disease as 'liver full of water' [1]. It is endemic in Mediterranean region and in Iraq it is a well-known health problem [2,3]. Although hydatid disease was uncommon in united State and Europe, now it is considered as

recognizable cause of morbidity and mortality in these regions due to travel and migration [4].

In general, hydatid disease affect liver in 50-70% of cases [1,5]. Of the most interesting complication encountered in 13-37% of patients with liver hydatid is the communication between cyst cavity and biliary radicles (CBC) secondary to rupture of the cyst into the biliary system [6-8].

Intrabiliary rupture could be an overt frank or silent occult [9]. In overt situations, a major cyst contents, like debris, daughter cysts and fragments of the laminated membrane may go down into the common bile duct [10]. Patients usually present with features of obstructive jaundice during the course of the disease [11]. Diagnosis in these patients is easy, and managed either surgically or by endoscopic retrograde cholangiopancreatography (ERCP) [12]. However, most CBCs are occult in nature without specific clinical, radiological or laboratory findings due to relatively small communication and discovered only during or after surgery as a postoperative biliary fistula (PBF) [13,14].

In this instance, undesired complications could be happened such as prolonged biliary leakage, biliary peritonitis and abscess, an increased morbidity, cost and hospital stay will ensure [15].

Bile usually not seen in the cyst cavity in cases of occult CBC due to high intracystic biliary pressure gradient (80 vs. 20 cm H_2O subsequently). After cyst evacuation, bile flow back into the cyst due to reversed pressure gradient [16,17].

Radical surgeries i.e. formal hepatectomy and pericystectomy have a rare incidence of postoperative biliary leakage and recurrence, at the same time they carry a more perioperative risk of bleeding and need expert hepatobiliary surgeon in a specialized center, on the contrary, conservative surgery; endocystectomy or partial pericystectomy, usual performed type of surgery in endemic regions, they have a high incidence of postoperative biliary leakage and local recurrence although less perioperative risk of bleeding [17].

ERCP has a beneficial role in showing the dilatation in the biliary duct and the relationship between the cyst and the bile ducts before operation. However, it is not usually possible to demonstrate minute CBC [14]. Moreover, it is not feasible to perform prophylactic ERCP in all patients. Accordingly, it is important to predict the CBC in asymptomatic patients. The sizes of cyst, levels of alkaline phosphatase (ALP), Gama Glutamyl Transferase (GGT) and Total Serum Bilirubin (TSB) have been reported as risk factors for CBC, it has been reported that ALP concentrations greater than 250 U L^{-1}, a total bilirubin level above 17.1 µmol L^{-1}, GGT higher than 34.5 U L^{-1}, and cyst size greater than 8.5 cm in the preoperative period were independent predictors of occult CBC [15,18].

GGT is present in hepatocytes and biliary epithelia lcells; its elevation is the most sensitive marker of hepatobiliary disease. However, its routine clinical use is not recommended, as it cannot by itself indicate a specific cause of liver disease, although measuring the GGT level can help determine a hepatic origin for an isolated elevation of alkaline phosphatase [19].

The presence of these risk factors should rise the suspicion of asymptomatic CBC and a clear hydatid fluid content without bile in the cyst does not mean an intact cyst wall, in such, the use of scolicidal agents should be avoided because septic complications and risk of sclerosing cholangitis [20].

Management should be planned in these patients, antibiotic chosen for prophylaxis, ERCP if possible and surgical intervention whenever possible. If the CBC is revealed during operation, an effort should be paid to suture with absorbable material, with or without cystic duct drainage (or T-tube insertion), otherwise if biliary opening cannot be identified, an adequate external drainage, preferably with suction drainage. Methylene blue injection into the common bile duct or

intraoperative cholangiogram can help in localization of communication [21].

Most of biliary fistulas close without intervention, failure of closure may occur in 4-27.5% of cases [22]. Endoscopic sphincterotomy is indicated if no evidence of healing within three weeks or persistent high output exceeding 300 mL/d [23].

This study aimed to identify and manage patients with occult CBC preoperatively, therefore, the clinical and laboratory parameters associated with such type of communication were studied in patients who developed biliary leakage after hydatid liver surgery.

Methods

A prospective study conducted at the Gastroenterology and Hepatology Diseases Teaching Hospital in Medical City, Baghdad, from the 1st of December 2013 to 29th of March 2016; (98) patients with liver hydatid cysts had been admitted to the hospital, (85) patients with uncomplicated hepatic cyst whether primary or recurrent, had been enrolled in the study, those with frank CBC (10) patients, and those underwent radical surgery (3) patients had been excluded from the study. Clinical assessment, laboratory and imaging studies had been done for all patients. Liver function tests with their reference normal value including TSB (0.2-1.2 mg/dL), AST (≤41 U/L), ALT (≤ 44 U/L), ALP (53-128 U/L) and GGT (5-35 U/L) were recorded in addition to WBC (4-10 * 10^9/L). The diameter, location, number and types of the cysts also reported utilizing the ultrasound and computerized tomography (CT) findings. The above data were analyzed as potential predictors of occult cystobiliary communication. Patients were prepared for surgery, preoperative Albendazol for two weeks, antibiotics at time of induction of anesthesia and deep venous thrombosis (DVT) prophylaxis. Access to the liver was through a midline, subcostal or right anterolateral 7th intercostals space thoracotomy incisions depending on the cyst location. Identification of the cyst, aqueous povidon socked packs or hypertonic soaked packs used to isolate cysts, injection of scolicidal agent was not done. Endocystectomy and or partial pericystectomy were undertaken with the control of suction. CBC had been looked for intra operatively for 5 minutes and sutured whenever found, drain was left inside cavity.

Postoperatively drains were removed on the 3rd to 5th postoperative day if they provided no biliary drainage. Patients who continued to have biliary drainage in the first 10 days postoperatively were considered to have biliary leakage. Longer biliary drainage was classified as biliary fistula. The fistulas were categorized into low and high-output types based on the fistula output, less or more than 300 mL/day, respectively.

Drains were kept in patients who developed biliary fistulas and amount was recorded daily. Sinogram then Endoscopic sphincterotomy was performed in patients in whom no reduction in biliary flow rate or a spontaneous termination of biliary leakage was unlikely within one month.

Included patients were divided into two groups, those who had no CBC; labeled as group A and those who had occult CBC as group B. Statistically the two groups were compared according to the clinical findings, laboratory tests, imaging and operative results.

The results are presented as the mean (and standard deviation SD), number (and %), significance was set at ($P<0.05$), sensitivity, specificity, positive predicative value, negative predicative values and accuracy.

Results

Eighty-five patients had been divided into group A 61 (72%) (Patients without CBC) and B 24 (28%) (Patients with occult CBC) based on intraoperative findings of bile spillage into the cyst cavity after endocystectomy or postoperative bile drainage through abdominal drain.

The overall CBC in this series was 34 (34.6 %) patients; those with frank communication had

been excluded as mentioned above 10 (10.2 %) patients and 24 (24.4%) patients had occult CBC.

The mean age was higher for those with CBC than those without communication, (41.4) versus (36.04) years. Female predominance was in both groups, A and B, (57.4%), (54.2%) subsequently although no significant relation to the development of CBC.

Patients from rural areas were predominating in both groups (A; 62.3%) and (B; 71%), still no statistical relation to the development of CBC. Abdominal discomfort was the presenting symptom in more than two third of patients, group A (83.6%) and group B (79.1%).

In relation to the size of the cysts, CBC were significantly associated with increased hydatid cyst size, most of those with CBC (79.9%) have cyst size more than 10 cm (P<0.001).

The cysts were located in the right lobe in 38(62.3%), in the left lobe in 17 (27.8%), and in both lobes 6 (9.8%) patients in Group A. The cyst locations were right, left and bilateral in 16 (66.6%), 5 (20.8%), 3 (12.5%) patients respectively in group B.

Majority of patients had single liver cyst, group A; 42 (68.8%) and group B; 18 (75%), usually they were unilocular. No significant statistical differences in relation to the other features of cysts in both groups as shown in (Table 1)

Table 1. Demographic and clinical data

Clinical features			Patients group A: 61 (72%)	Patients group B 24 (28%)	Significance *P* value
Age (Mean ± SD) year			36.04±12.9	41.4±15.5	0.5
Sex	Male		26 (42.6%)	11 (45.8%)	0.812
	Females		35 (57.4%)	13 (54.2%)	
Region	Urban		23 (37.7%)	7 (29%)	0.615
	Rural		38 (62.3%)	17 (71%)	
Presentation	Discomfort		51 (83.6%)	19 (79.1%)	0.744
	Fullness or swelling		29 (47.5%)	8 (33.3%)	
	Incidental		12 (19.6%)	3 (12.5%)	
Features of cyst	Size	< 10 cm	39 (63.9%)	5 (20.1%)	< 0.001
		≥ 10cm	22 (36.1%)	19 (79.9%)	
	Site	Rt. lobe	38 (62.3%)	16 (66.6%)	0.780
		Lt. lobe	17 (27.8%)	5 (20.8%)	
		Bilateral	6 (9.8%)	3 (12.5%)	
	No. of cyst	Single	42 (68.8%)	18 (75.0%)	0.792
		Multiple	19 (31.1%)	6 (25.0%)	
	Morph-ology	Unilocular	31 (50.8%)	14 (58.3%)	0.807
		Multilocular	22 (36.0%)	7 (29.1%)	
		Degenerative	8 (13.1%)	3 (12.5%)	
	Recurr-ence	No	48 (78.7%)	20 (83.3%)	0.768
		Yes	13 (21.3%)	4 (16.7%)	

Although the laboratory findings were not statistically different between two groups, the mean value for WBC, ALP, ALT, AST were lower in group A (7.3±2.39, 0.98±0.7, 85.4±41.5, 26.6±10.9, 26.6±10.9) than in group B (8.77±2.43, 1.7±0.82, 90.2±51.7, 39.7±20.2, 37.5±19.4) subsequently, except GGT and TSB levels were significantly different between the two groups (P< 0.001) as shown in (Table 2).

Table (3) shows the results of multivariate analyses for the risk factors of occult CBC presenting as biliary leakage after surgery. Cyst size ≥ 10, TSB >1.2 mg/dL and GGT >35 U/L levels were significant clinical factors that predict the risk of occult CBC with sensitivity of 79.1%, 70.1%, 83.3% subsequently, although positive predicted value was high only for the GGT 71.4%. Combination of these risk factors if present in patients with hydatid cyst; an accuracy of 95.2% can be yield in prediction of CBC.

Table 2. Laboratory tests

Test	Group A (mean ± SD)	Group B (mean ±SD)	P value
WBC (4-10 * 10^9/L)	7.3±2.385	8.77±2.43	0.072
TSB (mg/dL)	0.98±0.7	1.7±0.82	**<0.001**
ALP (U/L)	85.4±41.5	90.2±51.7	0.41
ALT (U/L)	26.6±10.9	39.7±20.2	0.53
AST (U/L)	23.9±11.3	37.5±19.4	0.31
GGT (U/L)	24.8±13.45	82.7±42.9	**<0.001**

Table 3. Risk factors associated with occult CBC on multivariate analysis

Variables	TP	TN	FP	FN	Sensitivity%	Specificity%	PPV%	NPV%	Accuracy%
Cyst size >10 cm	19	39	22	5	79.1	64	46.3	88.6	68.2
WBC >10000*10^9/L	8	52	9	16	33.3	85.2	47.1	76.4	70.5
TSB >1.2 mg/dL	17	42	19	7	70.1	68.8	47.2	85.7	69.4
ALP >45 U/L	8	47	14	16	33.3	77.04	36.3	74.6	64.4
GGT >35 U/L	20	53	8	4	83.3	86.8	71.4	92.9	85.8
If above risks are positive	6	75	2	2	75	97.4	75	97.4	95.2

TP = true positive, TN = true negative, FP = false positive, FN = false negative, PPV = positive predicted value, NPV = negative predictive value

Endocystectomy and or partial pericystectomy were done for the patients, 61 patients without CBC run a smooth postoperative period regarding absence of bile leak through their abdominal drains. Twenty four patients with occult CBC managed accordingly; 11 of them had an obvious bile coming from a visible biliray tributary adherent to the residual cyst cavity, intra operative suturing were under taken by long absorbable suture, there after they follow free postoperative period from any bile discharge, the remaining 13patients (15.3% of total number), no obvious intra operative biliray communication were detected, in 9 of them low output fistula developed that managed expectantly and almost closed spontaneously within 10 days, a high output fistula were developed in the remaining 4 patients to whom sinogram through draining tube to delineate anatomical relationship followed by ERCP successfully within one month from the surgery (Figures 1 and 2), the fistulas closed within two to three days later, (Table 4) showed the fate of those patients suffer CBC. The overall hospital stays were 4.3 days in group A, while10.2 days in group B ($P < 0.05$).

Discussion

Liver hydatid cysts may grow average 1-30 mm per year and cause compressive atrophy of surrounding hepatocytes and fibrosis. CBC due to spontaneous rupture may occur at the point of contact with a biliary duct [(18)].

The incidence of frank and occult CBC ranges 5-17% and 10-37% of cases, respectively [7,8, 24]. In this series, the frank and occult CBC were 10.2% and 24.4% subsequently.

Figure 1. Sinogram shows cysto-biliary communication

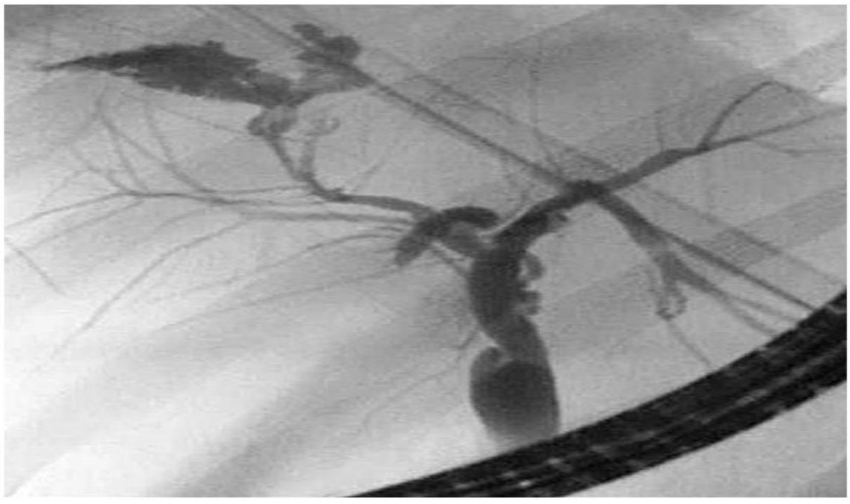

Figure 2. Endoscopic cholangiogram shows cystobiliary communication

Table 4. Fate of group B

Patients		No. (%)	Management
Intra operative leak		11 (46%)	Primary repair
Postoperative bile leak	<300 ml/d	9 (37.5%)	Expectant
	>300 ml/d	4 (16.5%)	ERCP

The mean age for group A was 36.4 years while 41.4 years for group B, Demircan et al. [29] found that a mean age for patients without CBC was younger than those with CBC 42.2, 44.4 years respectively. This explained by the longer the history and so the larger size of hydadit cyst is associated with increasing risk of complications.

Female preponderance was observed in both groups (Table 1), Sawady showed a female to male ratio of 1.5:1 in their study at Al Basrah city [3]. Khader Faheem et al. found in their study carried at Andhra Pradesh (India) a male to female ratio of 2.5:1, the differences in the reports were due to difference in socioeconomic, livelihood activities of farming, routine labor, animal breeding and agriculture variations in different regions in the world [25].

More than two third of patients in both groups were from rural area, which is expected as the disease is common where dogs and cattle are kept [26].

Although liver hydatid cysts are usually asymptomatic, in our series the most common symptoms were abdominal pain and swelling or fullness. A study done by Rukmangadha et al. showed that overall commonest presentation was incidental asymptomatic finding, while abdominal pain comes next, fever and jaundice may accompany complicated cysts [27].

The size of the cyst was one of the significant preoperative predictors of CBC with a cut-off value of ≥ 10 cm, (P <0.001). Zeybek et al. analysis also showed that a high preoperative cyst diameter (>10 cm) were significantly more common in patients who develop postoperative biliray fistula [28]. Demircan et al. found that cyst diameter greater than 8.5 cm were independent clinical predictors of occult CBC in multivariate logistic regression analysis [29].

Although majority of patient with CBC had their cyst location in the right lobe, no significant statistical relation was found with CBC. This applied for the other clinical findings of the cysts such as number, unilocular or multilocular, degenerative or recurrent. At Al-Basrah city, Sawady found that the frequency of biliary leakage was not affected by cysts being single or multiple, primary or recurrent or in which hepatic lobe they found (P >0.05) [3].

In the present study, whether or not the pre-operative laboratory findings are indicators for occult CBC was determined. None of the laboratory findings except GGT and TSB were useful as indicators of occult CBC (P <0.001).

Although most of patients with occult CBC were clinically not jaundiced, their TSB levels were mildly elevated biochemically. Sawady found that TSB level was upper normal in those patients with occult CBC [3]. GGT is a biliary enzyme that is especially useful in the diagnosis of obstructive jaundice, intrahepatic cholestasis, and pancreatitis. GGT is more responsive to biliary obstruction than are (AST) and (ALT). GGT is helpful to work up elevated ALP values and more specific for hepatic disease than is ALP [29]. Sing et al. found that the outcome of GGT was significantly higher in occult CBC group (P <0.05), they consider GGT level as useful for predicting of occult CBC in hepatic hydatid diseases preoperatively [30].

Cyst size, WBC, bilirubin, ALP and GGT have been reported as risk factors for CBC in literature [15]. We found that positive and negative predictive values were 46.3%, 88.6% for cyst size, 47.1%, 76.4% for WBC, 47.2%,

85.7% for total bilirubin, 36.3%, 74.6% for ALP and, 71.4%, 92.9% for GGT respectively.

Whenever a combination of these risk factors is present, positive, negative predictive value and accuracy of 75%, 97.4% and 95.2% will be obtained respectively. Unalp et al. [31] found in their series that the above parameters (except WBC) were independent risk factors for occult CBC in multivariate analysis, they found that, positive and negative predictive values were 41%, 95%for cyst size; 57%, 76%for total bilirubin; 43%, 96% for ALP and 50%, 97% for GGT respectively. Leukocytosis was a poor predictor, with positive predictive value of 17% and negative predictive value of 84%. However, if a combination of these 5 factors is present, the positive and negative predictive values increased to 100% and 90%, respectively.

The rate of external biliray fistula was 15.3%, 9 patients had a low output and closed spontaneously without intervention, the other 4 patients necissate ERCP (about one month from the surgery date) to close their high output fistula. Accordingly, the hospital stay was significantly higher for those with postoperative biliray fistula (10.2 days versus 4.3 days for those without fistula, P<0.05). Kemal et al found that the rate of external biliary fistulas was 22%, most of them closed spontaneously; they may persist in few cases. In their study, low-flow fistulas (< 300 mL/day) were present in 11 of 15 patients with fistulas; these fistulas closed spontaneously. The remaining 4 patients had high-flow fistulas and three of them closed after ERCP, whereas one patient underwent a fistula-enterostomy. Endoscopic sphincterectomy is performed after a 3-week waiting period, the overall hospital staying days were 5.3 days in patients without external biliray fistula and 21.2 days in patients with fistulas (p<0.05) [17].

This study concluded that a surgeon should suspect an occult CBC if asymptomatic patient has the above risk factors, endoscopic biliary interventions should be considered as a part of complementary treatment of those cases with refractory CBC and to be discussed in patients' consent preoperatively.

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Conflict of interest

The author has no conflicts of interest.

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Evaluation of Inflammatory State in Diabetic Patients by Measuring of Interleukin-6 and Tumor Necrosis Factor-α in Obese and Non-Obese Type 2 Diabetes Mellitus Patients as Compared with Control Subjects

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Abstract

Background Inflammation was one of the most important events in the biology of obesity; the obese subjects were recognized recently as characterized by low-grade chronic inflammation. It was thought that the mild inflammation associated with obesity, and particularly the production of inflammatory adipocytokines like interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), was important in the etiology of the diseases associated with obesity. In particular, insulin resistance (IR) and type 2 diabetes mellitus (T2DM).

Objective To investigate whether IL-6 and TNF-α play an important role in the etiology of IR and T2DM.

Methods This study enrolled 70 T2DM patients randomly assigned into two subgroups, 35 non-obese (body mass index (BMI) < 30) diabetic group 1 and 35 obese (BMI ≥ 30) diabetic group 2 with another 50 healthy control volunteers, divided into two subgroups, 25 non-obese (BMI < 30) control group 1 and 25 obese (BMI ≥ 30) control group 2. Levels of IL-6, TNF-α, fasting glucose, fasting insulin, HbA1c, homeostasis model assessment of IR (HOMA-IR), homeostasis model assessment of β-cell function (HOMA-B%) were examined.

Results The serum concentration of IL-6 of obese and non-obese diabetic patients was significantly ($p < 0.05$) lower as compared with obese and non-obese controls in contrast to the serum concentration of TNF-α, which was significantly ($p < 0.05$) higher in non-obese diabetic patients in comparison to non-obese controls. No significant correlation was observed for the levels of IL-6 and TNF-α with BMI of study population

Conclusion The proposed link between serum inflammatory cytokines (IL-6 and TNF-α) and T2DM was more related to insulin sensitivity, insulin secretion and/or glycemic control than to adiposity. Therefore, the inflammatory cytokines may play an important role in the etiology of IR and T2DM.

Keywords IL-6, TNF-α, type 2 diabetes mellitus, obesity, insulin resistance.

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List of abbreviation: BMI = Body mass index, CG1 = Control group 1, CG2 = Control group 2, DG1 = Diabetic group 1, DG2 = Diabetic group 2, HOMA-IR = Homeostasis model assessment of insulin resistance, HOMA-B% = Homeostasis model assessment of pancreatic function, IL-6 = Interleukin-6, IR = Insulin resistance, T2DM = Type 2 diabetes mellitus, TNF-α = Tumor necrosis factor-alpha, WHR = Waist to hip ratio.

Introduction

Type 2 diabetes mellitus (T2DM) is the most common metabolic disorder in man, affecting over 170 million individual over the world and, potentially, over 365 million in the year 2030 [(1)]. The etiology of T2DM involves abnormalities in both insulin action and secretion [(2)]. Although the precise pathological sequence which leads to insulin resistance (IR) was still unknown, sedentary lifestyle and excess nutrition leads to excessive lipid accumulation in adipose and peripheral tissues resulting in obesity.

Recent studies have shown that chronic low-grade inflammation is an important factor in the etiology of T2DM in humans [(3,4)]. Although liver and muscle show obesity-induced mild inflammation without significant changes of immune cells, adipose tissue was the most vulnerable target to mediate significant infiltration of the immune cells and inflammation contributing to systemic inflammatory response and IR in obese humans [(5)].

Several studies have observed the local expression of pro-inflammatory cytokines and activation of inflammatory cells in the liver and skeletal muscle [(5)]. Whether this results from local release of pro-inflammatory cytokines or systemic inflammation emanating from adipose tissue has yet to be established. Therefore, the aim of this study was to investigate the association of pro-inflammatory cytokines including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) with the pathogenesis of IR and T2DM.

Methods

A case – control study was conducted at Diabetes Centre of Al-Mawana Hospital in Basra from April 2014 till March 2015. The selected patients were informed about the aims of study before their written informed consent was obtained. The study was approved by the medical ethical committee of College of Medicine, Al-Nahrain University.

This study enrolled 70 T2DM patients (already diagnosed) randomly assigned into two subgroups, 35 non-obese (BMI < 30) diabetic group 1 and 35 obese (BMI ≥ 30) diabetic group 2. Patients were selected according to the diabetes diagnostic criteria of WHO (2011); fasting glucose level ≥ 7 mmol/L (≥ 126 mg/dl) and glycosylated hemoglobin (HbA1c) ≥ 6.5%. All patients had no other disease than T2DM. Another 50 subjects were enrolled in this study as healthy control volunteers, divided into two subgroups, 25 non-obese (BMI < 30) control group 1 and 25 obese (BMI ≥ 30) control group 2, control subjects were selected with glycemic control inclusion criteria were fasting glucose < 7.0 mmol/L (< 126 mg/dl) and HbA1c < 6.5%.

Subjects were excluded if they have: (1) macrovascular complications such as angina pectoris, myocardial infarction and peripheral vascular diseases. (2) history of hypertension or systolic blood pressure (SBP) > 140 mmHg and/or diastolic blood pressure (DBP) > 85 mmHg. (3) pregnancy.

Five ml blood samples were collected by venipuncture after overnight fasting (10-12 hr) and were divided into two portions, 2 ml in EDTA tube for measurement of HbA1c and 3 ml in plain tube, centrifuged for separation of serum, which was divided into two portions, one portion for assay of fasting serum glucose and the second portion of serum sample was frozen and stored at (–20 °C) for assay of IL-6, TNF-α and insulin.

Statistical analysis

All data were expressed as mean ± standard deviation. Student's t test was used to analyze sample averages. One-way analysis of variance (ANOVA) was used to evaluate differences of means between groups. Ratio was compared by the chi square test. Correlations between Homeostasis model assessment of insulin resistance (HOMA-IR), other parameters were analyzed by Pearson's correlation. $P < 0.05$ was accepted as statistical difference.

Results

The general characteristic of all study groups including age, age range, men/women, address, smoking, BMI, WHR, and physical activity were listed in table (1). All groups were matched for age, sex and number of smokers.

Table 1. General characteristics of control and diabetic groups

Parameter	Non-obese Control Group1 (CG1) n=25	Obese Control Group2 (CG2) n=25	Non-obese Diabetic Group1 (DG1) n=35	Obese Diabetic Group2 (DG2) n=35	P value
Age (years)	36.4±10.9	38.4±8.5	39.0±8.8	40.2±7.3	0.37
Age Range	23 - 58	28 - 58	26 - 62	29 - 54	
Men/women	13/12	13/12	18/17	17/18	0.9908
Address (center/rural)	13/12	23/2 a	22/13 b	20/15b	0.0055
Smoker/Non-smoker	4/21	9/16	11/24	7/28	0.20541
Body mass index (BMI)	27±2.1	37.4± 3.4 a	27±1.8b	36.7±3.4ac	< 0.001
Waist to hip ratio (WHR)	0.93±0.085	0.98±0.074 a	0.98±0.046 a	1±0.045 a	0.0005
Physical activity (min/wk)	456.4±77.2	290.0±164.8 a	239.7±92.8a	193.9±87.9abc	< 0.0001

All values were expressed as mean±SD
a Significant ($p < 0.05$) as compared with control group 1
b Significant ($p < 0.05$) as compared with control group 2
c Significant ($p < 0.05$) as compared with diabetic group 1

Group comparison using student t-test, as shown in table (2), the serum concentration of IL-6 of obese and non-obese diabetic patients was significantly ($p < 0.05$) lower as compared with obese and non-obese controls in contrast to the serum concentration of TNF-α, which was significantly ($p < 0.05$) higher in non-obese diabetic pt. in comparison to non-obese controls.

The serum insulin concentration of diabetic groups was significantly ($p < 0.05$) lower than control group 2 but no significant difference as compared with control group 1. Also, the serum insulin conc. of control group 2 was significantly ($p < 0.05$) higher than that of control group 1 as shown in table (3). The pancreatic β-cell function (HOMA-B%) of diabetic patients' groups was significantly ($p < 0.05$) lower than that of control subject groups with non-significant difference between diabetic groups (diabetic group 1 and diabetic group 2) while there was a significant ($p < 0.05$) difference between control subject groups as shown in table (3). The insulin sensitivity (HOMA-IR) of diabetic patients was significantly ($p < 0.05$) lower than that of control subjects (control group 1) with non-significant difference between diabetic groups (diabetic group 1 and diabetic group 2), and significant ($p < 0.05$) difference between control groups (control group 1 and control group 2) as shown in table (3).

Table 2. Results of serum pro-inflammatory cytokines (IL-6 and TNF-α) of control and diabetic groups

Parameter	Non-obese Control Group1 (CG1) n=25	Obese Control Group2 (CG2) n=25	Non-obese Diabetic Group1 (DG1) n=35	Obese Diabetic Group2 (DG2) n=35	P value
IL6 (pg/ml)	28.5±16.3	20.1±11.67 a	11.5±4.87ab	6.5±5.1abc	< 0.0001
TNF-α (pg/ml)	152.9±64.0	154.2±84.0	221.9±131.9ab	197.4±117.7	0.03345

All values were expressed as mean±SD
a Significant ($p < 0.05$) as compared with control group 1
b Significant ($p < 0.05$) as compared with control group 2
c Significant ($p < 0.05$) as compared with diabetic group 1

The correlation analysis revealed a significant negative correlation between IL-6 and fasting glucose, HbA1c and HOMA-IR with significant positive correlation between IL-6 and HOMA-B% in study population. Also, there was a significant positive correlation between TNF-α and fasting glucose and HbA1c with significant negative correlation between TNF-α and HOMA-B% in the study population as shown in figure (1), figure (2) and figure (3) respectively.

Table 3. Results of Glycaemic Control parameters and HOMA-indexes of control and diabetic groups

Parameter	Non-obese Control Group1 (CG1) n=25	Obese Control Group2 (CG2) n=25	Non-obese Diabetic Group1 (DG1) n=35	Obese Diabetic Group2 (DG2) n=35	P value
Fasting serum glucose (mg/dl)	99.1±8.4	99.6±9.3	218.7±72.7ab	234.5±90.1ab	< 0.0001
Disease duration (years)	-	-	4.8±3.9	2.8±2.2c	0.011
Family History (positive/negative)	17/8	13/12a	28/7b	27/8b	0.03
Family History Average scores (mean±SD)	0.7±0.48	0.5±0.51	1.6±1.5ab	1.6±1.36ab	< 0.0001
Insulin (µU/ml)	15.3±6.37	40.4±25.81a	14.5±12.28b	20.5±15.14b	< 0.0001
HOMA-IR	3.7±1.5	10.2±7.38 a	8.7±8.24a	12±11.07a	0.0021
HOMA-B%	164.4±94.7	395.1± 226 a	43.3±34.2ab	52.6±39.15ab	< 0.0001
HbA1c%	5.9±0.35	6.0±0.36	9.7±2.03ab	9.0±1.97ab	< 0.0001

All values were expressed as mean±SD
a Significant ($p < 0.05$) as compared with control group 1
b Significant ($p < 0.05$) as compared with control group 2
c Significant ($p < 0.05$) as compared with diabetic group 1

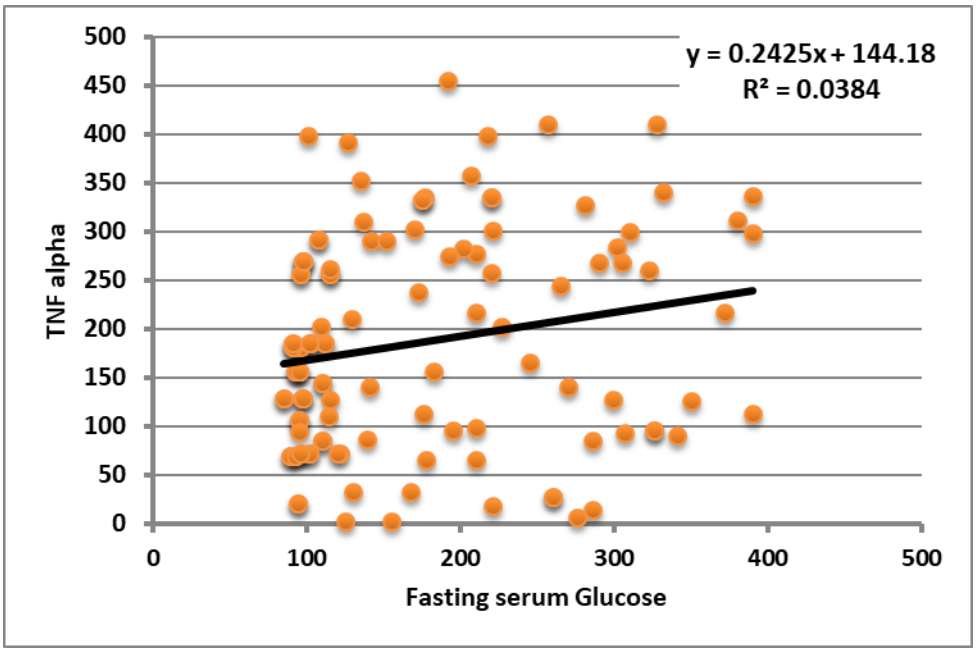

Figure 1. Correlation of TNF-α with fasting glucose of control and diabetic groups

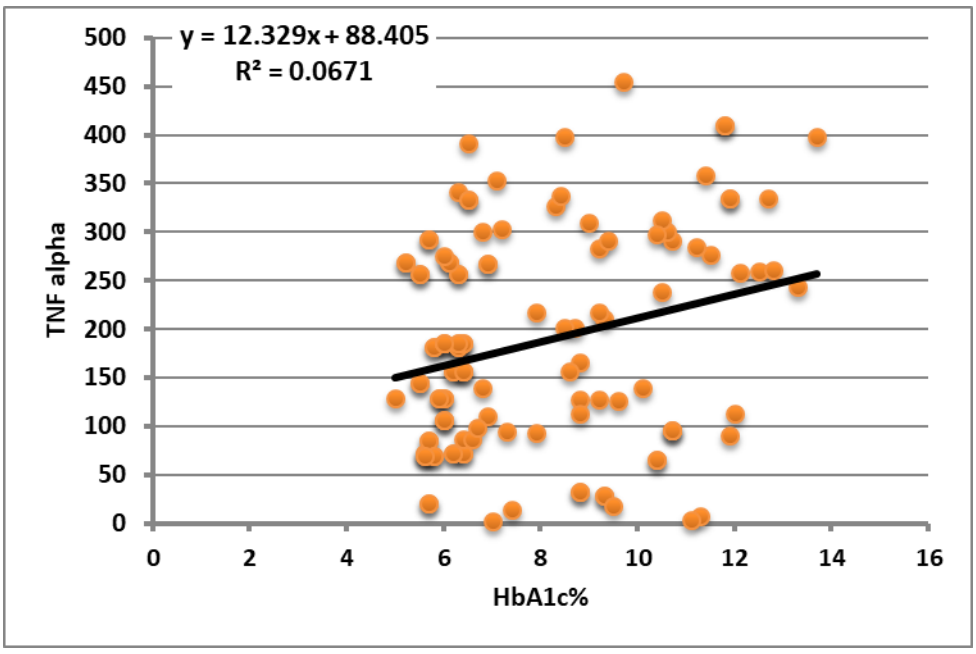

Figure 2. Correlation of TNF-α with HbA1c of control and diabetic groups

Discussion

The data of this study showed decreased levels of interleukin-6 and increased levels of TNF-α in type 2 diabetes mellitus compared to healthy controls. Decreased level of pro-inflammatory cytokine IL-6 was in agreement with Al-Shukaili et al. 2013 [6] who found that the levels of IL-6 were decreased in T2DM and were in disagreement with previous findings by Marques-Vidal et al. 2013 [7], Vidhate et al. 2013 [8], Al-Dahhan and Al-Dahhan 2015 [9],

those found that patients with T2DM had increased levels of IL-6 as compared to healthy control subjects and thus they suggest that lL-6 being a pro-inflammatory mediator might be responsible for some underline changes, which may contribute for the development of T2DM. This disagreement with the current study results of IL-6 could be attributed to the (I) duration of the disease (II) small sample size, and (III) the differences in age and sex of the studied groups Al-Shukaili et al. 2013 [6].

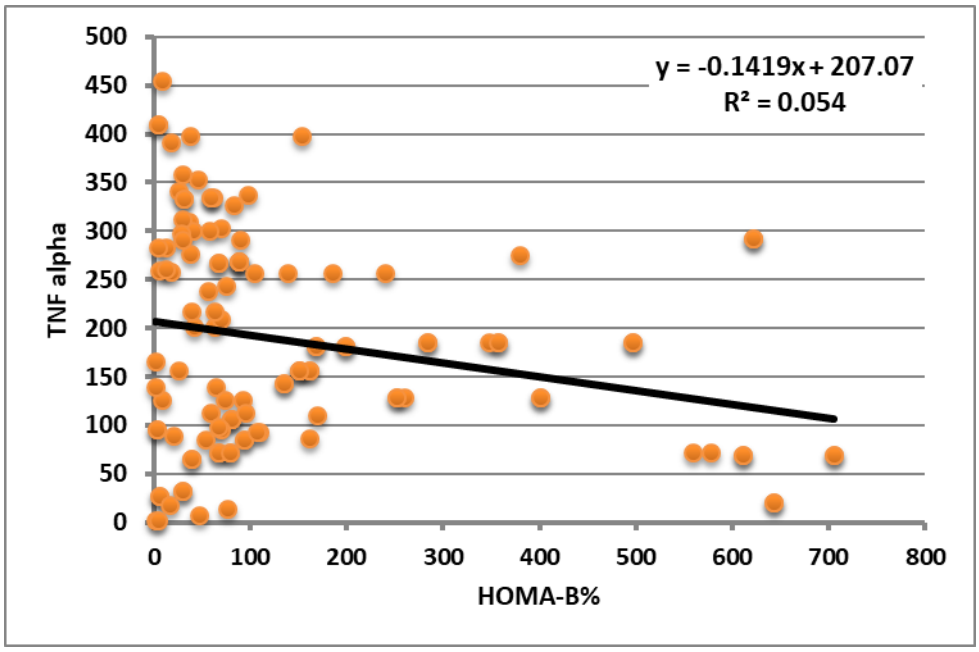

Figure 3. Correlation of TNF-α with HOMA-B% of control and diabetic groups

The role of IL-6 in insulin resistance is controversial with many findings suggesting a causative role in reduced glucose disposal, and others supporting the hypothesis that IL-6 might improve insulin sensitivity but the bulk of the previous findings suggest an insulin resistance inducing action of IL-6 [10].

Correlation and regression analysis of this study showed a significant negative correlation of IL-6 with fasting glucose, glycosylated hemoglobin and HOMA-IR and positive correlation with HOMA-B %, this may indicate a beneficial effect of IL-6 on insulin sensitivity and secretion in contrast to Hansen et al. 2010 [11] who found a significant positive correlation of IL-6 with HOMA-IR, fasting glucose and HbA1c indicating a negative effect on insulin sensitivity and glycemic control parameters and consistent with study done by Suzuki al. 2011 [12] who found that IL-6 acts directly on pancreatic β-cells and enhances glucose-stimulated insulin secretion.

The results of this study showed non-significant correlation between IL-6 and BMI of study population may be due to limited sample size, Darko et al. 2015 (13) observed a negative correlation between IL-6 and BMI of diabetic patients while Vozarova et al. 2001 [14] found positive correlation between them.

The current study revealed a significant elevation of TNF-α in diabetic patients as compared to controls consistent with Goyal al. 2012 [15] and Al-Dahhan and Al-Dahhan 2015 [9]; those observed an increase of TNF-α levels in T2DM patients as compared to controls. In obese individuals, the adipocytes enlarge, adipose tissue undergoes cellular alterations affecting systemic metabolism. First, increase in macrophage numbers in adipose tissue in obesity [16] where they apparently function to scavenge older adipocytes. Second, several

proinflammatory factors are produced in adipose tissue macrophages with obesity. In fact, almost all adipose tissue TNF-α expression originated from adipose tissue macrophages [17]. Concentrations of inflammatory markers such as TNF-α are also raised in diabetic patients. This finding has led to the proposal that elevated concentrations of pro-inflammatory cytokines may trigger much of the metabolic abnormalities due to obesity and diabetes mellitus. Therefore, it could be predicted that inflammation is thought to contribute to the development of insulin resistance, a significant outcome of obesity and is evident by studies describing role of TNF-α in mediating insulin resistance in obese patients [18].

Correlation and regression analysis of this study discovered a significant positive correlation between TNF-α and glycemic control parameters including fasting serum glucose and HbA1c indicating a probable role of TNF-α in impairment of insulin signaling and decrease cellular glucose uptake, which was consistent with Mahmoud et al. 2004 [19] and also there was a significant negative correlation of TNF-α with HOMA-B%, which indicate the inhibitory effect of TNF-α on islet β-cell secretory function consistent with Chen et al. 2007 [20]. The non-significant correlation of TNF-α with BMI and HOMA-IR might be due to limited sample size and was inconsistent with Swaroop et al. 2012 [21] who found a strong correlation between them indicating the association of elevated TNF-α with increasing adiposity and the role of pro-inflammatory cytokines in pathophysiology of insulin resistance. It has been demonstrated that TNF-α could inhibit insulin-responsive glucose uptake through a decrease in glucose transporter 4 gene transcription [22] and decrease tyrosine kinase activity of the insulin receptor [23].

This study concluded that the significant alterations in sera levels of IL-6 and TNF-α of diabetic patients as compared to controls were suggested a possible role of these pro-inflammatory cytokines with the presence of obesity, IR and T2DM and the assay and assessment of sera levels of IL-6 and TNF-α could be beneficial in early detection of T2DM and prevention of its unfavorable consequences especially the cardiovascular complications and atherosclerosis. It was still unclear whether altered inflammatory cytokines were a cause or compensatory mechanism to IR and T2DM.

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Author contribution

Dr. Fareed conceived and designed the study and preliminary analysis. Qasim collected, analyzed and interpreted the data and wrote the manuscript. Dr. Hassan did the statistical analysis of study.

Conflict of interest

None.

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Impact of Rapid Antigen Detection Tests in the Diagnosis of Streptococcal Tonsillopharyngitis in Children

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Abstract

Background Tonsillopharyngitis among children considered as a major public health problem in the community, because of post-streptococcal complications, cost, time consuming diagnostic tests and un-necessary antibiotics.

Objective To determine the validity of Rapid Antigen Detection Test (RADT) in the diagnosis of streptococcal tonsillopharyngitis in comparison with clinical and bacterial culture method.

Methods Throat swabs were taken from 214 children with tonsillopharyngitis in Baghdad from 20th November 2015 to 30th May 2016. Tonsillopharyngitis diagnosed by clinical, culture on blood agar and RADT.

Results *S. pyogenes* were recovered from 116 (54.2%) cases. *S. pyogenes* infection alone or with overgrowth of other bacteria is associated with recommendation for tonsillectomy, and indices for MCIsaac clinical prediction for Streptococcal pharyngitis. RADT detection of *S. pyogenes* revealed 83.62% sensitivity and 95.92% specificity. MCIsaac clinical prediction 89.66% sensitivity and 44.9% specificity. The combined MCIsaac-RADT algorithm had increased sensitivity with an increased point of score and specificity found to be inversely associated with points of score.

Conclusion For routine work, this study supports clinical screening with MCIsaac score for all children with tonsillopharyngitis and subsequent testing by Strep A RADT to facilitate the decision for antibiotic use.

Keywords Rapid antigen detection, streptococcus pyogenes, tonsillopharyngitis

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List of abbreviation: ASOT = Anti-streptolysin O antigen, GABHS = Group A ß Hemolytic Streptococci, RADT: Rapid Antigen Detection Test, RF = Rheumatic fever, RHD = Rheumatic heart disease

Introduction

Tonsillopharyngitis could be the most common reason among children attending primary health care centers [(1)]. It can be caused by wide spectrum of bacterial and viral pathogens [(2)]. Among them, Group A ß Hemolytic Streptococci (GABHS) or *Streptococcus pyogenes* (*S. pyogenes*) is the common cause of tonsillopharyngitis in 20-30% of children, which left untreated can cause rheumatic fever (RF) and rheumatic heart disease (RHD) [(3-5)].

Despite improvements in antimicrobial prescribing for tonsillopharyngitis among children, a substantial number of patients continue to receive inappropriate antibiotic therapy [(6,7)]. Therefore, physicians should be excluding the diagnosis of GABHS to prevent improper administration of antibiotics to large

numbers of patients. Such unnecessarily therapy exposes patients to several consequences to the development of antibiotic resistance among common pathogens [8].

Several diagnostic procedures have been applied for diagnosis of streptococcal tonsillopharyngitis worldwide, ranging from clinical, serological, conventional and molecular methods with varieties in their advantages and disadvantages [9-11]. Thus, high attentions have been paid worldwide for rapid and cost-effective method for proper diagnosis and effective antibiotic treatment were essential to prevent post-streptococcal complications [12-15]].

In Iraq, still difficult and time-consuming methods had been used in routine laboratory diagnosis of GABHS in health care centers and hospitals that associated with overuse/misuse of antibiotics from private pharmacies without physician consultation or definite diagnosis.

This study aimed testing rapid antigen detection test (RADT) as a valuable tool for diagnosis of GABHS in laboratory of Iraqi hospitals and health care centers to determine the diagnostic value of *S. pyogenes* by RADT in comparison with culture and clinical method.

Methods

Study design and population

A cross-sectional study was conducted on 214 children (3-18 years) suffering from tonsillopharyngitis at pediatric and ENT consultant clinic of (Al-Imamein Al-Kadhimein Medical City and Al-Zahra Primary Health Care Center during the period between (20th November 2015 to 30th May 2016). Inclusion criteria children from 3-18 years of age attending one of the ENT or pediatric specialist with evidence of fever, anterior tonsillar exudates or anterior cervical adenitis. Patients who had used antibiotics for the current illness were included in the study.

The study was approved by the Institutional Review Board of the College of Medicine, Al-Nahrain University (No.56, in 18/11/2015). Informed verbal consent for participation was obtained from the parents of the child.

Clinical pro-forma was completed for each participant who enter the study, in which socio-demographic and clinical features were recorded. Children were classified according to the McIsaac score [16], which comprises the following criteria: history of fever or temperature > 38 °C; absence of cough; tender anterior cervical adenopathy; tonsillar swelling or exudates; age ≤15 years scored +1 each, and age ≥ 45 years scored -1 While the age range between 15-45 years will be gives 0 score.

Sample collection and processing

Three throat swabs were taken from tonsillopharyngeal region, posterior pharyngeal wall and when patient permits, the swab rubbed with the rotation over one tonsillar area, then the arch of the soft palate and uvula, without touching the tongue or buccal surface to avoid contamination [17]. Then were processed as soon as possible for gram staining, culture on blood agar and RADT. The identification of *S. pyogenes* using RADT made by CerTest Strep A kit purchased from Certest Biotec® Spain. This test based on the qualitative immunochromatographic assay for the determination of Group A Streptococcal from throat swabs.

Statistical analysis

Data of the study sample were entered and analyzed by using statistical package for social sciences (SPSS) version 20. Descriptive statistics were presented as frequencies, proportion (%), means and standard deviation (SD). Chi-square test was used to estimate the association between two categorical variables. T-test was used to compare the actual difference between two means in relation to the variation in the data. Level of significance of < 0.05 was considered as significant.

Validity and predictability of different screening tests were assessed in relation to gold standard test by calculating sensitivity,

specificity, predictive value of positive and negative test results.

Results

Identification of microbial causes tonsillopharyngitis

The results of current study showed no significant association between *S. pyogenes* infection and sociodemographic characteristics of patients with tonsillopharyngitis like: age, gender, residence, maternal education, parent's occupation and crowding index. On the other hand, single *S. pyogenes* infection among tonsillopharyngitis patients was significantly associated with some clinical signs like: presence of exudate, tenderness of lymph nodes, body temperature above 38.5 °C and modified centor score (MCIsaac score). Recommendation for tonsillectomy was significantly associated with mixed *S. pyogenes* infection (Table 1).

Table 1. Association between *S. pyogenes* infection with demographic and clinical characteristics of tonsillopharyngitis patients

***S. pyogenes* (Culture)**		**Total**	**Positive**	**%**	**P value**
Duration of illness (days)	≤ 7 days	100	55	55.0%	0.981 NS
	> 7 days	114	61	53.51%	
Family history of tonsillopharyngitis	Yes	133	74	55.6%	0.590 NS
	No	81	42	51.9%	
Recurrent	Yes	169	96	56.8%	0.139 NS
	No	45	20	44.4%	
Antibiotic use	Yes	114	57	50.0%	0.187 NS
	No	100	59	59.0%	
Tonsillectomy	Yes	6	3	50.0%	0.834 NS
	No	208	113	54.3%	
Recommended for tonsillectomy	Yes	115	85	73.91%	<0.001**
	No	93	28	30.1%	
Exudate	Yes	147	95	64.6%	<0.001**
	No	67	21	31.3%	
Swelling	Yes	208	113	54.3%	0.834 NS
	No	6	3	50.0%	
Tenderness	Yes	141	88	62.4%	0.001**
	No	73	28	38.4%	
Cough	Yes	147	84	57.1%	0.201 NS
	No	67	32	47.8%	
Temp. over 38.5°C	<38.5	58	20	34.5%	<0.001**
	≥38.5	156	96	61.5%	
McIsaac score	Score 0	12	3	25.0%	<0.001**
	Score 1	19	3	15.79%	
	Score 2	25	6	24.0%	
	Score 3	37	11	29.73%	
	Score 4	62	40	64.52%	
	Score 5	59	53	89.83%	
Total		214	116	54.21%	

NS: None statistical significance (p>0.05).
**: Highly statistical significance (p≤0.001).

According to the culture characteristics and EPI 20 tests, *S. pyogenes* was isolated from 116/214 (54.2%) cases. *S. pyogenes* detected in 101/214 (47.2%) using Certest RADT (Table 2).

Validity of RADT and modified Centor score (MCIsaac) for detection of *S. pyogenes* infection were estimated in relation to the gold standard method (culture on blood agar). Regarding sensitivity, MCIsaac score (89.66%) and the lowest was RADT (83.62%). On the other hand, RADT was highly specific (95.92%), while, MCIsaac score had lower specificity (75.51% and 44.9% respectively). Similarly, RADT had the highest positive predictive value (96.04%) while, MCIsaac score shoed lowest PPV (65.82%) the lowest was MCIsaac score (78.57%) (Table 2). The results showed the validity of combined MCIsaac score and RADT (Table 3) in predicting *S. pyogenes* infection. The highest sensitivity had been increased with the increasing number of score in predicting *S. pyogenes* infection. Inversely, the specificity decreased by increasing the number of MCIsaac score.

Table 2. Diagnostic performance of different methods in detection of S. pyogenes

S. pyogenes		Culture		Total	Sensitivity	Specificity	PPV	NPV
		Positive	Negative					
RADT	Positive	97	4	101	83.62 (75.83-89.26)	95.92 (89.97-98.4)	96.04 (90.26-98.45)	83.19 (75.23-88.96)
	%	83.60	4.10	47.20				
	Negative	19	94	113				
	%	16.40	95.90	52.80				
MCIsaac score	Predict *S. pyogenes*	104	54	158	89.66 (82.79-93.98)	44.9 (35.43-54.75)	65.82 (58.13-72.76)	78.57 (66.18-87.29)
		89.66	55.10	73.83				
	Does not predict *S. pyogenes*	12	44	56				
		10.34	44.90	26.17				
Total		116 (54.04%)	98 (45.096%)	214				

PPV: positive predictive value. NPV: Negative predictive value.

Discussion

This study developed combined clinical and rapid point of care testing prediction guidelines in Iraqi primary health care centers and hospitals for improving clinical diagnosis of GABHS tonsillopharyngitis and prevention of post-streptococcal complications.

According to the results, the culture method reported a relatively high rate of isolation for GABHS among children with tonsillopharyngitis (116/214) 54.2%. This comes with agreement with Abd Al-kareem et al. 2014 whom reported comparable rate 142/376 (37.7%) [18]. Lower rate of *S. pyogenes* infection was also reported by Yousef et al., 2010 16/70 (22.9%) [19]. Unfortunately, few Iraqi studies were done on children with tonsillopharyngitis, or they utilize other methods for detection of *S. pyogenes* like Anti-streptolycin O antigen (ASOT) serum titer [20,21], or the study population were restricted to pharyngitis cases after tonsillectomy ([22,23]. Other studies restrict their objectives to investigate molecular aspects like multidrug resistance genes [24,25].

Rate of *S. pyogenes* isolation by culturing on blood agar like some Arabic studies Abu-Sabaah & Ghazi, 2006, in Saudi Arabia (19.4%) [26], Fourati et al. 2009, in Tunisia (20.2%) [27], Rimoin et al. 2010, in Egypt (24.8%) [28], and other global studies like Enright et al. 2011, in Scotland (21.5%) [29], Forward et al. 2006, in Canada (24.1%) [30], Subashini et al. 2015 in India (24.3%) [31], Altun et al. 2015, in Turkey (24.7%) [32], Buchbinder et al. 2007, in France (26.4%) [33], Rogo et al. 2010, in USA using Quickvue test kit (28.5%) [34], Tanz et al. 2009,

in Chicago (30%) [35], Finger et al. 1999, in Vietnam (30.5%) [36], Pitetti et al. 1998, in Russia (31.3%) [37], Flores Mateo et al. 2010, in Spain (34.1%) [38], Camurdan et al. 2008, in Turkey (38.1%) [39], Omurzakova et al. 2010, in Kyrgyzstan (40%) [40], Comparable results were reported by Sheeler et al. 2001 in New York, (54%) [41] and higher rate of *S. pyogenes* isolation reported by Kim, 2009, in Korea (66.5%) [42].

Table 3. Combined MCIsaac score and RADT diagnostic algorithm performance in detection of *S. pyogenes* infection

Score		Culture		Total	Sensitivity	Specificity	PPV	NPV
		Positive	Negative					
Score 0	RADT positive	0	0	0	0 (0-56)	100 (70-100)	0 (0-100)	75 (47-100)
	RADT negative	3	9	12				
Score 1	RADT positive	1	0	1	33.34 (1.7-88.2)	100 (80.6-100)	100 (5.1-100)	89 (67-98)
	RADT negative	2	16	18				
Score 2	RADT positive	3	0	3	50 (19-81)	100 (83.2-100)	100 (44-100)	86.4 (67-95.3)
	RADT negative	3	19	22				
Score 3	RADT positive	7	1	8	64 (35.4-85)	96.2 (81-100)	87.5 (53-99.4)	86.2 (70-94.5)
	RADT negative	4	25	29				
Score 4	RADT positive	38	2	40	95 (83.5-99)	91 (72.2-98.4)	95 (83.5-99)	91 (72.2-98.4)
	RADT negative	2	20	22				
Score 5	RADT positive	48	1	49	90.6 (79.6-96)	83 (44-99.2)	98 (89.3-100)	50 (24-76)
	RADT negative	5	5	10				
Total	RADT positive	97	4	101				
	RADT negative	19	94	113				

PPV: positive predictive value. NPV: Negative predictive value.

However, several broad studies reported lower Our results highlight the importance of GABHS as a potential human pathogen causing tonsillopharyngitis among Iraqi children. Furthermore, the incidence of *S. pyogenes* was the same in boys (55.6%) and girls (52.2%), affecting and no statistical significant association with any sociodemographic characteristics. In addition to that, no association have been reported with duration of illness or wither previously or recurrent infected. The incidence of S. pyogenes was associated among children whom recommended for tonsillectomy (73.9%), and clinical indices for MCIsaac clinical score like presence of exudate (64.6%), tenderness of cervical lymph nodes (62.4%), and body temperature over 38.5 (61.5%).

In clinical setting, MCIssac scoring indices and score failed to differentiate between Streptococcal and non-streptococcal bacterial tonsillopharyngitis, which is meaningfully assumed that MCIsaac clinical score is sensitive to diagnose bacterial tonsillopharyngitis. This finding is supported by Steinhoff et al. 1997 [43], Bisno et al. 2002 [44], Martin & Green 2006 [45], Ba-Saddik et al. 2014 [46] whom reported that clinical assessment of streptococcal pharyngitis is unreliable method because of their indices were none specifically present in most bacterial tonsillopharyngitis. Furthermore, MCIsaac clinical score could not be employed to stratify children for immediate antibiotic treatment and suggesting a higher need for rapid and specific method for diagnosis of streptococcal pharyngitis.

According to the RADT, a higher rate of *S. pyogenes* antigen was detected 101/214 (47.2%). In comparison with gold standard method (culture on blood agar), the RADT showed 83.62% sensitivity and 95.92% specificity. These values come in agreement with several Arabic and global studies (summarized in table 4). The higher specificity of the Certest kit attributed to that kit was designed to detect carbohydrate (CHO) antigens of streptococcus pyogenes, upon that several clinical practice guidelines have been recommended that all positive cases by RADT testing should not be cultured.

Table 4. Rate of streptococcus pyogenes infection according to references diagnosed by culture on blood agar and rapid antigen detection test

Study number	Country (Ref)	Culture	RADT
1	Canada [30]	24.1	21.0
2	New York [41]	54	46.0
3	Vietnam [36]	30.5	27.0
4	Russia [37]	31.3	27.0
5	Egypt [28]	24.8	26.4
6	Turkey [32]	24.7	20.4
7	Kyrgyzstan [40]	40	36.2
8	Turkey [39]	38.1	36.2
9	Scotland [29]	21.5	16.38
10	Spain [38]	34.1	45.0
11	Chicago [35]	30	21.3
12	Saudi Arabia [26]	19.4	23.5
13	France [33]	26.4	33.8
14	Tunisia [27]	20.2	24.7
15	India [31]	24.3	13.5
16	USA (Acceava) [47]	39.3	39.8
17	Korea [48]	66.5	66.5

RADT: Rapid antigen detection test.

Giving the advantages of rapid test (Point of care test), increase the number of appropriately treated child's with tonsillopharyngitis, not only for avoiding unnecessary treatment with antibiotics but also avoiding the post streptococcal complications by treating those with positive *S. pyogenes* results [47]. Furthermore, point of care testing for S. pyogenes appears to reduce long-term costs by lowering the incidence of post-streptococcal complications especially rheumatic fever and post-streptococcal glomerulonephritis.

A number of commercial kits have been marketed and evaluated globally for detection of *S. pyogenes* antigen from throat swabs were reviewed and used in statistical analysis (in addition to our results) for documentation of clinical utility of RADT to be used in clinical practice. However, the clinical utility score was 91.2% as an excellent for screening and good for case finding in clinical practice. With positive likelihood ratio 14.82. The authors believe this is the first study is that document the clinical utility of RADT for *S. pyogenes* among children with tonsillopharyngitis.

Many clinical diagnostic rules have been applied for identification of streptococcal tonsillopharyngitis since 1970 [44,48-53]. We used MCIsaac clinical score in this study to identify ability to define cases caused by *S. pyogenes*. However, its diagnostic performance showed

low positive predictive value (65.8%) and low specificity (44.9%) and without microbiological testing, is in accurate testing method and have wrong prediction for proper antibiotic prescription.

Several clinical prediction criteria have been mentioned in the literatures. The majority of them were generated based on adults based on clinical and epidemiological variables. They varied in their applications according to populations. Some of the original clinical prediction rules including those by Centor, Breese, and Wald, although originally developed among adult populations, have been validated and modified in different age and geographic populations [16,48,49,52]. Centor clinical scoring system is the best clinical prediction rule, based on presence of tonsillar exudate, swollen/tender cervical lymph nodes, absence of cough, and fever > 38.5 °C [5,60].

In the effort to make the diagnosis of streptococcal tonsillopharyngitis simple for use and selective diagnosis based on clinical findings, this study established combined clinical score and RADT results, in order to better predict infection. By another mean, the study claimed to introduce a simple point of care test method to increase the specificity of MCIsaac clinical score.

In this study, sensitivity, specificity, positive and negative predictive of RADT (Ceretest) where more the clinical score.

Conclusions

For routine work, our study supports clinical screening with MCIsaaq score for all children with tonsillo-pharyngitis and subsequent testing by Strep A RADT to facilitate the decision for antibiotic use.

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Author contribution

Hadi was responsible for sampling and experimental works. Dr. Ghazi works concept design and statistical analysis of study. Dr. Abdulla contributed by scientific advices and comprehensive editing and interpretation of data.

Conflict of interest

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Molecular Detection of Multidrug Resistant *Acinetobacter baumannii* from Different Clinical Samples

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Abstract

Background *Acinetobacter baumannii* (*A. baumanii*) has recently emerged as a major pathogen causing nosocomial infections in patients admitted to intensive care units with a surprisingly rapid acquisition of antibiotic resistance.

Objective To study the rate of occurrence of *A. baumanii* isolated from different clinical specimens and to study the rate of occurrence of multidrug resistance especially NDM-1 and qnrA genes.

Methods A total of sixty-two (62) clinical isolates of *A. baumanii* were tested against 14 antibiotics by disc diffusion method. Minimum inhibitory concentration was determined by agar dilution method for resistant isolates. Polymerase chain reaction (PCR) was performed to detect bla NDM-1 and qnrA genes.

Results All Acinetobacter isolates were complete resistant to Colistin and Tigacycline (100%), while high rate of resistance was to Aztronem (93.54%), Cefotaxime, (91.93%), Ceftriaxone (88.70%) and Meropinem (80.64%). Moderate - to- low rate of resistance was to Ceftazidime (77.41%), Cefepim (75.80%), Peperacillin and Ciprofloxacin (74.19%), Gentamicin (69.35%), Levofloxacin (64.51%), Amikacin (61.29%) and Impenim (50%). The highest minimum inhibitory concentration value 128 µg/mL was to Cefotaxime, Tigacycline and Colistin. While the lowest value 8 µg/mL was to Gentamicin, Imipenem, Ciprofloxacin and Levofloxacin. The PCR results showed that 50% of Metallo beta lactamase producers *A. baumannii* was carried bla NDM-1 gene in chromsomal DNA and 24 (48%) of Flouroquinolone resistance *A. baumannii* harbored qnrA gene in chromsomal DNA while the prevalence of qnrA gene was (60.6%) in plasmid DNA.

Conclusion There is a high prevalence of multidrug resistant *A. baumannii* in different samples from Baghdad with the high prevalence of bla NDM-1 and qnrA genes among this bacterium.

Keywords *Acinetobacter baumannii*, bla NDM-1, qnrA- gene, MDR

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List of abbreviation: bla NDM-1 = New Delhi Metallo beta-lactamase, MDR: Multi-drug resistance, qnrA = Quinolone resistance

Introduction

Acinetobacter baumannii (*A. baumanii*) is a gram-negative, non-lactose fermenting organism that is increasingly recognized as a major opportunistic pathogen causing nosocomial infections [(1)]. The organism is characterized by its tendency to acquire resistance to multiple classes of antimicrobials; including carbapenems, aminoglycosides, and fluoroquinolones [(2)]. Identified New Delhi MBL-1 (NDM-1) is a new type of carbapenemase belongs to the class B of Ambler β-lactamases

[3]. It was first reported in *Klebsiella pneumoniae* and *Escherichia coli* derived from a Swedish patient of Indian origin who was admitted to hospital in New Delhi, India in 2009 [4]. Carbapenem resistance caused by acquiring the Metallo-beta-lactamases (MBL) is considered to be more serious than other resistance mechanisms because MBLs can almost hydrolyse all beta-lactam antibiotics except monobactams [5].

Plasmid-mediated quinolone resistance (PMQR) genes, such as qnr family, can be horizontally transferred and contribute to reduced susceptibility to fluoroquinolones [6,7]. The qnr genes appear to be acquired from chromosomal genes in aquatic bacteria [8]. The gene was cloned and was found to produce a 219-aa protein belonging to the pentapeptide repeat family [9], members of which are involved in protein-protein interactions. Purified qnr protein was shown to bind to and protect both DNA gyrase and topoisomerase IV from inhibition by ciprofloxacin [9,10].

This study aimed to investigate the rate of occurrence of *A. baumanii* isolated from different clinical specimens and their resistance to different classes of antibiotics including Carbapenemes and Fluoroquinolone with the detection of (NDM-1) and (qnr) genes in this bacterium.

Methods

Clinical isolates

This study was conducted during the period from the first of November 2015 to the end of April 2016. Two hundred (200) of different clinical specimens include blood, urine, wound swabs, cerebrospinal fluid and sputum were collected from patients in Al-Imamein Al-Kadhimein Medical City in Baghdad and Child Protection Hospital. One hundred and thirty-two (132) swabs were obtained from injured soldiers and diabetic foot. The isolates were identified by conventional and biochemical methods.

Antibiotic susceptibility testing

Sixty-two of *A. baumannii* isolates were tested for their susceptibility to fourteen antimicrobial agents including; Amickacin (30 µg), Cefotaxime (30 µg), Ceftriaxone (30 µg) Ceftazidime (30 µg), Aztreonam (30 µg), Ciprofloxacin (10 µg), Cefepime (30 µg), Colistin sulphate (25 µg), Imipenem (10 µg), Gentamicin (30 µg), Meropenem (10 µg), Piperacillin (100 µg), Tigacyclin (15 µg), and Levofloxacin (5 µg) in accordance to Clinical and Laboratory Standards Institute (CLSI) 2014 recommendations [11].

Minimal inhibitory concentrations

The minimum inhibitory concentration was determined for *A. baumannii* by Vitik2-System and standard agar dilution method [12] according to the CLSI (2014) [11]. The antibiotic concentrations ranged from (0.125-128) µg/ml.

DNA Extraction

Chromosomal DNA were extracted using Genomic DNA extraction kit (Promega, USA), and analyzed on 1.5% agarose gel.

Plasmid Extraction

Rapid boiling method was used to extract plasmid DNA from clinical isolates according to Reischl et al. 2000 [13].

Molecular detection of bla NDM-1 gene and qnrA gene from *A. baumannii* isolates using PCR technique

Specific primer of NDM-1 gene was used as forward (5'-GGGCAGTCGCTTCCAACGGT) and reverse (5'-GTAGTGCTCAGTGTCGGCAT) [14]. While the qnrA specific primer forward was (5'-GATAAAGTTTTTCAGCAAGAGG) and reverse was (5'-ATCCAGATCGGCAAAGGTTA) [15] and Tri-phosphate isomerase genes were used as forward (5'-AAAGAAGCTACTAAGGGTACAAA) and reverse (5'-CATAATATTGGGTCTATTCCTAC) [16] that produce 475 bp, 593 bp and 230 bp respectively.

Twenty microliter of PCR mixture composed of 5 µl of PCR Master Mix (PCR Buffer, $MgCl_2$, dNTPs and Taq DNA polymerase) (Promiga,

USA), 2 µl of each 2 µM forward and reverse primer sequences (Alfa- DNA, Canada), 4 µl volume of chromosomal DNA sample, and nuclease free water to complete the volume to 20 µl. Internal control Triphosphate isomerase (tpi) 230 bp housekeeper gene was used as a positive control for compare with appositive results. The reaction was carried in Thermal cycler (Cleaver Scientific Thermal Cyclers-TC32/80, UK). PCR cycling program for detection of bla NDM, qnrA and tpi genes were showed in tables (1, 2 and 3) respectively. Of each amplified DNA, 10 µl were analyzed by electrophoresis in a 1.5% agarose gel at 70 V for 1 h in 1X Tris borate (TBE) containing 2.5% ethidium bromide. The samples were run alongside with 100 bp ladder (Promiga, USA) as molecular weight marker. The bands were visualized using UV Tran-illuminator (LKB, Sweden).

Table 1. PCR program for detection of bla NDM genes Amplification by thermal cycler

Steps	Temperature °C	Time	Cycles
Initial Denaturation	95 °C	5 minutes	1
Denaturation	95 °C	30 seconds	
Annealing	56 °C	30 seconds	40
Extension	72 °C	1 minute	
Final Extension	72 °C	15 minutes	1

Table 2. PCR program for detection of qnrA genes Amplification by thermal cycler

Steps	Temperature °C	Time	Cycles
Initial Denaturation	94 °C	10 minutes	1
Denaturation	94 °C	1 minute	
Annealing	57 °C	30 seconds	30
Extension	72 °C	1 minute	
Final Extension	72 °C	10 minutes	1

Table 3: PCR program for detection of tpi genes amplified by thermal cycler

Steps	Temperature °C	Time	Cycles
Initial Denaturation	95 °C	3 minutes	1
Denaturation	95 °C	30 seconds	
Annealing	60 °C	30 seconds	40
Extension	72 °C	30 seconds	
Final Extension	72 °C	10 minutes	1

Results

All Acinetobacter isolates were complete resistant to Colistin and Tigacycline 100%, while the high rate of resistance showed to Aztronem (93.54%), Cefotaxime (91.93%), Ceftriaxone (88.7%) and Meropinem (80.64%). Moderate-to-low rate of resistance to Ceftazidime (77.41%), Cefepim (75.80%), Peperacillin and Ciprofloxacin) 74.19%), Gentamicin (69.35%), Levofloxacin (64.51%), Amikacin (61.29%) and Impenim (50%) (Table 4).

The minimum inhibitory concentration (MIC) to detect the level of resistance was for Colitin and Tigacycline are ranged from (64-128 µg/ml), Cefotaxime (128 µg/ml), Gentamicin (8-

16 µg/ml), Ceftazidime (32-64 µg/ml), Ceftriaxone and Cefepim (16-64 µg/mL), Amikacin (62-125 µg/ml), Ciprofloxacin (2-8 µg/ml), Levofloxacin (4-8 µg/ml), Imipenem (8-16 µg/mL), Pipracillin (64- ≥128 µg/mL) and Meropenem (64-128 µg/ml).

By PCR essay, the amplified products of sequences of bla (NDM-1 gene) in chromosomal DNA, (qnrA gene) in both genomic and plasmid DNA and housekeeper gene (tpi) were of size 475 bp, 593bp and 230bp respectively (Figures 1, 2 and 3).

Table 4. Numbers and percentages of susceptibility of different isolates of *A. baumannii*

AB (14)	Blood (30)		Urine (6)		CSF (7)		Wound (15)		Sputum (4)		Total (62)	%	
	No	%	No	%	No	%	No	%	No	%		R*	S*
IMP	11	36.6	3	50	7	100	9	60	1	25	31	50	50
CTX	25	83.3	6	100	7	100	15	100	4	100	57	91.93	8.06
CAZ	20	66.6	5	83.3	7	100	12	80	4	100	48	77.41	22.58
CIP	20	66.6	3	50	7	100	12	80	4	100	46	74.19	25.80
LEV	20	66.6	3	50	5	71.4	8	53.3	4	100	40	64.51	35.48
PRL	21	70	5	83.3	6	85.7	13	86.6	4	100	49	74.19	25.80
GEN	19	63.3	4	66,6	7	100	9	60	4	100	43	69.35	30.64
FEP	20	66.6	5	83.6	6	85.7	12	80	4	100	47	75.80	24.19
CO	30	100	6	100	7	100	15	100	4	100	62	100	0
TG	30	100	6	100	7	100	15	100	4	100	62	100	0
MEM	25	83.3	4	66.6	6	85.7	11	73.3	4	100	50	80.64	19.35
Ak	18	60	2	33.3	6	85.7	8	53.3	4	100	38	61.29	38.70
AT	26	86.6	6	100	7	100	15	100	4	100	58	93.54	6.45
CTR	23	76.6	6	100	7	100	15	100	4	100	55	88.70	11.29

AT, Aztronem; CTX, Cefotaxime; CRT, Ceftriaxone; CAZ, Ceftazidime; CIP, Ciprofloxacin; LEV, Levofloxacin; TG, Tigacycline; PRL, Peperacillin; CO, Colistin; GEN, Gentamicin; Ak, Amikacin; FEP, Cefepim; IMP, Imipenem; MEM, Meropinem, R*, resistance; S*, sensitive

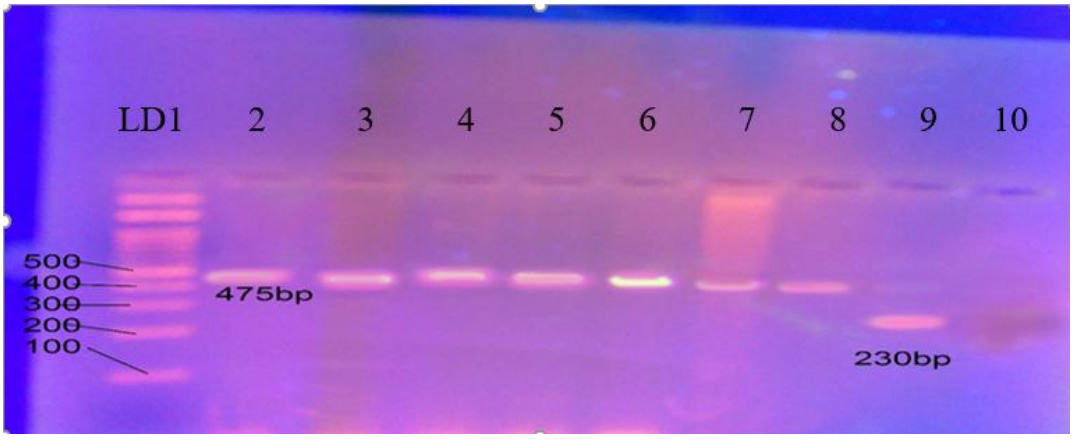

Figure 1. Gel electrophoresis of amplified PCR product (475 bp) for bla NDM-1 gene. Lane 1: 100 bp ladder. Lanes 2-8: Clinical isolates showing positive result. Lane 9: Internal control (tri–ph. iso.230 bp). Lane 10: Negative control. (1.5% agarose, 7 v/cm^2, 1.5 hrs)

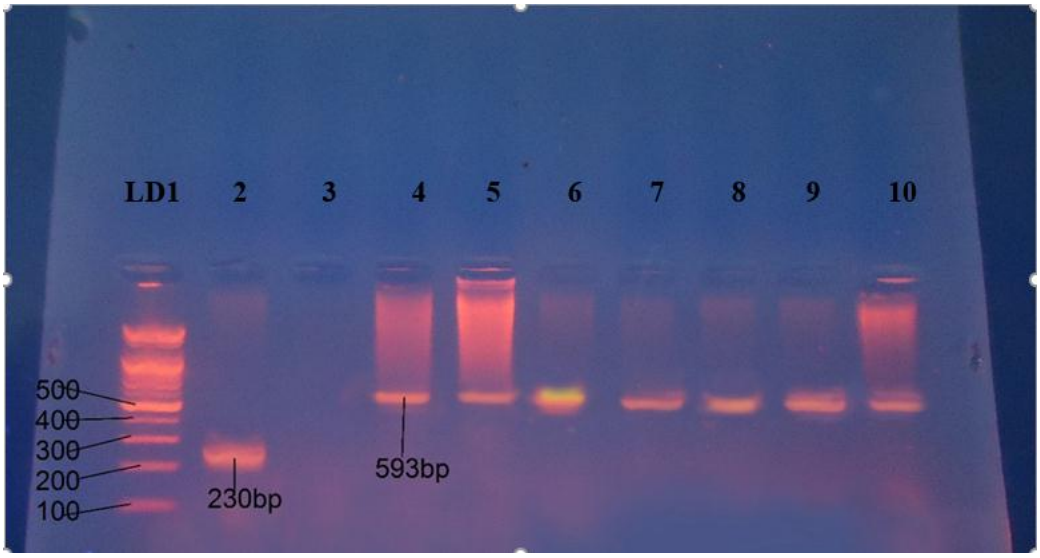

Figure 2. Gel electrophoresis of Amplified qnrA gene (593 bp) in genomic DNA using PCR with specific primers. Lane 1: 100 bp ladder. Lane 2: Positive control (tri-phosphotase 230 bp). Lane 3: Negative control. Lanes 4-10: Clinical isolates showing positive result. (1.5% agarose, 7 v/cm^2, 1.5 hrs)

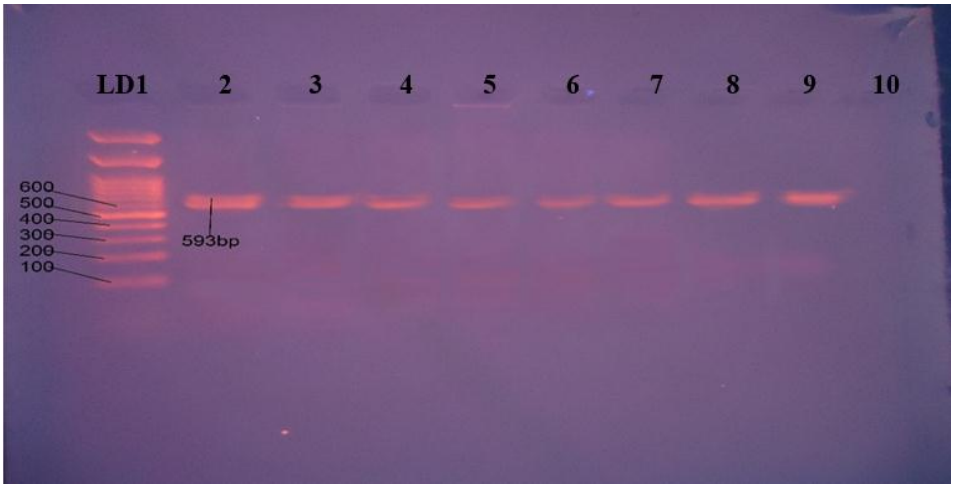

Figure 3: Gel electrophoresis of Amplified plasmid DNA for qnrA gene (593bp) using PCR with specific primers. Lane 1: 100 bp ladder. Lane 2-9: Clinical isolates showing positive result Lane 10: Negative control. (1% agarose, 7 v/cm^2, 1.5 hrs)

The present study also showed that the prevalence of bla NDM-1 was 25(50%) in chromosomal DNA, while the prevalence of qnrA gene was 24(48%) in chromosomal DNA and 20 (60.60%) in plasmid DNA (Table 5).

The presence of bla NDM-1 genes (48.0%) in *A. baumannii* was associated with high percentage of resistance to Carbapenems (Imipenim (53.6%) and Meropenim (60%). Also, the presence of qnrA gene (46.0%) in *A. baumannii* association with high percentage of resistance to Fluoroquinolones group (Ciprofloxacin (64.7%) and Levofloxacillin (60.7%) (Tables 6 and 7).

Table 5. The prevalence of bla (NDM-1 gene) and qnrA gene in Acinetobacter isolates

PCR-Results	NDM-1 gene (DNA)	qnrA gene (DNA)	qnrA gene (plasmid)
Positive No. (%)	25 (50.0%)	24 (48.0%)	20 (60.6%)
Negative No. (%)	25 (50.0%)	26 (52.0%)	13 (39.4%)
Total(No)	50	50	33

Table 6. Association between, Imipenim and Meropenim resistance and production of Metallo-β-lactamase (NDM-1gene) by Acinetobacter isolates

bla NDM-1 gene	Imipenem (10 µg)			Meropenem (10 µg)		
	R	S	Total	R	S	Total
Positive (No)%	15 (53.6%)	7 (36.8%)	22	24 (60.0%)	0 (0)	24
Negative (No)%	13 (46.4%)	12 (63.2%)	25	16 (40.0%)	10 (100%)	26
Total	28	19	47	40	10	50
P value		0.424			0.001*	

*Significant association (0.001)

Table 7. Association between, Ciprofloxacin and Levofloxacillin resistance with presence of qnrA-gene in Acinetobacter isolates

qnrA gene	Ciprofloxacin (5 µg)			Levofloxacin (5 µg)		
	R	S	Total	R	S	Total
Positive (No)%	22 (64.7%)	0 (0)	22	17 (60.7%)	0 (0%)	17
Negative (No)%	12 (35.3%)	13 (100%)	25	11 (39.3%)	15 (100%)	26
Total	34	13	47	28	15	43
P value		0.001*			0.001*	

*Significant association (0.001)

Discussion

The present study observed that 100% of the isolates of *A. baumannii* are multidrug resistant (MDR) when tested by standard disk diffusion method. The most prominent results of antibiotic sensitivity showed that this bacterium is completely resistant to Colistin and Tigacycline 100%. A study in Iraq done by Al-Samaree and Al-Khafaji in 2016 [17], they reported that, 30% of isolates resistant to Colistin. However, the current results are not in line with study in Thailand (2014), they found that *A. baumannii* isolates were susceptible to Colistin (97%) [18].

Modification of lipopolysaccharide outer membrane by addition of phosphor ethanol amine to the hepta-acylated lipid A structure has been suggested as a major mechanism of colistin resistance in *A. baumannii* [19-21]. While the resistance to Tigecycline due to over expression of a multi-drug efflux pump in *A. baumannii* [22]. In this study, a very high percentage resistance of *A. baumannii* to Aztronem. Also, similar findings showed by Al-Saleem 2013 [23] who found that *A. baumannii* clinical isolates developed 97.3% of resistance to Aztreonam and Ceftriaxone, 89.5% to Ceftazidime, 58.2% to Imipenem and Meropenem. Moreover, Al-Mashhadani in 2010 [24] studied the same bacterium revealed that, resistance percentage to Cefotaxime, Ceftazidime and Ceftriaxone were 100%. Carbapenem class represented by Imipenem and Meropenem. The current study was agreed with other study in Iraq; Al-Marjani in 2013 [25]

who found that a high level of resistance reached to 88.2% for Meropenem and 52.9% for Imipenem, with a resistance rate reached to 100% for Ceftriaxone, Cefepime and Azteronam, but not identical with the results obtained by Shareek et al. 2012 [26] whom found that (12.2%) of isolates were resistant to Cefotaxime.

Resistance to beta-lactams due to the presence of β-lactamases, which is the most prevailing mechanism of β-lactam resistance. These enzymes, at least partially, hydrolyze carbapenems along with other β -lactams [27]. Lately a new extended spectrum AmpC enzyme was identified *in A. baumannii*, which able to hydrolyze Ceftazidime, Cefepime and Aztroenam [28]. In regard to Fluoroquinolone resistance of *A. baumannii* is often caused by modifications in the structure of DNA gyrase secondary to mutations in the quinolone resistance-determining regions of the gyrA and parC genes [29,30]. These changes result in a lower affinity for the binding of the quinolone to the enzyme-DNA complex, a second mechanism of resistance to the quinolones is mediated by efflux systems that decrease intracellular drug accumulation [31]. In present study, the resistance of *A. baumannii* to Aminoglycoside is probably attributed to the fact that most clinical isolates of A. baumannii associated with Aminoglycoside-modifying enzymes or efflux pump mechanisms [32].

In the current study, these results are very close to study Done by Shali in 2012 [33] whom showed that MIC to Imipenem was ≥ 16, about 90% were resistant to Pipracillin/Tazobactam (MIC ≥ 128) whereas 85.71% of the strains showed resistance to Cefotaxim (MIC 16 - ≥ 64), Ceftazidime and Amikacin (MIC ≥ 64), but varied in Gentamicin (MIC ≥ 64) and Ciprofloxacin (64-320). However, this result disagreed with done by Al-Marjani in 2013 [25] whom found that *A. baumannii* isolates had MIC > 512 µg/ml for Cefotaxime. The current results showed that approximately 50% of *A. baumannii* have NDM-1 gene 50% in chromosomal DNA the percentage of NDM-1 gene in the current study was double of those in Egypt [34]. The present result was agreed with study done by Al-Harmoosh in 2015 whom found that (20%) of *A. baumannii* were harbored bla NDM-1 gene and (40%) bla NDM-2 [35].

A study on the dissemination of NDM-1 producing *A. baumannii* in Europe showed that the five isolates from Germany, France, Slovenia and Switzerland, which indicates a spread of NDM-producing clones in Europe [36]. Sixty percent (60%) Carbapenem-resistant *A. baumannii* strains isolated in 2012 in the Tripoli Government Hospital, Lebanon, from civilians wounded during the Syrian war (5.71%) were positive to NDM-1genes from Syrian isolates) and 1.42% (1 Lebanese isolate) [37]. However, two NDM-1-producing *Klebsiella pneumoniae* imported from Iraq were detected in 2010 [38].

A study done by Shali in 2012 [33] who detected that blaOXA-23-like in all of 21 strains of *A. baumannii* and resistant percentages for all isolates were recorded; highest resistant rate was against ampicillin (100%) while lowest rate was against imipenem (57.1%). The MICs of imipenem for the resistant isolates were ≥16. All isolates show multi drug resistance to different antibiotics. The bla NDM-1 positive bacteria have been disseminated worldwide [39]. This study noted, the presence of bla NDM-1 genes (48.0%) in *A. baumannii* was associated with high percentage of resistance to Carbapenems (Imipenim (53.6%) and Meropenim (60%)). The bla NDM-1 genes responsible for Carpapenems resistance by encoding to enzymes, these enzymes partially hydrolyze carbapenems along with other b-lactams [27].

Similar findings were reported in Brazil by Chagas et al. 2015 when they observed 37.5% of qnrA gene found in *A. baumannii* isolates [40]. Also, the presence of qnrA gene (46%) in *A. baumannii* association with high percentage of resistance to Fluoroquinolones group Ciprofloxacin (64.7%) and Levofloxacillin (60.7%). Members of which are involved in protein-protein interactions. Purified qnr

protein was shown to bind to and protect both DNA gyrase and topoisomerase IV from inhibition by Ciprofloxacin [9,10,41]. The qnr genes appear to be acquired from chromosomal genes in aquatic bacteria [8]. These results unlike some studies [42,43], that could not detect any qnrA in their clinical isolates of *A. baumannii*. None of the plasmid-mediated qnr determinants have been identified so far in non-enterobacterial Gram-negative species like *A. baumannii* in Iran [44]. On the other hand, these plasmid-mediated determinants have been identified in many Enterobacteriaceae species throughout the world [45]. The rapid emergence of multidrug-resistant (MDR) strains is generally because of their capacity to acquire and disseminate exogenous genes associated with mobile genetic elements such as plasmids, transposons, integrons, and genomic islands. Plasmid-mediated quinolone resistance (PMQR) genes, such as qnr family, can be horizontally transferred and contribute to reduced susceptibility to fluoroquinolones, these genes code for proteins of the pentapeptide repeat family that protects DNA gyrase and topoisomerase IV from quinolone inhibition [8].

This study concluded that *Acinetobacter baumannii* was the predominant species in patient with different infections in two hospitals in Baghdad. High frequency of *A. baumannii* infections was observed among neonates (<1 year). There was only a limited number of drugs sensitivity. Extended spectrum *B*-lactamase were increased in *A. Baumannii is* very alarming. Multidrug resistance *A. baumannii* to carbapenems (Impanel and Meropenim) and quinolones (Ciprofloxacin and Levofloxacin) was carried two genes have a role in the resistance. The bla NDM-1 genes is associated with the resistance to carbapenems specially IMP and MEM, and this gene is chromosomal- borne. The *qnrA*-gene is associated with the resistant to quinolones is chromosomal and plasmid-borne.

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Author contribution

Dr. Abdulrahman supervise this paper as part from a thesis. Mshachal and Dr. Khudair participated in sampling preparation. Mshachal, Dr. Abdulrahman and Dr. Hassan performed and did the tests. Dr. Abdulrahman interpret the results of the research

Conflict of interest

None.

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