Iraqi Journal of Medical Sciences

IRAQI JMS

المجلة العراقية للعلوم الطبية

Volume 13, Number 2, 2015
April - June

ISSN 1681-6579

Volume 13 (2) 2015 ISSN 1681-6579

IRAQI JOURNAL OF MEDICAL SCIENCES

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Iraqi Journal of Medical Sciences

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Issued Quarterly

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Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq

Facial Skin Lines

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Abstract

Throughout ages, skin lines had been examined by anatomists and surgeons with a great controversy about the consistency of each. None of them is considered to be the gold standard for surgical incisions. Furthermore, skin lines are not often fully understood and being misquoted over and over in literatures. Various methods had been adopted; from a simple pinch to 3D scan computerized models. Different explanations had been given for the causes of formation and variation of those lines.

Keywords: Skin lines, Langer's lines, tension lines.

List of Abbreviation: RSTL: Relaxed skin tension lines, SHO: Senior House officer

Introduction

Over a long time, skin lines were the interest of surgeons as well as the anatomists for elective incisions to achieve the best aesthetic scar. Throughout a century, thirty six differently named lines had been described as guidelines [1] none of them is consistent but the Relaxed Skin Tension Lines (RSTL) by Borges may be the best and the most popular one. Wide range of techniques had been used to determine those line from simple stab on cadaveric tissue to 3D scans models [2]. Despite the fact that resulted lines share a common pattern in the most areas in the face, but areas of controversy still exist. In other words, when several linesare applied at a specific region of the face they will cross other lines pattern in right angles rather than being parallel.

Techniques used to determine skin lines

Karl Langer (1819 to 1887), a professor of Anatomy at Joseph's Academy in Vienna [3] conducted extensive investigations on fresh cadavers by puncturing the skin using a round awl (Fig. 1). The resulted holes were elliptical rather that circular, an explanation had been given that was due to the action of the underlying muscle which was been repeated over and over in literatures [4].

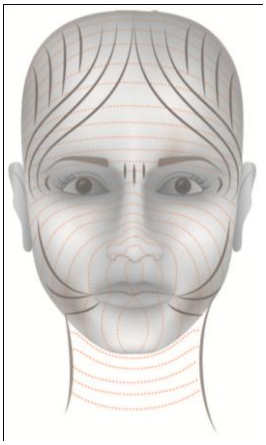

Fig. 1. Langer's line

Eventually, Langer was not the first scholar who noticed this property of the skin but Guillaume Dupuytren (1834), who is well known for his contracture, precisely described

this phenomena when he encountered a patient with multiple suicidal stabbing wounds on the chest with a round tool, he got the same pattern of ellipses rather than circles [5].

A year later, Langer performed a second set of experiment on the extremities by marking a 3cm circular template on the flexor aspect of a flexed elbow and stated that skin relaxed longitudinally while after excision of that circle the skin retracted transversely [6].

Cox , during his MD study in England 1941, re-examined the cleavage lines using pointed marlinespike again on cadavers but choosing only average body build people to overcome profounder factors [7] (Fig. 2).

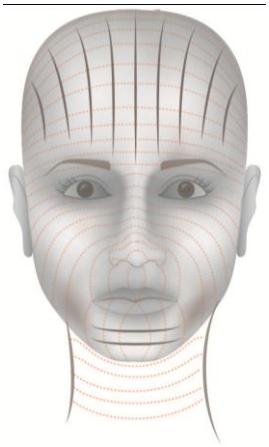

Fig. 2. Cox's Lines

At 1947 Rubin, from the Kings Country Hospital in Brooklyn, used a police device, like that used for finger print (coloured material is swiped on skin then stamped on white paper), to determine the tension lines of the skin [8] (Fig. 3).

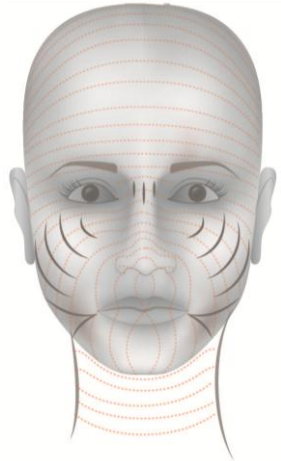

Fig. 3. Rubin's Lines

The photography and sketching were the methods used by Kraissl, from New York, how took a photograph for an old man after contraction of facial muscle, the wrinkles were exaggerated and composite sketch was achieved [9] (Fig. 4).

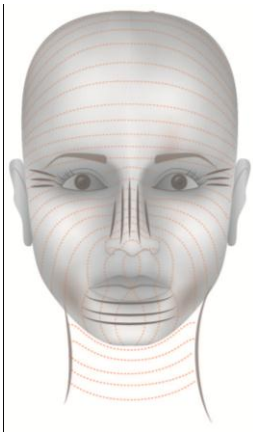

Fig. 4. Kraissl's lines

Straith et al in 1961, from Detroit, presented a paper on subcuticular suture with depiction of skin tension lines but no explanation had been given about the method they used to produce that scheme of lines [10] (Fig. 5).

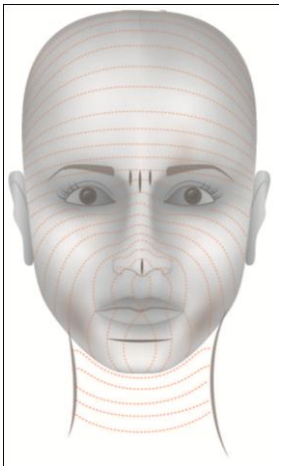

Fig. 5. Straith's Lines

With the repetition of Langer second set experiment, Bulacio, from Argentina 1961 and 1974 presented what he named new procedure while he use the same principle of Langer's second experiment when drawing extension lines from holes in skin of fresh

cadavers or holes made by flaps and grafts [11] (Fig. 6).

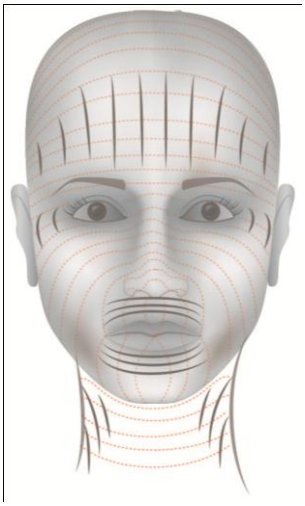

Fig. 6. Bulacio's Lines

Finally, in 1984 Broges described simple method to determine the Relaxed Skin Tension Lines (RSTL) by pinching skin and observing the formed furrows and ridges rather than furrows formed by muscle contraction and joint mobilisation which might give false lines depending on degree and direction of mobilization and muscle contraction [12] (Fig. 7).

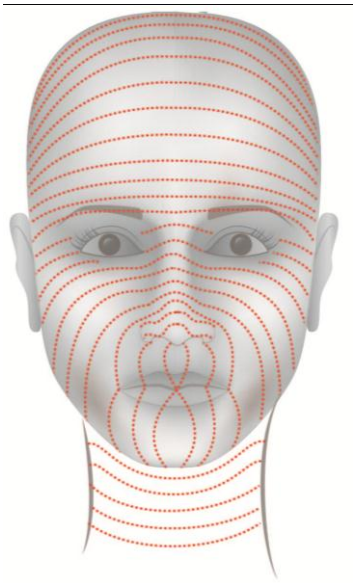

Fig. 7. Borge's Relaxed Skin Tension Lines

Sarifakioglu et al in 2004 describe new skin lines called them "sleep lines" which are affected by position of sleep and pillow type and referred as "buried pillow" but these line should not be confused with line followed for surgical incision as they are perpendicular to RSTL and Langer's lines [13].

Skin lines were considered to be a static feature of the skin till Bush et al conducted a research on 175 punch skin excisions on the face and neck which revealed significant differences in the degree of rotation of Langer's lines on facial expression by comparing long axis of each wound in relation to the previously marked vertical line preoperatively [14].

Furthermore, dynamic skin tension lines had been estimated by 3D scansmodel based on the kinematic analysis of skin with computational automatic identification, it is less invasive, repeatable and claimed to be less erroneous measurement [2].

Result and Discussion

Huge debate about the Langer's lines which are drawn in almost all books of plastic surgery. Furthermore, "Langer's lines" and "RSTL" might be used interchangeably by mistake. A search by librarians at the Royal College of Surgeons of Glasgow revealed that original Langer's lines were falsely redrawn in various textbook, in addition to that, his work was not well translated from German to English [3]. Further dilemma was merged that Langer did not perform his work as a guide for surgical incisions. Moreover, his line cannot be called as" relaxed line" because the tissue he used was cadaveric in rigor mortis which is never relaxed [12].

Going over through these lines, they share a common pattern in most area of the face while other areas show 90 degree crossing between them.

If we take Borges lines as a background, we can elicit the "Anti-RSTL" in table 1 and fig. 8.

Table 1. Anti-RSTL areas

Lines type	Anti-RSTL areas
Langer's lines	Scalp, forehead, lateral to the eyelids, glabella and middle of the cheeks.
Cox's lines	Scalp, forehead and chin
Rubin's lines	Glabella, middle and lower cheeks
Kraissl's lines	Nose, crow's feet and chin
Straith's lines	Columella, glabell and mentolabial fold
Bulacio's lines	Forehead, lower crow's feet, upper and lower lips upper neck
Sleep lines	Forehead, crow's feet, nasolabial fold and glabella

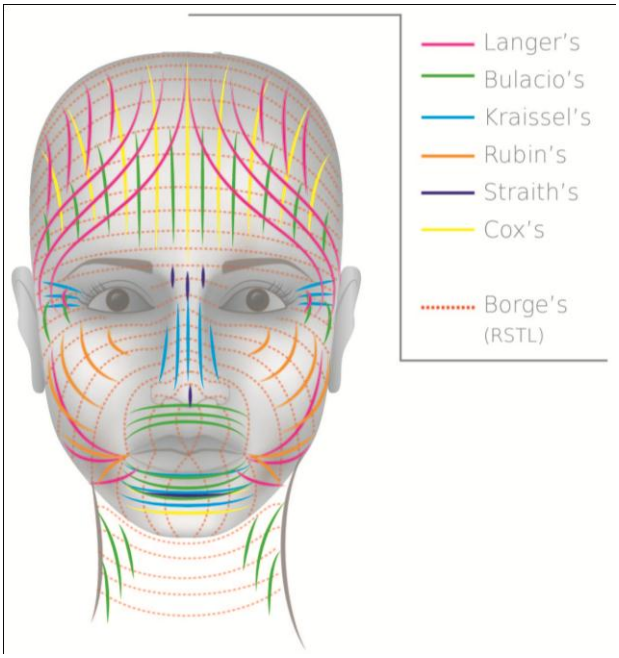

Fig. 8. Areas of right-angle cross of Borges' line with other lines.

In conclusion, various methods had been used over more than a century to determine these lines, the simplest and most applicable one is that adopted by Borges as simple pinch of the skin. Among the diversity of the directions of the skin lines, none of them is consistent guidelines for surgery but the most preferred and acceptable lines are Borges' (RSTL) and Kraissl's lines [(15)]. While Langer's line are often misquoted, the response of plastic surgeons showed different compliance to follow guidelines of "gold standard" for consultants and registrars from those who were SHO [(16)].

Explanations had been given for the cause of the formation of those lines ,the well-known widely accepted is the action of the underlying muscles which run perpendicular to the lines [(12)]. Another explanation had been made after examining skin under scanning electron microscope revealed that skin tension lines are formed by the interrelation between elastic and collagen fibres as well as fixed attachments between collagen fibres while Langer claimed that there was no elastin in the skin during his investigations [(17)]. Furthermore, "sleep lines" are claimed to be related to the position of the head regardless skin structure and muscles action [(13)].

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Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq

Self-rated Health and Medical Conditions in Refugees and Immigrants from the Same Country of Origin

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Abstract

Background Research suggests that refugees are at an increased risk for poor health outcomes as compared to immigrants. However, prior studies have compared refugees and immigrants from different countries and have failed to isolate specific war-related factors.

Objective To compare health outcomes and their determinants in refugees and immigrants from the same country of origin.

Methods A cross-sectional study based on a convenient sample and on self-report participants were conducted at Southeast Michigan during the period September to December 2009. A validated survey was used to examine refugees (n = 75) and immigrants (n = 65) from Iraq. The survey covered socioeconomics, lifestyle, violence exposure, self-rated health, and number of medical conditions (high blood pressure, fatigue, and backache, shortness of breath, gastrointestinal disorders, skin problems, and musculoskeletal problems). Group differences and predictors of health outcomes were assessed.

Results Refugees reported significantly more violence exposure than immigrants ($p < 0.001$). There were no significant differences in self-rated health or medical disorders between groups; however, violence exposure was the main predictor of health outcomes in refugees, whereas age was the main predictor in immigrants. Other predictors also varied by migratory group.

Conclusion Even though migration status did not directly influence health outcomes, results suggest that factors associated with migration status, e.g., violence exposure and age, do impact health. Future studies need to more carefully define and control for country-specific variables.

Key Words Health, Trauma, Violence, Emigrating, Iraq

List of abbreviation: PTSD = posttraumatic stress disorder.

Introduction

Refugees, individuals forced to leave their country of origin due to fear of persecution [(1)] are reported to be at an increased risk to suffer from adverse somatic, psychosomatic, and mental health as compared to immigrants, those who leave their country of origin voluntarily, and the host country population [(2-6)]. Refugees also more frequently rate their health as fair-to-poor as compared to immigrants [(7)]. In terms of somatic chronic diseases, there is a range of studies reporting a greater risk for refugees to suffer from cardiovascular disease, diabetes, arthritis, respiratory diseases, skin diseases and other medical illnesses as compared to non-war exposed immigrants [(8-10)]. Refugees are also more likely to suffer from depression, posttraumatic stress disorder (PTSD), and anxiety [(11-13)].

A major limitation in studies to date is the fact that refugees are typically compared to

immigrants originating from different countries, to the host country population, or to no control group [14,15]. When refugees and immigrants representing different countries are compared, it is more difficult to isolate war-related adverse health effects from factors related to other country-specific risks. For example, cultural, lifestyle and environmental factors of relevance for somatic, mental, and psychosomatic health might differ between the countries of origin for refugees and immigrants [16,17]. Failure to acknowledge such possibilities represents a gap in the literature about the possible differences in health of persons emigrating from the same country of origin but under different circumstances, either as immigrants or as refugees. Individuals from the same war-torn country are not necessarily equivalent in their exposure to the war, which can be examined by explicitly measuring violence exposure; however, other health-relevant factors are more likely to be similar as compared to reference populations coming from entirely different countries. Research suggests that factors which affect health may well differ across countries. For example, persons from different countries often make different attributions of illness, health, disease, symptoms, and treatment [18,19]. Such differences therefore play an essential role in the formation of beliefs concerning health and illness.

It is thus imperative to study persons emigrating from the same country – but during different civil circumstances. This will enable a better understanding of the causality between specific exposures and health among refugees. Furthermore, such a design will address the question of whether there are inequalities in post-migration health between refugees and comparable immigrants. The current study is part of a larger program to investigate health inequalities and factors affecting such differences among refugees that have been forcibly displaced as opposed to immigrants whom voluntarily left their country of origin. This study focuses on self-rated health and reported medical conditions (e.g. high blood pressure, fatigue) in refugees as compared to immigrants, both of whom were from Iraq.

The aims of this study were to:

1. Investigate and explore possible differences in self-rated health and number of medical conditions in refugees versus immigrants.
2. Determine possible contributing factors behind such differences.

We anticipated that refugees would report worse self-rated health and more medical conditions than immigrants, as prior studies have consistently reported such results [6,8,9,12,20]. Furthermore, we predicted that refugees would report higher pre-migration violence exposure. Finally, controlling for migration status, we predicted there would be an association between violence exposure, self-rated health, and number of medical conditions, such that higher violence exposure would predict poorer self-rated health and an increased number of medical conditions.

Methods

Participants and Procedures

A cross-sectional study based on a convenient sample and on self-report participants were conducted at Southeast Michigan during 2010. The sample documented Iraqi immigrants (n=65) and Iraqi refugees (n=75) were compared. Sample sizes in specific analyses may be slightly different due to small amounts of missing data. Migration status was determined by asking participants about their immigration status in the United States. According to United Nations High Commissioner for Refugees, refugee is defined as any person who: owing to a well-founded fear of being persecuted for reasons of race, religion, nationality, membership of a particular social group, or political opinion, is outside the country of his nationality, and is unable to or, owing to such fear, is unwilling to avail himself of the protection of that country". Immigration is defined as the movement of people into a country to which they are not native in order to settle there, especially as permanent or future citizens. Immigrants are motivated to leave their native countries for a

variety of reasons, including a desire for economic prosperity, political issues, family re-unification, escaping conflict or natural disaster, or simply the wish to change one's surroundings. In this paper, we include Iraqi who reports themselves as refugees or immigrant or others.
Inclusion criteria included being at least 18 years of age, being born in Iraq, and having left Iraq to immigrate to the U.S., either as an immigrant or refugee.
Participants were recruited in 2010 through community organizations that provide assistance and services to immigrants and refugees in Southeast Michigan, U.S.A. Several methods of recruitment were used to ensure a representative sample. All participants were Arabic speaking and were recruited from the Arab American and Chaldean Council (ACC; a non-profit organization serving immigrants and refugees), Chaldean and Assyrian Churches, as well as from the Islamic Center of Detroit. Moreover, repeated Arab language announcements by means of mass media (newspapers, organization newsletters) were used for recruitment.
A short notice describing the study, in both written and oral Arabic, was presented to all prospective participants at the various community centers. Interested individuals were scheduled for participation. Prior to data collection, potential participants again received a detailed written and oral description of the study, and were given the opportunity to ask questions. Subsequently, they were asked by the research staff to sign a consent form, and were clearly informed that participation was voluntary. The research staff then administered the survey, with participants responding to the questions in the survey. Once the participant completed the survey, they received a $30 gift card. The study protocol was approved by the Human Investigation Committee of Wayne State University.

Measures

Participants completed a survey comprised of questions regarding demographics (e.g. age, gender) and self-reported exposure to violence [(21)]. In addition, respondents rated their health, and indicated whether or not they had been diagnosed or suffered from a list of medical conditions [(22)]. The survey was translated from English into Arabic by a bilingual professional. The Arab version of the survey was then back-translated by an independent, dual-speaking community translator to ensure accuracy.

Socio-demographics: The socio-demographic section asked participants for background information including migration status, gender, and age, number of years in the U.S., education, health insurance, weight, smoking behavior, and employment status. We categorized educational attainment as “high school or less” and “more than high school” and current employment status as “employed” and “unemployed.”

Self-rated health: Self-rated health was assessed using a five-point scale ranging from poor to excellent. Research suggests that this measure represents a good proxy for prospective health status, including that of minority populations [(22-24)]. The scale was dichotomized as good health (excellent, very good, good) versus poor health (fair, poor). The dichotomized scale was more appropriate for our analyses than treating it as continuous, given that one item measured on a Liker scale represents only ordinal level data.

Medical conditions: Prevalence of medical conditions was assessed by asking a series of questions regarding whether a participant had been diagnosed with or experienced problems with the following medical conditions: high blood pressure, fatigue, and backache, shortness of breath, gastrointestinal disorders, skin problems, and musculoskeletal problems. For instance, the participant was asked “Have you ever been diagnosed with or experienced problems with high blood pressure?” Participants were provided three response options, past, current, or never. Current and past diagnoses of the disorder or problem were counted as equivalent. These conditions were

chosen because they provide a general overview of the participants' health [25]. All affirmative responses, present and past, were summed to create a total health score, which ranged from 0-7, and represented the number of medical conditions afflicting the individual.

Exposure to violence: Pre-migration exposure to violence was assessed using a modified measure based on the Survey of Children's Exposure to Violence [26]. The instrument has been modified and validated for use with adolescent and adult refugees [21], and provides a comprehensive assessment of violence exposure, such as having been arrested, kidnapped, threatened, attacked, sexually abused, or having experienced an explosion. Again, affirmative responses were summed, thereby creating a total pre-migration violence exposure score.

Data Analysis

Chi square analyses and t-tests were used to assess group differences between refugees and immigrants on demographic variables, self-rated health, prevalence of specific medical conditions, and pre-migration violence exposure. The sample sizes used in this study provided adequate power to find an effect between refugees and immigrants on our outcome variables (1-β = 0.84), self-rated health and number of medical conditions.

Three sets of logistic regressions were computed to calculate the odds ratios (OR) and to test the possible associations between the independent variables – migration status and pre-migration violence exposure – and the outcome variable – self-rated health. The first logistic regression tested this association while controlling for gender, age, education, employment, weight, smoking, and years in U.S. for the whole sample. The second and third logistic regressions tested whether associations differed across migration status. In a similar fashion, three linear regression analyses were conducted to explore the association between the independent variables and the number of medical conditions. All statistical analyses were carried out with Statistical Package for the Social Sciences (SPSS) version 19 with a two-tailed statistical significance set at $\alpha = .05$.

Results

Table 1 shows the demographic characteristics of the sample by migration status. A greater proportion of immigrants was male, employed, older, and had lived longer in the U.S than refugees. Refugees, however, reported significantly more pre-migration exposure to violence. No significant differences between the two groups were found with respect to level of education, health insurance, weight, and smoking behavior.

There were no statistically significant differences between the two groups in the prevalence of each of the 7 medical conditions. Overall, 35% of participants reported having high blood pressure, 41% had backache, 21% had gastrointestinal disorders, 63% were suffering from fatigue, 24% suffered from shortness of breath, 29% had musculoskeletal problems, and 10% had skin problems. The groups also did not differ in the number of medical conditions reported (M = 2.50, SD = 1.89; t (137) = 0.47, p = 0.64). Furthermore, no significant differences were found in self-rated health between the two groups. Overall, 35% of the entire sample reported fair or poor health.

Table 2 shows the results of the logistic regression analysis for the predictors of self-rated health. For the combined sample, being younger, having never smoked, and having lower violence exposure were significant predictors of good self-rated health (excellent, very good, and good). However, when the groups were examined separately, pre-migration exposure to violence was the only significant, albeit inverse, predictor of good self-rated health among refugees, while older age was the only significant predictor, also inverse, among immigrants.

Table 3 shows the results of the linear regression analysis predicting the number of medical conditions. Pre-migration exposure to violence and being older in age were significant

predictors of the number of medical conditions in the combined model. In refugees, pre-migration exposure to violence and being older in age were significant predictors for reporting a higher number of medical conditions. Additionally, education emerged as a protective factor for refugees, i.e., higher education predicted fewer reported medical conditions. Of these variables, violence exposure was the strongest predictor for this group, accounting for 16% of the variance in reported number of medical conditions. For immigrants, older age, being female, and having smoked were significant predictors of a higher number of medical conditions. In this group, age was the strongest predictor, accounting for 21% of the variance in reported medical conditions.

Table 1. Socio-demographic Characteristics by Study Group

		Refugee N (%)	Immigrant N (%)	χ2	df	*P*
Gender	**Male**	52(37.1)	31(22.1)	6.76	1	0.009
	Female	23(16.4)	34(24.3)			
Insurance	**Insured**	19(13.7)	25(18.0)	3.01	1	0.08
	Not Insured	56(40.3)	39(28.1)			
Education	**< High School**	32(23.9)	29(21.6)	0.40	2	0.82
	High School	29(21.6)	21(15.7)			
	College	12(9.0)	11(8.2)			
Employment	**Employed**	11(8.1)	29(21.5)	16.16	1	<0.001
	Unemployed	62(45.9)	33(24.4)			
Smoking	**Has Smoked**	62(44.3)	48(34.3)	1.61	1	0.21
	Never Smoked	13(9.3)	17(12.1)			
Self-Rated Health	**Excellent**	9(12.7)	7(10.8)	1.12	4	0.89
	Very Good	14(19.7)	13(20.0)			
	Good	26(36.6)	21(32.3)			
	Fair	15(21.1)	14(21.5)			
	Poor	7(9.9)	10(15.4)			
		***M* (*SD*)**	***M* (*SD*)**	***t***	***df***	***P***
Age		40.93(12.55)	48.5(16.94)	2.98	134	0.003
Years in U.S.		2.69(3.13)	12.52(9.63)	8.35	138	<0.001
Violence		18.12(5.98)	14.15(4.81)	4.28	138	<0.001
No. Medical Conditions		2.57(1.73)	2.42(2.08)	0.47	137	0.64
Weight		146.45(52.89)	153.26(40.98)	0.82	132	0.41

Table 2. Logistic Regression: Predictors of Good Self-Rated Health

Group	Predictor	B	Wald	*P*	O.R.	Lower CI	Upper CI
Combined Sample	Age	-0.06	10.26	0.001	0.94	0.91	0.98
	Gender (male = 0)	-0.37	0.40	0.53	0.69	0.22	2.15
	Years in U.S.	0.02	0.23	0.63	1.01	0.94	1.09
	Weight	-0.01	1.98	0.15	0.99	0.98	1.00
	Smoking (never smoked = 0)	1.46	4.07	0.04	4.30	1.04	17.72
	Employment (unemployed = 0)	1.14	2.84	0.09	3.11	0.83	11.67
	Education (≤ high school = 0)	0.73	1.68	0.19	2.07	0.69	6.24
	Violence Exposure	-0.03	4.00	0.04	0.96	0.93	0.99
	Migration status (immigrant = 0)	0.90	1.52	0.21	2.47	0.58	10.43
Refugees	Age	-0.05	3.79	0.05	0.94	0.89	1.00
	Gender (male = 0)	0.01	0.00	0.99	1.01	0.10	9.73
	Years in U.S.	0.01	0.002	0.96	1.00	0.78	1.30
	Weight	-0.005	0.28	0.59	0.99	0.97	1.01
	Smoking (never smoked = 0)	1.16	1.30	0.25	3.20	0.43	23.53
	Employment (unemployed = 0)	2.20	2.40	0.12	9.10	0.55	148.53
	Education (≤ high school = 0)	0.60	0.42	0.51	1.83	0.29	11.22
	Violence Exposure	-0.05	5.41	0.02	0.94	0.90	0.99
Immigrants	Age	-0.06	5.70	0.02	0.94	0.89	0.99
	Gender (male = 0)	-0.73	0.92	0.33	0.47	0.12	2.15
	Years in U.S.	0.04	1.13	0.29	1.04	0.96	1.13
	Weight	-0.01	0.66	0.14	0.99	0.97	1.01
	Smoking (never smoked = 0)	1.29	1.46	0.22	3.66	0.44	30.13
	Employment (unemployed = 0)	1.07	1.33	0.24	2.93	0.47	18.17
	Education (≤ high school = 0)	1.06	1.59	0.20	2.90	0.55	15.16
	Violence Exposure	0.03	0.57	0.44	1.04	0.94	1.15

Note: df for each component of this analysis is 1; when no reference category is indicated continuous measures were used.

Discussion

In the current study, we examined factors associated with somatic aspects of psychiatric complaints outcomes in refugees as compared to non-refugee immigrants originating from the same country. The study design allowed for a better control of the influence of non-war related exposures on health in refugees in that it allows for control of country-specific factors possibly contributing to poor health. Country-specific factors could include health care systems, general health, behavioral attitudes,

environmental factors, and belief systems. Indeed, prior studies have reported that refugees generally exhibit worse health when compared to immigrants [7]. However, a major limitation in those studies has been the comparison of refugees and immigrants from different countries of origin, since factors other than war-exposure may contribute to health differences [15]. Our study is the first to compare refugees to a control group of immigrants coming from same country, which makes them more likely to share similar traditions, beliefs, and language. Despite differences in pre-migration violence exposure, refugees and immigrants did not differ significantly in self-rated health or reported number of medical conditions. This suggests that factors other than war and conflict contribute to both outcomes.

Table 3. Linear Regression: Predictors of Number of Medical Conditions

				CI				
Group	**R^2**	**Predictor**	**β**	**Low**	**High**	**High**	**t**	***P***
Combined Sample	0.37	**Age**	0.44	0.03	0.08	4.88	<0.001	0.14
		Gender (male = 0)	0.16	-0.10	1.36	1.70	0.09	0.01
		Years in U.S.	-0.06	-0.06	0.03	-0.59	0.55	<.01
		Weight	0.17	<0.01	0.01	1.93	0.06	.02
		Smoking (never smoked = 0)	0.03	-0.61	0.92	0.40	0.68	<0.01
		Employment (unemployed = 0)	-0.17	-1.49	0.04	-1.87	0.06	0.02
		Education (≤ high school = 0)	-0.08	-1.03	0.32	-1.04	0.29	<0.01
		Violence Exposure	0.23	0.01	0.05	2.65	0.01	0.04
		Migration status (immigrant = 0)	-0.15	-1.43	0.21	-1.47	0.14	.01
Refugees	0.36	**Age**	0.26	0.01	0.07	2.30	0.03	.06
		Gender (male = 0)	-0.09	-1.52	0.80	-0.62	0.53	<0.01
		Years in U.S.	0.10	-0.09	0.20	0.80	0.42	<0.01
		Weight	0.15	-0.01	0.01	1.06	0.29	0.01
		Smoking (never smoked = 0)	-0.20	-1.92	0.21	-1.61	0.11	0.03
		Employment (unemployed = 0)	-0.18	-2.02	0.23	-1.59	0.11	0.03
		Education (≤ high school = 0)	-0.26	-1.87	-0.05	-2.11	0.04	0.05
		Violence Exposure	0.44	0.02	0.07	3.86	<0.001	0.16
Immigrants	0.54	**Age**	0.58	0.04	0.11	4.59	<0.001	0.21
		Gender (male = 0)	0.23	0.03	1.97	2.06	0.04	0.04
		Years in U.S.	-0.17	-0.09	0.02	-1.42	0.16	0.02
		Weight	0.12	-0.01	0.02	1.07	0.28	0.01
		Smoking (never smoked = 0)	0.24	0.11	2.28	2.21	0.03	0.05
		Employment (unemployed = 0)	-0.21	-2.08	0.23	-1.61	0.11	0.03
		Education (≤ high school = 0)	0.05	-0.79	1.26	0.46	0.64	<0.01
		Violence Exposure	-0.15	-0.10	0.02	-1.34	0.18	0.02

Note: r = semi-partial correlation; when no reference category is indicated continuous measures were used.

These factors are largely unknown and need further investigation, although post-traumatic stress disorders could be one of the problems developed in patents facing disasters; however, refugees, who have been exposed to violence, may be more inclined to express reactions to trauma through psychological disorders, including PTSD and depression [25]. Therefore, refugees may be reacting to the increased violence exposure via psychological reactions as opposed to somatic reactions [25], which may account for the similarities in self-rated health and number of medical conditions found in the present study.

Predictors of both self-rated health and number of medical conditions differed across groups. Violence exposure was the strongest predictor of poor self-rated health and higher number of medical conditions for the full sample and for the refugees independently. For immigrants, on the other hand, the strongest predictor of poor self-rated health and higher number of medical conditions was increased age. Furthermore, violence was not a significant predictor of poor health for immigrants. This result suggests that the results for the combined model were driven by refugee violence exposure, and that there may be a critical level of violence exposure that must be reached before it causes adverse health effects. The refugee group is much more likely to have reached this critical level of exposure, which may be why violence was only a significant predictor for this group in the present study.

Immigrants and refugees reported similar numbers of medical conditions after adjusting for age, gender, education, employment, smoking behavior, weight, and length of time in the U.S. However, the limited predictive strength of violence exposure on the number of medical conditions (accounting for 16% of the variance) seems to indicate that there are other factors contributing to health outcomes in war-exposed populations, e.g., exposure to hazardous materials, contaminated food, poor quality air, poor mental health, etc. This is consistent with research comparing individuals residing at different distances from a war environment reporting that those closer to a war zone were at higher risk for poor health [27]. Another study examined housing conditions in a refugee camp and found that poor housing conditions (presence of dust, smoke, and mold; burning of biomass fuels; overcrowding; poor ventilation, etc.) were directly related to poor health outcomes [28]. Future studies could examine this possibility more directly, as this was outside of the scope of the present study; however, the participation of immigrants and refugees from the same country of origin make it unlikely that these factors would influence the results of the present study. Recent research also suggests that mental health has an impact on physical health [29]. The low predictive power of violence exposure could therefore be due to mental health particularly posttraumatic stress disorder, not being accounted for in the present study. It may be that individuals exposed to violence develop poor mental health which subsequently causes declines in physical health [29]. Indeed, the medical conditions that were examined in this study were somatic aspects of common mental disorders, albeit not all of the same disorder. This makes it possible that for the refugee sample, participants are expressing mental anguish through somatic complaints. This is unlikely for the immigrant sample, given that violence exposure was not a significant predictor but age was the strongest predictor.

Despite the association between poor health outcomes and higher pre-migration violence exposure in refugees but not in immigrants, both groups had similar self-rated health and objectively measured medical conditions after controlling for demographic variables. Results differ from previous studies which report that refugees are at higher risk for poor health as compared to immigrants [12,30]. Iraqi refugees, in particular, have been found to report more health problems than other Arab immigrants. A recent study had demonstrated that Iraqi refugees reported greater medical complaints in comparison to non-war exposed Yemeni immigrants [20]. Another study reported that

refugee populations in general frequently report greater psychosomatic complaints than other immigrants [6]. Furthermore, previous studies have not measured violence exposure as specifically as the present study, yet those studies concluded that health differences were due to war-exposure [20].

Other studies have also suggested that acculturation processes might contribute to worsening health with increasing time in the host country [31,32]. However, in the current study, years in the U.S., which may be considered a proxy for acculturation [33], was not a significant predictor for our various health measures. In fact, the slopes for time in all models were almost flat when controlling for age. Therefore, time in the U.S. cannot account for the differences across groups, or lack thereof, in any of our models. While the present study is cross-sectional, these results suggest that both self-rated health and number of medical conditions is likely stable over time. Theoretically, it might take a longer time to develop poor health among immigrants due to lifestyle factors in the U.S., as opposed to war exposure in refugees through a mechanism not simply related to years in the U.S. In that case, health status would be similar between the two groups; however, this theory requires prospective data in which both groups have arrived in the host country at the same time to allow for more formal testing. Such data would allow for the separation of war versus post-migration acculturation factors, including lifestyle.

Finally, in each model we statistically controlled for individual characteristics (age, gender, education, employment, weight, and smoking behavior) that are known or purported to be related to health outcomes. Our findings are similar to observations in numerous prior studies of diverse populations [21,34]. For instance, higher education levels have been shown across many studies to be related to better health outcomes [34]. This coincides with the results of the present study, in which education was found to significantly predict number of medical conditions in refugees. Gender was a significant predictor of the number of medical conditions for immigrants, with women reporting worse health than men in this group. This is consistent with prior research in large community based samples, which suggests that women tend to report more somatic health problems than do men [35]. Smoking behavior was also a significant predictor of an increased number of medical conditions reported by immigrants. Smoking has been linked to many negative health outcomes such as, hypertension [36], heart disease [37], and respiratory diseases [38]. Weight, on the other hand, was not found to be a significant predictor of the number of medical conditions or self-rated health for either group. This is contrary to a number of studies which suggest that not maintaining an appropriate weight can have a negative impact on health [39,40].

Strengths, Limitations, and Conclusions

To the best of our knowledge, this is the first study comparing health among individuals from the same country but with different migration status. While some research has examined region of origin, such work has not directly compared the effects of migration status on health outcomes within groups from the same country [6,41]. Our research design allowed for the examination of two groups from same country of origin and thereby reduced the potential impact that country of origin could have on comparative refugee-immigrant health studies.

There were also some limitations to the present study. First, data were collected from convenience samples. The participants were recruited through local community organizations and other gathering places for Iraqis in Michigan. Therefore, it is not clear to what degree the samples are representative of the populations from which they were drawn. Ideally, studies should be based on random samples of newly arrived refugees and immigrants from the same country. A random sample could have provided more accurate measurement of health status among refugees as compared to immigrants

since random sampling reduces systematic bias. Additionally, as in most refugee studies, health outcomes in the present study were based on self-report as opposed to objective clinical assessment and may have been subject to reporting bias; however, there is no reason to believe health status was differentially reported by refugees and immigrants. The samples used in this study could be considered small; however, power analyses revealed that there was sufficient power (1-β = 0.84) to detect differences between these two groups for both self-rated health and number of medical conditions. Sample size was therefore not considered a limitation.

This study contributes important new information related to risk factors among refugees for self-rated and overall health. The fact that refugees reported a similar prevalence of medical conditions compared to immigrants, despite a substantially higher degree of violence exposure, possibly points to the importance of resilience in refugees. Previous research has suggested that high resiliency can be protective following highly aversive events [42].

By comparing persons from the same country, we limit some of the potential factors influencing health and well-being; however, the fact that our models nevertheless were quite poor in predicting outcomes of interests, suggests a need to delve further into the determinants of health in these populations. We do not have sufficient information to conclude that immigrants and refugees were altogether homogenous in terms of cultural background; however, they are more likely to be homogenous, and thus comparable, in this sample compared to prior studies that included refugees and immigrants from different countries. We did, as many other studies have done, control for socioeconomic and behavioral factors.

Acknowledgments

We would like to thank the staff of the Arab American and Chaldean Council (ACC), Chaldean and Assyrian Churches, the Islamic Center of Detroit and all others agents who help in disseminating the flyer of the project in the Southeast Michigan.

Author contributions

Dr. Jamil: supervis data collection, study design and revising the manuscript; Dr. Barkho: collection of data and writing the draft of the manuscript; Drs. Broadbridge and Ventimiglia: acquisition of data, analysis and interpretation of data, statistical analysis; Drs. Arnetz and Lami: participated in the interpretation of the result and revising the manuscript and Dr. Arnetz: study concept or design, interpretation of results and revising the manuscript

Conflict of interest

There are no conflicts of interest for any of the authors of this manuscript and its potential publication.

Funding

Funding of all authors except Dr. Faris Lami was partially supported by Award Number R01MH085793 from the National Institute of Mental Health. The content is solely the responsibility of the authors and does not necessary represents the official views of the National Institute of Mental Health or the National institutes of Health.

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Received 24th Jun. 2014: Accepted 8th Apr. 2015

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq

Clinical and Urodynamic Study of Adult Female Patients with Urinary Incontinence

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Abstract

Background Urodynamics provide objective pathophysiological explanations for symptoms and/or dysfunction of the lower urinary tracts. It reproduce patient symptoms during the performance of the study and assist clinicians in determining the precise cause, aid in diagnostic process and follow up of patients with urinary incontinence.

Objective To evaluate the role of urodynamic study in confirming the diagnosis of urinary incontinence in Iraqi women. Differentiate the types of urinary incontinence and assess the importance of risk factors in its development.

Methods This study was performed in the Urology Department, Al-Sader Medical City, Holly Najaf between March 2013 and March 2014. One hundred and twenty female subjects aged 20 to 60 years were studied. They comprised 60 patients and 60 control subjects. Medical history, clinical examination and urodynamic tests were performed for them.

Result Thirty four patients presented with stress urinary incontinence, nineteen with urge type, five with mixed type and only two patients presented with overflow urinary incontinence. The patients were complaining of cough, constipation (most of them in stress type) and presence of cystocele (most of them in stress urinary incontinence patients). In addition, there was positive history of hypertension and positive family history of urinary incontinence. The strong desire to urination and the maximum urinary bladder capacity of patients was significantly smaller than those of control subjects, specifically those patients with stress and urge urinary incontinence.

Conclusion Age, Parity and body mass index significantly affect the prevalence of urinary incontinence in women who have given birth vaginally. Stress urinary incontinence is the most common type of UI among women regardless the small sample size in the study. Cystometric changes of urodynamic study were markedly evident in the stress urinary incontinence patients as compared to the healthy women.

Keywords Urinary incontinence, Urodynamic study, Cystometry, Adult females.

List of Abbreviation: SUI = stress urinary incontinence, UUI = urge urinary incontinence, MUI = mixed urinary incontinence, OFI = overflow incontinence, UI = urinary incontinence, LUT = lower urinary tract, UTI = urinary tract infection.

Introduction

Urinary incontinence (UI) is defined by the International Continence Society as involuntary loss of urine that cause a social or hygienic problem. The reported prevalence varies, with up to 33% of younger women and over 50% of women over 60 years old affected [(1)].

Population studies estimate that 20-30% of women are affected but only 7-12% perceives it as a problem [(2,3)]. UI is a pelvic floor disorder leading to an involuntary loss of urine that commonly affects older adults [(4)]. It is an extremely common complaint in every part of

the world. It causes a great deal of distress and embarrassment, as well as significant costs, to both individuals and societies [5,6].

The main types of UI are stress incontinence (SUI) which is defined as the complaint of involuntary loss of urine on effort or physical exertion (e.g., sporting activities), or on sneezing or coughing [7].

Urge incontinence (UUI), presenting as an involuntary passage of urine associated with an overwhelming urge to urinate as a consequence of abnormal detrusor activity and not connected with increased intra-abdominal pressure [8,9].

Mixed incontinence (MUI) which is defined as involuntary leakage of urine associated with urgency on exertion, effort, sneezing, or coughing.

It has been reported that between 30% and 50% of incontinent women experience MUI [10]. A less common form of UI in women is overflow incontinence (OFI) which is associated with overdistension of the urinary bladder (UB) and can be caused by obstruction, i.e., pelvic organ prolapse or a neurological condition, i.e., spinal cord injury [11].

The most important risk factor is being female [12]. The incidence of UI increases with increasing age [13], pregnancy, child birth [14,15], an increase of body mass index (BMI) [16] and smoking [17].

Since 1976, through the influence of the International Continence Society (ICS), urodynamics has been promoted as an important, objective aspect of the diagnostic evaluation of patients with such symptoms [18].

In the case of incontinence, the most relevant of these tests are directly related to the incontinence itself; that is, they aim to demonstrate involuntary leakage in the test setting. In the last 30 years, cystometry has been established as the gold standard for detailed assessment of lower urinary tract (LUT) symptoms, particularly SUI and UI [19].

The objective of our study is to evaluate Iraqi women with different types of UI and its relation to some confounding risk factors.

Methods

The study was approved by the Institute Review Board of the College of Medicine, Al-Nahrain University. A cross sectional study conducted in Al-Sader Medical City, Holly Najaf for the period from the March 2013 to March 2014. Sixty controls females and 60 patients with UI were involved in the study. Pregnant women or those within six weeks postpartum were excluded. Women with neurologic diseases like spinal cord injury, history of trauma or medications that could cause incontinence, urinary tract malformations, pelvic tumors and those who already undergone corrective surgery for UI were also excluded from the study. General urine examination was made for all participants to exclude urinary tract infection (UTI).

Standardized urodynamic assessment was performed with multichannel urodynamic instrument, Model 10318, Mediwach, Medtronic, UK, including retrograde water cystometry at a filling rate of 50 ml per minute. Filling cystometry performed with infusion of distilled water. The urodynamic procedure was in accordance with the standards recommended by the International Continence Society.

In the beginning of urodynamic test, the patient was asked to empty her UB after she was positioned in lithotomy position on the examination table by 16 F Folly's catheter to remove any residual urine.

Provocative cough testing was carried out initially to assess the correct placement of urodynamic machine lines. Abdominal, vesical and detrusor pressure were measured using external pressure transducers, which were calibrated before each procedure and zeroed to atmospheric pressure using the level of the symphysis pubis as the reference height. A 12Fr microtip catheter was placed in the subject rectum to record abdominal pressure (pabd).

An 8Fr dual microtip catheter with sideway holes, 30 cm long and an infusion port was placed in the UB to record intravesical pressures (pVes). Detrusor pressure (pdet) was measured with a continuous subtraction (pdet = pVes-pabd). A cough and stress maneuvers performed

every 100 cc until maximum cystometric capacity (MCC) was attained.

Cystometrogram parameters including first sensation to void (the patient was asked to mark once she feel any desire to urination), normal desire (when she can tolerate the examination), strong desire to void (when she have severe desire to void), MCC (when the patient can't tolerate the examination any more). The patient was then asked to cough and bounce up and down. Any rises in the detrusor pressure or leakage of urine in response to these activities were recorded. The presence of detrusor overactivity with or without incontinence was annotated. Valsalva leak point pressures were assessed at a minimum volume of 200 mL. At the end, the subject was seated upright and she attempted to void. After voiding was complete the patient was catheterized and post-voiding residual urine was recorded.

Data Analysis

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 20. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as mean and standard deviation 95% confidence interval. Independent sample t-test was done to find the diffidence between two groups. A *P* value of ≤ 0.05 was considered as significant.

Results

The age of the patients and the control group was matched with no significant difference (37.53±12.54 versus 35.27±10.19 years). The BMI of the patients was 34.42±5.62, which is significantly higher (*P* < 0.001) than 26.13±5.20 of the control group. Regarding the parity, majority (60.0%) of the patients had more than 3 children compared to 40.0% of control group (*P* = 0.001). Thirty four (56.6%) patients presented with SUI, nineteen (31.70%) with UUI, five (8.30%) with MUI and only two patients (3.40%) presented with OFI. History of obstetric surgery (cesarean section), chronic cough, constipation, genitourinary prolapse, hypertension and family history of UI were significantly different between the two groups but not the smoking habit (Table 1).

Table 1. Socio-demographic data of patients with urinary incontinence using Chi square test

Variable		Control (N %)	Urinary Incontinence Patients (N %)	*P* value
Caesarean section	Yes	15 (25.0)	6 (10.0)	0.031
	No	45 (75.0)	54 (90.0)	
Chronic Cough	Yes	1 (1.7)	20 (33.3)	< 0.001
	No	59 (98.3)	40 (66.7)	
Constipation	Yes	3 (5.0)	17 (28.3)	0.001
	No	57 (95.0)	43 (71.7)	
Genitourinary Prolapse	Yes	0 (0.0)	29 (48.3)	< 0.001
	No	60 (100.0)	31 (51.7)	
Hypertension	Yes	9 (15.0)	22 (36.7)	0.007
	No	51 (85.0)	38 (63.3)	
Family History of UI	Yes	2 (3.3)	13 (21.7)	0.002
	No	58 (96.7)	47 (78.3)	
Smoking Habit	Yes	2 (3.3)	6 (10.0)	0.272
	No	58 (96.7)	54 (90.0)	

Cystocele, smoking habit, chronic constipation, and chronic cough were present in high percentages in the SUI patients as compared to the other UI types, whereas history of cesarean section was higher in UUI patients (Table 2).

Table 2. Socio-demographic data of urinary incontinent patients

Variable	Urinary Incontinence patients				Control group N (%)
	SUI N (%)	MUI N (%)	UUI N (%)	OFI N (%)	
Cystocele	18 (52.9%)	2 (40%)	9 (47.3%)	none	0 (0.0)
Smoking	4 (11.76)	2 (40%)	none	none	2 (3.3%)
Constipation	10 (29.4%)	5 (100%)	2 (10.52%)	none	3 (5.0%)
Cough	12 (35.29%)	5 (100%)	3 (15.78%)	none	9 (15.0%)
Cesarean section	5 (14.7%)	3 (60%)	6 (31.57%)	1 (50%)	6 (10.0%)

SUI = stress urinary incontinence, MUI = mixed urinary incontinence, UUI = urge urinary incontinence, OFI = overflow incontinence.

LUT symptoms like cough induced UI, UI before arriving to the water cycle, UI at nighttime, wetting bed, continuity of UI, repeated UTI, using of pack, psychological effect of UI was present in the majority of patients with significant statistical difference as compared to the control group. On the contrary, minority (21.7%) of UI patients have feeling of complete bladder empting after urination versus 68.3% of the control group. None of the control group had positive cough test compared to 39 UI patients (Table 3).

Table 3. Lower urinary tract symptoms in urinary incontinent patients and control group

Variable		Patients (N %)	Control (N %)	*P* value
Cough-induced urinary incontinence	Yes No	48 (80.0) 12 (20.0)	0 (0.0) 60 (100.0)	<0.001
Urinary incontinence before arriving bath	Yes No	52 (86.7) 8 (13.3)	0 (0.0) 60 (100.0)	<0.001
Frequent urination at nighttime	Yes No	46 (76.7) 14 (23.3)	4 (6.7) 56 (93.3)	<0.001
Bed wetting	Yes No	23 (38.3) 37 (61.7)	0 (0.0) 60 (100.0)	<0.001
Continuity of urinary incontinence	Yes No	48 (80.0) 12 (20.0)	0 (0.0) 60 (100.0)	<0.001
Complete bladder emptying	Yes No	13 (21.7) 47 (78.3)	41 (68.3) 19 (31.7)	<0.001
Repeated urinary tract infection	Yes No	34 (56.7) 26 (43.3)	0 (0.0) 60 (100.0)	<0.001
Pack Usage	Yes No	40 (66.7) 20 (33.3)	0 (0.0) 60 (100.0)	<0.001
Psychological effect of urinary incontinence	Yes No	58 (96.7) 2 (3.3)	0 (0.0) 60 (100.0)	<0.001
Cough Test	Positive Negative	39 (65.0) 21 (35.0)	0 (0.0) 60 (100.0)	< 0.001

Table 4 shows the cystometric findings in UI patients and the controls. The strong desire to urinate equals to (388.38±158.78) mL water in UI patients, which is significantly reduced (P < 0.001) as compared to (500.30±103.48) mL water of the control women. Similarly, UI patients exhibit maximum cystometric capacity of (427.78±157.94) mL water in, a value that was significantly less than (537.57±97.57) mL water of the control group (P < 0.001).

Table 4. Cystometric findings in stress urinary incontinence patients and control group using T test

Cystometric findings	Group	Mean ± SD	*P* value
1st Desire to urinate (mL water)	SUI Patients Control	188.45 ± 104.49 219.38 ± 76.51	0.067
Normal desire to urinate (mL water)	SUI Patients Control	297.32 ± 144.95 330. 53 ± 101.03	0.148
Strong desire to urinate (mL water)	SUI Patients Control	388.38 ± 158.78 500.30 ± 103.48	< 0.001
Maximum cystometric capacity (mL water)	SUI Patients Control	427.78 ± 157.94 537.57 ± 97.57	< 0.001

SUI = stress urinary incontinence

Discussion

In the current study, majority of MUI and UUI patients were older (≥ 50 years) than patients with SUI (30-50 years). This finding agree with most of the researches dealing with UI that reveals an increase in the prevalence of MUI and UUI up to middle age and then leveling off at age of 50-70, followed by steady increase among aged population [(15)]. Furthermore, and in harmony with the findings of many researchers [(20)], SUI was predominant in younger and middle age women.

Since collagen is the most important component of the connective tissue and because the age range of the patient of the current study ranges from 27 to 60 years; it is speculated that reduced collagen content in the anterior vaginal wall with aging might contribute to the SUI of those patients. This also noted by Keane *et al* [(21)] who found urodynamic SUI even in premenopausal nulliparous women.

UI is an unwelcome and unacceptable outcome of childbearing. A significant correlation was demonstrated between parity and the prevalence of UI in the present study where 60.0% of the patients had more than 3 children. This would suggest that pregnancy itself might cause mechanical changes, hormonal changes or both, leading to UI [(20)].

Continence changes may be due to injuries, such as perineal tears, muscle trauma, or damage of the pudendal nerve. Notably, incontinence commencing in pregnancy can double the risk of developing postpartum UI, and pre-pregnancy incontinence can quadruple the risk of UI in the postpartum period [(22)]. In a study conducted in Australia reported in one out of three women who have ever had a baby, and six in 10 pregnant women, involuntarily leak urine. This prevalence rate means that from the 41% of Australian women giving birth for the first time each year, there is a potential of 30,900 new cases of childbirth related UI annually [(23)].

The majority of patients in this study presented with SUI followed by UUI and MUI while the minority shows OUI type, a finding, which is in accordance with those reported by Pregazzi *et al* [(24)] and Casey *et al* [(25)]. The small percentage of the patients who reported OUI was also reported by Chancellor *et al* [(26)] who stated that this type was more common in males than females.

Obesity emerged as an important risk factor for both SUI and UUI in this study. This is consistent with the observation of Melville *et al* [(27)] who

noticed that high BMI is a risk factor for UI. An association between obesity and pelvic floor disorders is a relatively consistent finding in epidemiologic studies [28]. Obesity is a potentially modifiable risk factor for bladder symptoms: Women can be encouraged to maintain a healthy weight as strategy to reduce the risk of both SUI and UUI [29].

In recent years, there has been increasing interest in elective cesarean delivery to reduce the long-term maternal risk of pelvic floor disorders and UI [30]. In the present study, most of patients have history of more than three vaginal deliveries. In contradiction to these findings, Chou *et al* [31] demonstrates no statistical difference in the prevalence of UI between vaginal and cesarean delivery after labor, yet, this study followed the patients for 1 year postpartum in primiparas which is a short period for follow up.

In this study, 17% of the patients had history of constipation; about 29% of these patients were diagnosed to have SUI, while all MUI patients reported history of chronic constipation. These findings were in consistence with other studies [32,33]. There is a close relationship between the muscles and nerves that control bladder functions and those that control bowel movements; moreover, the bladder and the colon are close together in the body. Large amounts of stool in the colon can put pressure on the bladder which can cause the bladder to not fill as much as it should, or cause the bladder to contract when the bladder is not supposed to contract. This large amount of stool can also cause the bladder to not empty well [34]. All of these problems can lead to daytime wetting, nighttime wetting, UTIs, and in some cases vesicoureteral reflux. Constipation and straining may weaken pelvic floor muscles, predisposing to SUI [33].

Chronic cough emerge as a risk factor in the study group. Twenty patients reported history of chronic cough; most of them were in the SUI group. The majority of patient with UI reported history of hypersensitive airway as a cause of chronic cough and only six patients reported history of smoking as a cause of chronic cough.

Smoking is an important risk factor for development of SUI among women in European countries [17]. In our society, the smoking is not a public habit, while the air pollution could be the risk factor for development of hypersensitive airway. Many studies reported cough as contributing factor for increasing intra-abdominal pressure leading to development of SUI [35,36].

Near half of the patients enrolled in the present study had have cystocele on the examination; most of them were in the SUI group (52.9%) followed by UUI group (47.3%). Cystoceles, which lead to loss of bladder neck and urethral support represent only one component of anterior vaginal wall prolapse. Cystoceles have been reported to develop in up to 52% of women after their first vaginal delivery [37].

In the present study, patient's psychological mode and limitation in their social and public life are positively associated with incontinence. Women living with UI have been shown to have a significantly lower quality of life compared with those who are continent. Muslim women suffered from additional disruption from UI because of cleanliness requirements for religious obligations.

Different theories have been proposed to explain the association between depressive mood disorders and incontinence. Altered neurotransmitter function in depressed patients could affect the complex bladder innervation, leading to UI. One possible explanation is that a decreased serotonin activity can lead to depression [38] which also has an effect on bladder function [39]. A different theory suggests that the increased activity of the hypothalamic-pituitary axis seen in depressed individuals may determine physiological changes in the bladder, causing incontinence. Alternately, the embarrassment from urine loss may lead to progressive social isolation and subsequent depression over time [40].

About 20% of the patients of the current study reported positive family history of UI; most of

them were in the SUI group. This might disclose a genetic predisposition to the development of UI in women. Other researchers also reported that the daughters of mothers with incontinence had an increased risk for UI [41,42].

Out of the sixty women enrolled in current study, only 22 women answered "yes" to the question "Do you have hypertension"; those women significantly older in age, with high BMI, and pervious urinary disease. This was in agreement with the findings of Chang *et al* [43] who assess the associated risk factors and the prevalence of UI among women with hypertension. In women with hypertension, UI is significantly related to risk factors such as age, DM, BMI, and urinary diseases, in addition, BMI is considered a key risk factor for hypertension.

Regarding the control group, the cystometric values of the first desire, normal desire, strong desire, and maximum cystometric capacity were within the normal ranges recorded by other researchers [44,45].

The values of all types of sensation were lower in UUI and MUI women that are in harmony with the results of other researchers [45,46]. Patients with UUI and MUI demonstrated significantly high volumes of Pdet.max reflecting detrusor instability; this was in consistence with the results of Mardon *et al* [47].

On the contrary, those with SUI and MUI recorded near normal cystometric values, which might signify normal filling sensations in these two groups; a finding also reported by Irwin *et al* [48] and Al-Taee *et al* [45]. This can be explained on the basis of the pathophysiology of these types of incontinence as they results from a sudden increase of abdominal pressure in the absence of detrusor contraction and associated with both intrinsic sphincter dysfunction and urethral hypermobility [49]. SUI is characterized by normal urodynamic finding except identifying leakage from the urethra coincident with increased abdominal pressure [15]. OUI also characterize by normal urodynamic finding apart from presence of post-voiding residual urine due to incomplete bladder emptying [50].

In conclusion, age, parity and BMI significantly affect the prevalence of UI in women who have given birth vaginally. SUI is the most common type of UI amongst women regardless the small sample size in the study. Cystometric changes of urodynamic study were markedly evident in the SUI patients as compared to the healthy women.

Acknowledgment

We would like to thanks to members of Urodynamic Unit at Al-Sader Medical City, Holly Najaf for their help during the period of samples collection.

Author Contribution

The authors share the responsibility in preparing and completing this work.

Conflict of interest

The authors declare no conflict of interest.

Funding

The work not funded by any mean.

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Received 8th Jan. 2015: Accepted 27th Apr. 2015.

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq

Effect of *Glycyrrhiza glabra* on Antigen Induced Arthritis in Mice Model

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Abstract

Background Rheumatoid arthritis is a chronic inflammatory autoimmune disease represents the most common form of chronic inflammatory joint diseases. *Glycyrrhiza glabra* (*G. glabra*) was widely known to have several pharmacological activities, which might be beneficial in preventing and treating both acute and chronic inflammatory conditions.

Objective To study the effect of aqueous extract of *G.glabra* on antigen induced arthritis model in mice.

Methods Forty-eight male Swiss albino mice were used in this study. Group 1 arthritic mice without treatment (positive control); group 2 arthritic mice treated with *G. glabra* aqueous extract 750 mg/kg/day; group 3 arthritic mice treated with *G. glabra* aqueous extract 300 mg/kg/day and group 4 negative control (non-immunized, non-treated mice). Antigen induced arthritis was induced by Methylated bovine serum albumin in Imject Alum adjuvant. The mice were given the drug orally and the treatment was started from day 1 of the induction of arthritis until day 20. At day 20 of arthritis all mice were sacrificed and serum TNF-α was measured using ELISA technique. Biopsies of the left knee joint were taken for histopathological evaluation.

Results The results indicate that *G.glabra* caused inhibition of histopathological features of antigen-induced arthritis in dose dependent manner. *G.glabra* also caused reduction of serum TNF-α concentration in antigen-induced arthritis model in dose dependent manner.

Conclusions *Glycyrrhiza glabra* can significantly inhibit antigen-induced arthritis in mice. This effect seems to be in dose dependent manner.

Key words Rheumatoid arthritis, *G. glabra*, TNF-α, antigen induced arthritis.

List of abbreviation: RA = Rheumatoid arthritis, TNF-α = tumor necrosis factor alpha, NSAIDs = non-steroidal anti-inflammatory drugs, DMARD = disease-modifying antirheumatic drugs, ELISA = Enzyme Linked Immunosorbent Assay, H&E = Hematoxyline and Eosin, LE = Liquorice extract, CIA = collagen-induced arthritis.

Introduction

Rheumatoid arthritis (RA) is a common autoimmune disease that is associated with progressive disability, systemic complications, early death, and socioeconomic costs [(1)]. The epidemiological ratio of arthritis in female: male is 3:1 and the prevalence is 1% of the world population [(2)]. Synovial inflammation underlies the cardinal manifestations of this disease, which include pain, swelling, and tenderness followed by cartilage destruction, bone erosion, and subsequent joint deformities [(3,4)].

Despite intensive research, the precise cause of RA remains elusive. Although a variety of cells play a role in RA disease progression [(5)]. Macrophage-derived cytokines, such as tumor necrosis factor alpha (TNF-α), appear to play a critically important role in the induction and perpetuation of the chronic inflammatory processes in rheumatoid joints as well as in the systemic manifestations of this disease [(6)]. Medications that used to treat rheumatoid arthritis are divided into three main classes: non-

steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying antirheumatic drug (DMARD) (both synthetic and biologic) [7].

The majority of patients with newly diagnosed RA are started on disease-modifying antirheumatic drug (DMARD) therapy within 3 months of diagnosis such as Methotrexate [6]. Non-steroidal anti-inflammatory drugs (NSAIDS), glucocorticoid joint injections, and/or low-dose prednisone may be considered for control of symptoms [6,8].

Furthermore, the past decade have seen the introduction of seven new DMARDs which include leflunomide, the highly specific and efficacious anticytokine agents, including adalimumab, etanercept, and infliximab, and recently, abatacept, and rituximab, and others [9,10]. These therapies are emerging as important and successful therapeutics for patients with early disease [6,9,10]. Although effective in many patients, they are not without their drawbacks. Methotrexate and Leflunomide require careful monitoring and can cause serious hepatic and pulmonary toxicities [6]. The anti-TNF-α biological agents are costly, require parenteral administration, and have been associated with serious and opportunistic infections and lymphoma [6,9,10]. Furthermore as there are no 'cures', patients will require 30-40 years of ongoing therapy; although most of these agents do not remain effective in an individual for longer than 5 years. Thus, despite major recent advances in the treatment of RA, there is still need for convenient, safe, and effective therapies for many patients [6]. With these difficulties, the field of arthritis research has progressed exponentially towards herbal therapies that have been considered safe and effective in all elevating chronic pain associated with arthritis [2].

Glycyrrhiza glabra (Liquorice) has been used in medicine for more than 4000 years. The plant is distributed in the subtropical and warm temperature region of the world. The root and rhizome of the plant has been used as anti-inflammatory, anti-oxidant, anti-spasmodic, and expectorant [2].

In the present study, aqueous extracts of *G. glabra* was administered to mice with antigen induced arthritis to investigate the suggested benefit on this model as a animal model close to RA in human. Mouse models of antigen arthritis have been used extensively to study efficacy of biologies and the role of specific cytokines in the various aspects of disease pathogenesis [15-17].

The objective of our study is to study the effect of *G.glabra* on antigen induced arthritis model in mice.

Methods

Animals

Forty-eight male Swiss albino mice were used in this study; the animals were obtained from the animal house of the Al-Nahrain University. The animals aged 8 to 10 weeks and weighing 20 to 25 g, housed at a maximum of six per cage on wood shavings with free access to food and water. Before starting study, the animals were left for 48 hours to acclimatize to the animal room conditions and were maintained on an environment of controlled temperature, with a 12 hours light-dark cycle and standard pellet diet and tap water.

Arthritis induction

Mice were immunized subcutaneously with Methylated bovine serum albumin (mBSA) in Imject Alum in a concentration of (100 µg) mBSA/ 0.1 ml Imject Alum. The immunization was done on day zero and booster dose given after 7 days by subcutaneous route. After fourteen days of the second immunization (day 21 of experiment), arthritis was induced by intra-articular injection of 100 µg of mBSA mixed with the Imject Alum adjuvant in 1:1 ratio, in the left Knee joint [18].

Experimental design

The mice were divided into four experimental groups each group consist of 12 mice as follows:

Group 1: Arthritic mice without treatment (positive control).

Group 2: Arthritic mice treated with *G. glabra* aqueous extract 750 mg/kg/day [19].

Group 3: Arthritic mice treated with *G. glabra* aqueous extract 300 mg/kg/day [20].
Group 4: Negative control (non-immunized, non-treated mice).
All mice were weighed, and the drugs were measured according to the weight of each mouse. The mice were given the drug orally and the treatment was started from day 1 of the induction of arthritis (day 21 of experiment) until day 20 (day 41 of experiment) which is the end of the experiment.

Measurement of serum TNF-α

Mice of all groups were sacrificed at days 20 from induction of arthritis (day 41 of experiment). Blood was collected by cardiac puncture; serum was obtained to measure serum TNF-α level using Enzyme Linked Immunosorbent Assay (ELISA) technique.

Histopathological evaluation

Mice of all groups were sacrificed at day 20 from induction of arthritis and hitopathological changes of left knee joint of each mouse were evaluated.

Assessment of histopathological changes

Biopsies of the left knee joint were taken at day 20 of arthritis from the sacrificed mice of all groups to study the histopathological changes in them. The histopathological severity of arthritis was graded on a scale of 0-3 [21], where Zero = normal; One = minimal synovitis, cartilage loss, and bone erosions limited to discrete foci; Two =synovitis and erosions present, but normal joint architecture intact; and three = synovitis, extensive erosions, and disrupted joint architecture.

Biopsies were fixed by formalin 10 % for 4 hours and decalcified by HCL 10% for 6 hours. Dehydrated by using different concentrations of ethanol 70%, 80%, 95% and 100% (2 hours for each concentration), then specimen treated with xylol (2 steps two hours for each) before dipping in liquid paraffin at 55-60 ^{o}C, then tissue was embedded in paraffin (two steps for 2 hours for each), and Paraffin blocks were made. Sections were made with 5-µm thickness by using microtome. Finally, sections were stained by Hematoxyline and Eosin (H&S).

Statistical analysis

Statistical analysis was performed with the SPSS 19.0 statistical package for social sciences and Excel 2010. Descriptive statistics for the numerical data were formulated as mean and standard error (SE). Numerical data were analyzed using independent sample t-test for comparison between two groups. While histological score comparison between each group were done by Wilcoxon Mann whitney test. The level of statistical significant difference (*P*-value) is below (0.05).

Results

Serum TNF-α level

G.glabra seems to reduce serum TNF-α level in dose dependent manner. Mean serum TNF-α level of group 3 was 73.83±1.82 pg/ml which was significantly lower ($P \leq 0.001$) than 173.61±2.88 pg/ml of group 1. Moreover, serum TNF-α level was 53.23±1.82 pg/ml of group 2 which was significantly lower ($P \leq 0.001$) than 73.83±1.82 pg/ml of group 3. However, serum TNF-α level of group 2 and group 3 remain significantly higher ($P \leq 0.001$) than 18.22 ±1.29 pg/ml of group 4 (Table 1).

Table 1. Serum TNF-α level of study groups on day 20 of arthritis induction

Study Group	Serum TNF-α level (Mean± S.EM)
Group 1	173.61±2.88 (B*,C*,D*)
Group 2	53.23±1.82 (C*,D*)
Group 3	73.83±1.82 (D*)
Group 4	18.22±1.29

B: Comparison with group 2, C: Comparison with group 3, D: Comparison with group 4, S.EM: standard error mean, TNF-α: Tumor necrosis factor alpha, *= $P < 0.05$.

Histopathological score evaluation

Glycyrrhiza glabra also seems to reduce the histopathological score in our model of arthritis in dose dependent manner. Histopathological scores of group 3 showed a significant reduction ($P < 0.05$) compared to group 1. Moreover, histopathological scores of group 2 showed a

significant reduction ($P < 0.05$) compared to group 3 (Table 2).

Table 2. Histopathological scores of study groups

Study Group	Histological score Median
Group 1	$2.75^{(B*C*)}$
Group 2	$0.75^{(C*)}$
Group 3	1.00

B: Comparison with group 2, C: Comparison with group 3, *= $P < 0.05$.

Histopathological section taken from group 1 reveals loss of joint architecture with heavy chronic inflammation of the synovium, pannus (an abnormal layer of fibrovascular tissue or granulation tissue), marked erosion of bone and cartilage, hyperplasia and hypertrophy of the synovial lining (Figure 1).

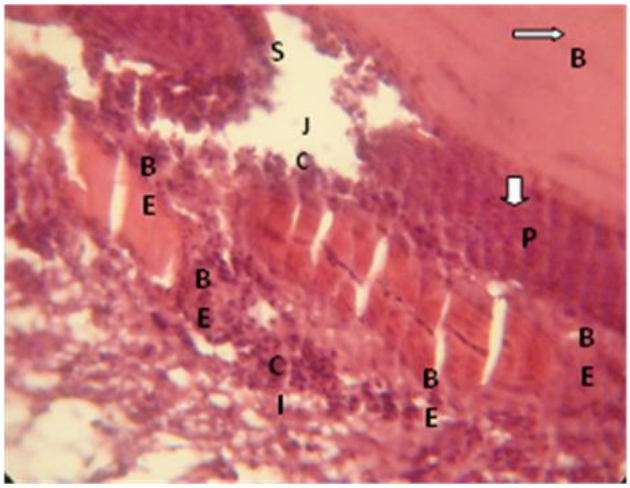

Fig. 1. Arthritic knee joint of group 1 on day 20 of arthritis showing loss of joint architecture with heavy chronic inflammation of the synovium, pannus, marked erosion of bone and cartilage, hyperplasia and hypertrophy of the synovial lining. (S) synovium, (JC) joint cavity , (p) pannus, (B) bone, (BE) Bone erosion, (CI) chronic inflammation. H & E stain (4X).

Histopathological slide examination of group 2 mice shows preservation of the joint architecture, with minimal chronic inflammation and minimal erosion in the bone (Figure 2) and for group 3 showed preservation of the joint architecture, focal synovitis, and pannus caused cartilage and bone erosion limited to discrete foci (Figure 3). Histological section taken from group 4 showed normal joint architecture with normal cartilage, bone and synovium, no inflammatory cell infiltration (Figure 4).

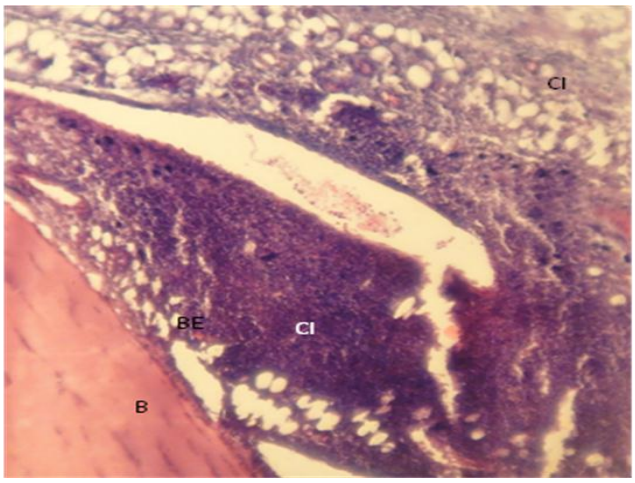

Fig. 2. Arthritic knee joint of group 2 on day 20 of the arthritis showing preservation of the joint architecture, with minimal chronic inflammation and minimal erosion in the bone. (B) Bone, (CI) chronic inflammation, (BE) bone erosion. H & E stain (40X).

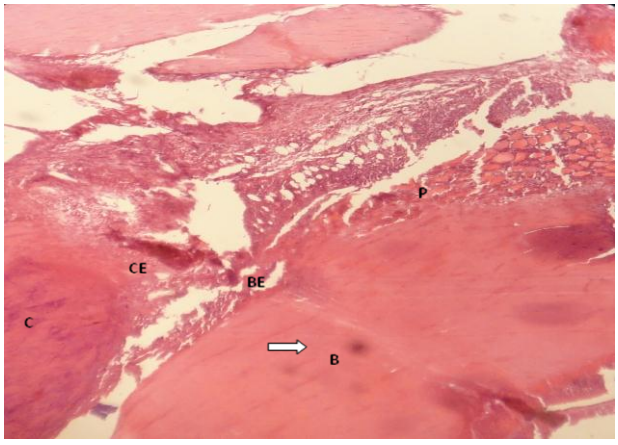

Fig. 3. Arthritic knee joint of group 3 on day 20 of the arthritis showing preservation of the joint architecture, focal synovitis, and pannus caused cartilage and bone erosion limited to discrete foci. (B) Bone, (BE) Bone erosion, (C) cartilage, (CE) cartilage erosion. (P) Pannus. H & E stain (4X).

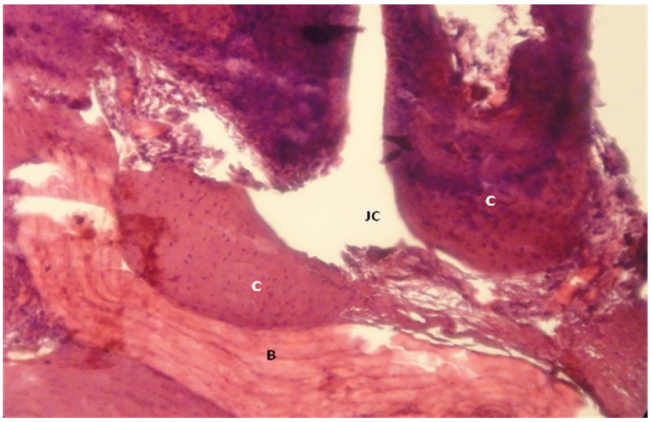

Fig. 4. Left knee joint of group 4 showing normal joint architecture with normal cartilage, bone and synovium, no inflammatory cell infiltration. (C) Cartilage, (JC) Joint cavity, (B) bone. H & E stain (4X).

Discussion

Antiarthritic therapeutic potential of *G. glabra* aqueous extract on mice antigen induced arthritis has been studied in the present study. The results of present study indicate that *G. glabra* was significantly effective in inhibiting serum levels of TNF-*α* in the present model of arthritis in dose dependent manner. The results of present study come in agreement with other study in which Liquorice extract (LE) oral administration to collagen-induced arthritis (CIA) mice result in significant reduction in the serum levels of TNF-*α* compared to CIA mice treated with vehicle.

G. glabra was effective in inhibiting histopathological changes in the present model of arthritis (inflammatory cells infiltration, synovitis, cartilage erosion, bone erosion, loss of joint architecture and pannus formation) in dose dependent manner.

In other study, LE oral administration to CIA mice cause noticeable reduction of the histopathological changes in the joint (including CIA-characteristic synovial hyperplasia, infiltration of inflammatory cells into the joint cavity, and extensive pannus formation) this come in agreement with results of present study [22].

It has been concluded that aqueous extracts of *G. glabra* is the good source of anti-oxidants. In addition, in *G. glabra* anti-oxidant contents were high. Therefore, it is more beneficial for the treatment of various diseases, which are caused due to the oxidative stress [14]. Oxidative stress is involved in the pathogenesis of autoimmune rheumatological diseases, including RA, systemic lupus erythematosus, and systemic sclerosis [23]. Epidemiological studies have demonstrated an inverse correlation between the dietary intake of antioxidants and the incidence of RA [24]. The disease activity correlates inversely with antioxidant levels and positively with the presence of oxidative stress in patients with RA [25,26]. Oxidative damage to proteins, lipids, DNA, cartilage, and extracellular collagen has been demonstrated in patients with RA [28].

Some drugs used to treat RA such as methotrexate; etancercept and infliximab are known to play essential roles as antioxidative agents [29]. Lipid peroxidation markers such as serum malondialdehyde and urine isoprostane are reported to be elevated in CIA compared with those in controls [30,31]. The beneficial effects of antioxidants have been demonstrated in mice with CIA [32-37]. An important mechanism of anti-arthritic activity is the membrane stability modulating effect [38]. *G. glabra* may exert its effects by modifying the lysosomal membrane in such a way that it is capable of fusing with the plasma membrane and there by preventing the discharge of acid hydrolase or by inhibiting the release of lysosomal enzymes [39]. The activity of *G. glabra* may be due to presence of flavonoids i.e. liquiritin and isoliquiritin [2]. Lysosomes are membrane enclosed cytoplasmic organelles, which possess an acidic interior that contain many hydrolytic enzymes. Lysosomal enzymes are widely distributed in tissue and circulating blood cells and are responsible for intracellular breakdown of complex macromolecules. They also degrade endothelial membrane glycol-conjugates. The altered enzyme activities in arthritis can be regarded as an index of lysosomal enzyme activation occurring in response to metabolic need of degrading various

constituents of cells such as mucopolysaccharides and glycoproteins accumulated in tissue due to arthritis associated with vasculopathies [2].

Another mechanism may explain *G. glabra* inhibition of arthritis in the present model can be reducing autoantigen production by inhibition protein denaturation [2]. Denaturation of proteins as one of the causes of rheumatoid arthritis is well documented. Production of autoantigens in certain rheumatic diseases may be due to in vivo denaturation of proteins. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding [2]. The results from previous study revealed that *G. glabra* is capable of controlling the production of autoantigens due to in vivo denaturation of proteins in rheumatic diseases [2]. Proteinases have been implicated in arthritic reactions. Neutrophils are known to be a rich source of proteinases, which carry in their lysosomal granules many neutral serine proteinases. Leukocyte proteinases play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors. *G. glabra* exhibited significant anti-proteinase activity in previous study and this may explain anti-arthritic effect of this herb in the present study [2].

Inhibition of some important proinflammatory cytokine(s) may be additional effect of *G. glabra* on the present model of arthritis. However, there is evidence that antioxidants reduce the activation of NFκB, which is involved in the production of inflammatory cytokines, including TNF-α and IL-17 [40,41]. Taken together, these data indicate that *G. glabra* may inhibit the production of TNF-α and IL-17 by T cells by inhibiting NF-κB [42]. Suppression of osteoclastogenesis also may be another mechanism accounted for *G. glabra* effect in the present experiment, because previous studies, reveal that antioxidants cause suppression of osteoclastogenesis [37,43,45].

Acknowledgement

To the Deanery and Departments of Pharmacology and Pathology of College of Medicine/Al-Nahrain University.

Author contributions

Authors contribute in organizing the idea and protocol of the research, performing the practical aspects and accomplishing writing the final outcome of this work.

Conflict of Interest

No.

Funding

College of Medicine/Al-Nahrain University.

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Received 21st Dec. 2014: Accepted 14th Apr. 2015

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq

Where and Why do we Select the Type and Site of Colostomy in Children below Two Years

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Abstract

Background Alexis Litter (1710) may be called the father of colostomy, when he made an incision in the belly and opened the ends of closed bowel to the belly surface where it never closed and preformed the function of anus for an infant suffered from intestinal obstruction due to congenital malformation of the return, probably an imperforate anus. This operation was known as Litter's operation.

Objective To decrease the incidence of colostomy complications through selection of proper site and type of colostomy.

Methods Two hundred temporary colostomies made for 200 neonates, infants and children below 2 years old in Al-Kadhemiya Pediatric Hospital and Al-Imamain Al-Kadhemain Medical City over a period from September 2008 to September 2013.

Results Imperforate anus was the most common indication for colostomy in 59% of cases and Hirschsprung's disease in 33.5%, which were done mainly in neonatal period (57%) especially for male imperforate anus without fistula. Prolapse was the most common and challenging complication following colostomy creation in 25% followed by severe skin excoriation 24% which mainly happened with loop transverse, while declining incidence in divided sigmoid and descending colostomies.

Conclusions Divided and separated descending and sigmoid colostomies were the stoma of choice for most clinical situations requiring colostomy because of complete fecal diversion with the least complications prolapse and skin excoriation.

Keywords Colostomy complications, prolapse, skin excoriation

List of abbreviation: HD = Hirschsprung's disease, IA = imperforate anus, PSARP = posterior saggital ano-rectoplasty.

Introduction

Colostomy as a diverting procedure has its origins in antiquity. The first successful colostomy was performed in 1798 by Durret for a four day old neonate with anorectalagenesis [(1)]. A colostomy is an artificial opening made in the large bowel to the exterior in order to divert its contents, where it can be collected in an artificial appliance.

Most of the colostomies in pediatrics are temporary and indicated for decompressing obstructed large bowel with fecal diversion to protect a distal anastomosis following resection of a ganglionic segment, tumors, injured or perforated colonic lesions and conditions that require definitive pelvic operations. The most common indications for temporary colostomies in children below 2 years old are imperforate anus (IA) and Hirschsprung's disease (HD) [(2)].

Right loop transverse and loop sigmoid colostomies were routinely performed for most cases of IA and HD. In HD, the colostomy must be created in the ganglionic part of large bowel defined by frozen section at time of operation at centers where it is available [(3)]. Sometimes colostomy is created above the transitional zone depending upon barium enema and macroscopic findings. In IA, the colostomy is usually

performed in sigmoid or descending colon (loops or divided).

In divided colostomies, mucus fistula is usually irrigated to release the impacted meconium to avoid mega sigmoid and at the same time prepare it for further definitive pelvic operation (pull through or posterior sagittal anorectoplasty) (PSARP) [4]. Distal colostogram was done to identify the fistula and the extent of distal colon. PSARP definitive surgery in imperforate anus is performed between 4-8 weeks and pull through for HD can be done even in neonatal period [5].

The objectives of this study were to make an early diagnosis, management and prevention of colostomy complications through performing proper site and type of colostomy.

Methods

A total number of 200 temporary colostomies were done for 200 neonates, infants, and children below 2 years old age in Al-Kadhemiya Pediatric Hospital and Al-Imamain Al-Kadhemain Medical City from September 2008 to September 2013 .

A standardized data was prepared for collection of information; included, age, sex, body weight, natal history, family history, age of presentation, associated lesions, type of lesion, history of abdominal trauma, age at which colostomy was performed and site or type of colostomy performance.

Patients were fully examined clinically and investigated according to their diseases. These investigations included erect plain abdomen, invertogram, abdominal ultrasonic exam, computerized tomography scan, magnetic resonance image, echocardiogram, cystoscopy, cystourethrogaphy, intravenous pyelography, barium enema, rectal biopsy and multiple colonic biopsies.

Most of our patients presented with intestinal obstruction and they were prepared prior to surgical intervention, through fluid and electrolytes replacement. Also they were covered by broad spectrum antibiotics.

Results

The commonest indications for temporary colostomy in our study were IA in 118 patients (59%) and HD in 67 patients (33.5%), while the other least indications were necrotizing enterocolitis in 8 patients (4%), rectal atresia in 4 patients (2%), pseudo intestinal obstruction in 2 patients (1%) and one patient (0.5%) had colonic perforation by bullet injury (Table 1).

Table 1. Colostomy indications with sex distribution

Disease	Colostomies		
	Male	Female	Total
Imperforate anus	85	33	118
Hirschsprung's disease	39	28	67
Necrotizing Enterocolitis	5	3	8
Colonic Atresia	2	2	4
Pseudo intestinal obstruction	2	0	2
Colonic Injury	1	0	1
Total	134	66	200

Most of the colostomies were performed in the neonatal period (114) patients (57%) with ratio of male to female 3:1 (Table 2). Seventy (70) patients (61%) of them had IA while 31 patients (27%) had HD. Eight patients (7%) had necrotizing enter colitis, 4 patients (3.5%) had colonic atresia and one patient (1%) had colonic perforation.

Table 2. Age at colostomy creation with sex distribution

Age at stoma	Imperforate anus		Hirschsprang's disease		Others		Total
	M	F	M	F	M	F	
Neonatal Period 1 day – 1 month Infancy	65	5	19	12	9	4	114
Period 1-12 month	0	18	34	20	2	2	76
Childhood Period 1 month-2 year	0	2	4	2	2	0	10
Male to Female ratio	2.6: 1		2.6:1		2:1		200

The most common type and site of colostomy performance was right loop transverse in 58 patients (29%) and 55 of them (96.5%) had HD. Therefore, the most common colostomy in HD was right loop transverse colostomy, while most common colostomy in IA was loop sigmoid.

The second most common type was loop sigmoid which was done for (55) patients (27.5%), while divided sigmoid was performed for 48 patients (24%) and 24 patients (12%) had divided descending colostomy while 15 patients (7.5%) had divided transverse colostomy. Most of patients with divided sigmoid and descending colostomies had IA.

The complications of colostomy were:

1. Colostomy prolapsed which is noticed in 50 patients (25%). Twenty five patients (50%) of them had right loop transverse colostomy. Ten patients (20%) with divided transverse developed prolapse while 10 patients (20%) with loop sigmoid had colostomy prolapse. Only four patients (8%) with divided sigmoid and one patient (2%) with descending colostomies developed prolapse. The prolapse occurred most commonly in the distal limb of colostomy. Forty patients (80%) who developed prolapse had HD and the distal prolapsed more frequently due to redundancy of distal limb, while it declined in divided colostomies and least in divided descending due to fixed distal limb.
2. Severe skin excoriation was the second most common complication. It happened in 48 patients (24%) and from these 24 patients (50%) had right loop transverse colostomy, while 15 patients (31%) had divided transverse. Only 2 patients (4%) of them with divided sigmoid colostomy developed severe skin excoriation. Most of the patients with this complication had HD. They were mainly from rural areas of low educational state and less nursing care in addition to deficiency of appliance and stomal bag in addition to high output fluid leading to severe skin excoriation.
3. Wound sepsis was the third common complication; it included local infection, abscess, fistula and dehiscence. Twenty four patients (12%) development wound sepsis. Eighteen patients (75%) of them had HD because of their low immunity and failure to thrive in comparison to other patients. Only 6 patients (3%) with IA developed wound sepsis due to bad technique and wound contamination during separation and fixation of colostomy limbs. Two patients (1%) had wound dehiscence and needed secondary suturing (Table 3).
4. Intestinal obstruction developed in 12 patients (6%). Eight 8 patients of them had loop transverse colostomy, while only 2 patients with divided transverse and 2 patients with loop sigmoid colostomy developed intestinal obstruction. Ten patients (83%) of them had HD because during colostomy creation we need some times taking multiple colonic biopsies to identify the extent of the aganglionic segment.
5. Stomal retraction developed in 7 patients (3.5%). It occurs due to small size incision in the abdominal wall creation in comparison to colonic diameter leading to ischemia and necrosis of the colostomy endings, in addition to recurrent trauma of the appliance to the colostomy. Three patients with divided transverse, two patients with divided sigmoid and one with divided descending colostomy developed stomal retraction.

6. Stomal stenosis happened in 5 patients (2.5%). It developed in divided transverse, sigmoid and descending colostomies due to ischemia to colonic vessels and improper using of colostomy appliance.
7. Stomal dysfunction developed in 4 patients (2%), three of them had HD and the other one with IA had sepsis and died.
8. Parastomal hernia. It developed in 2 patients (1%), one with loop transverse and the other one with divided transverse colostomy.
9. Bleeding happened in 2 patients (1%) one with divided transverse and the other with divided sigmoid colostomy (Table 4).

Table 3. Complications following colostomy creation

Type and Site of Colostomy	Colostomy Prolapse	Sever skin exponent	Wound sepsis	Intestinal obstruction	Colostomy retraction	Colostomy stenosis	Colostomy dysfunction	Parastomal hernia	Bleeding
Right loop transverse	25	24	5	8	0	0	3	1	0
Divided transverse	10	12	10	2	3	2	0	1	1
Loop sigmoid	10	8	6	2	1	1	1	0	0
Divided sigmoid	4	3	2	0	2	1	0	0	1
Divided Descending	1	1	1	0	1	1	0	0	0
Total	50	48	24	12	7	5	4	2	2

Table 4. Comparison our results with collected studies about morbidity rate and colostomy complications

Complication	Mollitt et al [8] N=146 1980	Lister et al [9] N=156 1983	Al-Salem et al [10] N=77 1992	Nour et al [11] N=108 1994	Sheikh et al [13] N=16 2006	Our study 2013
Prolapse	**11.6%**	**12%**	**18.9%**	**5%**	**20%**	**25%**
Severe skin excoriation	-	**21.6%**	-	**2%**	**25%**	**24%**
Wound sepsis	-	**17%**	-	**1%**	-	**12%**
Intestine obstruction	-	**4%**	-	**3%**	**12%**	**6%**
Stomal retraction	**3.4%**	**1.9%**	**2.7%**	-	-	**3.5%**
Stomal stenosis	**6.2%**	**6.4%**	**2.7%**	-	**3%**	**2.5%**
Stomal dysfunction	-	-	-	-	**2%**	**2%**
Parastomal hernia	-	-	-	-	**5%**	**1%**
Bleeding	-	**5%**	-	-	**1.5%**	**1%**

Discussion

The basic principles of constructing a good temporary colostomy as an initial management for a variety of diseases in pediatric age group including appropriate positioning, a viable bowel segment without tension and a tunnel in the abdominal wall to ensure complete diversion of bowel contents. Although great advances have been made with regard to stoma formation and management, both early and late complications are common [6]. The overall morbidity from colostomy has been reported to be as high as 50% [7,8] and a retrospective study was (42-75%) [2,8].

In our study, the morbidity rate was higher than other studies Lister et al [9] and Nour et al [10].

The reasons of our high percentage of complications in comparison to other studies are due to more frequent occurrence of prolapse and severe skin excoriation in our study in addition to the fact that some studies did not record or undeestimated bleeding, severe skin excoriation [8,11].

The most common complication of colostomy in our study was prolapse. Our results were in harmony with those of Sheikh et al (20%) but they were more than other studies [8,9,10,11,13].

The second most common complication was severe skin excoriation. It was more frequent with right loop transverse colostomy, because bowel motion is more frequent and the fluid and minerals are not fully absorbed yet as in sigmoid and descending colostomies. Our results reported a percentage less than Soomro et al [12] and in close proximity to Sheikh et al [13]; yet, more than others. The reasons of high percentage in our study were due to the shortage in supplying colostomy appliances; the colostomies, being most of loop type which were associated with more soiling than divided ones on which appliances fit better, in addition to not recording this complication by many studies or underestimating it by others [10,13].

The third common complication was wound sepsis (infection, stich abscess, fistula and dehiscence). It was higher than other studies due to imperfect nursing care. Lister et al [9] had also high incidence of wound sepsis (17%). This complication was more frequent with divided type due to the technical error by doing big abdominal wall incision and contamination of the wound by immediate opening of the bowel before fixing it to abdominal wall and not well forming bridge in between the colostomy limbs.

The fourth complication was intestinal obstruction which was lower than that reported by Nour et al [10], but higher than that of Lister et al (4%) [9]. It occurred more in loop transverse colostomy and more in cases of HD because during laparotomy we need to take multiple colonic biopsies and creation of colostomy leading to extensive bowel manipulation and sometimes contamination of the peritoneum during biopsies taking.

Considering the less frequent complications of stomal retraction and stenosis; they were noticed more with divided colostomies due to technical errors through creating small abdominal wall incision in the presence of dilated and hypertrophied bowel that leads to ischemia and necrosis of colostomy endings and later stenosis and retraction. Additionally, frequent trauma to the colostomy endings by improper appliances could be another cause. Our results were in accordance to those reported by Al-Salem et al [14] but lower than other studies.

On the other hand, stomal dysfunction and parastomal hernia reported in this study were in agreement to that recorded by Nour et al [12]. Bleeding was the least frequently reported complication noticed in our patients, yet, it is less than that shown by Lister et al [9] and Nour et al [10].

In conclusion, divided stomas must be the stoma of choice for most of clinical situations requiring colostomy creation, because of complete fecal diversion and least complications especially prolapse and skin excoriation. It is advisable to reduce the length of time the child having colostomy, by doing the definitive operation as early as possible particularly to those having prolapse and severe skin excoriation.

Acknowledgments

I am grateful to the permanents in pediatric surgery; Dr. Fatima Haitham and Dr. Noor Talib for their best doing in collecting the reports.

Author contribution

Dr. Mohammed did and writes the paper; Dr. Abolhab and Dr. Mohamed collect the data and share in writing parts of the research.

Conflict of interest

We declare no conflict of interest.

Funding

None.

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Received 7th Jan. 2015: Accepted 4th May. 2015

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq

Age- and Strain-related Changes in the Mutant Albino Swiss/ Anatomy Glasgow University Rats: A comparative Study of Lipofuscin and Calbindin D-28k Levels in Cerebellar Purkinje Cells

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Abstract

Background A spontaneous recessive mutation in the gene coding for protein kinase gamma has created a new rat strain called Albino Swiss/ Anatomy Glasgow University (AS/AGU). It characterized by disordered locomotion due to impaired dopamine release from certain areas of the brain and eventual neurodegeneration. The mutant rats also have a shorter life span.

Objective The present study investigates Lipofuscin level as an indicator of age and calbindin D-28k level as an indicator of neuronal integrity in cerebellar Purkinje cells of the mutant AS/AGU strain in comparison to the normal AS strain.

Methods Eighteen rats (9 AS and 9 AS/AGU) were selected to study the Lipofuscin and calbindin in relation to age and strain using multiple histological techniques including fluorescent microscopy.

Results Fluorescent microscopical study has shown early age related Lipofuscin accumulation in the AS/AGU rats compared to the normal strain. Moreover, Calbindin D-28k showed age related increase in both strains but marginally significant decline in the AS/AGU strain.

Conclusions Our study presents AS/AGU as an animal model of early aging in addition to its value as a model of neurodegeneration.

Keywords Aging, Albino Swiss Rat, Calbindin D-28k, Fluorescent Microscopy, Lipofuscin.

List of abbreviation: LF = Lipofuscin, CNS = central nervous system, AS = Albino Swiss, AS/AGU = Albino Swiss/Anatomy Glasgow University, ICC = immunocytochemistry, FM = Fluorescent Microscopy, PBS = Phosphate Buffer Saline solution, PKC-γ = Phosphate Kinase C - gamma.

Introduction

Lipofuscin (LF) is a brown pigment that accumulates with age in postmitotic cells and cells with low mitotic activity [1,2], but not in actively proliferating cells except where division has been inhibited [3,4]. While LF accumulates in a time dependent linear fashion, ceroid accumulation is fast and age-independent [7].

Electron microscopy shows LF as dense material bounded by a single layer membrane (typical for lysosomes) often containing vacuoles [8]. LF is formed predominantly by un-categorized proteins and lipids with traces of carbohydrates and metals [6,8,9]. It auto-fluoresces in a narrow wavelength range in intact cells (440-460 nm) but in a wider range when extracted [8,10]; while the source of fluorescence in LF is unknown, several studies have managed to produce similar auto-fluorescent material from limited combinations of molecules [11,12].

The mechanism of LF formation is unclear, but most theories assume dysfunction of lysosomal

mechanisms in which there is a mismatch between formation and degradation, and LF forms from residual macromolecular and cellular components [8,13-17]. One of the factors which may precipitate lysosomal dysfunction, is the formation of excessive reactive oxygen species which leads to mitochondrial disorganization and proteosomal accumulation of non-degradable LF [8,18,19]. It is noteworthy that LF still exhibits lysosomal enzyme activity [9].

Calbindin D-28k is one of the intracellular calcium binding proteins that regulate the critical intracellular level of calcium ion for normal cellular functions [20] and it may be altered by cell injury [21]. Many studies have shown that calcium binding proteins have neuroprotective effects in acute brain injury [22], in Parkinson's disease and after calcium induced toxicity [23]. Age related changes of Calbindin D-28k are therefore a potential field of study for many researchers in both health and disease conditions. Calbindin D-28k is highly expressed in most central nervous system (CNS) neurons in both humans and animals including the Purkinje cells of the cerebellum.

The Albino Swiss/Albino Glasgow University (AS/AGU) rat has a recessive mutation in the gene ***agu*** coding for PKC-gamma [24] which leads to disordered locomotion [25,26] and an inability to release dopamine and serotonin in the striatum [27]. Mutant rats also reach a lower body weight than the parent AS strain, and have a shorter life span. They may therefore be useful as a spontaneous model of accelerated aging and neuronal injury. Since LF accumulation is inversely related to remaining life span [28] and Calbindin D-28k changes might have effects on vulnerability to neuronal dysfunction, this present study was carried out to examine LF deposits and Calbindin D-28k levels in the brain of AS/AGU rat and compare them with the AS control rat, using Purkinje cells of the cerebellum as a large and easily defined population. Purkinje cells are a class of GABAergic neurons located in the cerebellum of human and other mammalian brains including rats [29,30]. They are characterized by large number of dendritic spines and by their large size, which make them easily identifiable. They are found within the Purkinje layer in the cerebellum and aligned linearly stacked one in front of the other [31]. Purkinje cells send inhibitory projections to the deep cerebellar nuclei, and constitute the sole output of all motor coordination in the cerebellar cortex [32,33].

This study aimed to investigate changes in the AS/AGU rat strain to demonstrate early aging and state of neuroprotection.

Methods

A total of 18 male rats were used in this study and fixed with 4% paraformaldehyde by cardiac perfusion post-mortem. The CO_2 method used to sacrifice the animals was followed and according to the ethical standards employed by the University of Glasgow. These rats included six animals aged 6 months (3 AS and 3 AS/GU) and twelve animals aged 12 months (6 AS and 6 AS/AGU). In each case, the brain was extracted from its fixative and cut into three parts: forebrain, midbrain and cerebellum as shown in Fig. 1.

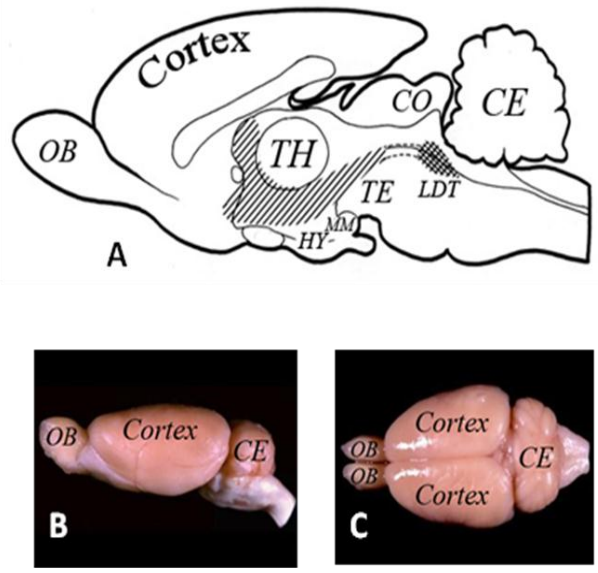

Fig. 1. Gross Anatomy of rat brain showing (A) sagittal section with major brain areas illustrated (B) lateral view and (C) axial top view of the rat brain. CO = colliculi, HY = hypothalamus, LDT = laterodorsal tegmental nucleus, MM = mammillary bodies, TH =

thalamus, TE = tegmentum.

For fluorescent microscopy and immunocytochemistry (ICC), these parts were put in a tissue processer for 24 hours to prepare them for paraffin embedding. After paraffin embedding, cerebellar blocks were selected and cut using a Jung Biocut-2035 rotatory microtome and the slides were standardized to 8 micron thickness for consistency in measurement and for ordinary visualization. The sections were fixed to APES-coated slides and kept overnight to dry.

Fluorescent Microscopy (FM) and Immunocytochemistry (ICC)

A total of 12 cerebellar blocks (6 AS and 6 AS/AGU) from two age groups (half at 6 month age and half at 12 month age) were chosen for LF and Calbindin D-28k measurement. Two widely separated sections (8 micron thickness) from each cerebellar block were selected for LF measurement and another two were used for Calbindin D-28k measurement.

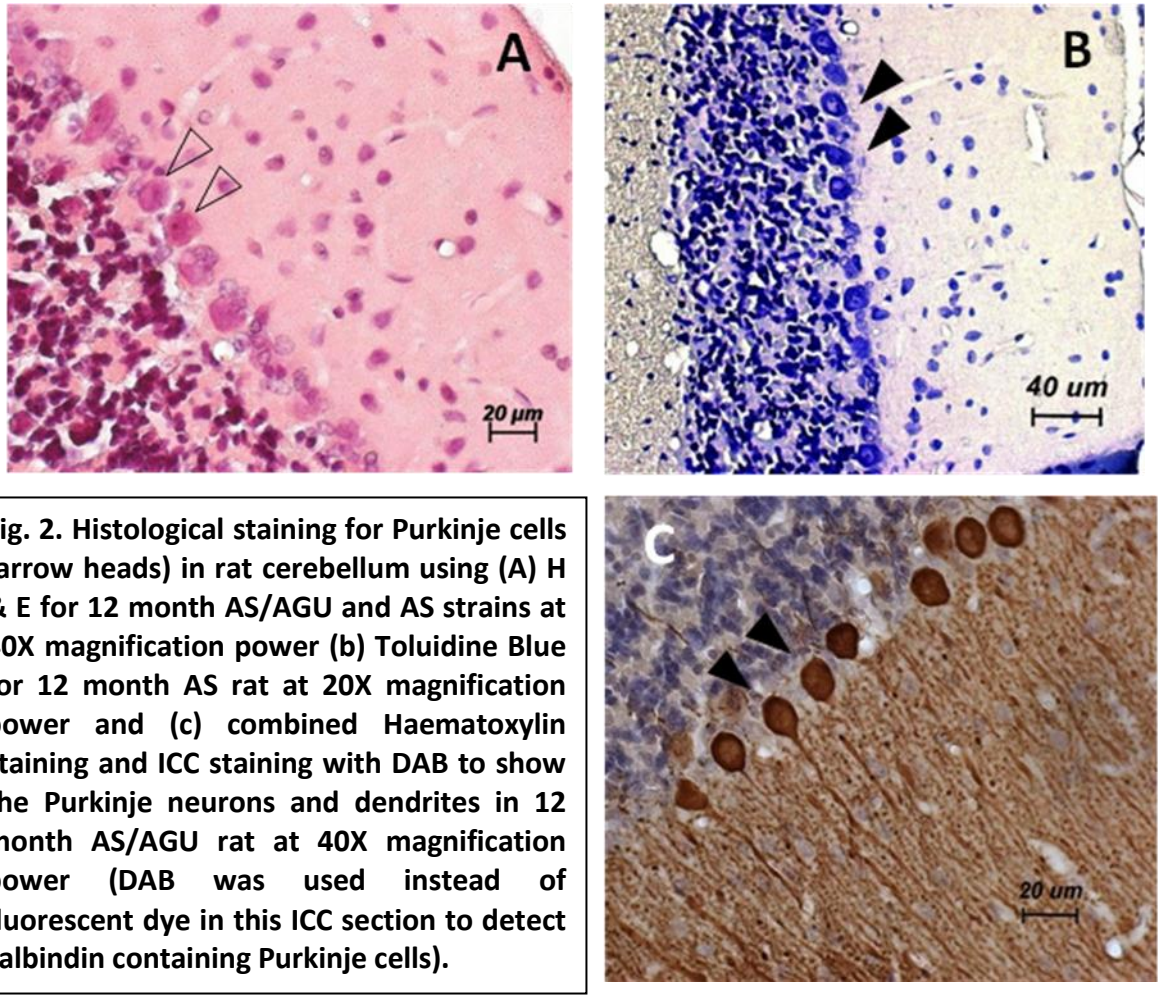

Fig. 2. Histological staining for Purkinje cells (arrow heads) in rat cerebellum using (A) H & E for 12 month AS/AGU and AS strains at 40X magnification power (b) Toluidine Blue for 12 month AS rat at 20X magnification power and (c) combined Haematoxylin staining and ICC staining with DAB to show the Purkinje neurons and dendrites in 12 month AS/AGU rat at 40X magnification power (DAB was used instead of fluorescent dye in this ICC section to detect calbindin containing Purkinje cells).

The LF sections were deparaffinised in Histo-Clear solution for 15 minutes and mounted. The ICC slides for calbindin, were deparaffinised and boiled for 30 minutes in citrate buffer (pH=6) to unbind the cellular proteins (a critical step in ICC), then they were rinsed three times using 0.2M Phosphate Buffer Saline solution (PBS).

Blocking serum (1% normal goat serum in 0.3% Triton/PBS) was prepared and added on the slides to prevent nonspecific binding of calbindin specific primary antibody.

Then, the primary antibody against Calbindin D-28k (Swant® monoclonal Ab CB300) was used at a dilution of 1:500 in the blocking serum to continue blocking during primary

antibody application.

Next day, a fluorescent secondary antibody (at 1:100 dilutions in PBS) was added after three rinses in 0.2M PBS and kept overnight.

Finally, slides were washed three times using 0.2M PBS and mounted with hard fluorescent mounting material (VectaShield© Hard set™ H-1400).

Both LF and ICC slides were measured spectrophotometrically using a LEITZ LABRORLUX Fluorescent Microscope.

The fluorescent microscope parameters were standardized to 650 volts of the UV power source and an objective lens with 40X magnification power was used in all fluorescent microscope measurements.

Each slide was examined and 50 Purkinje cells in row with an obvious nucleus were selected for measurement providing that the appearance of the nucleus indicated that we were roughly in the centre of the Purkinje cell.

Measurement of fluorescence (autofluorscence in LF slides and fluorescent dye in ICC slides) were recorded in tables designed for this purpose. In addition, five background fluorescence measurements were taken and their mean value was subtracted from the cells' records to eliminate the effect of background emission resulting from nonspecific binding of fluorescent antibody or other tissue emission.

Statistical Analysis of Variance (ANOVA) was carried out using Statistica© software package (version 8) to compare the AS and AS/AGU rats in both age groups.

Results

Lipofuscin Results

Under fluorescent microscope, LF showed two kinds or arrangements: polar and dispersed aggregations (Fig. 3).

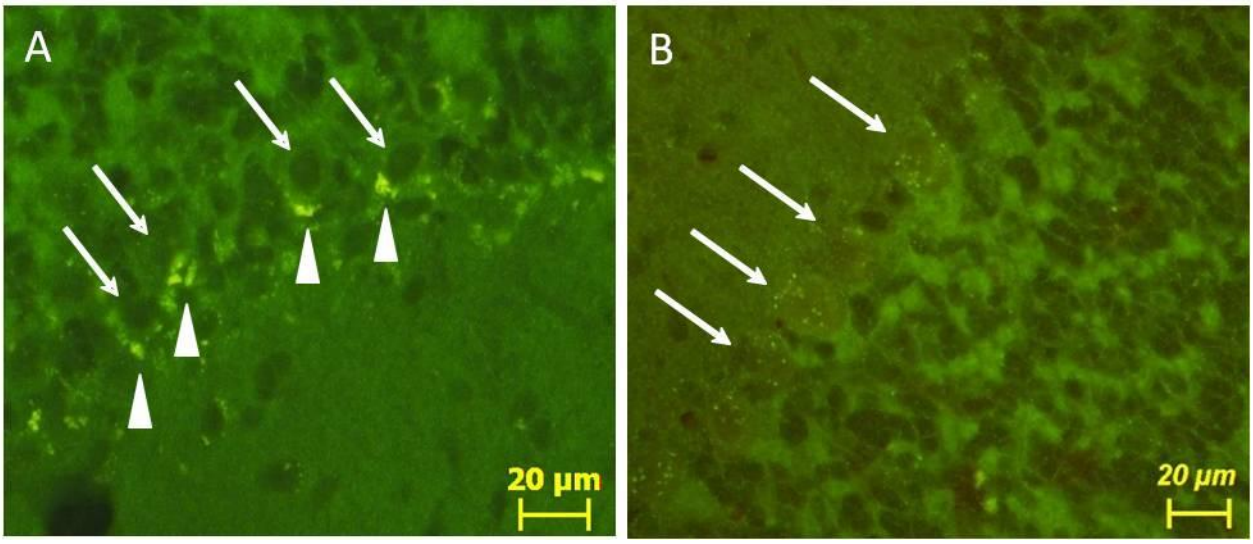

Fig. 3. Age difference and Patterns of Lipofuscin accumulations in Purkinje cells using fluorescent microscope (green filter). (A) 6 month aged Albino Swiss rat showing scanty and dispersed distribution of Lipofuscin in Purkinje cells (arrows). (B) Twelve months Albino Swiss/Albino Glasgow University rat showing Purkinje cells (arrows) with great amount of Lipofuscin accumulation in polar distribution (arrow heads), (40X).

LF was seen through a wide range of wavelengths since it was even clear through the red filter of the fluorescent microscope.

More importantly, analysis of LF auto-fluorescence showed an age related increase (Fig. 4).

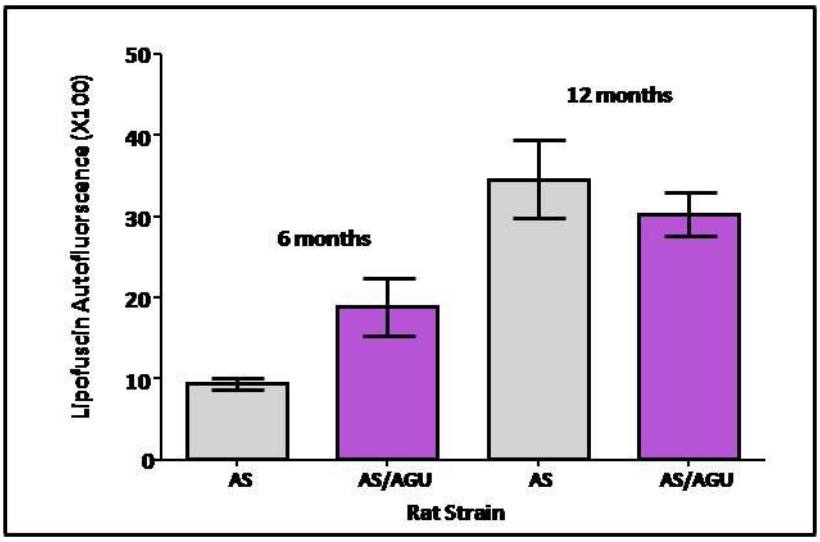

Fig. 4. Age- and Strain-related changes in autofluorescence levels of Lipofuscin measured spectrophotometrically. There is a clear trend toward an age-related increase of Lipofuscin and strain difference in young animals. Each column in the graph represents a mean value of 3 animals in the study with standard error.

There was a significant increase (*P* = 0.006) in LF accumulation in the AS strain with ageing (Table 1). The AS/AGU strain showed a twofold increment of LF at the 12 months age compared with 6 months age however, the statistical significance was only marginal (*P* = 0.061).

Table 1. Age and strain statistical comparisons of Lipofuscin autofluorescent results.

Group 1	Group 2	P value
9.4	34.6	0.006*
9.4	18.9	0.056‡
18.9	30.2	0.061‖
34.6	30.2	0.471‼

* = AS 6month vs. AS 12month, ‡ = AS 6month vs. AS/AGU 6month, ‖ = AS/AGU 6month vs. AS/AGU 12month, ‼ = AS 12month vs. AS/AGU 12month.

The cut off significant values for the analysis of variance are: significant level $P < 0.05$, marginal or weak significant level $0.10 > P > 0.05$, not significant $P > 0.1$

Comparative Lipofuscin analysis between AS and AS/AGU strains has demonstrated interesting figures (Table 1 and Figure 4). The level of LF was very close and statistically not different at age 12 month between the two strains; while 6 month AS/AGU rats showed approximately double the autofluorscence of AS rats. The small sample size has contributed to the marginal significant values.

Calbindin D-28k Results

The immunecytochemistry and fluorescent microscopy investigations revealed an overall increase of Calbindin D-28k at the 12 months age compared with 6 months age in both strains (AS and AS/AGU) (Fig. 5 and 6).

There was a highly significant increase of Calbindin D-28k between age 6 months and 12 months in the AS rat strain (*P* = 0.001) with an almost 10-fold increase (Table 2). However, the statistical evidence was weaker in the AS/AGU strain with *P* value of (0.093). There was no significant difference between strains at either age (Table 2).

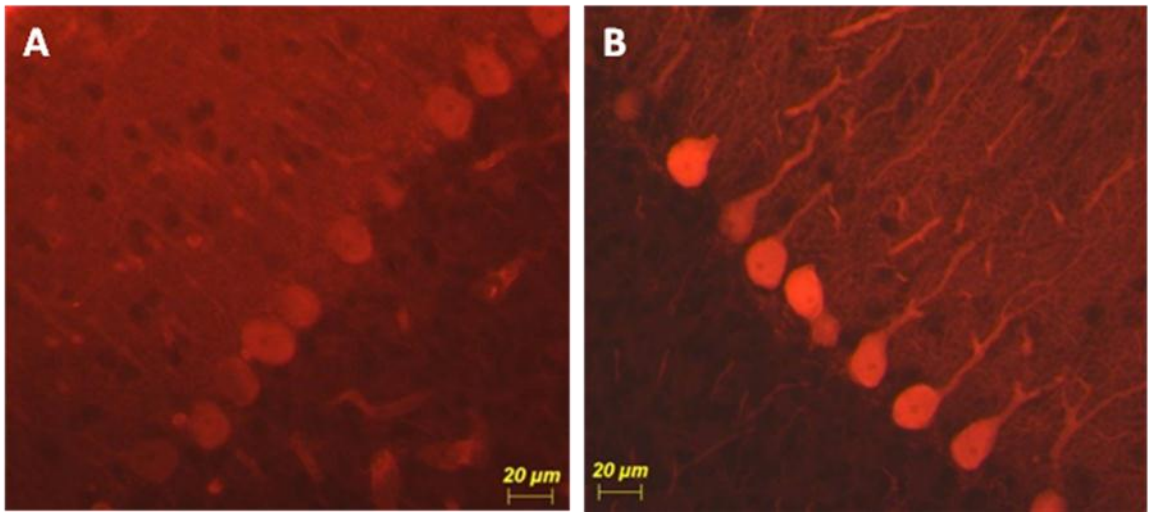

Fig. 5. Age- and strain-related differences in Calbindin D-28K levels using immunocytochemistry and fluorescent microscop (red filter). (A) six months aged Albino Swiss rat showing low immunoreactivity of Purkinje cells (faint color and no dendrites were visible). (B) Twelve months Albino Swiss/Albino Glasgow University rat showing high calbindin immunoreactive cells (40X).

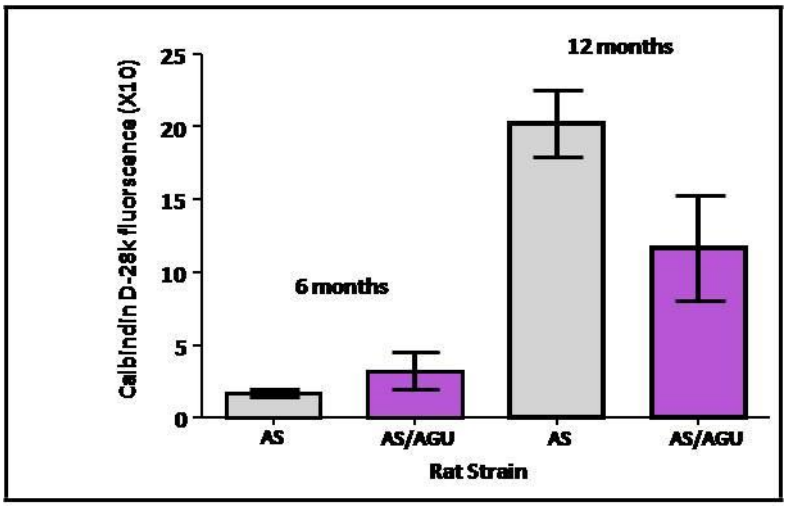

Fig. 6. Age and strain related changes of Calbindin D-28k levels in cerebellar Purkinje cells. Overall trend is an increase with age in both strains and in between 6 month age till 12 month age. The levels were relatively similar in the two strains. Each column in the graph represents a mean value of 3 animals in the study with its standard error

Discussion

Lipofuscin measurements

The age related increase in Lipofuscin is consistent with all previous LF studies in which LF has been reported to increase as a function of age (its alternative name is age pigment) [34-36]. Moreover the marginally significant difference between 6 and 12 months old AS/AGU rats could be explained by either a slow rate of accumulation in the AS/AGU strain in comparison to AS strain or the AS/AGU strain at 6 month having an already high intracellular LF level and the LF concentrations are levelled off at age 12 months. The first possibility has been excluded since Lipofuscin levels were similar at 12 month age.

Table 2. Age- and Strain-related changes in Purkinje cell Calbindin D-28k levels measured using immunocytochemistry and fluorescent microscopy.

Group 1	Group 2	P value
1.7	20.2	0.001*
1.7	3.7	0.303ǂ
3.7	11.7	0.093‖
20.2	11.7	0.116‼

* = AS 6month vs. AS 12month, ǂ = AS 6month vs. AS/AGU 6month, ‖ = AS/AGU 6month vs. AS/AGU 12month, ‼ = AS 12month vs. AS/AGU 12month

The overall trend is an increase with age and no strain difference. The cut off significant values of the analysis of variance are: significant level ($p < 0.05$), marginal or weak significant level ($0.10 > p > 0.05$), not significant level ($p > 0.1$) and $t > 2.77$.

If there is a true slow accumulation of Lipofuscin in AS/AGU strain, LF levels would be lower than in the AS strain at 12 month age which is not a finding of this study. Furthermore, we would expect a lower LF level at age 6 months in the AS/AGU strain because of slow LF accumulation before 6 months of age, which is again not obtained in results of this study.

Therefore, there was tendency to accept the second possibility since LF level was statistically similar at age 12 month in both strains indicating that they reach similar adult LF level at the same age (12 months).

Additional supportive evidence is the relatively significant increase of Lipofuscin level in AS/AGU strains at 6 months age inferring earlier LF accumulation in the mutant strain. Since LF is related to aging, one simple explanation is that AS/AGU rats are aging faster than AS rats.

The onset of dopaminergic neuronal loss at 6 month [37] and an early onset of motor and behavioral changes in this mutant strain is additional evidence [38].

Then, the AS/AGU LF levels would reach similar levels at age 12 months to their counterpart rats from AS strain. This could be simply because the Purkinje cells were not the target cells for ***agu*** mutation effects and the case in other brain regions may be different. Campbell et al [31,37] has pointed out a substantial loss of extracellular dopamine at age 3 month and expected that these changes already started at the early weeks of life. Consequently, the eventual cell death is at the end of a long pathway of cellular injury that may involve free radical or neurotoxin formation.

This might support a common cell death and aging pathway theory that claims involvement of oxidative stress and dysfunctional mitochondria and lysosomes, both of them are involved in LF formation [8]. Consequently, LF accumulation, whether it is a major step or by-product of the common cell death and aging pathway, is probably a suitable aging indicator and could be useful in further studies.

Calbindin D-28k measurements

Calbindin D-28K results are consistent with the findings of Amenta et al [20] who used radioimmunoassay to measure intracellular Calbindin level and showed that Calbindin D-28k increases from birth till old age in rats (12 months).

The insufficient statistical evidence of age-related difference in AS/AGU rats, implies that there is no or slow rise of Calbindin D-28k level in AS/AGU rats. However, there was no statistical evidence that AS rats have higher Calbindin D-28K level than AS/AGU strain at age 6 months and age 12 months (Table 2).

An older age group (16 months or 18 months) and a larger sample size are necessary to decide whether the Calbindin D-28k curve of AS/AGU is different from that of AS strain. Providing that many studies showed the old age group (24 -27 months) has the lowest Calbindin D-28k level, we tend to believe that

12 month age groups will reveal no statistical difference between both strains. One of the weaknesses of this study is the small sample size and further large-scale study is vital to fortify the results of this study and to fully differentiate between actual and confounding data.

This study did not investigate the Calbindin D-28k level at old age group (18-24 months) but Amenta et al [20] argued that after 12 month Calbindin D-28k starts to decline to reach its lowest level at 24 months. Reaching the lowest level will make the cell vulnerable to intracellular Calcium ion rises and probably neurodegenerative cell injury and death [20,39].

Intracellular calcium ion rise is thought to be the final step in aging and in some neurodegenerative processes [20]. Similarly Iacopino et al [40] pointed out the same trend in mice, where the maximum Calbindin D-28k level is reached at age 4-8 weeks and starts to decline gradually till old age (120 weeks) reaching its birth level again. Lacopino et al [40] supported their experiment by measuring Calbindin D-28k gene expression which shows a 3-4 fold rise in the first week and a steady state level at age 4-8 weeks before its regression to its birth level at 120 weeks of age.

However, Dutar et al [41] argued that Calbindin reactive cells are decreasing in number between age 4 months (young) and 24-27 months (old). They provided no data about the adult age and they estimate the Calbindin level using staining intensity. Similarly, another two studies [42,43] have shown the same age related decrease in Calbindin immunoreactive cells in human cerebral and hamster cerebellar cortices respectively.

Moreover selective age related decreases of Calbindin D-28k were reported in basal forebrain cholinergic neurons of human by Wu et al [39] and Geula et al [21]. They put this decrease as a cause of the selective vulnerability of basal forebrain cholinergic neurons for degenerative changes in old aged people and Alzheimer diseased patients.

It is worth noting that some studies have found no or little changes in Calbindin D- 28k with age. In studies on rat CNS [44], on Gerbil duodenal neurons [45] and on the cochlear nucleus of impaired hearing mice [46], the number of calbindin reactive cells remains unchanged with age. However, these studies either chose other nervous system area or other animal models, hence comparison of the current results with these studies would be most probably biased especially there is still a possibility of selective neuronal changes in the study animal as in case of selective cholinergic neuronal loss in basal forebrain of human with age [21,39].

In conclusion, the results clearly showed an age related pattern of increase in both age pigment (LF) and the neuroprotective Calbindin D-28k between the ages of 6 months and 12 months. The strain specific difference in Lipofuscin accumulation indicates that AS/AGU rats aged faster than their counterpart AS rats.

Nevertheless, more extensive investigation and large scale studies are required to fully establish this hypothesis; the AS/AGU strain is therefore a potential model for early aging investigations. With respect to Calbindin D-28k, the age related rise is not consistent with all previous studies; however, the statistical and immunocytochemical proof in this increase cannot be questioned.

The logical reason for this discrepancy is the different techniques and animal ages used in these studies. However, this study did not claim any changes between strains or beyond the 12 month age of AS and AS/AGU strains. Further study with larger sample size and wider age range is recommended to establish the calbindin age- and strain-related differences between AS/AGU mutant rats and control group (AS rats).

Acknowledgment

Grateful acknowledgement is due to Mr. D. Russell for his skillful assistance and guidance throughout all the study stages and in utilization of laboratory instruments. Many thanks are due to Professor J. Kusel for kindly

providing us the use of Fluorescent Microscope facility in biomedical building. Special thanks to Professor A. Payne for allowing us to use the specimens held by the University of Glasgow and for his insightful help and guidance.

Author contribution

This study was conducted in the Department of Anatomy at the University of Glasgow. The author has the main role in dissection of the preserved samples, histological staining, measurements using the immunocytochemical and fluorescent techniques, recording the data, analysis of the results and preparation of the discussion.

Conflict of interest

The author declares that there was no conflict of interest in this study as the researcher has any personal or financial relations with the organization funding the research. The research was undertaken as a master project for which the funding organization required no benefits apart from addition to the scientific literature in the topic studied.

The researcher declares that this study will bring him no personal benefits whether direct or indirect.

Funding

This research was funded by the Department of Anatomy at the University of Glasgow. The sponsor provided the specimens, histological laboratory, materials, chemicals and facilities used in the research. The funding organization provided no support for the preparation of this article, neither in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

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Email: hayderjalil2002@yahoo.com
Received 2nd Dec. 2014: Accepted 18th My 2015

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq

Association between Asn142Asp Genetic Polymorphism of GSTO2 and Susceptibility to Bladder Cancer

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Abstract

Background The glutathione-S-transferases (GSTs) comprise a class of enzymes that detoxify carcinogenic compounds by conjugating glutathione to facilitate their removal. Polymorphisms in glutathione S-transferase Omega 1,2 (GSTO1, GSTO2), and GSTP1 genes have been related to risk for bladder cancer.

Objective To assess a comprehensive picture of the relationship between smoking and GSTO2 gene Asn142Asp variant (rs156697) with bladder cancer

Methods A case control study was conducted at Chemistry and Biochemistry Department, College of Medicine and DNA Research and Training Center, Al-Nahrain University from February 2014 to September 2014. Forty one bladder cancer patients and 41 age matched apparently healthy controls were participated in this study. Genotyping of the GSTO2 Asn142Asp polymorphism was evaluated using a polymerase chain reaction fragment length polymorphism (PCR-RFLP) method. The odds ratio (OR) and 95% confidence interval (CI) were calculated as a measure of the combined effect of cigarette smoking and the GSTO2 Asn142Asp polymorphism on bladder cancer risk.

Result It was found that subject with the GSTO2 Asp/Asp genotype have significantly increased bladder cancer risk (OR 4.92; 95% CI =1.32 - 18.30). A statistically highly significant increased the bladder cancer risk was also found in ever smoker of the GSTO2 (Asn/Asn) (OR =11.8; 95% CI=2.43 - 57.84) and (Asn/Asp +Asp/Asp) (OR =12.8; 95% CI=3.23 - 51.41) compared with never smoker Ala/Ala genotype.

Conclusion The study suggests that smokers having GSTO2 Asn/142Asp polymorphism could play an important role as risk factor for the development with bladder cancer.

Keywords Bladder cancer, single nucleotide polymorphism, glutathione S-transferase, GSTO2, Asn142Asp, smoking, rs156697.

List of Abbreviation: GST = Glutathione transferases, GSH = glutathione, GSTO2 = glutathione S-transferase Omega 2, ROS = reactive oxygen species.

Introduction

Glutathione transferases (GST) are detoxification enzymes that play a role in the conjugation of endogenous or exogenous xenobiotic toxins to glutathione (GSH); however several GSTs function as GSH peroxidases [(1)].

Human cytosolic GST super family contains at least 16 genes subdivided into eight distinct classes designated as: Alpha, Kappa, Mu, Omega, Pi, Sigma, Theta, and Zeta [(2,3)].

GSTs catalyze the conjugation of GSH to a wide variety of endogenous and exogenous electrophilic compound [(4)].

Unlike other GSTs, glutathione S-transferase Omega (GSTO) has an active site cysteine that is able to form a disulfide bond with GSH and

exhibits glutathione dependent thiol-transferase and dehydroascorbate reductase activities, reminiscent of thioredoxin and glutaredoxin enzymes [5].

Human Omega class GST contains two expressed gene hGSTO1 and hGSTO2 [6]. The hGSTO1 and hGSTO2 are 12.5 and 24.5 kb, respectively, and lie 7.5 kb apart on chromosome 10q24.3, between the markers D10S603 and D10S597.

Three polymorphisms in hGSTO genes: hGSTO1*Ala140Asp, hGSTO1 and hGSTO2*Asn142Asp have been identified in ethnic groups [7] but their relationship with bladder cancer are not yet fully understood.

Recently studies reported that carcinogens in the cigarettes like hydrocarbons, polycyclic aromatic, aromatic amines and N-nitroso compounds could be one of the major causes of bladder cancer [8].

Tobacco smoking has been identified as a major lifestyle risk factor for developing transitional cell carcinoma (TCC). It has been estimated that around 50% of all TCC cases can be attributed to tobacco smoking, with considerable variation in groups of former and current smokers [9,10].

Chemicals and carcinogens contained in cigarettes require the detoxification by phase II enzymes like Glutathione-S-transferases, this leads to formation of less toxic and more hydrophilic derivatives, which is more readily excreted.

However, the deficiency in detoxification-related enzymes generates oxidized products including reactive oxygen species (ROS) which can cause DNA damage and the accumulation of genetic mutations [11].

hGSTO2*Asn142Asp is a single nucleotide polymorphism of GSTO2 gene causing variations in enzyme activity and may influence individual susceptibility to bladder cancer [12].

This study was aimed to investigate the joint effect of smoking on GSTO2 Asn142Asp polymorphism susceptibility in patient with bladder cancer.

Methods

This case control study was approved by the Ethical Committee of College of Medicine Al-Nahrain University, Baghdad, Iraq. The study was carried out during the period from (February 2014 to September 2014). It included 82 subjects, 41 subjects (30 males, 11 females) with bladder cancer mean age ± SD (60.95 ± 10.74) and 41 subjects, (23 males and 18 female) healthy volunteers (control) mean age ± SD (59.35 ± 9.914).

This study was conducted at Chemistry and Biochemistry Department, College of Medicine Al-Nahrain University and Forensic DNA Research and Training Center, Al-Nahrain University, Baghdad.

All patient were first diagnosed with bladder cancer and investigated by urologist and underwent cystoscopy examination for transurethral resection of bladder tumor or underwent cystoscopy with biopsy of bladder lesion for histopathological examination.

Urine cytology was requested for all patients to detect the presence of bladder cancer in all patients

The patients were recruited at Gazi Al-Harery Hospital for Specialized Surgery. The main exclusion criteria were as follows: subjects with history of urinary tract infection, bladder stones, benign bladder tumor and prostate cancer.

All participants provided informed consents and then were interviewed by a well-trained interviewer using a structured questionnaire to collect information including a history of cigarette smoking.

Study subjects who had smoked more than 100 cigarettes during their lifetime were regarded as ever smokers, while those who had smoked less than 100 cigarettes were defined as never smokers.

Genomic DNA was isolated from the whole fresh blood sample using the Geneid Genomic DNA Mini Kit Vogelstein [13]. The DNA was made into aliquot and store at -60^{o}C for future use.

Genotyping was determined using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Briefly, GSTO2 Asn142Asp polymorphism was determined by PCRRFLP according to Marahatta et al. [14].

Primer sequence ->3')

Forward primer: (5'AGG CAG AAC AGG AAC TGG AA 3')

Reverse primer: (5'GAG GGA CCC CTT TTT GTA CC3')

The PCR conditions were obtained by making an optimization using multiple samples and multiple annealing temperatures.

PCR conditions were: one cycle at 95 ^{o}C for 5 min; 35 cycles of 95 ^{o}C for 30 sec, 58 ^{o}C for 30 sec and 72 ^{o}C for 45 sec, and a final extension at 72 ^{o}C for 10 min.

The amplified PCR product was 181 bp and visualized by electrophoresis in a 2% agarose gel as shown in fig. 1.

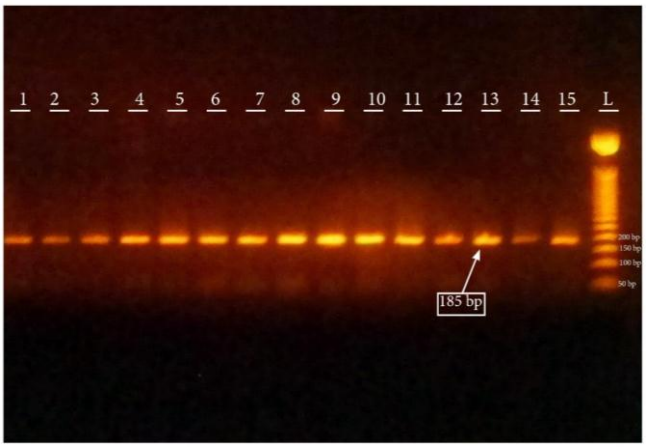

Fig. 1. PCR product for the of GSTO2 rs156697 polymorphism using Promega master mix on 1% agarose, 70V, and for 60 minute (7 µl of DNA loaded in each well). Lane L: 50 bp Ladder, Lane 1-15: PCR product

After the complete digestion with the restriction enzyme MobI at 37 ^{o}C for 18 h, the resulting DNA fragments which represented the Ala142Asp polymorphism of GSTO1 gene were analyzed by electrophoresis in a 3% agarose gels as shown in fig. 2.

To ensure quality, a random 10% of the samples were genotyped repeatedly.

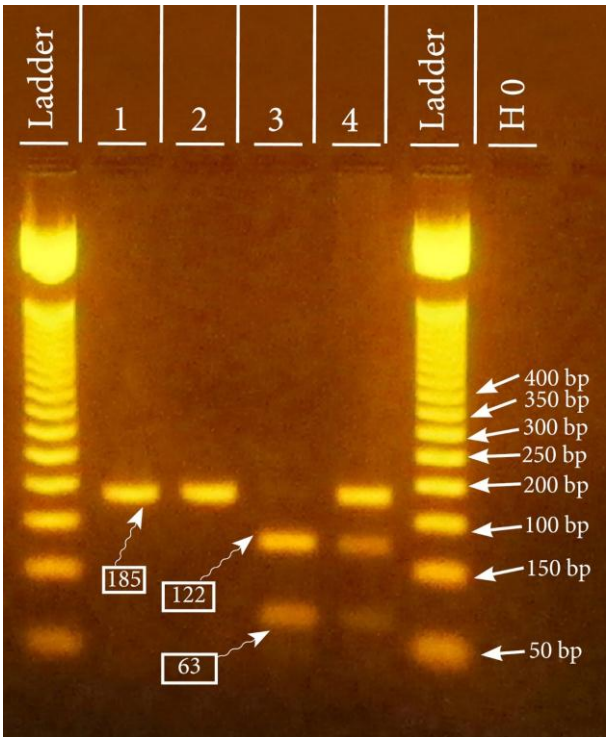

Fig. 2. Restriction digestion of PCR products of GSTO2 rs156697 polymorphism demonstrated the patterns of digestion in different genotypes of GSTO1 rs4925 polymorphism in 3% agarose, 70V, and for hour (7 µl of DNA loaded in each well). Lane L: 50 bp Ladder, Lane 1 and 2 show 1 band (185); Asn/Asn Genotype, Lane 3 show 2 bands (63 and 122); Asp/Asp Genotype, Lane 4 show 3 bands (68,122 and 185); Asn/Asp Genotype

Statistical Analysis

The data of the study were stored in Microsoft excel spread sheet and analyzed on the computer using (IBM SPSS Advanced Statistics 20.0 software) and Microsoft excel program (2013). Numeric variables were expressed as mean ± SD. Students t-test was used for comparison of mean between two groups. Chi-square test was used to compare frequency. Chi- square test was performed to determine if the control samples demonstrated Hardy-

Weinberg equilibrium for GSTO2 polymorphism. Logistic regression was used to calculate ORs and 95% CI for bladder cancer risk associated with the genetic polymorphisms of GSTs as well as the joint effects of cigarette smoking and the GSTO2 Asn142Asp polymorphisms on bladder cancer risk.

Results

The Basic characteristics for bladder cancer patients and controls are shown in table 1. There were no significant differences between age, weight, height and body mass index ($P > 0.05$) between bladder cancer patient and control.

Table 1. Basic characteristics of study groups

Feature	Patient group N = 41	Control group N = 41	*P* value
Age (year)	60.95 ± 10.74	61.0 ± 9.91	0.47
Weight (Kg)	74.42 ± 12.0	75.0 ± 9.9	0.113
Height (Cm)	169.5 ± 7.1	169.0 ± 6.9	0.06
BMI (Kg/m^2)	26.27 ± 4.46	26.0 ±3.7	0.12

Table 2 shows that there were no significant differences ($P > 0.05$) in the distribution of age between bladder cancer patients and control.

On the other hand a highly significant increased ($P < 0.001$) bladder cancer risk was notice in ever smokers subjects (OR=7.44; 95%CI =2.79- 19.75).

Table 2. Demographic characteristics of the patients and controls

Variable		Patient group (n=41)	Control group (n=41)	OR	95% CI	*P* value
Age	< 55	9 (21.95)	14 (34.14)	1		
	55-65	20 (48.78)	17 (41.46)	1.83	0.63-5.27	0.26
	> 65	12 (29.26)	10 (24.39)	1.03	0.57-6.10	0.30
Gender	Female	11 (26.82)	18 (43.90)	1		
	Male	30 (73.1)	23 (56.09)	1.6	0.84-5.3	0.1086
Cigarette smoking	Never	11 (26.82)	30 (73.17)	1		
	Ever	30 (73.1)	11 (26.82)	7.44	2.79- 19.75	0.0001

GSTO2 Asn142Asp polymorphism distribution of the observed genotype frequencies among control group was consistent with Hardy–Weinberg equilibrium (HWE), ($P = 0.4$). The gene frequency of Asn142 in control group was 0.73 and was 0.27 for allele Asp142, in bladder cancer patients gene frequency of Asn142 was 0.52 and for Asp142 was 0.47.

Table 3. GSTO2 rs156697 polymorphism Genotypes and Allele frequency among patients and control groups

GSTO2	Genotype, n (%)		Gene frequency				*P* value
	No.	Asn142/Asn142	Asn142/Asp142	Asp142/Asp142	Asn142	Asp142	
Control	41	23 (56.09)	14 (34.14)	4 (9.75)	0.73	0.27	0.4
Patients	41	14 (34.14)	15 (36.58)	12 (29.26)	0.52	0.47	0.08

As shown in table 4, study subjects who carried the Asp/Asp genotypes of GSTO2 gene had a significantly higher BC risk of 4.92 (95% CI = 1.32 - 18.30) comparing to individuals who carried the Asn/Asn genotype (P = 0.01), While a non-significant higher BC risk 1.7 (95% CI = 0.65 – 4.71) was found in subject carried the Asn/Asp comparing to individuals who carried the Asn/Asn genotype. Furthermore there was a significantly higher BC risk of 2.46 (95% CI = 1.0 - 6.0) in subjects who carried the combination of Asp/Asp and Asn/Asp genotypes of GSTO2 gene when compared to individuals who carried the Asn/Asn genotype (*P* = 0.04).

Table 4. Distribution of GSTO2 Asn142Asp polymorphism in patients and control groups

GSTO2 genotype	Patient group No. (%)	Control group No. (%)	OR	95% CI	P value
Asn/Asn	14 (34.14)	23 (56.09)			
Asn/Asp	15 (36.58)	14 (34.14)	1.76	0.65- 4.71	0.26
Asp/Asp	12 (29.26)	4 (9.75)	4.92	1.32 - 18.30	0.01
Asn/Asn	14 (34.14)	23 (56.09)			
Asn/Asp+Asp/Asp	27 (56.85)	18 (43.90)	2.46	1.0 to 6.0	0.04

To Find the Joint effect of cigarette smoking and the Asn142Asp polymorphism of GSTO2 gene on the development of bladder cancer

As shown in table 5, comparing with never smoking who carried Asn/Asn genotype of the GSTO1 gene as a reference group, it was found a non-significant higher BC risk (OR = 3.4; 95% CI = 0.84 - 14.16) in never smokers who carried Asp/Asp and Asn/Asp genotypes of the GSTO1 gene; a very high significant bladder cancer risk in ever smoking group carried Asp/Asp and Asn/Asp genotypes of 12.8 (95% CI = 3.23 – 51.41) (*P* = 0.0003); and a very high significant bladder cancer risk in ever smoking group carried Asn/Asn genotypes of 11.8 (95% CI = 3.23 – 51.41) (*P* = 0.0022).

Table 5. Combined effect of GSTO2 Asn142Asp polymorphism and cigarette smoking on bladder cancer risk

Smoking	GSTO2 genotype	Patient group	Control group	OR	95% CI	P value
Never	AsnAsn	4 (9.75)	19 (46.34)	1		
	AsnAsp+AspAsp	8 (19.51)	11 (26.82)	3.4	0.84 - 14.16	0.0851
Ever	AsnAsn	10 (24.39)	4 (9.75)	11.8	2.43 - 57.84	0.0022
	AsnAsp+AspAsp	19 (46.34)	7 (17.07)	12.8	3.23 - 51.41	0.0003

Discussion

In the present study we have shown that mutant heterozygous GSTO2 Asn142Asp or homozygous mutant GSTO2 Asp142Asp genotypes are associated with development bladder cancer. GSTO2 as genetic markers may have a prognostic or pharmacogenomics role in patients with muscle invasive bladder cancer [15]. Recently, many studies have investigated the association between single nucleotide polymorphisms on glutathione S-transferases and susceptibility to bladder cancer [19]. previous studies described that cigarette smoking can induce Single-nucleotide polymorphisms in the detoxification enzymes like GSTs, which may cause a substitution in amino acid leading to a changing in the activity

of these enzymes and affecting the biological metabolism [7,12].

The combined effect of polymorphisms in GSTO2/rs156697, gene on the risk of bladder cancer was studied as well as the joint effect of smoking and polymorphisms on bladder cancer risk.

In this study, it was found a highly significant increased (P < 0.001) bladder cancer risk in ever smokers cancer patients (OR=7.44; 95%CI =2.79- 19.75) (as shown in Table 2) these results were in consistent with previous studies that showed that ever smokers had a significantly increased risk of bladder cancer [10,16].

The GSTO2 enzymes are encoded by the omega class glutathione S-transferase (GST). GSTO2 widely expressed in all tissues, it has been seen in liver, kidney, skeletal, muscle with lower expression in the heart and high levels in testis [17].

GSTs are involved in the metabolism of xenobiotics and carcinogens, in human; the GSTO2 is polymorphic with an Asn142Asp substitution in the coding region [18].

Previous study reported that the GSTO2 Asn142Asp polymorphism may have an effect on individual susceptibility to many multifactorial diseases, however Marahatta reported that there is no association between GSTO2 Asn142Asp polymorphism and the risk of the hepatocellular carcinoma, cholangiocarcinoma, colorectal cancer and breast cancer as well [14].

Recently, a study demonstrated that GSTO2 polymorphism may significantly increase cancer risk in Caucasian population and is associated with elevated risk of breast cancer [12]; other study shows that GSTO2 could use as genetic markers may have a prognostic or pharmacogenomics role in patients with muscle invasive bladder cancer [19].

In this study, the gene frequency of GSTO2 Asp142 in healthy control was 0.27 as shown in table 3. The gene frequency of GSTO2 ASP142 has been reported, 0.31 among European Australians in Canberra, 0.86 among Bantu Africans in Durban, and 0.27 among Chinese from Hong Kong [19].

The present study revealed that the control group exhibited GSTO2 ASP142 gene frequency, which was similar to Chinese, reflecting resemblance in Asian population; however the allele frequency of Asp142 in total bladder cancer was 0.47 and was higher than the control group.

Moreover, there was higher significant bladder cancer risk of 4.92 (95% CI = 1.32 - 18.30) for subjects who carried the Asp/Asp genotypes of GSTO2 comparing to individuals who carried the Asn/Asn genotype (P = 0.01).

Furthermore, there is a significantly higher BC risk of 2.46 (95% CI = 1.0 - 6.0) in the subjects who carried the combination of Asp/Asp and Asn/Asp genotypes of GSTO2 gene when compared to individuals who carried the Asn/Asn genotype (P = 0.04).

GSTO2 Asn142Asp creates a non-conservative amino acid change from uncharged polar to acidic amino acid.

It is unfortunate that the insolubility of GSTO2 has prevented its characterization and comparison with GSTO1 Pervious study reported that the GSTO2 142Asp (142Asp) variant allozyme showed 20% reduction in level of expression compared with the level of the GSTO2 wild type (142Asn) allozyme [20].

The effect of tobacco smoking with formation of the Asn142Asp genotype GSTO2 polymorphism, we find A very high significant bladder cancer risk in ever smoking subjects carring Asp/Asp and Ala/Asp genotypes of 12.8, (P = 0.0003) and a very high significant bladder cancer risk of 11.8 in ever smoking subject who carring Ala/Ala genotypes, (P = 0.0022).

In conclusion, GSTO2 rs156697, polymorphism is associated with bladder cancer risk but the gene-environmental factor (GSTO2-smoking) may increase the bladder cancer risk.

Acknowledgments

The authors with like to express their sincere appreciation to the lab staff of DNA Research

and Training Center at Al-Nahrain University for their help.

Author contribution

Literatures survey, manuscript preparation by Mr. Mahmood; manuscript writing and editing by Dr. Abdul-Rasheed; sample choosing and collection by Dr. Al-Nasiri; and genetic studies by Dr. Al-Awadi and Al-Zubaidi.

Declaration of interest

The author declare no conflict of interest.

Funding

Self-funding.

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Tel. +9647704218786
Received 25th Mar. 2015: Accepted 10th Jun. 2015

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq

Histopathological Changes of Male Mice Kidneys Treated with Fresh *Aloe vera* whole Leaf Extract

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Abstract

Background *Aloe vera* is an evergreen perennial plant widely used in modern herbal practice and is often available in proprietary herbal preparations. Evidence of efficacy is strongest for the laxative effects of *Aloe vera* latex; however the anthraquinones in the latex are associated with considerable risks.

Objective To investigate the histopathological changes of kidney tissues of male mice administered low dose of whole leaf fresh *Aloe vera* extract.

Methods Forty immature male Swiss Webster mice divided into two equal groups (experimental and control) (G and C respectively). The experimental group (G) was given 20 µl of *Aloe vera* extract orally for 21 days. While the control groups (C) were given by the same dose and route of administration with normal saline only. After six weeks (around puberty), the male were sacrificed to get their kidneys, then fixed with 10% formalin, and histological sections with a thickness of 5 microns were prepared.

Results Histological studies of mice kidneys from groups that consume low dose fresh *Aloe vera* whole leaf extracts showed disrupted entire structure of kidneys including degenerative changes in most parenchymatous elements in comparison with control.

Conclusions Using low doses of fresh *Aloe vera* whole leaf extract induce adverse effects on the histological features of mice kidneys and impaired their entire structure.

Key words *Aloe vera*, Kidney, anthraquinones, whole leaf extract.

List of Abbreviations: IASC = International *Aloe* Science Council, CIREP = cosmetic ingredient review expert panel, FDA = Food and Drug Agency, LOAEL = lowest-observed-adverse-effect level, GFR = glomerular filtration rate, PCTs = proximal convoluted tubules, DCTs = distal convoluted tubules, ROS = reactive oxygen species, LMWF: Low molecular weight fraction, PMN = polymorphonuclear.

Introduction

The kidney is one of the most vital bodily organs. It has a former role in the whole body homeostasis, controlling the electrolyte concentrations, acid-base balance, extracellular fluid volume, as well as playing a crucial role in the blood pressure regulation. It eliminates a wide variety of waste products. It secretes a group of hormones, such as erythropoietin. It also secretes the enzyme renin and calcitriol which is activated form of vitamin D [1,2].

Aloe plants have been used medicinally for centuries. Among them, *Aloe barbadensis*, commonly called *aloe vera*, is one of the most widely used healing plants in the history of mankind [3]. The molecular studies put *Aloe* in the order *Asparagales*. Within *Asparagales*, it is either in the family *Asphodelaceae* or the family *Xanthorrhoeaceae* [4]. The species is used widely in the traditional herbal medicine of China, Japan, Russia, South Africa, the United States, Jamaica, Latin America and India [5]. Briggs, 1995 [6] described the leaf of *Aloe* plants as consisting of two main parts. One part, the pericyclic cells, is found just below the plant's skin. The pericyclic cells produce a bitter, yellow latex known as *Aloe* juice, or latex. When this juice dries it forms a dark

brown solid material. The main active constituents of the latex are anthraquinones, which include aloins A, and B, barbaloin, isobarbaloin, and emodin. Also included are aloe-emodin, resins, aloesin and its aglycone, aloesone, and chromone derivatives [7]. *A*loe juice is approximately 99% water [8], and the remainder consists of minerals, vitamins, polysaccharides, lipids, phenolic compounds, and organic acids [9]. The second part, the inner central area of the leaf, contains the thin walled parenchymal cells that produce the clear slightly viscous (mucilaginous) fluid known as *Aloe* gel or inner gel. This gel contains the polysaccharides and three malic acid acylated carbohydrates [10]. Other potentially active constituents are lipids, amino acids, sterols [11] and high concentration of mannose 6-phosphate [12].

Many biological activities, including *Aloe* species have been used for centuries for their laxative, antiinflammatory, immunostimulant, antiseptic, wound and burn healing activities [13]. There have been reports, also, on the antidiabetic activity of *Aloe* extracts [14]. Additionally, numerous constituents within *Aloe vera* have demonstrated enhancement of immune system functioning within the body [15]. Leaf pulp extract also showed hypo-glycaemic activity in type I and II diabetic rats [16]. *Aloe vera* is a common ingredient in cosmetics and pharmaceutical industries [17].

In addition to the well-documented positive effects, there have been also reports of negative actions. There is, however, little scientific evidence of the effectiveness or safety of *Aloe vera* extracts for either cosmetic or medicinal purposes, and what positive evidence is available is frequently contradicted by other studies [18]. The International *Aloe* Science Council (IASC) distinguished between the anthraquinones found in the outer cell layer of the aloe leaf and the rest of the plant. According to this group, the maximum allowable aloin content in aloe-derived material for non medicinal use is 50 ppm or lowers [19]. The laxative effect of the anthraquinone glycosides found in *Aloe vera* latex is well established [20]. The Cosmetic Ingredient Review Expert Panel (CIREP) (2007) concluded that *Aloe* latex, but not the polysaccharide material derived from the inner gel, is cytotoxic [21].

Oral use may cause diarrhea or vomiting [22]. Many of these reactions appear to be associated with anthraquinone contaminants of the gel product. Moreover use of *Aloe vera* as a laxative during pregnancy may pose potential teratogenic and toxicological effects on the embryo and fetus [20]. Pregnant women are advised not to take *Aloe* latex because of its cathartic action, which may cause severe uterine contractions and increase the risk of miscarriage. It should also not be ingested by nursing mothers because of the possibility of causing severe cramps and diarrhoea in the infant [23].

In 1998, 27 adverse events due to *Aloe vera* were reported to the Food and Drug Agency (FDA) [24]. Adverse effects of *Aloe* whole-leaf powder have been reported at concentrations of 2 g/kg BW, and the lowest-observed-adverse-effect level (LOAEL) for aloin is estimated at 11.8 g/kg BW [25]. The distribution and/or accumulation of aloen in the stomach, liver, and kidneys indicated that aloin and its metabolites accumulated in the liver and the kidneys. In fact, liver and kidney were the only organs that had higher concentrations of aloe-emodin than plasma [26], leading to nephro-toxicity [27]. Nephrotoxicity is a poisonous effect of some substances, both toxic chemicals and medication on the kidneys decreased urine concentrating capacity, tubular proteinuria, lysosomal enzymuria, mild glucosuria, decreased ammonium excretion and lowering of glomerular filtration rate (GFR) [28]. Until now, there are no published controlled *in vivo* toxicology studies of *Aloe vera* in humans; further research in humans is required to confirm these effects [29]. Due to vital function of kidneys, the present study was, therefore, designed to investigate the histopathological changes of kidney tissues of male mice

administered low dose of fresh *Aloe vera* whole leaf extract.

Methods

Forty immature Swiss Webster male mice (3) weeks old were divided into two equal groups: experimental (G) and control (C), twenty animals each.

Fresh leaves of plant having a length of approximately 25 to 50 cm were washed with fresh water then cut from the middle. The whole leaf extract was separated by scratching with a spoon. The obtained substance is then divided into 2 ml volume tubes, kept at 4 ^{o}C overnight, before being used. The aloe juice was prepared daily to get fresh extracts [30].

Immature male mice were obtained from animal house of the High Institute for Infertility Diagnosis and ART/ Al-Nahrain University randomly selected. The mice weighed 15-18 g and were about 3 weeks old. They were kept in metal cages at room temperature (27 ^{o}C - 30 ^{o}C) in the animal room and exposed to photo-periodicity 12:12. The mice, divided into 2 groups of twenty mice each, were fed on mice pellet and had access to water *ad libitum*. The experimental group (G) was given 20 µl of fresh *Aloe vera* whole leaf extract orally for 21 days. While the parallel control group was given normal saline by the same rout and dose as that used in the experimental group. After six weeks (around puberty) the mice were sacrificed to get their kidneys, cleared of adhering tissues, then fixed with 10% formalin, and histological sections with a thickness of 5 microns were prepared using the routine histological technique [31].

Results

Light microscopic study

Control mice

The histological section of control mice revealed normal renal parenchyma. Cortical portion of the kidney constitute of renal corpuscle and convoluted tubules. Acidophilic cells distinguish the proximal convoluted tubules (PCTs) from the distal convoluted tubules (DCTs), whose smaller, less intensely stained cells (Fig. 1).

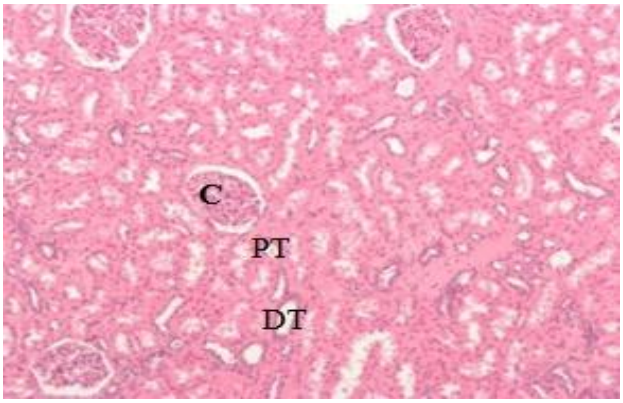

Fig. 1. Section in control mice kidney showing normal renal tissue. Normal renal corpuscle (C), the glomerular capillary loops are thin and delicate. The surrounding proximal (PT) and distal renal tubules (DT) are normal. The renal corpuscle is surrounded by Bowman's capsule. A urinary space (which appears as a clear space) is visible (20X, H&E).

The renal corpuscle exhibits the glomerular capillaries, parietal layer and visceral epithelium of the glomerular (Bowman's) capsule and the capsular space [32,33] (Fig. 2).

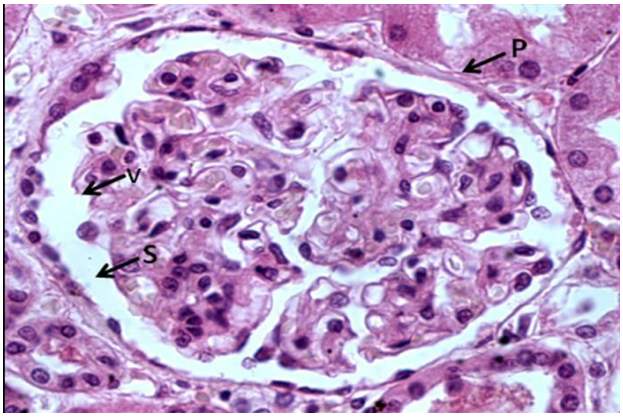

Fig. 2. Renal corpuscle of control adult male rat, P: parietal layer, V: visceral layer and S: urinary space (40 X, H & E) [34].

Histopathological changes

The kidney revealed degenerative changes in most of its entire structure in comparison with control. Atrophy of renal corpuscle with

shrinked glomeruli were represented by decrease in glomerular cellularity (Fig. 3).

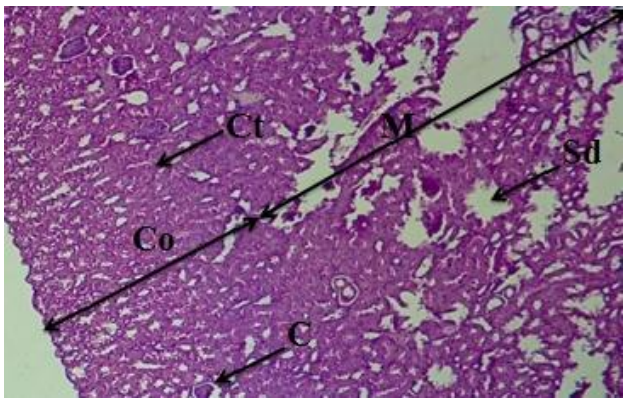

Fig. 3. Section of *Aloe vera* treated mice kidney showing atrophy of renal corpuscle (C) (arrow) with shrinked glomeruli in renal cortex (Co) (double head arrow) with degenerative cortical renal tubules (Ct). Sloughing of necrotic areas (Sd) (arrow) in most parenchymatous elements in renal medulla (M) (double head arrow) (4X, H&E).

Proximal tubules and distal renal tubules showed histological changes in the form of widening of tubular lumen with hyalinization. Glomeruli appears to be degenerative with necrosis (Fig. 4).

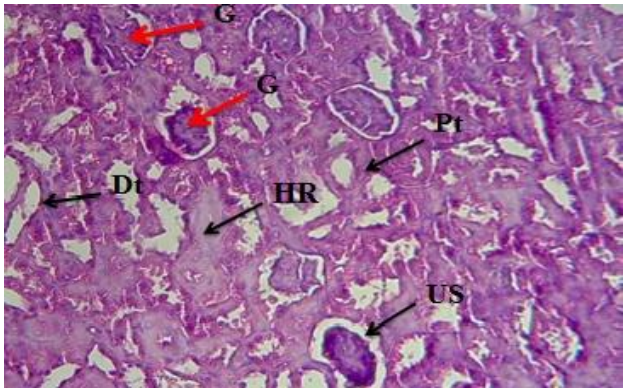

Fig. 4. Section of *Aloe vera* treated mice kidney showing widened deformed proximal renal tubules (Pt) and distal renal tubules (Dt) (arrows), along with hyalinization of renal tubules (HR). Glomeruli (G) reveal features of degenerative and necrosis (Red arrows). Widened urinary space (US) of the Bowman's capsule was observed (arrow) (20X, H&E).

Other observation demonstrates both proximal and distal tubules showing degenerated epithelial lining. The cells showed decrease in height, the cytoplasm was stained deeply acidophilic. The nuclei were small and deeply stained (pyknotic). The rest of the tubules showed dilated lumen. Massive regions of necrosis in renal parenchyma markedly observed (Fig. 5).

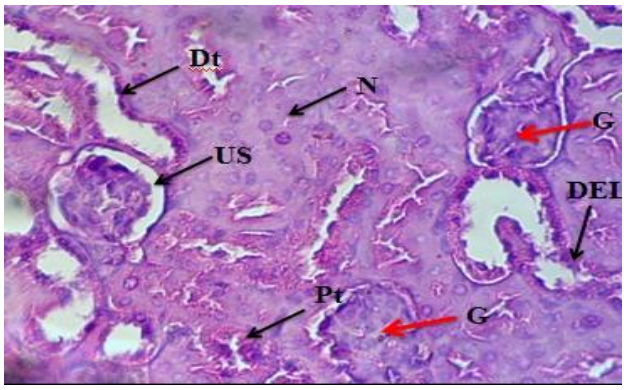

Fig. 5. Micrograph of mouse kidney treated with 20 µl *Aloe vera* showing degenerative and necrotic glomeruli (G) (Red arrows) with widened urinary space (US) of the Bowman's capsule (arrow). Both proximal (Pt) and distal tubules (Dt) showed degenerated epithelial lining (DEL). The cells showed decrease in height, the cytoplasm stained dark acidophilic, and the nuclei are small, pyknotic and dark stained. Distal tubules showed wide lumina. Massive regions of necrosis in renal parenchyma markedly observed (N) (40X, H&E).

Discussion

Estimation of the renal excretion of the waste metabolites and histological changes in the kidney has provided useful information on the health status of the kidneys [35]. Renal systems actively involved in drug elimination from the body through renal filtration process, proximal tubule secretion and distal tubule reabsorption. It is well known that most of drugs, including: antibiotics, nonsteroidal anti-inflammatory, radiographic contrast media and some of cancer remedies, may be the cause of renal failure. Although damage may be

reversible, it may cause chronic changes in kidney parenchyma [36].

The histological observations from this study illustrate atrophy of renal corpuscle with shrinked glomeruli represented by decrease in glomerular cellularity (Fig. 3 and 4). The decrease in glomerular cellularity may be explained by the fact that *aloe vera* leaves contain phytochemicals such as anthraquinone [37] and it was suggested that the presence of yellow sap (rich in anthraquinones) in *Aloe vera* gel reduces cell growth [38], while dilation of the urinary space of the renal corpuscles in *Aloe vera* treated mice may be due to basement membrane alterations and epithelial changes in the PCTs, decreasing the functional properties of PCTs and resulting in a decrease in the glomerular filtration rate (GFR) and the accumulation of urine in the urinary space. It is documented that normality of the basement membrane of glomerulus is essential for normal GFR and any change in this structure leads to proteinuria [39].

One finding of the present study was disorganization and necrosis of renal convoluted tubules, wide spacing of tubules, and atrophy of the lining epithelium along with hyalinization in most renal tissues (Fig. 3-5). This histopathological alterations could lead to nephrotoxicity which characterized by direct tubular necrosis [40,41]. The toxic effect of *Aloe vera* gel could be due to the generation of anthraquinones formed by oxidation of low molecular weight components such as aloin which are present in the plant leaves [42]. Aloin accumulates in proximal straight and distal convoluted tubules and promotes cellular damage, by multiple mechanisms including oxidative stress, DNA damage and apoptosis [43,44]. Furthermore the cytotoxic effects could be masked by the production of reactive oxygen species (ROS) by redox cycling induced by anthraquinones of the low molecular weight fraction (LMWF). Indeed, 'tHart *et al.* (1990) observed that the (LMWF) seems to have a stimulatory effect on the O_2 consumption by resting polymorphonuclear leukocytes (PMN), due perhaps to the mentioned mechanism of redox cycling. At longer times the toxic effect predominates and becomes evident. Likewise, it is also possible that the inhibitor is present in *Aloe vera* extracts, although in different amounts. It is apparent that LMWF obtained from *Aloe vera* gel has cytotoxic activities [45].

Another histopathological change is degeneration and necrosis of glomeruli (Fig. 4 and 5) and massive regions of necrosis with loss of renal parenchyma in some areas was clearly observed (Fig. 3 and 5). The degenerative alterations mentioned in the present study may be due to that anthraquinones, which are poorly absorbed from the GIT, are cleaved by gut bacteria to produce aloe-emodin, which is more readily absorbed and responsible for the purgative properties of these preparations [27]. The liver and kidney were the only organs that had higher concentrations of aloe-emodin than plasma [26]. In addition to that, oxidative stress has also been proposed to play a role in the pathogenesis of renal and hepatic tissue damage [46,47]. Several lines of evidence suggest the role of ROS in the pathogenesis of nephrotoxicity [48]. Moreover elevated generation of free radicals may lead to disruption of cellular functions and oxidative damage to membranes and may enhance susceptibility to lipid peroxidation [49]. Likewise abnormal production of ROS may damage some macromolecules to induce cellular injury and necrosis via several mechanisms including peroxidation of membrane lipids, protein denaturation and DNA damage [50].

This study suggests that continuous consumption of *Aloe vera* whole leaf extract in the tested dosage range result in histopathological changes of the renal tissue in mice. It is important to note that the amount of *Aloe vera* used in many previously published studies were very high, in contrast to the present study which showed evidence of organ injury at relatively lower doses, suggesting that further research is warranted to examine the safety profile of this widely used food additive.

Moreover, herbs have a variety of complex chemical constituents that act on the body as a whole or on specific organs and systems. Some of the chemical constituents are mild and safe even in large doses while, some act more strongly or are toxic in large doses or when taken continuously [51].

In conclusion, using low doses of *Aloe vera* whole leaf extract induce adverse effects on the histological features of mice kidneys and impaired their entire structures.

Acknowledgment

I would like to express my deep gratitude to Allah for helping me to complete this study.

Conflict of interest

None.

Funds

None.

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Tel. + 964 7705327555
Received 8th Jan. 2015: Accepted 10th Jun. 2015

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq

A comparative study of Serum Malondialdehyde and Hexanoyl-Lysine Adduct in Preterm and Post-term Deliveries

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Abstract

Background High oxidative stress reflects the state when the production of reactive oxygen species exceeds their removal. Malondialdehyde (MDA)and Hexanoyl-Lysine Adduct (HEL) are indicators of oxidative damage of lipids caused by free radicals in blood. The high level of these biomarkers has been implicated in early pregnancy complications.

Objectives This study aimed to investigate the correlation between serum oxidative stress biomarkers in preterm and post-term deliveries.

Methods A case-control study was designed to recruit 90 pregnant women with 30 women delivering at term (control group), 30 women at preterm (first case group) and 30 women at post-term (second case group). All women underwent elective cesarean section. Blood samples were collected before admission to operation theatre. Women's age, body mass index, lipid profile, renal function test and random glucose were measured. Serum oxidative stress biomarkers (Malondialdehyde and Hexanoyl-Lysine Adduct) were measured as indicators of lipid peroxidation.

Results The results showed that study groups were significantly different in serum MDA and HEL ($P \leq 0.01$). Both serum MDA and HEL levels were significantly higher in preterm women group and their level steadily decreased as pregnancy progressed. Serum MDA was not significantly different between term and post-term women groups (P>0.05).

Conclusions Oxidative stress biomarkers may be important contributors of premature birth. Low level of serum HEL may play a role in delayed onset of labor. The causal relationship between oxidative stress biomarkers and pregnancy outcome may be further investigated by longitudinal studies.

Key words Oxidative Stress, Preterm women, Post-term women, Malondialdehyde, Hexanoyl-Lysine Adduct.

List of Abbreviation: MDA = Malondialdehyde, HEL = Hexanoyl-Lysine Adduct, OS = Oxidative stress, ROS = Reactive oxygen species, BMI = body mass index, TG = triglyceride, VLDL-C = Very Low Density Lipoprotein Cholesterol (VLDL-C), HDL-C = High Density Lipoprotein Cholesterol (HDL-C), LDL-C = low Density Lipoprotein cholesterol.

Introduction

High Oxidative Stress (OS) takes place when the production of reactive oxygen species (ROS) exceeds the physiological level in blood and soft tissue [(1)]. These ROS may cause tissue injury resulting in cytotoxic damage to cellular proteins, lipids and DNA which has been implicated in early pregnancy complications such as preterm labor, preeclampsia and ante partum hemorrhage [(2)]. Body defense response involves enzymatic and non-enzymatic antioxidant buffering pathways which reduce the effect of these free radicals [(3)]. Previous studies have shown that increasing level of OS biomarkers in blood may lead to preterm labor [(4)]. Other studies showed that the administration of antioxidant vitamins during pregnancy was associated with decreased

incidence of spontaneous preterm labor with a positive dose-response across all groups [5]. However, the oxidative pathways linking low antioxidants level in serum to preterm labor and premature rupture of membrane have not been fully explained, but such a relationship have been hypothesized by several investigators [6,7].

Several OS biomarkers have been described as indicators to measure OS level in serum. Malondialdehyde (MDA) and Hexanoyl-Lysine Adduct (HEL) have been widely used as estimates of oxidative damage of lipids caused by free radicals in blood [8]. MDA has been confirmed as one of the advanced lipid peroxidation products. Another study was also conducted among Chinese women showed that pregnancy induced hypertension may be a predisposing factor for increasing the oxidative stress biomarker, MDA, which may eventually lead to premature labor [9]. Similarly, HEL is an important biomarker for initial stage of lipid peroxidation [8]. HEL is formed by oxidative modification of oxidized omega-6 fatty acids such as linoleic acid or arachidonic acid [8].

However, the present research has been done to examine the association between OS level and pregnancy outcome among Iraqi women. It intended to assess the association between OS level in serum and the type of delivery (Preterm, term or post-term) among Iraqi women. The purpose of this paper is to provide evidence reflecting the influence of OS level and whether it can predict pregnancy duration.

Methods

Study Design and participants

A case-control study design was used in this study. Ninety pregnant women were recruited (30 term women were recruited as the study control, 30 preterm women as the first case group, and 30 post-term women as the second case group). Women were approached from hospitals in Baghdad (Al-Hakeem and Al-Imamain Al-Kadhimain Medical City) during the period from 12th of January to 19th of September 2014. The sample size was estimated to achieve a statistical power of 0.80 (at $P < 0.05$, 95% confidence interval, assuming R is equal to 3). The exclusion involved women with the following criteria: hypertension, thyroid disease, diabetes mellitus, smoking, evidence of active infection, fever, chronic inflammatory diseases (including rheumatoid arthritis, joint pain, osteoarthritis, abdominal complain, inflammatory bowel disease); currently taking any medication, cytomegalovirus and toxoplasmosis infection. These conditions were excluded by gynecologist. The ethical approval was obtained for this study from Al Nahrain University and permission was sought from these women before enrolling in this study.

Sampling and Methods

Prior to admission of enrolled women to operation theatre, blood samples were collected. These women were not in fasting state. Ten millimeters of venous blood had been withdrawn and left to clot in a tube, centrifuged for 10 minutes at 3000 rpm to collect serum. These samples were used to measure serum lipids, random glucose, urea and creatinine to affirm the biochemical similarity between study groups and to exclude women who had abnormal biochemical measures. The weight and height of women were also measured to estimate the body mass index (BMI). Serum was used to determine glucose, lipid profile including total cholesterol, triglyceride (TG), very low density lipoprotein cholesterol (VLDL-C), and high density lipoprotein cholesterol (HDL-C) [measured by the precipitation of chylomicrons] done using colorimetric enzymatic method. Low density lipoprotein cholesterol (LDL-C) was calculated by the formula of Friedewald *et al.* 1972 [10]. Blood samples were also used to measure MDA and HEL levels by OxiSelect MDA Adduct ELISA Kit (CELL BIOLABS, Inc, Canada) and Hexanoyl-Lys adduct (HEL) ELISA Kit (Japan Institute for the Control of Aging JICA).

Statistical Analysis

Data were encoded and entered into SPSS statistical software (v. 22). Descriptive analysis

of biochemical and anthropometric characteristics of study sample was first presented. The mean and standard error (SE) were presented to describe study variables. The difference between study groups in anthropometric and biochemical criteria was assessed utilizing Analysis of Variance (ANOVA) test.

Pearson correlation test was performed to examine the correlation between OS level and types of pregnancy. ANOVA analysis was also performed to assess the difference between study groups in OS levels. If significant, independent samples t-test was performed to assess the statistical difference between preterm and post-term women groups (as case groups) and term women group (as study control). Alpha index (p value) of less than 0.05 was considered significant.

Results

The present study enrolled 90 women (30 pre-term, 30 post-term and 30 term). The mean age of study participants was 29.73 ±SE: 0.54 years. The summary of biochemical criteria for study participants is summarized in Table 1. The results from ANOVA analysis showed that study groups were not significantly different in age, BMI, lipids, random glucose, urea and creatinine ($P > 0.05$). Data were presented as mean and standard error.

Table 1. Anthropometric and biochemical criteria of the studied groups

Variables	Preterm N=30 Mean ± S.E	Post-term N=30 Mean ± S.E	Term N=30 Mean ±S.E	*P* value
Age (years)	28.23 ± 0.43	31.71 ± 0.63	29.31 ± 0.73	0.115
BMI (kg/m^2)	31.32 ± 0.33	28.71 ± 0.91	29.46 ± 0.43	0.111
Total Cholesterol (mg/dl)	184.03 ± 1.32	178.07 ± 0.79	179.80 ± 0.72	0.091
HDL-C (mg/dl)	67 ± 0.96	65.60 ± 0.81	65.93± 1.92	0.738
LDL-C (mg/dl)	89.73 ± 2.31	86.63 ± 1.11	87.13 ± 1.73	0.421
Triglyceride (mg/dl)	136.70 ± 3.77	129.16 ± 2.12	133.77 ± 6.51	0.495
LDL-C/HDL-C ratio	1.39 ± 0.03	1.38 ± 0.04	1.31 ± 0.03	0.124
TC/HDL-C ratio	2.76 ± 0.04	2.73 ± 0.03	2.79 ± 0.08	0.529
Random Serum glucose (mg/dl)	143.60 ± 2.12	146.83 ± 2.01	151.3 ± 1.92	0.101
Urea (mg/dl)	17.22 ± 0.93	19.10 ± 0.49	17.52 ± 1.11	0.274
Creatinine (mg/dl)	0.59± 0.007	0.67 ± 0.003	0.64 ± 0.003	0.071

BMI = Body Mass Index; HDL-C = High Density Lipoprotein Cholesterol, LDL-C = Low Density Lipoprotein Cholesterol, S.E. = standard error.

Oxidative stress biomarkers (MDA and HEL) and pregnancy types

ANOVA analysis was utilized to assess the difference between study groups in serum MDA and HEL. Data were expressed as mean ± (SE). Table 2 depicts the analysis results.

Table 2. Comparison between study groups in oxidative stress biomarkers in blood

Oxidative stress biomarkers	Preterm (N=30) Mean ± S.E	Post-term (N=30) Mean ± S.E	Term (N=30) Mean ± S.E	*P* value
MDA (pmol/l)	62.37 ± 2.99	49.90 ± 3.60	51.70 ± 2.52	0.010*
HEL (nmol/l)	373.13 ± 4.95	184.37 ± 3.88	297.10 ± 5.76	<0.001*

* = $P < 0.05$, MDA = Malondialdehyde, HEL = Hexanoyl-Lysine Adduct

As for the results presented in table 3, pre-term, post-term and term women groups were significantly different in both MDA (F= 8.11, df: 2, *P* = 0.01) and HEL (F= 24.11, df: 2, *P* < 0.001). Both OS biomarkers levels were the highest among preterm women, and the lowest among post-term women. After affirming the difference between study groups in OS levels, independent samples t-test was performed to examine the difference in serum MDA and HEL levels between preterm and post-term women groups in comparison to term women.

Table 3. The difference in oxidative stress biomarkers in blood between preterm, post-term in comparison to term women

Oxidative stress biomarkers in blood	Preterm		Post-term	
	T value	P value	T value	P value
MDA (pmol/l)	-2.73	0.008*	-0.41	0.684
HEL (nmol/l)	-10.01	<0.001*	-16.24	<0.001*

* = p<0.05, MDA = Malondialdehyde, HEL = Hexanoyl-Lysine Adduct.

The results showed that the preterm and term women groups were significantly different in serum OS biomarkers (t: -2.73, *P* = 0.008 and t: -10.01, *P* < 0.001 for MDA and HEL respectively). Moreover, the direction of the relationship was also examined by interpreting the t value for the difference between preterm and term women groups. The relationship between pregnancy types and both MDA and HEL seems to be negative. In other words, preterm pregnancy was associated with higher levels of both MDA and HEL, which seemed to decrease with increasing gestational age.

Similarly, the difference between post-term and term women groups was also examined. The results showed a significant difference in HEL between post- term and term women (t: -16.24, *P* < 0.001). By examining the t value, the direction of the relationship showed that both MDA and HEL were negatively associated with the gestational age.

Association between OS biomarkers and pregnancy types

Pearson correlation analysis was performed to assess the association between serum MDA and HEL levels and pregnancy types. Both preterm and post-term groups were compared to term women group as the study control.

According to the results, MDA level in blood was significantly correlated with gestational age in preterm women group only. While serum HEL was significantly associated with gestational age in both preterm and post-term women groups. The results also confirmed the negative direction of the relationship between serum OS and pregnancy type. The relationship between OS biomarkers and pregnancy is depicted in fig. 1 and 2.

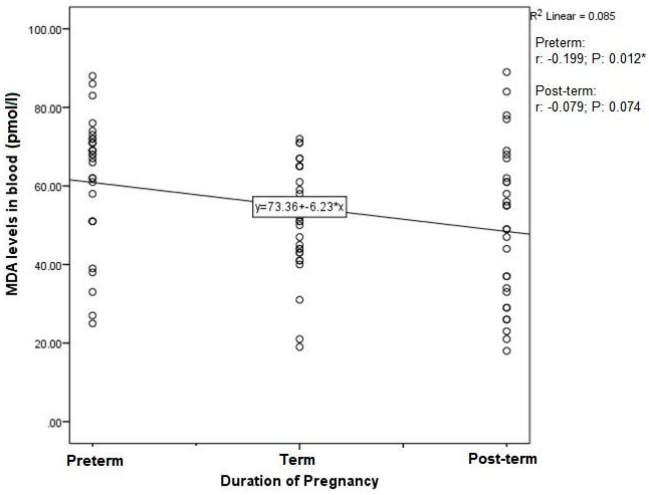

Fig. 1. The correlation between MDA level in blood and pregnancy duration

Discussion

The results of the present study showed that OS biomarkers were negatively correlated with duration of pregnancy among preterm women group, but the only significant correlation was that of HEL in the post-term women. The

findings of our results are consistent with previous studies that ascertained similar relationship in different settings. Kasat et al. (2013) reported a higher level of serum MDA among preterm women as compared to term women group [11]. This is further evidenced by Cinkaya et al. (2010) who reported lower total antioxidant status among preterm delivering women as compared to term women group [12]. The high MDA level was also implicated in neonatal complications.

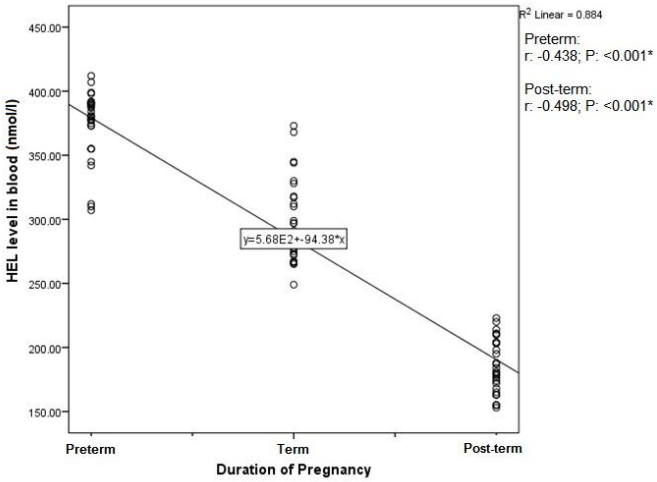

Fig. 2. The correlation between HEL level in blood and pregnancy duration

One study conducted in Tikrit University showed that serum level of MDA is correlated with low birth weight and having neonate complications such as respiratory distress syndrome [13]. Although the mechanism through which high OS levels may affect pregnancy outcome is still unclear, Menson (2014) suggested that high OS level has damaging effect on the intrauterine tissues particularly the fetal membrane of the placenta which may result in fetal cell aging [14]. Aging cells form uterotonic biomolecular signals enhancing and promoting the labor process [14]. This is also confirmed by the results of the current study which showed that preterm women had significantly higher levels of serum MDA and HEL in comparison to term and post-term women.

On the other hand, the present study reported that only serum HEL was inversely associated with post-term pregnancy. This was also supported by evidence that showed the decreasing level of OS markers in post-term pregnancy may play a role in delaying the onset of labor [15]. This may also confirm Menson's theory about the effect of OS on fetal tissue aging which triggers early premature labor [14]. At last, it could be said that high serum MDA and HEL levels may be an important contributor to the pathophysiology of premature labor. The OS levels were the highest among preterm women and lowest among post-term women. Further investigation of the causal relationship between OS and pregnancy duration is recommended. The effect of anti-oxidants as a mean to reduce the risk of premature labor may also be evaluated.

Acknowledgment

We are extremely grateful to all the authorities for granting the permission to conduct the study and the pregnant women from Al-Hakeem Hospital and Al-Imamain Al-Khadimain Medical City; special thanks to the nurses who have made a collect samples. Finally, I owe especial appreciation to my college and friends for their help and support.

Author Contribution

Zeena Conception and design, data collection, analysis, interpretation, writing and revision of the manuscript were performed; Dr. Risala help in sampling and Dr. Rayah and Dr. May supervise this paper as part from a thesis.

Conflict of Interest

None

Funding

None

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P.O. Box 70036
Received 23rd Feb 2015: Accepted 14th Jun. 2015.

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq

The Role of Tumor Necrosis Factor α (TNF-α) and Intracellular Adhesion Molecules-1 (ICAM-1) in Atherosclerotic Coronary Heart Disease

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Abstract

Background Tumor necrosis factor-α elaborated soon after myocardial ischemic injury. The intracellular adhesion molecule-1 is required for polymorphonuclear emigration, the primary cause of inflammatory tissue damage due to ischemia-reperfusion.

Objective Detect the serum level of tumor necrosis factor-α and to look for the percentage of expression of intracellular adhesion molecule-1 in atherosclerotic coronary heart disease.

Methods Fifty patients (40 males and 10 females) were enrolled in this study with age range (42-80) years, and fifteen, age and sex matched, apparently healthy individuals. The patients group was further classified into acute and chronic cases. Blood sample was taken from each subject and divided into 2 parts. One part used for lymphocyte separation by using immunocytochmistry to detect intracellular adhesion molecule-1 and the other one for serum separation by using ELISA technique to detect tumor necrosis alpha-α.

Results Significant difference in the concentrations of tumor necrosis factor-α was found between patients and control groups and it was elevated in acute cases compared to chronic cases. Similarly, intracellular adhesion molecule-1 was elevated in patients compared o control groups and more in acute than chronic cases.

Conclusions TNF-α is an important marker that acts on coronary arteries which may contribute to the development of congestive heart disease. Elevation of intracellular adhesion molecule-1 level correlates well with the development of acute events in the disease.

Keywords Atherosclerotic coronary heart disease, TNF-α, ICAM-1, ELISA, immunocytochemistry technique.

List of abbreviation: **CHD** = Coronary heart disease, TNF-α = tumor necrosis factor-α, ICAM-1 = intra cellular adhesion molecules-1, PBLs = peripheral blood lymphocytes, ELISA = enzyme linked immuno sorbent assay.

Introduction

Atherosclerosis is a progressive inflammation disorder of the arterial wall (large and medium size arteries) that is characterized by focal lipid-rich deposits of atheroma that remain clinically silent until they become large enough to impair arterial perfusion or until ulceration or disruption of the lesion results in thrombotic occlusion or embolization of the affected vessel [(1)]. Accumulating experimental evidences support a key role for inflammation as a link between risk factors for atherosclerosis and the biology that underlies the complications of this disease [(2)].

Coronary heart disease (CHD) is the most common form of heart disease and the single most important cause of early death in all regions of the world. In United Kingdom 1 in 3

men and 1 in 4 women die from CHD, an estimated 300000 people have a myocardial infarct each year and approximately 1.3 million people have angina [(1)].

Atherosclerosis might, at least partly, be an inflammatory condition. Inflammation which is an immune response to injury characterized by swelling and redness which involves the production of proteins called "cytokines," that attract cells of the immune system to the site of injury. In atherosclerosis, damage to the artery walls seems to trigger inflammation, which helps the atherosclerotic plaques grow. Because of the potential involvement of inflammation in atherosclerosis, increased levels of circulating cytokines might be associated with an increased risk of CHD.

Tumor necrosis factor-α (TNF-α), a pivotal cytokine in the inflammatory cascade, is directly involved in vascular pathophysiology [(3)]. The inflammatory phenomenon and immunological mediators have been identified to positively correlate with the underlying disease load. They regulate every stage of the inflammatory cascade, from endothelial activation to the adhesion of inflammatory cells and platelets, and subsequent remodeling of the internal vascular environment [(4)]. Inflammation is thus moving from a theoretical concept to a tool that provides practical clinical utility in risk assessment and targeting of therapy.

Cytokines are key regulatory glycoproteins allied to inflammatory/immunological processes which modulate all aspects of vascular inflammation by altering the proliferation, differentiation and function of an extensive array of cell types. They are intimately associated with atherogenesis and modulate plaque morphology and stabilization [(5)].

Cytokines act by binding to specific receptors an interaction which has clarified their involvement in atherosclerosis, and highlighted potential new ways for therapeutic intervention [(6)].

TNF-α increases the risk of coronary artery disease by interfering with the thrombotic process by enhancing procoagulant activity (PAI-1, von Willebrand factor) and suppressing the antithrombotic protein C pathway in endothelial cells. The impact of TNF-α on vascular injury in both acute and chronic inflammatory conditions has made it is an important therapeutic target [(7)].

Hence this study tries to investigate the expression of activation markers, i.e., intracellular adhesion molecules-1 (ICAM-1) on peripheral blood lymphocytes (PBLs), and estimation of TNF-α serum levels in atherosclerotic patients.

Methods

Fifty patients with CHD (40 males and 10 females), their age ranges from 42-80 years old, were included in this study. Eleven patients (7 males and 4 females) with acute myocardial infarction were admitted to the Cardiac Care Unit at Al-Imamain Al-Kadhimain Medical City and 39 patients (33 males and 6 females) were attendant as outpatient clinic of Ibn Al-Baitar Hospital in Baghdad with history of atherosclerotic chronic coronary insufficiency. The period of sample collection was from May to July 2008. Consent for the participation in the research was obtained from each patient.

The diagnosis was done by cardiologist based on clinical presentation and history of ischemic heart disease, which was confirmed by electrocardiography, cardiac enzymes and coronary artery catheterization. Fifteen, age and sex matched, apparently healthy individuals, were included in this study as healthy control group.

Sample collection: From each patient and control, five ml venous blood was aspirated from a suitable vein after efficient disinfecting over the injection site.

Blood samples were divided into two parts, three ml were immediately transferred to sterile heparinized vacutainer tube for lymphocyte separation, and two ml of blood

immediately transferred to a sterile plain tube for serology.

The unheparinized blood in plain tube was left to clot and then centrifuged at 1000 rpm for 5 minutes to separate the serum and dispensed into tightly closed Eppendorf tubes in 0.1 ml aliquots and stored at -20^{o}C until assayed.

Detection of serum TNF-α

The procedure of Enzyme Linked Immuno Sorbent Assay (ELISA) was done according to the manufacturer instructions, as it was written in the kit guideline, product code: KAC1751; while the standardization of the kit to produce a standard curve was done by using a duplicate sample of each standard and the mean of them was used.

Detection of PBLs ICAM-1

Primary monoclonal antibody (USBiological$^{(R)}$) specific for Human ICAM-1 protein were applied on slides; then immunoperoxidase Secondary Detection system that were used from Dako Cytomation Company, USA, (Ref K0673). Positive results were identified by presence of brown colored precipitate.

Statistical analysis

Statistical analysis was performed with the SPSS 15.01 Statistical Package for Social Sciences and also Excel 2003. Data analysis was done using independent sample t-test for tables with means and standard deviations. *P* value of ≤ 0.05 was used as the level of significance and *P* value of ≤ 0.001 was used as the level of highly significance. Descriptive statistics for the clinical and laboratory results were formulated as mean and standard deviation.

Results

Serum levels of TNF-α of all acute and chronic cases was 43.659±6.374 which is significantly higher (*P* = 0.000) than 16.340±3.645 of the control group. Moreover, significant difference (*P* < 0.025) was found between the acute and chronic cases (44.934±12.421 and 40.847±10.872, respectively) as seen in table 1.

Table 1. Serum tumor necrosis factor-α in the coronary heart disease patients and control group

		Tumor necrosis factor-α	*P* value
Group	Patients (N=50) Control (N=15)	43.65±6.37 16.34±3.64	< 0.001
Disease phase	Acute (N=11) Chronic (N=39)	44.93±12.42 40.85±10.87	0.025

The ICAM-1 expression on PBLs was equal to 56.571 ±16.434 which is significantly higher (*P* = 0.000) in the patients as compared to 24.500±7.623 of the control group. Similarly, ICAM-1 expression on PBLs was statistically higher (*P* < 0.043) in the acute cases compared to the chronic cases (65.364±14.583 and 54.026±16.226, respectively) as shown in table 2.

Table 2. Expression of intracellular adhesion molecule-1 on peripheral blood lymphocytes in the coronary heart disease patients and control group

		Intracellular adhesion molecule-1	*P* value
Group	Patients (N=50) Control (N=15)	56.57±16.43 24.50±7.62	<0.001
Disease phase	Acute (N=11) Chronic (N=39)	65.36±14.58 54.03±16.22	0.043

Discussion

The current study showed that serum level of TNF-α in CHD patient was higher than healthy control group; such findings come in agreement with Cybulsky et al [6] who found that many cytokines, including, IL-1, TNF-α, and IFN-γ, have been implicated in the induction of an array of adhesion molecules and chemokines in the vascular wall and Natanson *et al* [7] and Ridker *et al* [8]. The difference between acute and chronic cases was in agreement with Deten et al [9]; although they did their study on experimental rats. IL-1 and TNF-α stimulate membrane expression of leukocyte adhesion molecules ICAM-1, ICAM-2, VCAM-1, E-selectin, and P-selectin by endothelial cell. These molecules interact with specific ligands expressed by neutrophils, lymphocytes, and circulating monocytes.

Unfortunately, results done by Chung et al [10]; Muller-Ehmsen and Schwinger [11] allied to TNF neutralization therapies have been inconsistent thus far. Trials using TNF-α antagonist, such as infliximab (ATTACH trial), failed to show any improvement in cardiac failure, while the ATTACH trial was associated with an increased all and cause mortality. Although there have been no studies focusing on anti-atherogenic therapeutic interventions in peripheral arterial disease, a study by dePalma et al workers [12] indicated that there were no marked differences in TNF-α levels in peripheral arterial disease patients receiving/not receiving statin therapy.

Our results showed the expression of ICAM-1 on PBLs was higher in patients than in controls and there was a significant difference between acute and chronic cases of patients; a findings which is in agreement with studies done by Blann and McCollum [13], Hwang *et al* [14] who reported significantly higher values of circulating ICAM-1 in patients with peripheral vascular disease and ischemic heart disease than in healthy control subjects. Caroline *et al* [15] showed that patient with cardiovascular events during follow-up had higher ICAM-I and VCAM-I than those without events.

Squadrito *et al* [16] reported significantly higher levels of circulating ICAM-1 and E-selectin in patients with acute myocardial infarction than in patients with chronic stable angina and healthy control subjects.

There was a consistent relationship between the levels of circulating ICAM-1 and incident CHD. One possible explanation for these data is that levels of circulating ICAM-1 are more closely related to the activity of atherosclerosis. Increased levels of ICAM-1 may be important in migration of increased numbers of T lymphocytes into active lesions [17]. Other possibility is that patients with higher circulating levels of ICAM-1 have an increased number of plaques prone to rupture, thrombi, or other events leading to clinical CHD. The interaction between fibrinogen and ICAM-1 observed in *in vitro* study provides evidence suggesting an association between ICAM-1 and thrombosis/ischemic events [18].

This study revealed that TNF-α is considered as an important marker on coronary arteries which may contribute to the development of (CHD), and ICAM-1 levels correlates well with the development of acute events in CHD.

Acknowledgment:

We would like to appreciate with special thanks all staff members of Microbiology Department for their help in performing this work.

Conflict of Interest

There were no personal or financial conflicts or problems raised during the performance of this work.

Author contribution

All authors participated in performing this work worked as one team.

We shared nearly the same responsibilities regarding data collection preparing and performing the lab work, writing and printing and other steps were necessary to complete this work.

Funding

No governmental or other sponsors financially supported this work. All funds needed in this work were paid by the authors and on their own expense.

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Received 2nd Dec. 2014: Accepted 14th Jun. 2015

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq

Experimental Study on the Effect of Air-Drying on Durability of Embalmed Human Cadavers

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Abstract

Background The embalmed human cadavers used for teaching anatomy in the medical colleges could be preserved for a very long time, the crucial factors for acheivement of this prolonged preservation was not fully discussed.

Objectives This is an experimental study to evaluate the veracity of using paraffin sectioning feasibility as a test for the degree of damage in the embalmed cadaveric tissue affected by recurrent and prolonged air drying in anatomy laboratories.

Methods Routine paraffin sections were done for tissues obtained from cadaveric organs exposed to recurrent and prolonged air drying and tissues from cadaveric organs preserved most of the time in a hydrated condition.

Results The results of this study showed unblemished paraffin sectioning of the emblamed cadaveric tissue preserved with good hydration. The cadaveric tissues exposed to reccurent air drying could not be sectioned properly, the sectioned tissues were hard and brittled.

Conlcusion The experts dealing with the embalmed cadavers evidently necessitude maintancance of cadaveric hydration as a requisite to prolong durability of the demonstrative details in the dissected cadavers. Accordingly; the comparably more proper paraffin sectioning of the well hydrated cadaveric tissue may be considered as a sign of more durable embalmed cadaveric organs.

Keywords cadaver, paraffin, anatomy, fixation, formalin.

Introduction

The human cadavers in medical colleges usually were preserved for a very long time in a fixative solution which contains formalin as a major constituent, in addition to acohol, phenol, water, and glycerin [(1)].

Routinely, many of the laboratories in the departments of human anatomy underestimated the harmful effect of air dryness on the cadaveric samples. The human cadavers are not easily provided for teaching purposes in many countries, a fact that encourage this study.

The avialable references fulfilled no simple description to the process of tissue fixation, and the aims of good fixation are not fully fulfilled with any of the fixative materials used routinely. The routine methodologies for tissue fixation could preserve the histological criteria as much as it is necessary [(2,3)].

The effects of tissue fixation with a low-formalin embalming fluid on the histology of organs obtained from embalmed cadavers were debatable. Many histopathologists believed that the use of specimens from embalmed cadavers is good enough for investigative research and forensic medicine, especially in determining the cause of death at

autopsy [4]. Hardening is one of the difficulties predictable if the embalmed cadaveric tissues were proccesed for paraffin sections, the formalin fixatives used overharden the cadaveric tissue due to the long time of exposure [5].

In the ultimate methods for histological preparations, the tissues should be fixed directly and completely from the living status. This is rarely accomplished in practice due to many factors. The extracted tissues often exposed to anoxia for a period, and the penetration of the fixatives requires relatively long duration [6]. Anoxia brings about changes which are visible with electron microscope within minutes [7], the enzymes are lost within few hours of anoxia [8].

The postmortem tissues have various adverse factors disturbing the use of these tissues for paraffin histological sectioning. There might be agonal changes in tissue, the body is usually left for some time at room temperature before it is transffered to the mortuary and refrigerated. These factors might give rise to artefacts. The tissues will undergo dryingif left in air before fixation, also the tissue will undergo shrinkage and bacteriological changes with autolysis. There are many damaging chemical reactions taking place by the cellular enzymes within the tissue [9].

This study is an experimental appraisal of utilizing the paraffin sectioning feasibilty as an indicator for the severity of the damaging effect resulting from prolonged and recurrent air drying on the embalmed cadavers.

Methods

The cadaveric tissues used for this experimental study were obtained from the Department Of Human Anatomy, College of Medicine, Al-Nahrain University. All the cadaveric tissues were primarly embalmed by the same procedure and materials [1], also all these tissues were preserved in the same constituents of the preservative solution since 2-3 decades.

The tissue used for this study were macroscopically intact and not affected by fungal infection, cadaveric organs having a damaged gross anatomical configuration were excluded .

Small pieces of 0.5 cubic centimeter size were taken from the tissues of the quadrate lobe of the liver, the descending colon, and the left ventricle of the heart.The tissues used in this experimental study were of two groups:

Group A: Include tissues taken from extracted cadaveric organs that were routinely preserved in containers filled with the preservative solution immediately after each anatomy session, which usually lasting about 2-3 hours. Three specimens were taken from each of the organs used, two extracted organs of each type were selected.

Group B: Include tissues taken from organs that were part of the whole cadaver. These cadavers were frequently exposed to recurrent and prolonged air dryness (specially during the hot seasons) because it is difficult to return the whole cadavers in the preservative solution daily. These cadavers were placed routinely on the bench in the laboratory of human anatomy for a week or more, and thus they were exposed to dryness due to the long time exposure to atmospheric temperaturethat may reach up to 45 ^{o}C in summer. The poor ventillation in the anatomy laboratory exaggerated the effect of high temprature on the cadaveric tissue.

Also, three specimens from each of the organs used were taken, and two cadaveric organs of each type were selected.

The tissues of both group A and B were placed in the preservative solution routinly used in the anatomy laboratory for preservation of the cadaveric organs. The tissues were stored in this solution at room temperautre for one week inorder to ensure proper hydration. The tissues were then transferred into three changes of 70% ethonol (two days for each), after that, the tissues were dehydrated by higher concentration of ethanol alcohol, cleared in xylene, and impregnated in a 58 ^{o}C

melted paraffin and parafin blocks were prepared. The wax impregnation was used in three changes with a total impregnation time of three hours. The paraffin blocks sectioned using a rotary microtome (Riechert Jung) at 7, 10, 14, and 20 microns tissue thickness. Sections were float in an albumen solution on a glass slides. The sections were stained with hematoxylin and eosin [10].

Results

The process of sectioning the paraffin blocks obtained from the two groups of cadaveric tissues were compared. The paraffin blocks of group B could not being sectioned easily. The tissues were hard and brittling of the tissues occurs during sectioning. Few of the sections of this group contain pieces of the tissues, most of the sections show holes in the strips of the paraffin at the region of the falling brittled tissues. The sectioned liver tissue produced few fairly intact sections.

The hematoxyline and eosin staining of the tissues obtained from group B showedhazy histological boundaries of the cells and tissue fragmentation with complete loss of the histological tissue architecture of each type of tissue.The low power microscopic examination of the tissues of group B showed irregular section margins (Fig. 1).

The sectioning of the paraffin blocks of group A was much easier in comparison to group B. All the paraffin strips of group A sectioned contain intact tissues.

The histological examination of the sections of group A showed delineated cellular boundaries, however; the histological criteria of each tissue were distorted and the microscopic features of each type of tissue were not ideally demonstrated. The low power microscopic examination of these sections showed a linear cutting margin compared to that of section of group B (Fig. 2 and 3).

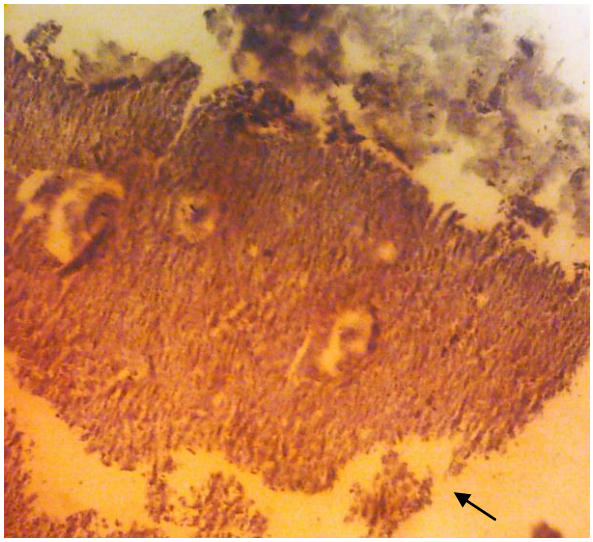

Fig. 1. The paraffin section of liver tissue, group (B). The section showed irregular margins (arrow). H&E 100X.

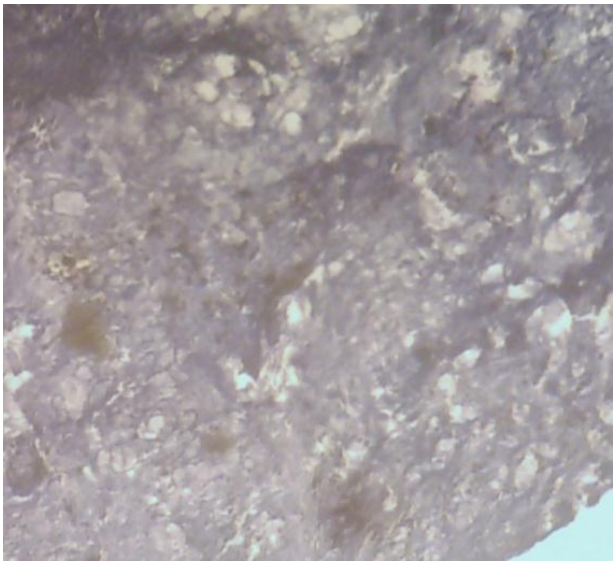

Fig. 2. Paraffin section of cardiac muscle tissue from group (A). H&E 400X

Discussion

It was logically concluded from the practice in anatomy teaching that preserving the extracted human cadaveric organs in the preservation solution after each anatomy session kept the samples beneficial for teaching purposes for a much longer duration. This logic conclusion was not previously tested experimentally, a theme which this study established.

Therefore; the results of this study may suggest that the comparably more easy and the proper paraffin sectioning of the tissues obtained from the extracted cadaveric organs that were moslty kept hydrated in the preservative solution (group A) could be considered as a sign for the relatively more durable and sound tissue.

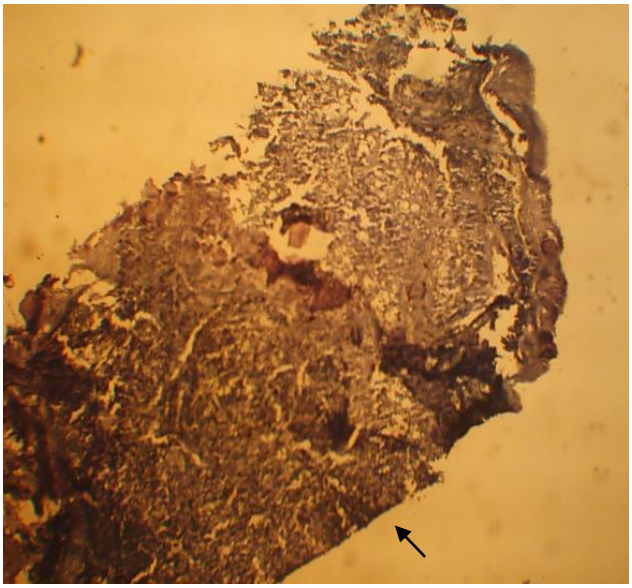

Fig. 3. Paraffin section of cardiac muscle tissue from group (A) stained with hematoxylin and eosin. Linear cutting margins are seen (arrow). 50X.

The comparison of normal histology of the human liver, colon, and heart [11] with histological features of the sections obtained from the extracted organs of group (A) markedly proveddistorted morphology. This distortionmay be attributed to the effect of the embalming solution on these tissues. The embalming solution was not prepared for histological tissue processing as it contains materials that do not fit with the criteria of proper tissue fixation [1,10]. The prolonged exposure of the extracted organs of group A to preservative solution (for 2-3 decades) may also be a contributing factor to the distortion of the histological features of these tissues.

This interpretation about the effect of the composition of embalming solution on histological quality of the specimens was a supportive conclusion to the previously reported experiemntal studies [12].

The histologist and pathologist were always obsessive from the bad processing of the samples as the proper tissue processing usually leads to easy and good paraffin sectioning and produces comprehensive histological details [13]. This attentiveness was the bases of the proposal done in this study considering the feasibility of paraffin sectioning as an experimental procedure that could evaluate the extent of tissue damage by the effect of exposure to air drying.

The traditional practice in teaching anatomy in the laboratories of human anatomy considered an implication that the dried human cadaveric tissue could be returned back to its beneficial status by returing the cadaver into the preservative solution. The results of this study may present an evidence that recurrent air drieness of the cadaveric tissue results in its advanced damage. This assumption was established from the difficulties noticed during paraffin sectioning of the cadaveric tissue of group B compared to that of group A. Also, the loss of the histological appearance of the sections of group B with loss of cellular boundaries and tissue fragmentation may be considered as feature of the evident tissue damage by the effect of recurrent air drying.

The laboratory in the Department of Human Anatomy in the College of Medicine Al-Nahrain University stored the cadaveric samples for many years with a minimal tissue damage and with minimal loss of the cadaveric organs that kept the details of the anatomical descriptions. This prolonged period of preserving the anatomical samples is a fact that may be considered as a supportive evidence to the results of this study asthe staff members in the department used to routinely protecting the cadaveric samples from being left exposed to air for a long time.

In agreement with results of this study, it was reported that the aim of embalming is to achieve perfusion of the fixative solution throughout all parts of the body. The low

formalin fluid used for embalming is designed to preserve the tissue, made the cadaver suitable for dissection, and prevent bacterial and fungal growth. The low formalin embalming fluid made the cadavers suitable for histological sectioning, however; some organs showed distorted microscopic achitecture that is considered to be more close to normal architecture than others [(4)].

The mantainance of the embalmed human cadaveric organs used for teaching for a longer duration carries an importance in many various considerations as financial, humanitarian, and scientific respects. The results of this study may objectively provided a technical method to evalute the durability of the apparantly intact cadaveric organs and detecting the organsexposed to neglection, application of this methodmaydesignate the embalmed cadaveric organs that are damaged should to be replaced within a short peroid.

Aknowlegments

I would like to express my gratfulness to the members of the Department of Human Anatomy for their help and for keeping the human cadaveric sample in a very organized way.

Conflict of interest

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

Funding

The research working funding was provided by the authors.

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Tel.: + 964 7904772919
Received 26th Mar. 2015: Accepted 4th Jun. 2015.

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq

Medial and Lateral Percutaneous Fixation versus Lateral Fixation for Treatment of Gartland Type II, III Supracondylar Fracture of Humerus in Children

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Abstract

Background Operative treatment of supracondylar fractures with reduction and percutaneous pinning is so effective and safe. The great majority of displaced fractures should be treated operatively. There is little controversy that all closed Gartland type II and III fractures should have an attempt at closed reduction and pinning.

Objective To compare the efficacy of medial and lateral entry pinning with lateral entry pinning for percutaneous fixation of displaced (Gartland type II and III) extension type supracondylar fractures of the humerus in children.

Methods A prospective study conducted at a single centre from December 2008 to November 2011. Eighty patients who satisfied the inclusion and exclusion criteria were enrolled in the study, with 40 patients in each group. All the pinning was done according to a uniform standardized technique. The patients were re-evaluated as outpatients at three weeks, six weeks and three months after the surgery.

Results No significant differences were found between the two groups with regard to base-line characteristics, withdrawals, complication rate and various outcome measures such as carrying angle, passive range of elbow motion, Flynn grading, Baumanns angle, change in the Baumann angle and loss of reduction grading.

Conclusions If a uniform standardized operative technique is followed in each method, then the result of both methods will be same in terms of safety and efficacy.

Keywords Supracondylar fractures, Humerus, Children, Percutaneous fixation

List of abbreviations: K-wires = kurschner wires, AP = anteroposterior, ORIF = open reduction and internal fixation, gm = gram, IV = intravenously, Deg = degree.

Introduction

Supracondylar fractures of the humerus are the most common type of elbow fracture in children and adolescents. They account for 50% to 70% of all elbow fractures and are seen most frequently in children between the ages of 3 and10 years [(1)].

Supracondylar fractures are produced by forcibly hyperextending the elbow. The level of the fracture is determined by the olecranon forming a fulcrum in the supracondylar region [(2)]. Prevention of cubitus varus or valgus or loss of flexion and extension by obtaining as anatomical a reduction as possible is necessary. The Gartland classification is useful for determining appropriate treatment for supracondylar fractures: type I, undisplaced; type II, displaced with intact posterior cortex; and type III, displaced with no cortical

contact[3].

Posterior displacement and tilt is the commonest (95% of all cases), suggesting a hyperextension injury, usually due to a fall on the outstretched hand. The jagged end of the proximal fragment pokes into the soft tissues anteriorly, sometimes injuring the brachial artery or median nerve. Anterior displacement is rare, but may result from over-reduction of the usual posterior displacements [4].

Treatment

The initial evaluation of these fractures should include a careful evaluation of the medial distal humerus, with consideration of the need for contralateral comparison radiographs. Subtle comminution of the medial distal humerus in an otherwise minimally displaced fracture can lead to cubitus varus [5].

Attempts have been made to correlate various radiographic measurements with adequate fracture reduction Baumann angle is the most frequently cited method of assessing fracture reduction and has been reported to correlate well with the final carrying angle, not to change significantly from the time of initial reduction to final follow-up, and not to be obscured or invalidated by elbow flexion or pronation [3].

Reported normal values range from 9 to 26 degrees. A common rule of thumb is that a Baumann angle of at least 10 degrees is acceptable [3].

Studies of the pin configuration for supracondylar fractures have compared the use of medial- and lateral entry crossed pins with the use of lateral-entry pins alone. Biomechanical studies found that crossed pins are stronger in torsion than a lateral-entry construct. Proponents of lateral-only pins cite a lower incidence of iatrogenic nerve injury with these pins [5].

The objectives of this study was to compare the efficacy of medial and lateral entry pinning with lateral entry pinning for percutaneous fixation of displaced (Gartland type II and III) extension type supracondylar fractures of the humerus in children.

Methods

This study is a prospective, randomized controlled clinical trial, conducted in Department of Orthopaedics and Traumatology in Al-Imamain Al-Kadhimain Medical City from December 2008 to November 2011. Written informed consent was obtained from the study participants.

The study included 80 patients with age range from two to twelve years who had supracondylar fractures. The patients divided into two groups 40 patients in each.

Inclusion criteria: (1) age between two and twelve years (2) unilateral fracture (3) extension type (4) Gartland type II and type III (5) patients presenting within seventy two hours after the injury (6) no other associated injury in the same limb (7) no previous fracture in the same limb.

Exclusion criteria: (1) open fractures (2) fractures that required open reduction and (3) patients with neurovascular abnormalities that were found at the time of presentation.

At three months follow-up visit, following information were recorded as outcome measures: (1) Carrying angle (degree) (2) passive range of elbow motion (degree) (3) Flynn's criteria for grading, based on the loss of carrying angle and loss of total range of elbow motion. (4) Baumanns angle (degree) (5) Change in Baumann angle (degree) between the Intraoperative radiographs after the surgery and radiographs at three months follow-up visit (6) loss of reduction grading, based on the change in the Baumann angle.

Surgery was done under general anaesthesia by more than one surgeon. All the patients were positioned supine on a fracture table and closed reduction were performed under the fluoroscopic control. The method of reduction was initial traction in an extended position of the elbow joint, followed by flexion and dorsal pressure with the thumb on the distal fragment in extension-type fractures and simultaneously pronating the forearm. Fracture reduction with flexed elbow joint was evaluated in a position of 90° external rotation, in the anteroposterior

view, and in 90° internal rotation .Fractures are fixed either by two lateral wires (Figure 1 & 2), or by medial and lateral wires as in figure 3.

The patients were re-evaluated as outpatients at three weeks, six weeks and three months after the surgery. The same surgeon throughout the trial did follow-up assessment of each patient.

At three months follow-up visit, the following information were recorded as outcome measures: (1) carrying angle (degree) (2) passive range of elbow motion (degree) (3) Flynn's [6] criteria for grading, based on the loss of carrying angle and loss of total range of elbow motion (Table 1) (4) Baumann angle (degree), calculated on the anteroposterior view of elbow (5) The Change in Baumann angle (degree) between the Intraoperative radiographs after the surgery and radiographs at three months follow-up visit (6) loss of reduction grading, based on the change in the Baumann angle.

The major loss of reduction (defined as a change in the Baumann angle of > 12° between the Intraoperative radiographs and radiographs at three months was selected.

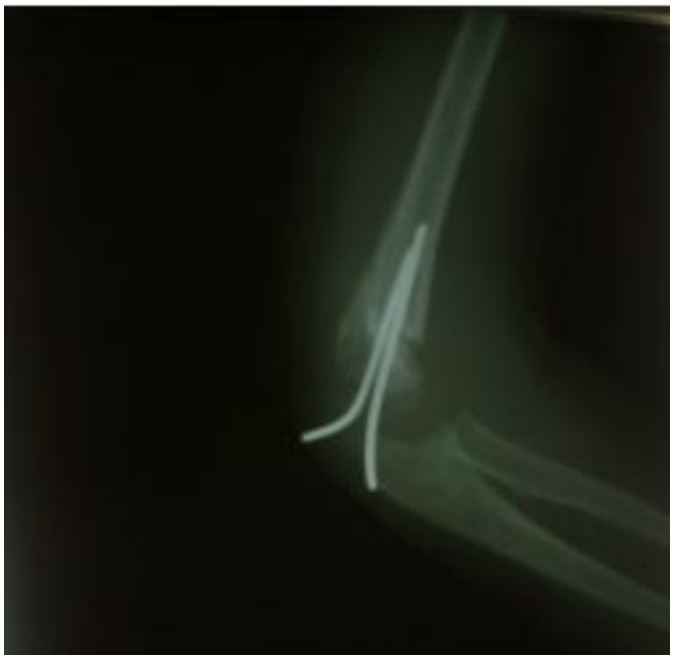

Fig. 1. Lateral x-ray of 6 years old patient with supracondylar fracture humerus treated with closed two lateral K wires

Table 1. Flynn's criteria for grading

Result	Rating	Carrying angle loss (Degrees)	Total range of elbow motion loss (Degrees)
Satisfactory	Excellent Good Fair	0-5 5-10 10-15	0-5 5-10 10-15
Unsatisfactory	Poor	Over 15	Over 15

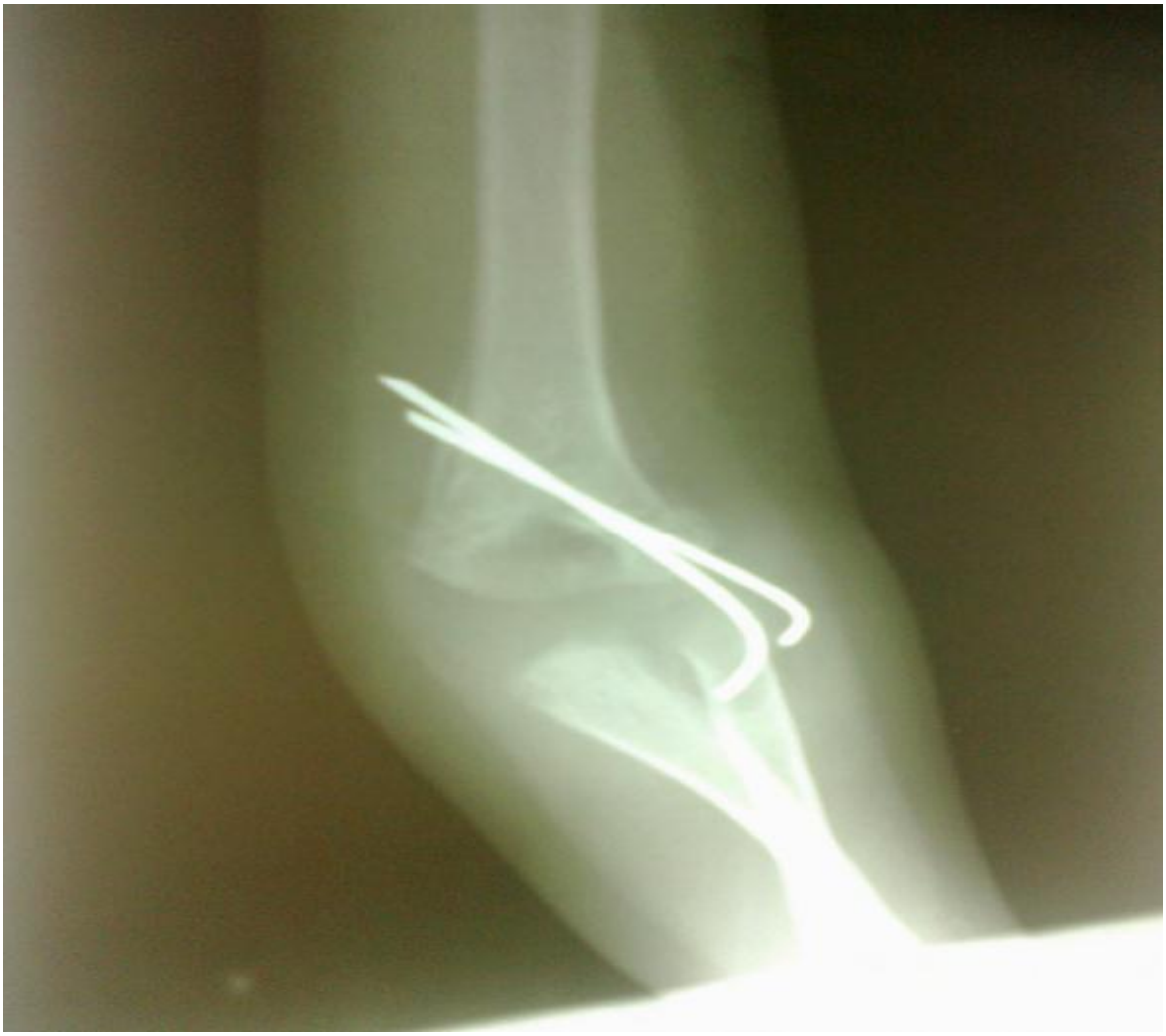

Fig. 2. AP x-ray of the same patient

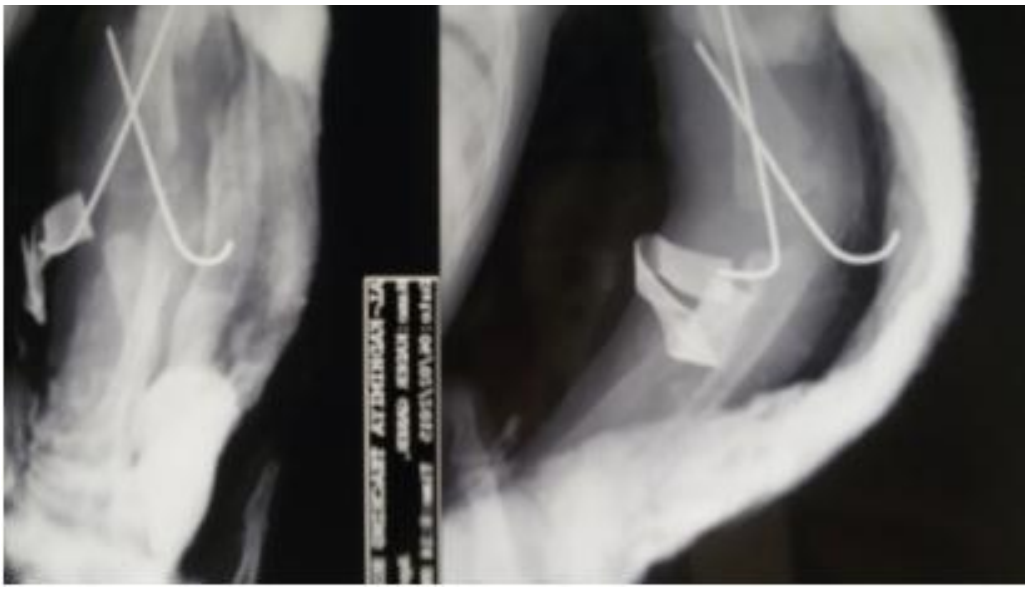

Fig. 3. AP & Lateral x-rays of 3 years old patient treated with closed medial & lateral k wires

Statistical analysis

Chi-square test was used for comparison between the groups. *P* value of < 0.05 was considered statistically significant.

Results

There were no significant differences between the two groups regarding base-line characteristics such as age, male sex, side, types of displacement, and types of fracture, interval from injury to admission and interval from admission to surgery (Table 2).

Complications

There were no significant differences between the two groups regarding neurovascular complications at the time of admission, iatrogenic ulnar nerve injury, and pin track infection (Table 3).

At three months follow-up visit, patients were evaluated by recording the various outcome measures. There were no significant differences between the two groups with regard to the various outcome measures such as carrying angle, passive range of elbow motion, Flynn grading, Baumann angle, and change in the Baumann angle and loss of reduction grading (Table 4).

Table 2. Baseline characteristics of 80 patients with displaced (Gartland type II and III) extension type supracondylar fractures of humerus

Baseline Characteristics		Crossed medial-lateral pin entry group (N = 80)	Two-lateral pin entry group (N = 80)	*P* value
Age (years)		6.24 ± 1.77	6.12 ± 1.82	0.67
Male sex [11] (% of patients)		38 (95)	37 (92)	0.74[v]
Side [11] (% of patients)	Left	26 (65)	28 (70)	0.53 [v]
	Right	14 (35)	12 (30)	
Types of Displacement [11] (% of patients)	Posterolateral	28 (70)	26 (65)	0.71 [0]
	Posteromedial	9 (22)	9 (22)	
	Posterior	3 (7)	5 (13)	
Types of fracture according to Gartland [41 (% of patients)] [11]	Type II	28 (70)	17 (42)	0.75 [v]
	Type III	12 (30)	20 (50)	
Interval from admission to surgery (hours)		25.4 ± 1026	23 ± 8.78	0.11
Interval from injury to admission (hours)		27.8 ± 16.12	29.47 ± 11.74	0.45

t: the data are given as the mean ± standard deviation. 11: the data are given as the number (%) of patients. Independent-sample student t test. V = Fisher's exact, 0: Chi-square test.

Table 3. Complications of the 80 patients with (Gartland type II and III) extension type supracondylar fractures of humerus randomly assigned to receive percutaneous fixation with either crossed medial-lateral pin or, two-lateral pins

Complications	Crossed medial-lateral pin entry group t (n = 40)	Two-lateral pin entry group* (n = 40)	*P* value
Neurovascular complications at the time of admission [w]			0.75 [11]
Radial nerve injury	6 (15)	5 (12.5)	
Median nerve injury	9 (22.5)	12 (30)	
Pulse less pink hand	7 (17.5)	6 (15)	
latrogenic ulnar nerve injury [w]	0	0	1.0 [p]
Pin track infection at three weeks follow-up visit	2 (5)	3 (7.5)	1.0 [p]

W: The datas are given as the number (%) of patients. 11: Chi-square test. P: Fisher's exact test

Table 4. Comparative outcome measures at three months after the surgery in both groups

Outcome measure		Crossed medial-lateral pin entry group t (n = 34)	Two-lateral pin entry group * (n =36)	*P* value
Carrying angle (degree)n		5.52 ± 3.77	5.56 ± 4.62	0.95
Loss of Carrying angle (degree) [n]		3.58 ± 3.08	3.86 ± 3.33	0.62
Passive range of elbow motion (degree) [n]	Flexion	128.3 ± 12.67	127.96 ± 438	0.75
	Extension	-2.6 ± -0.13	-2.56 ± -0.16	0.12
	Total range of motion	130.58 ± 3.9	129.39 ± 4.48	0.111
Loss of total passive range of elbow motion (degree) [n]		3.4 ± 2.9	3.8 ± 3.21	0.45
Flynn grading (% of patients) [11]	Excellent	27 (80)	26 (73)	0.84 [Ay]
	Good	3 (9)	4 (12)	
	Fair	4 (11)	6 (15)	
	Poor	0	0	
Loss of reduction grading (% of patients) [11]	Major	0	0	0.94 [v]
	Mild	5 (15)	4 (12)	
	None	29 (85)	32 (88)	
Baumann angle (degree) [n]		772 ± 435	76.2 ± 3.51	0.15
Change in the Baumann angle (degree) [IT]		3.57 ± 2.43	3.71 ± 2.1	0.72

n: the datas are given as the mean ± standard deviation, 11: The datas are given as the number (%) of patients, IT: independent-sample student t test, Ay; Chi-square test. V: Fisher's exact test.

Discussion

The standard treatment for displaced (Gartland type II and III) extension type supracondylar fractures of the humerus in children is closed reduction and percutaneous pin fixation. But, controversy persists among authors regarding optimal method of percutaneous pin fixation. Swenson [(7)], Casiano [(8)] and Flynn et al [(6)] used two crossed medial-lateral pins. Arino et al [(9)] used two lateral pins.

Though crossed medial-lateral pin configuration provides good biomechanical stability, but

simultaneously it carries the increased risk of iatrogenic ulnar nerve injury due to placement of the medial pin. Conversely, though the two-lateral pin configuration carries less risk of iatrogenic ulnar nerve injury, but it provides less biomechanical stability

In this study comparison of the efficacy of medial and lateral entry pinning with lateral entry pinning for percutaneous fixation of displaced (Gartland type II and III) extension type supracondylar fractures of the humerus in children.

In the present study, there was no significant difference between the two groups with regard to iatrogenic ulnar nerve injury and loss of reduction grading.

Though several studies [10-16] have been done so far to compare the efficacy of medial and lateral entry pinning with lateral entry pinning for percutaneous fixation of displaced (Gartland type II and type III) extension type supracondylar fractures of the humerus in children but, it is very difficult to compare between them because: (i) pinning technique, pin size, position of elbow during pinning differs in various studies, (ii) only one study [10] consists of more than 40 patients in each group but, that was a retrospective study, (iii) Most of the studies were retrospective and uncontrolled [10-15]. Only two studies [13,15] were randomized controlled but, these studies consist of less than 40 patients in each group. All of these studies found no significant difference between the two methods in terms of loss of reduction and six studies found no significant difference between the two methods in terms of iatrogenic nerve injury. Only one shows significant difference in favour of lateral entry pinning method in terms of iatrogenic nerve injury. So, convincing evidence of the optimal method of percutaneous pin fixation is lacking in various literature overviews.

Brauer et al [17] performed a systematic review using pooled data of 2054 children from 35 previous studies: 2 randomized trials, 6 retrospective studies and 25 case series. They found no significant difference between the two groups in terms of loss of reduction and iatrogenic nerve injury.

Therefore, the results of present study are consistent with the results of most of the previous studies consists of the same clinically relevant question.

The major strength of the present study is its prospective randomized design. All of the patients in each group were operated on according to a uniform standardized well-accepted technique. In addition, thorough follow-up assessment of each patient was done with the use of various clinical and radiological outcome measures at standardized intervals. Follow-up assessment of each patient was done by the same surgeon throughout the trial.

The major limitation of present study is that, both the surgeon and the patients were not blinded of the treatment received throughout the trial. Another weakness of this study is the number of patients who did not complete the three-month follow-up visit. However, as the rate of the patients lost to follow-up in this study is comparable with that in other studies, we do not believe that it hampers present results.

In conclusion, we found that if a uniform standardized operative technique is followed in each method, then the result of both the percutaneous fixation methods will be same in terms of safety and efficacy.

Acknowledgments

Great thanks to Dr. Zaid Al-Shimmary who assisted me in the follow up of cases; my residents who assisted me in collecting and the follow up of cases.

Conflict of interest

The authors declare no conflict of interest.

Funding

None.

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Received 25th Mar. 2015: Accepted 14th Jun. 2015

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq

Detection of Epstein Barr Virus in Renal Transplant Recipients: Two Centers Study

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Abstract

Background Viruses are among the most common causes of opportunistic infections after transplantation. The risk for viral infection is a function of the specific virus encountered and the intensity of immune suppression used to prevent graft rejection.Epstein-Barr virus infection has also been implicated as co-factor in acute and chronic rejection syndromes.

Objective Detection of Epstein-Barr viremia in renal transplant recipients.

Methods Fifty seven (57) renal transplant recipients were enrolled in this study. Plasma samples were taken from all renal transplant subjects. Screening of Epstein-Barr virus was first done by serology viamono spot test, then, viral DNA of Epstein-Barr viruswas extracted from 200 µl plasma samples and Epstein-Barr virus DNA was detected and measured by Taqman quantitative real-time PCR.

Results 19/57 (33 %) of renal transplant subjects had Epstein-Barr virus viremia and the viral load ranged from 7100 to 16.165 copies/ml. Serology of all RT subjects showed negative heterophil antibody except for one patient had positive hetrophil antibody.

Conclusion The current study showed that Epstein-Barr virus might be considered as an important cause of renal impairment and allograft loss in renaltransplant subjects. And Epstein-Barr virus seems associated with post transplantation renal impairment and/or kidney rejection. Real-time PCR is a very sensitive and specific method for the detection of Epstein-Barr viremia in renal transplant subjects.

Key words Epstein-Barr virus, Renal transplantation, real-time PCR

List of abbreviation: EBV = Epstein-Barr virus, PTLD = post-transplant lymphoproliferative disease, RT = renal transplant, CSA = cyclosporine A, MMF = mycophenolate, TAC = tacrolimus.

Introduction

Epstein-Barr virus (EBV) is a double stranded DNA virus belonging to the family of herpes viruses. EBV causes a disease that can be intensified by the immunosuppressive agents used to prevent rejection of the allograft [1,2]. The virus persists long-term as a latent infection. EBV is capable of driving B cell proliferation *in vitro* to form immortalized cell lines and also *in vivo* when immune surveillance is inadequate [3,4].

In the setting of allogeneic transplantation when iatrogenic immunosuppressant is used to prevent graft rejection, an unintended consequence is failure to suppress active EBV infection, which is accompanied by a heightened risk of developing Post-transplant lymphoproliferative disease (PTLD) [5-7].

An EBV-negative renal transplant (RT) from an EBV-positive donor is at increased risk for developing PTLD [8]. EBV is one of the most prevalent viral infections of early reactivation occurring from the first week after the initiation of immunosuppressive therapy, suggesting that EBV reactivation may induce a

T cell response through the phenomenon of allo-cross-reactivity which could play a critical role in graft rejection [9].

The Kidney Disease Improving Global Outcomes (KDIGO) Transplant Work Group recommends that high-risk renal transplant patients should be tested for EBV nucleic acid once within the first week after transplant then at least monthly for 3 to 6 months, and then every 3 months for the rest of the first year. Additional EBV testing is recommended after treatment for acute rejection [10].

In Iraq, active kidney transplantation program was started in 1973 at Al-Rasheed Military Hospital; and since then, renal transplantation is being successfully done at several centers in Iraq [11-13]. Very few studies are conducted for the detection of viral infections or reactivation in Iraqi RT recipients using real time PCR [14,15], or urine cytology [16], however, to the best of our knowledge, this study is the first to investigate the incidence and the role of EBV viremia in RT subjects and its relationship to kidney impairment using quantitative RT-PCR.

Methods

Renal transplant subjects and blood sampling

This cross-sectional study was conducted from November 2013 to March 2014. A total of 57 RT recipients (including 42 males and 15 females) who attended the (Center of Kidney Diseases and Transplantation) in the Medical City of Baghdad and Al-Karama Teaching Hospital, were enrolled in the study. A consent letter was signed by each patient, and the study was approved by the ethical committees of the Ministry of Health and Al-Nahrain University.

The mean age of RT subjects was 35.95 year (ranging from 18-74 years), and the mean post-transplantation time of presentation was 161.4 days.

Renal function was decided according to the levels of serum creatinine that were measured in the hospital laboratories at the time of sampling, and accordingly, these RT subjects were divided into two groups. The first group is called control group where RT subjects with normal renal function (serum creatinine ≤ 1.2 mg/dl) [10,17]. The second group is the test group where RT subjects had biopsy proven either acute renal impairment and/or allograft rejection (biopsy results were taken from the patients' reports).

Relying on kidney transplantation specialists, the most suitable cut off time that separates between early and late presentation of RT subjects is 6 months (which was also considered in dividing the presentations into early and late renal impairment) [10,17].

3 ml blood samples were collected from these 57 RT subjects, plasma was then separated from blood and DNA was extracted from 200 µl of plasmain accordance to the manufacturer of DNA extraction kit, namely DNA-sorb-B (Sacace, Italy). DNA extraction steps included disruption/lysis of plasma sample, removal of the contaminants and recovery of the nucleic acid. The concentration and purity of the DNA were measured using the nucleic acid measuring instrument Analytica-Gena (USA) nanodrop.

Detection of EBV DNA and quantification of its DNA loadusing quantitative real-time PCR

The kit used was EBV Real-TM Quant Kit (Sacace, Italy) for the detection of LMP gene in EBV genome. The procedure was done according to the manufacturer guidelines. EBV DNA amplification was detected on FAM (Green) channel and exogenous internal control (IC) was detected on Rox (Orange)/Texas red channel.

The quantity of reactants for one reaction was 10 µL of PCR-mix-1 and 1.5 µL of PCR-mix-2 buffer and 0.5 µL of hot Start DNA polymerase. Then, DNA from sample/standard/positive or negative control was added to the mix. The final volume per reaction tube was 25 µL.

The RT-PCR instrument used in the study was STRATAGENE MxPro QPCR (Agilent Technologies, USA). The thermal protocol for Sacace Quantification Kit is composed of an initial denaturation for activation of the

HotStarTaq DNA Polymerase at 95 °C for 15 min; then, five cycles of thermal cycling 95 °C for 15 sec, and 60 °C for 20 sec, and 72 °C for 15 sec, and finally 40 cycles composed of 95 °C for 10 sec, and 60 °C for 40 sec, and 72 °C for 15 °C.

Statistical analysis

Data were analyzed using SPSS version 12.0.01 software. Qualitative frequency data were subjected to Chi square test for association while parametric quantitative data were subjected to ANOVA and t-test for measuring significance of difference. Relative risk (RR) and correlation coefficient (r) were also used in accordance to results, $P \leq 0.05$ was considered statistically significant.

Results

The results of this study are based on the analysis of fifty seven patients with renal transplantation. EBV viremia was detected in 19/57 (33 %) of RT subjects. The age of RT subjects ranged from 16 to 58 years with mean ± SD age of 35.95 ± 12.50 year. There was obvious predominance of males over females among RT subjects. Male: female ratio was 3.75: 1.

The findings showed that about three quarters of the RT subjects were studied early in this research, less than 6 months after kidney transplantation, while one quarter of RT subjects were studied late, more than 6 months after kidney transplantation (Figure 1).

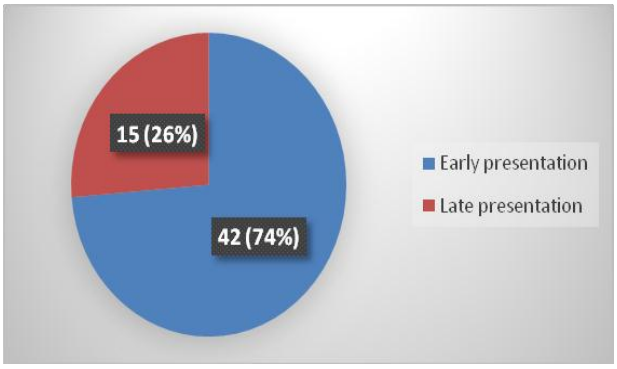

Fig. 1. Distribution of RT subjects according to the post-transplant period (cutoff 6 months) The association of positive EBV viremia with age and gender of RT subjects

Age distribution among RT subjects in relation with positive EBV viremia was non-significant ($P > 0.05$). However, the age group older than 40 years showed a bit higher percentage (40%) of EBV infection than others. The quantitative analysis of the load of EBV viremia in regard to age groups showed no significant difference ($P > 0.05$).

Positive EBV viremia was associated with gender of RT subjects involved in this study ($P > 0.05$). It was found that 18/45 males were shown to have positive EBV viremia with much higher percentage of positive EBV viremia, 40%, than that in females, 8.3% (Table 1). On the other hand, the quantitative analysis of the load of EBV viremia in regard to gender type showed no significant difference ($P > 0.05$), (Table 2).

Table 1. The association of gender of RT subjects with EBV viremia in real time PCR

Gender type		EBV		Total
		Negative	Positive	
Female	No. (%)	11 (91.7)	1 (8.3%)	12 (100.0)
Male	No. (%)	27 (60.0)	18 (40.0)	45 (100.0)
Total	No. (%)	38 (66.7)	19 (33.3)	57 (100.0)
P value		0.036*		
RR for males as risk factor		4.8 : *P* = 0.1		

Table 2. The quantitative analysis of the load of EBV viremia in regard to gender type

Gender type	No.	Mean	Std. Deviation	Std. Error Mean	*P* value
Female	1	5025.00	1984.11	563.7	0.771
Male	18	5946.33	3012.22	709.99	

The association of positive EBV viremia with post-transplant period

The findings of this study indicated a high association between EBV positivity and late presentation (> 6 months) of RT subjects ($P < 0.05$) in that 66.7% of late presenters versus 21.4% of early presenters showed positive EBV viremia (Table 3). The quantitative analysis of the load of EBV viremia in regard to the time of presentation showed no significant difference ($P > 0.05$) Table (4). The mean ± SD of the post-transplantation time till presentation in this study was 161.40 ± 130.34 days.

Table 3. The association between time of presentation of RT subjects and EBV positivity

Post-transplant period		EBV		Total
		Negative	Positive	
Early-post- transplant*	No. (%)	33 (78.6)	9 (21.4)	42 (100.0)
Late-post- transplant	No. (%)	5 (33.3)	10 (66.7)	15 (100.0)
Total	No. (%)	38 (66.7)	19 (33.3)	57 (100.0)
P value	0.002**			

* The cutoff is 6 months, ** = $P < 0.05$.

Table 4. The quantitative analysis of the load of EBV viremia in regard to the time of post-transplant period.

Presentation	No.	Mean	Std. Deviation	Std. Error	*P* value
Early post-transplant	9	5799.44	3118.31	1039.44	0.894
Late post-transplant	10	5986.40	2926.83	925.54	

The association of positive EBV viremia with the level of serum creatinine in RT subjects

It was found that 61% of RT subjects had abnormal high levels of serumcreatinine, namely renal impairment, versus 39% with normal levels of creatinine (Figure 2).

The association between creatinine levels and EBV viremia were remarkably significant. It was shown that 50% of RT subjects with abnormally high creatinine levels were with positive EBV viremia while none of the RT subjects with normal creatinine level showed EBV viremia.

This indicates the strong association between EBV viremia and renal impairment after kidney transplantation (Table 5).

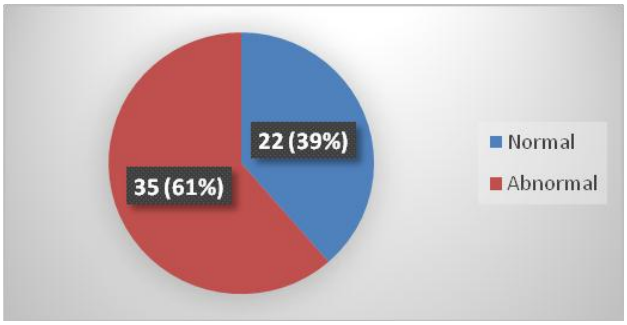

Fig. 2. The distribution of RT subjects according to the level of serum creatinine (cutoff normal serum creatinine ≤1.2 mg/dl)

Table 5. The association between positive EBV viremia and serum creatinine levels*

Blood creatinine level		EBV		Total
		Negative	Positive	
Normal	No. (%)	19 (100.0)	0 (0.0)	19 (100.0)
High	No. (%)	19 (50.0)	19 (50.0)	38 (100.0)
Total	No. (%)	38 (66.7)	19 (33.3)	57 (100.0)
P value	0.001**			
RR for high blood creatinine as a risk indicator			20 : *P* = 0.033**	
RR for positive EBV viremia as a risk factor for high blood creatinine			1.95 : *P* < 0.0001***	

* = cutoff normal serum creatinine ≤ 1.2 mg/dl, ** = *P* < 0.05, *** = *P* < 0.001.

As an interesting result, the high blood creatinine level was a grave risk indicator for the presence of EBV viremia with RR equal to 20 (*P* < 0.05) implying to the notion that RT subjects with abnormally high creatinine level are 20 times more prone to develop EBV viremia.

Moreover, considering EBV viremia as a risk factor for the development of abnormally high serum creatinine level, it was found that positive EBV viremia doubled the chances for RT subjects to have high serum creatinine; the interesting issue in this result, the confidence of EBV viremia as risk factor for high blood creatinine was too high (*P* < 0.0001) rendering EBV viremia as a remarkable risk for developing serious renal impairment (Table 5). However, the quantitative analysis of the load of EBV viremia in regard to the positivity of creatinine levels showed no significant difference (*P* > 0.05) (Table 6).

Table 6. The quantitative analysis of the load of EBV viremia in regard to the positivity of blood creatinine

Blood creatinine	No.	Mean	Std. Deviation	Std. Error	*P* value
Positive	18	5858.83	3014.99	710.64	0.814
Negative	1	6600.00	3543.38	737.18	

The association between EBV viremia and renal impairment in renal transplant recipients

The RT subjects were categorized into three groups: under control, acute renal impairment, and chronic renal impairment groups. The findings showed highly significant association between renal impairment, whether acute or chronic, and EBV viremia (P<0.05) (Table 7). Moreover, none of the control group had EBV viremia (*P* < 0.05). However, both chronic and acute renal impairment groups showed very close percentages of RT subjects with positive EBV viremia, 51.7 and 50.0 %, respectively (*P* > 0.05).

Table 7. The association between renal impairment and positive EBV viremia

Renal impairment		EBV		Total
		Negative	Positive	
Acute renal impairment	No. (%)	4 (50.0)	4 (50.0)	8 (100.0)
Chronic renal impairment	No. (%)	14 (48.3)	15 (51.7)	29 (100.0)
Total	No. (%)	18 (48.65)	19 (51.38)	37 (100.0)

Association between EBV viremia and the type of immuno-suppressive regimen used in RT recipients

Two main standard immunosuppressive regimes are mainly followed; the first regimen includes cyclosporine A (CSA), mycophenolate (MMF), and prednisolone, the second regimen includes tacrolimus (TAC) instead of CSA, in addition to MMF and prednisolone (Figure 3).

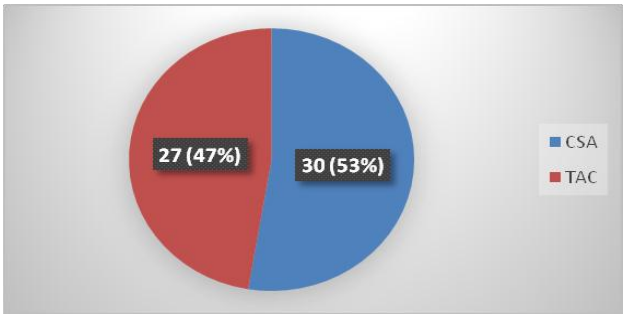

Fig. 3. Distribution pattern of the immuno-suppressive regimens among RT subjects

This study showed that CSA-based regimen is significantly associated with positive EBV viremia when compared to TAC-based regimen (P<0.05) (Table 8). Calculating the relative risk for RT subjects treated with CSA-based regimen showed that CSA acted as a significant risk factor for the development of EBV viremia (P<0.05) (Table 8).

Such results highlight that the potent immunosuppressive regimen using CSA might aggressively lead to extensive immuno-suppression which in turn favors EBV reactivation of latent infection or contraction more easily of external EBV infection. However, the quantitative analysis of the load of EBV viremia in regard to the type of immunosuppressive regimen showed no significant difference (*P* > 0.05) Table (9).

Table 8. The association between the type of immunosuppressive regimen and positive EBV viremia

Drugs		EBV		Total
		Negative	Positive	
CSA	No. (%)	16 (53.3)	14 (46.6)	30 (100.0)
TAC	No. (%)	22 (81.5)	5 (18.5)	27 (100.0)
Total	No. (%)	38 (66.7)	38 (66.7)	19 (33.3)
P value			0.023*	
RR for CSA as a risk factor			2.5 : *P* = 0.039	

CSA = cyclosporine A, TAC = tacrolimus * = p<0.05

Table 9. The quantitative analysis of the level of EBV viremia in regard to the immunosuppressive drugs used

Drug	No.	Mean	Std. Deviation	Std. Error	*P* value
CSA	14	5484.93	3091.18	826.15	0.318
TAC	5	7054.00	2332.53	1043.14	

CSA = cyclosporine A, TAC = tacrolimus

Discussion

In the current study, 57 Iraqi RT subjects were involved. EBV viremia was detected in 19/57 (33 %) of RT subjects. About 50 and 51.7% of RT subjects involved in the current study had acute and chronic renal impairment, respectively. Interestingly, in the current study, RT subjects with positive EBV viremia were commonly with high risk for developing both acute and chronic renal impairment (*P* = 0.0001) as well as high levels of serum

creatinine in RT subjects showed significant relative risk to have EBV viremia.

These findings agree with a study done earlier revealing that a higher rate of graft loss was observed in RT subjects had a positive EBV PCR during the first 6 months post-transplant [18]. Association of EBV viremia with acute/ chronic renal impairment indicates serious type of relationship.

EBV primary infection or reactivation in RT subjects might impose cause-effect relationship with renal impairment [1,4]. A study conducted in Germany found that EBV viremia is an underestimating cause of renal impairment and maybe rejection of transplanted kidneys [19]. In this instance, the exact driving cause for EBV infection to develop renal impairment is still not well known. However, several explanations were presented in the literature of the field and as follows:

First, EBV-induced cytotoxic T lymphocyte response contains clones that are reactive to self-MHC/peptide complexes that show strong allo-cross-reactivity against allo-MHC–presented peptides [5,10,20,21].

Second, EBV is implicated in counteracting immune suppression of T cells as EBV-driven induction of T cell immune response would be a limiting step for the immunosuppressive effect of drugs taken after kidney transplantation [22].

Third, EBV replicates mainly in B lymphocytes; this results in the induction of B cells' signaling pathway of immunoglobulins production which in turn results in excessive formation of heterophil antibodies. Heterophil antibodies are suspected to be another factor for targeting tissues of the transplanted kidney through complement activation leading to destruction of renal glomeruli [23].

The frequency and quantitative analysis of EBV viremia in regard to age groups showed no significant difference ($P > 0.05$). However, several previous studies showed a positive correlation between age and EBV viremia [24,25], but could be in agreement with previous reports demonstrated that recipient age did not affect the incidence and severity of acute rejection or graft survival [26,27].

In the current study, males were shown to have more positive EBV virema, 40%, than in females, 8.3 %. A previous study found that femalekidney transplant recipients have better 8 year graft and patient survival than male recipients [28].

A previous study found that EBV viremia is detected mainly in the first year of RT subjects [29]. However, the current study revealed high association observed between EBV positivity and late presentation (> 6 months) of RT subjects indicating that EBV viremia takes several months after kidneytransplantation to be detectable.

EBV load serves as a functional marker of the degree of immunosuppression and that undetectable EBV implies under immunosuppression and associated risk of rejection [30]. Unbalance between too little and too much immunosuppression given to RT subjects results in organ rejection and high risks of opportunistic infections, respectively [31].

The current study indicated a strong relationship between the type of immunosuppressive regimen with CSA and EBV positivity. Thisfinding is indirectly congruous with that of a previous study which found that cyclosporine levels after kidney transplantation were highly predictive of acute cellular rejection episodes [30,32]. Related to the point, another study showed that CSA, despite of its strong immunosuppression, results in significantly higher median creatinine compared to other drugs and long-term CSA use may cause glomerular sclerosis, arteriolar hyalinosis and tubular atrophy and interstitial fibrosis [32].

In conclusion, the findings of 33% positive EBV viremia among renal transplant recipient and all of them had impaired renal function; indicate a possible relationship between EBV positivity and impaired renal allograft function and may recommend good screening for this virus in these patients.

Acknowledgement

Authors would like to acknowledge the Center of Kidney Diseases and Transplantation in The Medical City of Baghdad and Al-Karama Teaching Hospital, for their cooperation in accomplishing this study

Author contributions

Sahar A. Shams-aldein, Ahmed S. Abdlameer did the DNA extraction and RT-PCR; Asmaa B Al-Obaidi and Haider S Kadhim collect the specimens; Ali J.Al-Saedi Providing patient's Data sheet

Conflict of Interest:

Authors declare no conflict of interest.

Funding

There is no funding source for this research

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Received 26th Mar. 2015: Accepted 14th Jun. 2015

Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq

Non-Specific Peritonitis due to High-Voltage Electrical Shock: Case Report

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Case Report

Abstract An electrical burn is a burn that results from electricity passing through the body causing rapid injury. Electrical burn differs from thermal or chemical burns in that they cause much more sub dermal damage. They can exclusively cause surface damage, but more often tissues deeper underneath the skin have been severely damaged. As a result, electrical burns are difficult to accurately diagnose, and many people underestimate the severity of their burn. In extreme cases, electrical injury can cause damage to the brain, heart, and injury to other organs like abdominal viscera and lining peritoneum causing aseptic peritonism and peritonitis.

Keywords peritonitis, electrical shock, burn

Introduction

An electric shock occurs when a person comes into contact with an electrical energy source. Exposure to electrical source can cause a variety of injuries from a very nil to very serious devastating injury or to death. Young adults are more prone to high electrical current voltage shock caused by mischievous exposure at work [1,2]. Many variable scans determine what type of injury can occur, if any. These variables include the amount of the current (affected by the resistance of the involved organs and the type of current whether it is AC or DC) and the electrical pathway through its course [1-3]. Low voltage electricity usually its harmless but high electrical voltage (greater than 500 volts) have very serious and significant injury [4-6]. A victim who has fallen from a height or sustained a severe shock causing multiple jerks may have a serious spinal cord injury and should not be moved without protecting the spine. A person who has suffered an electric shock may have very little external evidence of injury or may have obvious severe burns. The person may die immediately from cardiac standstill. In addition, other than organ can be injured like, abdominal viscera, pulmonary system urinary system [7-9] and so on.

Case report

The condition started on 7th of December 2012 where 34 years old patient presented in emergency department as a case of very high tension electrical shock (direct contact of both hands with very high tension voltage). It resulted in severe burn of both hands, with thrombosis of the main vessels of forearm ended in bilateral amputation from elbow joint and because he thrown very fast at the moment of accident he developed paraplegia due to fractured last dorsal vertebra with spinal cord injury. During first few days of injury the patient was fully conscious, all investigation done at moment of injury where within normal limit. He had soft abdomen,

normal vital signs, he passed his bowel motion beside that he had normal appetite. Three weeks later he started to complain of mild abdominal discomfort, mild abdominal distention, and infrequent vomiting. An US examination had showed a lot of clear ascetic fluid in peritoneal cavity.

The Patient was kept on conservative therapy but his abdominal tenderness increased, with unhealthy look. Plain abdominal X-ray didn't show any feature of intestinal obstruction. The patient was operated upon and more than 3 liters of ascetic fluid was drained out. It was turbid in color, odorless no bile or intestinal fluid was seen; culture and biochemical analysis of ascetic fluid show high protein content with no bacterial growth; all small bowel was matted together with slimy fibrinous adhesions and edematous wall that easily separated but in some areas there were multiple areas of coagulative adhesions with difficulty in adhesiolysis. In some other areas there where a white particles like boiled egg due to passage of high voltage currency that caused protein coagulation, all loops intestine were checked out to exclude any perforations. The abdomen closed with tube drain in pelvis and in the second day, he resumes oral fluid diet

Discussion

The outcome of electrical burn, determine the severity and degree of burn beside the type of current, voltage and the resistant, the severity of damage also depends on the pathway of the current through the body and which it usually takes the pathways of least resistance in the body [1-3]. At the first start in the vascular system then nervous system then internal viscera muscles, skin, fat and lastly the bone which is the most resistant tissue in the body [1-3]. As the body comes into contact with an electrical source, it becomes part of the electrical current. As such, the current has two points, an entry and an exit at two different points on the body. The entry point tends to be leathery and depressed whereas the exit wound is typically more explosive and extensive [1-5]. It is hard to accurately diagnose an electrical burn fully like this case because only the entry and exit wounds are visible and the internal damage is not. For this reason his abdominal complain is not clear at the moment of accident it may appear latter with other serious visceral injuries like colonic perforation, sold organ burn or even thrombosis of the internal organ vessels. The most accepted explanation for his problem is that, the high voltage current burn cause coagulation of peritoneal fluid protein, this in turn stimulated peritoneal membrane to secreted ascetic fluids.

In conclusion, the health provider must take in his consideration that many serious complications related to burn patient that may developed later on.

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Received 1st Jan. 2015: Accepted 14th Jun. 2015

المجلد الثالث عشر، العدد الثاني، 1436 هـ، 2015م

المجلة العراقية للعلوم الطبية

عنوان المراسلات إلى المجلة العراقية للعلوم الطبية، صندوق بريد 70044 بغداد، العراق. تلفون (07717516090).

رقم الإيداع في دار الكتب و الوثائق ببغداد 709 لسنة 2000

Iraqi Journal of Medical Sciences

IRAQI JMS

المجلة العراقية للعلوم الطبية

Volume 13, Number 2, 2015
April - June

ISSN 1681-6579

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