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ORIGINAL ARTICLE

Conditions of Oral Mucosa due to Takhzeen al-Qat

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Abstract:

Takhzeen al-Qat (Qat chewing habit) in Yemen is widely spread and practiced by millions. Adverse health effects of this habit on body systems and oral tissues have been reported.

Objective: The objective of this study was to assess the effects of Qat chewing on buccal and gingival oral mucosa among Yemeni Qat chewers.

Methods: This was a cross-sectional study in which subjects were randomly recruited from patients attending dental clinics in two hospitals in Sana'a, Yemen. Subjects were interviewed for chewing and smoking habits and examined clinically for the effects of Qat chewing on buccal and gingival mucosa.

Results: A total of 650 subjects satisfied the inclusion criteria. Subjects were 515 (79.20%) males and 135 (20.80%) females. Among these, 490 (75.38%) were Qat chewers. White patch on buccal mucosa recorded in 486 (99.7%) among Qat chewers. White patch on gingival mucosa recorded on 442 (89.8%) among Qat chewers. Gingival recession recorded on 370 (76.0%) among Qat chewers. Differences between chewers and non chewers were statistically significant ($p<0.05$).

Conclusion: This study concluded that mucosal white lesions occurred more frequently in Yemeni Qat chewers.

Key words: Takhzeen al-Qat, Qat chewing, Khat, Adverse health effect, Buccal and gingival mucosal conditions, Yemen.

1. Introduction

Takhzeen al-Qat (Qat chewing) is a term extensively used in Yemen to describe the habit of Qat chewing (1). Qat plant identified first to belong to the *Species Catha Edulis Forsk* by the European expedition of plants origin visited Yemen in 1761 to 1763. The plant name was given in memory of Forskal, member of the mission who died in Yemen (2). Qat grows in some countries of East Africa such as Ethiopia, Kenya, Tanzania, Madagascar and South Africa. It also grows in South Arabia, mainly in Yemen and South West part of Saudi Arabia. In Yemen Qat use and cultivation spread slowly over time to cover the whole country (3). A recent survey revealed that 90% of adult men and 20% of adult women were regular chewers (4). Also Qat consumption is increasing in Europe and the west where emigrants from Qat countries are living (5). The adverse effects of this habit on different human body systems were extensively reviewed (6-8). The effect of Qat chewing on different oral tissues including periodontal tissues, hard tissues of teeth, temporomandibular joint, muscles of mastication and salivary glands were also extensively reviewed (9-12,1). Regarding the effects on oral mucosa, studies available in the literature reported inconsistent effects ranged between simple white keratotic effects to the occurrence of oral cancers. Tumors of the oral cavity including lower maxilla, buccal mucosa and lateral surface of the tongue were reported in Hodeida, Yemen (13). A study conducted over two years period in Asir region reported 28 head and neck cancers (14). Ten cases were in the mouth mostly in the anterior two thirds of the tongue and floor of the mouth. Seventeen cases of head and neck carcinoma were diagnosed in one year period among chronic Qat chewers in Hajjah, Yemen (15). The previous studies did not confound for tobacco smoking and shamma (snuff), despite the area where the three studies done is well known epidemic of smoking and shamma use. On the other hand, no oral cancers were reported by many studies (1,11,12,16-19). Mucosal changes or some degrees of oral keratosis found at site of chewing among 50% of Qat chewers (12). In Kenya the association between leukoplakia, smoking, alcohol consumption and Qat chewing have been studied (16). Results showed that Qat chewing was not significantly associated with leukoplakia compared to tobacco and alcohol consumption. In a cross sectional hospital based study recruited 431 subjects (325 chewers and 106 non-chewers) reported buccal and gingival white lesions among the chewers (1). 342 mild keratotic white lesions among Yemeni Qat chewers were reported among 2500 subjects evaluated (17). Oral white lesions reported among Yemeni Jew male chewers (18,19). Similarly, the histopathological studies available in the literature did not report any leukoplakia and revealed only changes such as acanthosis, papillomatosis, ortho- and para-keratosis and intercellular edema (20-22).

The aim of this study was to elaborate further on oral mucosa at Qat chewing sites among Yemeni Qat chewers.

2. Materials and Methods

The design of the study was hospital based cross sectional type. Inclusion criteria included; age 18-60, Qat chewer for a minimum of 3 years and chewed on one side of their mouths only. A direct pre coded interview questionnaire included detailed information about personal data, chewing and smoking information was filled. Clinical assessment for buccal and gingival mucosa was done carefully. For buccal mucosa, the cheek was reflected and the mucosa was inspected for any changes in color (whitening, redness), changes in texture (ulcer, erosion, vesicles) and for any other changes or lesions. This was followed by careful

bimanual examination to feel the mucosal surface and to detect any underlying structure. Gingiva was also assessed carefully by inspection for changes in color (redness, whitening or keratinization), texture (presence or absence of stippling) density and level of attachment (presence of a disease or recession). This was followed by surface palpation and light rubbing for the free and attached gingiva to reveal the degree of stippling or degree of keratinization. The examination was done at the chewing and opposite sides among chewers and at both sides of non chewers. All changes were recorded. Data collected was analyzed by means of Chi square test. Level of significance was P<0.05 and CI at 95%. Various 2×2 or contingency cross tabulation was preformed to estimate Relative Risk (RR) approximated by the Odds Ratio (OR).

3. Results

650 healthy Yemeni subjects (490 (75.38%) chewers and 160 (24.62%) non chewers) were recruited and divided into 515 (79.20%) male and 135 (20.80%) females with M/F ratio of 4:1. Their mean age and range were 29 and 18-60 respectively. Distribution of age, sex and chewing habit is present in table 1. Majority of subjects 378 (77.14%) chewed on their left side and 112 (22.85%) chewed on their right side. Chewing duration ranged from 3 to 45 years and the mean was 19 years. Frequency of chewing days/week categorized into; 358 (73.06%) chewed every day, and the remaining 98 (20.00%) and 34 (6.93%) chewed 1-2 and 3-5 days/week. Average chewing session/hours were 377 (76.93%) chewed 3-5 hours/session and the remaining 74 (15.10%) and 39 (7.95%) chewed more than 6 and 1-2 hours/session respectively. Information on cigarette and water pipe smoking are available in tables 2 and 3. Conditions of buccal mucosa are shown on table 4. White lesions were recorded on 486 (99.7%) subjects at chewing sites compared to only 14 (8.5%) detected among non chewers and the difference was statistically highly significant (P<0.05%). Other conditions such as; red patches, ulcer/erosion and vesicles were recorded among 12 (2.5%), 3 (0.6%) and 2 (0.3%) chewer subjects respectively. In the same time, these conditions was not recorded among non chewers and differences were statistically highly significant (P<0.05%). Conditions of gingival mucosa are shown on table 5. White gingival lesions detected among 442 (89.8%) subjects at chewing sites compared to only 14 (8.5%) detected among non chewers and the differences was statistically highly significant (P<0.05). Gingival recession recorded on 370 (76.0%) subject at chewing sites compared to 13 (7.5%) among non chewers, and the difference was statistically highly significant (P<0.05). Red patch recorded on only 26 (5.2%) subjects at chewing sites compared to nothing among non chewers with statistically highly significant difference (P<0.05).

Table 1. Distributions of age, sex and chewing habit among study subjects

Age groups	Chewers		Non chewers		*P*
	M	F	M	F	
18-27	64(16.4%)	16(16.0%)	20(16.0%)	10(28.5%)	
28-37	108(27.8%)	36(36.0%)	30(24.0%)	8(22.9%)	
38-47	110(28.1%)	29(29.0%)	40(32.0%)	7(20.0%)	<0.05
48-57	81(20.8%)	11(11.0%)	26(20.8%)	8(22.9%)	
58-60	27(6.9%)	8(8.0%)	9(7.2%)	2(5.7%)	
Total	390(100%)	100(100%)	125(100%)	35(100%)	

Table 2. Cigarette smoking among study subjects

	Chewers		Non chewers		Total
	M	F	M	F	
Smokers	143(36.7%)	5(5.0%)	6(4.8%)	00.0	154(23.7%)
With Qat	57(14.6%)	5(5.0%)	00.0	00.0	62(9.5%)
Non smokers	190(48.7%)	90(90.0%)	119(95.2%)	35(100%)	434(66.8%)
Total	390(100%)	100(100%)	125(100%)	35(100%)	650(100%)

Table 3. Water pipe smoking among study subjects

	chewers		Non chewers		Total
	M	F	M	F	
Smokers	16(4.1%)	12(12.0%)	2(1.6%)	00.0	30(4.6%)
With Qat	50(12.8%)	47(47.0%)	00.0	00.0	97(14.9%)
Non smokers	324(83.1%)	41(41.0%)	123(98.4%)	35(35.0%)	523(80.5%)
Total	390(100%)	100(100%)	125(100%)	35(35.0%)	650(100%)

Table 4. Conditions of buccal mucosa among chewers and non chewers

Description	Chewers		Non chewers	
	N	%	N	%
Normal	00.0	00.0	145	91.5
Ulcer/erosion	3	0.6%	00.0	00.0
White patch	486	99.7%	14	8.5%
Red patch	12	2.5%	00.0	00.0
Vesicles	2	0.3	00.0	00.0

Table 5. Conditions of gingival mucosa among chewers and non chewers

Description	Chewers		Non chewers	
	N	%	N	%
Normal	00.0	00.0	141	88.7
White patch	442	89.8%	12	7.5%
Red patch	26	5.2%	00.0	00.0
Recession	370	76.0%	13	7.5

Figure I. Buccal mucosal whitening at Qat chewing site

Figure II. Gingival recession at chewing site

4. Discussion

In Yemen, Takhzeen al-Qat is a widespread social habit practiced by men and women and for decades. The continuous process of chewing and storing Qat leaves until the cheek grows into a noticeable ball will definitely lead to some change on the surrounding tissues. Many adverse health changes were reported in the literature. The aim of this study was to elaborate more on the oral mucosal white lesions at the site of chewing by applying cross sectional study design. In this study, results obtained from chewing habit information revealed that the majority of subjects chewed on their left sides, chewed every day and for 3-5 hours a day. Similar results had been

reported in the literature (1,11,12). The distribution of smoking among chewer subjects revealed that 51% and 10% were cigarettes smokers among males and females respectively. In the same time, 17% and 59% were water pipe smokers among males and females respectively. This result is in agreement with study done by Sayem, who reported that Yemeni women prefer to smoke water pipe than cigarettes (23). Clinical results revealed that white lesions reported were 99.7% and 89.9% on buccal and gingival mucosa among chewers respectively. Gingival recession was reported on 76% of subjects at chewing sites. Also, no oral cancer or leukoplakia was found among chewer subjects in this study. These results are in agreement with many studies done earlier (1,11,12,16-19). Once we discuss the aetiology of white lesions in this study, it seemed to be due to the effect of tobacco use and not consequences of Qat chewing. This is because more than 60%of our chewer subjects were either cigarettes or water pipe smokers. Both types of tobacco are well known to induce white lesions on oral mucosa and known to develop into malignancy (24). Therefore, white lesions detected in this study could not be attributed to Qat chewing unless this effect of tobacco use is diminished or removed statistically. When results were adjusted for tobacco risk removal by stratification, risk remained among Qat chewers indicating that white lesions were due to Qat chewing only. White lesions among cases were also statistically examined in correlation with the duration of chewing, cigarette smoking and water pipe smoking. Qat chewing duration was highly significant with P=0.000. No significant results found regarding smoking and water pipe durations. Additionally mucosal conditions recorded were found at the site of Qat chewing. If these lesions were due to any type of smoking, they will be present anywhere in the mouth and not at the site of chewing. All these statistical results supported that white lesions reported in this study were attributed to Qat chewing and not to tobacco. The mechanism by which Qat chewing induced white lesions on the oral mucosa might be due to its mechanical and/or chemical effects. The daily application of Qat on the oral mucosa is expected to cause mechanical and/or chemical irritation leading to an increase in the thickness and keratinisation as a defence mechanism. Similar changes were reported under the traumatic focal (frictional) hyper keratosis leading to increased thickness and colour changes of the oral mucosa due to continuous trauma (25). Tannins, the phenolic compound present in Qat, and the chemical additives in pesticides may have caused the changes seen in the oral mucosa. In conclusion, Qat chewing habit caused buccal, gingival white lesions and caused gingival recession at sites of chewing among Yemeni Qat chewers.

References

1. Alsharabi AKK: Oral and Para-oral lesions caused by Takhzeen Al-Qat. PhD Thesis, Khartoum University, Khartoum, Sudan, 2003.
2. Forskal P: Flora Aegypto Arabia. In, *Niebuhr*. C. Paris, 1775. Weir S: Qat in Yemen: Consumption and Social Changes. British Museum Publications, London, 1985.
3. National Conference on Qat: Towards formulation of comprehensive Qat policy in Yemen, Technical field study, gender and Qat consumption, 6-7 April Sana'a, Yemen, 2002, page 67.
4. Warfa N Klein A Bhui K et al: Khat use and mental illness: a critical review. Soc Sci Med Jul, 2007, 65, 309-318.
5. Al-Habori M. The potential adverse effects of habitual use of katha Edulis (Khat). Expert Opinion on Drug Safety, 2005, 4, 1145-54.
6. Al-hebshi N Skaug N: Khat (*Catha Edulis*) an updated review. Addiction Biology, 2005, 10, 299-307.
7. Hassan NAGM Gunaid AA and Murray-Lyon IM: Khat (Catha Edulis): health aspects of khat chewing. Eastern Mediterranean Health Journal, 2007, 13, 706-13.
8. Halbach H. Medical aspects of the chewing of khat leaves. Bulletin of the World Health Organization, 1972, 47, 21-29.
9. Luqman W and Danowski TS. The use of Qat in Yemen: social and medical observations. Ann Int Med, 1976, 85, 246-9.
10. Kennedy J Teague J Rokwa W et al: A medical evaluation of the use of Qat in North Yemen. Social Science and Medicine, 1983, 17, 783-93.

11. Hill CM and Gibson A: The oral and dental effects of Qat chewing. J O Surg O Path and O Med, 1987 63, 433-6.
12. Makki I. Schleimhautkarizonome der Mundhole. Qat and Shamma abuses. MD Thesis, University of Heidelberg, FRG, 1975.
13. Sufi HE Kameswaran M and Manatani T: Khat and oral cancer. J Laryngo Oto, 1991. 105, 643-45.
14. Nasr AH and Khatri ML: Head and neck squamous cell carcinoma in Hajjah Yemen. Saudi Med J, 2000, 21, 595-98.
15. Macigo FG, Mwaniki DL and Guthua SW. The association between oral leukoplakia and use of tobacco, alcohol and khat. Euro J Oral Sci. 1995, 103, 268-273.
16. Ali AA Al-Sharabi AK Aguirre JM and Nahas R: A study of 342 oral keratotic white lesions induced by Qat chewing among 2500 Yemenis. J Oral Pathol Med, 2004, 33, 368-72.
17. Gorsky M, Epstein JB, Levi H and Yarom N. Oral white lesions associated with chewing khat. Tobacco Induced Diseases, 2004, 2, 145-150.
18. Yarom N, Epstein J, Levi H, et al. Oral manifestations of habitual khat chewing: a case- control study. Oral Surg, Oral Med, Oral Path, Oral Radiol and Endod, 2010, 109, 60-6.
19. Al-Sharabi AK Ali AA and Hassan NA: Histopathological differences in oral mucosa of chewing and opposite sides due to Takhzeen Al-Qat. Sana'a University J of Medical Sciences, 2004, 1, 32-6.
20. Ali AA Al-Sharabi AK and Aguirre JM: Histopathological changes in oral mucosa due to Takhzeen al-Qat: a study of 70 biopsies. J Oral Path Med, 2006, 35, 81-5.
21. Ali AA: Histopathological changes in oral mucosa of Yemeni addicts to water-pipe and cigarette smoking in addition to Takhzeen al-Qat. Oral Surg, Pathol, Radiol and Endod, 2007, 103:55-9.
22. Sayem N: Motivating factors and changes in Qat use amongst women. National conference on Qat, Conference discussing materials, April, Sana'a Yemen, 2002, 36-7.
23. Pindporg JJ, Roed-Petersen B and Renstrup G. Role of smoking in floor of the mouth leukoplakia. J Oral Pathol, 1972; 1: 22-29.
24. Regezi JA and Sciubb J. (ED) Oral Pathology: Clinical Pathological Correlations, 2nd edition, 1993, 30-119. W.B. Saunders, Philadelphia.

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ORIGINAL ARTICLE

Comparative Study between Plant and Animal Sources of Omega-3 Fatty Acid in Their Potential Role of Regulating Blood Glucose and Lipid Profile in Healthy Volunteers

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Abstract:

Objective: This study is aimed to compare Omega-3 fatty acid of animal source (Shark liver oil=SLO) and plant source (Nigella Sativa=NS) in their potential hypoglycaemic and hypolipidemic effects through measuring total cholesterol, triglycerides, low density lipoprotein and other possible effect on serum electrolytes including serum calcium, sodium and potassium in healthy volunteers.

Methods: The current study was conducted in the Department of Pharmacology –UST. Eighteen healthy students (age 23-31 years) participated as volunteers. Nigella Sativa (Phenomenale 1200 mg/day) was taken for 2 weeks. Washout period for 2 weeks was done. Then SLO (500mg/day) was taken for another 2 weeks.

Results: The effect of Nigella Sativa (NS) and shark liver oil (SLO) on different parameters was measured. It was shown that NS had significantly ($P< 0.05$) reduced Fasting Blood Sugar level from 4.80±0.065 mmol/l in control group to 4.46±0.153 mmol/l in NS group. Both NS and SLO produced insignificant change ($P> 0.05$) in total cholesterol and LDL-c as compared with control. On the other hand, SLO resulted in significant increase in FBS and Total Glycerides as compared with control and NS group. Moreover, NS had resulted in significant increasing in serum sodium as compared with the control and SLO groups.

Conclusion: Both drugs have *omega-3 fatty acid* in their component; in addition NS has also omega-6 fatty acid. NS showed antidiabetic activity as it significantly reduced FBS. There is no change in lipid profiles of NS group in normal volunteers.

Key words: Nigella Sativa, Shark liver oil, Omega-3, blood glucose, lipid profile and serum electrolytes.

1. Introduction

Nigella Sativa is a flowering plant, native to southwest Asia that grows to 20-30 cm tall with linear leaves. The flowers are delicate, and usually colored pale blue and white, with 5-10 petals. The fruit is a large and inflated capsule composed of 3-7 united follicles, each containing numerous seeds. The seed is used as a spice. It belongs to the Plant family of Ranunculaceae (buttercup family). Nigella seeds have little odour, but when ground or chewed they develop a vaguely oregano-like scent. The taste is aromatic and slightly bitter (1).

The seeds contain numerous esters of structurally unusual unsaturated fatty acids with terpene alcohols (7%); furthermore, traces of alkaloids are found which belong to two different types: isochinoline alkaloids are represented by nigellimin and nigellimin-N-oxide, and pyrazol alkaloids include nigellidin and nigellicin. However, in the essential oil (avr. 0.5%, max. 1.5%), thymoquinone was identified as the main component (up to 50%) besides p-cymene (40%), a-pinene (up to 15%), dithymoquinone and thymohydroquinone. Other terpene derivatives were found only in trace amounts: Carvacrol, carvone, limonene, 4-terpineol, citronellol. Furthermore, the essential oil contains significant (10%) amounts of fatty acid ethyl esters. On storage, thymoquinone yields dithymoquinonene and higher oligocondensation products (nigellone).

The seeds also contain a fatty oil rich in unsaturated fatty acids, mainly linoleic acid (50 – 60%), oleic acid (20%), eicodadienoic acid (3%) and dihomolinoleic acid (10%) which is characteristic for the genus. Saturated fatty acids (palmitic, stearic acid) amount to about 30% or less. Commercial nigella oil ("Black Seed Oil", "Black Cumin Oil") may also contain parts of the essential oil, mostly thymoquinone, by which it acquires an aromatic flavour (1,2).

On the other hand, the most widely available source of *fish oil* is cold water oily fish such as salmon, herring, mackerel, anchovies, sardines and sharks. Oils from these fish have a profile of around seven times as much *n*−3 as *n*−6. Other oily fish such as *tuna* also contain *n*−3 in somewhat lesser amounts (3). Some research suggests that *fish oil* intake may reduce the risk of ischemic and thrombotic *stroke*. However, very large amounts may actually increase the risk of hemorrhagic stroke (4-6). In addition, omega-3 fatty acids have been known as essential to normal growth and health since the 1930s, awareness of their health benefits has dramatically increased in the past few years (7). Several studies report possible anti-*cancer* effects of *n*−3 fatty acids (particularly breast, colon and prostate cancer) (8-10). In addition, shark liver oil is used along with usual cancer drugs to treat leukaemia and other cancers; to prevent radiation illness from cancer X-ray therapy; to prevent the common cold, flu, and swine flu; and to boost the body's immune system. It is also used for increasing white cell counts during treatment with anti-cancer drugs (chemotherapy). White cells are important in fighting off infection, but many are killed by chemotherapy (11). However, alkylglycerols (AKGs) in the shark liver oil have a unique antioxidant effect whereby it has the ability to enter the cell to attack, prevent and reduce those free radicals which have penetrated cell membranes and tissues. Free radicals within the cell itself are the most dangerous. These free radicals can damage the DNA and cause healthy cells to mutate leading to grave health problems. AKGs also inhibit an essential protein which may be directly involved in abnormal cell growth (12).

This study is aimed to compare between animal source (shark liver oil) and plant source (Nigella Sativa) omega-3 fatty acid in their potential regulation of some parameters especially:

Fasting blood sugar, lipid profiles like total cholesterol, triglycerides (TG) and low density lipoprotein (LDL-c). In addition to serum electrolytes like: calcium, potassium and sodium.

2. Materials and Methods

- **Volunteers**

18 male Yemeni individuals participated in this study. Their ages range from 23-31 years old, healthy and did not take any medications or herbal remedies.

- **Materials**

  - Natural Nigella (Black seed) Sativa oil from Phenomenal®, Damascus, Syria Alpha Company, Pharmaceutical industries, Aleppo.
  - Shark Liver oil from Vitex Pharmaceuticals Pty Ltd. Weld St Prestons NSW 2170 Australia.
  - Food and drinks were kept in fixed situation.
  - Studied parameters were measured before trials were started, then after 14 days.
  - Nigella Sativa tablets in a dose of (3 capsules per day= 400 mg each) were taken for 14 days.
  - Washout period for two weeks.
  - Fish oil tablets in dose of (2 capsules per day= 250 mg each) were taken for 14 days. Biochemical tests including fasting blood sugar (FBS) (13), total cholesterol (14), LDL-c (15), triglycerides (16), electrolytes (calcium, sodium and potassium) (17) were measured before and after using medications and comparison was done.

Results were analyzed by using SPSS version 13.0 statistical programme using paired T-test with a significance level of less than 0.05.

3. Results

Table 1 and figure 1 have shown that intake of Nigella Sativa produced a significant reduction (P value = 0.022) in fasting blood sugar from 4.80±0.065mmol/l in control group to 4.46±0.153 mmol/l in NS group, while shark liver oil produced significant increase (P-value = 0.001) in FBS from 4.80±0.065 mmol/l in control group to 5.16±0.098mmol/l in SLO group.

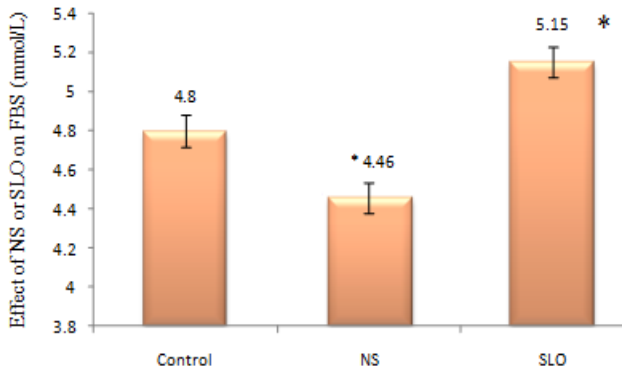
Figure 2 showed that intake of SLO produced a significant elevation (P-value= 0.029) in triglycerides from 125.63±12.0mg/dl in control group to 143.34±13.32mg/dl in SLO group. No change on lipid profiles was shown after using NS.

Figure 3a and b showed that intake of NS or SLO did not cause a significant change on both calcium and potassium. On the other hand it was found that NS produced significant increase in the level of sodium from 140.5±0.43 mg/dl in control group to 143.0±0.87 mg/dl in NS group.

Table 1. Effect of Nigella Sativa (NS)(1200mg/d) or Shark liver oil (SLO)(500mg/d) on (mean±SE) Fasting blood sugar (FBS) (mmol/l) , lipid profiles(mg/dl) and serum electrolytes (mmol/l)for14 days in volunteers (n=18)

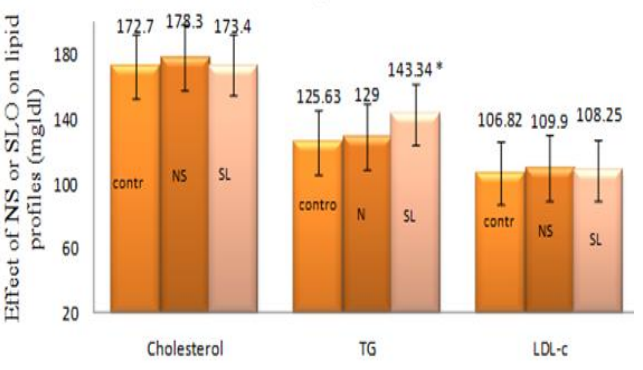
	M±SE	M±SE	P-value	M±SE	P-value
1-FBS(mmol/l)	4.80±0.065	4.46±0.153*	0.022	5.16±0.098*†	0.001
2-Lipid profile					
-C(mg/dl)	172.67±7.15	178.34±6.35	0.119	173.39±6.95	0.831
-TG(mg/dl)	125.63±12.0	129.0±12.7	0.639	143.34±13.32*	0.029
-LDL-c(mg/dl)	106.82±5.53	109.9±5.15	0.335	108.25±5.80	0.643
3-Serum electrolytes					
-Ca+2(mmol/l)	1.197±0.008	1.25±0.014	0.161	1.20±0.067	0.894
-Na+(mg/dl)	140.5±0.43	143.0±0.87*	0.001	140.9±0.76	0.436
-K+(mmol/l)	4.75±0.08	4.64±0.11	0.392	4.58±0.089	0.185

*Significant as compared with control at P‹0.05
†Significant as compared with NS group at P‹0.05



*Significant as compared with control at P‹0.05

Figure 1. Effect of Nigella Sativa (NS)(1200mg/d) or Shark liver oil (SLO)(500mg/d) on (mean±SE) Fasting blood sugar (FBS) (mmol/l) for 14 days in volunteers (n=18).



*Significant change as compared with control at P‹0.05

Figure 2. Effect of Nigella Sativa (NS) (1200mg/d) or Shark liver oil (SLO)(500mg/d) on (mean±SE) lipid profile for 14 days in volunteers (n=18)

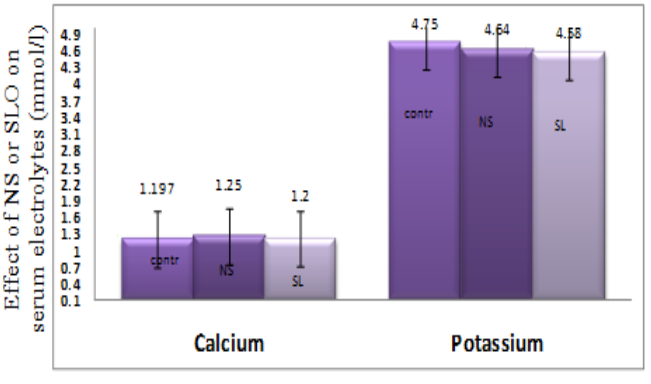
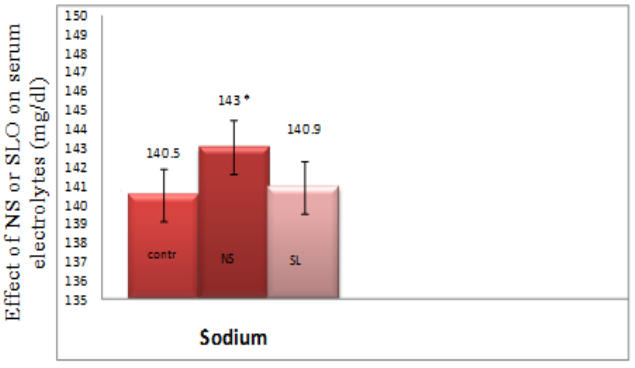


Figure 3a. Effect of Nigella Sativa (NS) (1200mg/d) or Shark liver oil (SLO)(500mg/d) on (mean±SE) serum electrolyte for 14 days in volunteers (n=18)



*Significant change as compared with control at P‹0.05

Figure 3b. Effect of Nigella Sativa (NS)(1200mg/d) or Shark liver oil (SLO)(500mg/d) on (mean±SE) serum electrolyte for 14 days in volunteers (n=18)

4. Discussion

Essential fatty acids (EFA) are necessary in proper brain and nervous system function and for building new cells. Although they are essential for good health, these fatty acids cannot be made by the body. We must obtain EFA from dietary sources. The two primary EFAs are the omega-3 and omega-6 (18).

The role of EFAs in human nutrition has long been recognized. Research links EFA intake with reduced risk for coronary heart disease (CHD), improving blood cholesterol levels and regulating the inflammation (19).

In the present study, Omega-3 fatty acid of animal source (Shark liver oil=SLO) and plant source (Nigella Sativa=NS) were compared in their potential hypoglycaemic and hypolipidemic effect. NS showed significant hypoglycaemic effects (P‹ 0.05) compared to control and SLO. Both drugs have omega-3 in their components, but Plant derived omega-3 fatty acid is called alpha-linolenic acids, marine derived omega-3 is also known as eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA). In addition, NS has

also Omega-6 fatty acids which is called linoleic acid in its structure (19).

Meddah B et al. in 2008 supported our findings. They found that Nigella Sativa L. (Ranunculaceae) seeds have been used traditionally for centuries, notably for treating diabetes. The aqueous extract of Nigella Sativa (0.1 pg/ml to 100 ng/ml) exerted dose-dependent inhibition of sodium-dependent glucose transport across isolated rat jejunum. An oral glucose tolerance test was carried out in rats after the initial dose and after a 6-week treatment of Nigella Sativa (2 g/(kg day)), and compared to metformin (300 mg/(kg day). Chronic Nigella Sativa treatment improved glucose tolerance as efficiently as metformin. Nigella Sativa and metformin also reduced body weight without any toxic effect. So NS inhibits intestinal glucose absorption and improves glucose tolerance (20).

Fararh K, Atoji Y, Shimizu Y and Takewak T in 2002 found that NS has insuliotropic effect. Significant decrease in blood glucose level together with significant stimulatory effect on β cell function with consequent increase in serum insulin level were observed after treatment with *N. Sativa* oil For 4 weeks (21). In addition, Al-Hader A, Aqel M, Hassan, 1993 and Zubaida A. Hawsawi; Basil A;. Bamosa A , 2001showed that hypoglycemic effect of NS may referred to its active constituent thymoquinone that showed its hypoglycemic effect after 4-6 days (22,23).

Houcher et al., 2007, showed other explanation. Many factors can influence the black cumin seed essential oil, like heredity, age of plant, climatologic environment, harvesting time, fertilization, distillation procedure and isolation methods. Both, N. Sativa extract and its commercial oil, produced significant antihyperglycaemic effect in diabetic rats precisely after 10 days of treatment. This effect was partly dependent on decrease in liver gluconeogenesis and was neither mediated through elevating plasma insulin levels nor by suppression of the intestinal absorption of glucose. We suggest that these results provide strong evidence in support of the ethno pharmacological use of N. Sativa seeds as an antidiabetic complement in cases of type II diabetes in Arabic world (24). On the other hand, in this work NS –treated group showed no significant change in lipid profiles including cholesterol, triglycerides and low density lipoprotein in normal persons. Most studies showed that omega-3 fatty acids either from plant or animal sources may reduced the elevated lipid profiles. Patients with high triglycerides and poor coronary artery health were given 4 grams a day of a combination of EPA and DHA along with some monounsaturated fatty acids. Those patients with very unhealthy triglyceride levels (above 500 mg/dl) reduced their triglycerides on average 45% and their VLDL cholesterol by more than 50%. VLDL is a bad type of cholesterol and elevated triglycerides can also be deleterious for cardiovascular health (20).

Schoub BD, 1999 studied the benefits of eicosapentaenoic acid (EPA). This study involved over 18,000 patients with unhealthy cholesterol levels. The patients were randomly assigned to receive either 1,800 mg a day of EPA with a statin drug or a statin drug alone. The trial went on for a total of five years. It was found at the end of the study that those patients in the EPA group had superior cardiovascular function. Non-fatal coronary events were also significantly reduced in the E-EPA group. The authors concluded that EPA is a promising treatment for prevention of major coronary events, especially non-fatal coronary events (25).

Controversially, shark liver oil in this study showed significant increasing in FBS and TG. This may be due the components of this oil. Shark liver oil may be obtained from several species of sharks, including the deep sea shark (*Centrophorus squamosus*), the dogfish shark (*Squalus acanthias*), and the basking shark (*Cetorhinus maximus*). All these types contain the same component including alkylglycerols, squalene, pristine, vitamins A and D, esters of fatty acids, glycerol ethers, triglycerides, cholesterol and fatty acids (26).

Navarro-Garcia G, 2002, showed that triacylglycerols (TAG) accounted for 56.9% of the total lipid in the shark liver oil (27). High proportion of this is triacylglycerols. The process of fat digestion begun in the stomach by acid-stable gastric or lingual lipases, the extent of which depending on species but may be important for efficient emulsification (28). However, this is insignificant in quantitative terms in comparison to the reaction with the colipase-dependent pancreatic lipase, which occurs in the duodenum. Entry of triacylglycerol degradation products into the duodenum stimulates synthesis of the hormone cholecystokinin and causes the gall bladder to release bile acids, which are strong detergents and act to emulsify the hydrophobic triacylglycerols so increasing the available surface area. In turn, cholecystokinin stimulates the release of the hydrolytic enzyme pancreatic lipase (29).

The process of hydrolysis is regiospecific and results in the release of the fatty acids from the 1(3) positions of the triacylglycerols and Formation of 2-monoacyl-*sn*-glycerols. Isomerization of the latter to 1(3)-monoacyl-*sn*-glycerols occurs to some extent, and these can be degraded completely to glycerol and free acids. Within the intestines, triacylglycerols are incorporated into lipoprotein complexes termed chylomicrons. These particles are secreted into the lymph and thence into the plasma for transport to the peripheral tissues For storage or structural purposes. Adipose tissue in particular secretes appreciable amounts of the enzyme lipoprotein lipase into the surrounding blood vessels, where it rapidly hydrolyses the triacylglycerols at the cell surface, releasing free fatty acids, most of which are absorbed into the adjacent adipocytes and re-utilized for triacylglycerol synthesis within the cell. The chylomicrons remnants eventually reach the liver, where the remaining lipids are hydrolysed and absorbed. The fatty acids within the liver can be utilized for a variety of purposes, from oxidation to the synthesis of structural lipids, but a proportion is re-converted into triacylglycerols, and some of this is stored as lipid droplets within the cytoplasm of the cells (30). Excessive accumulation of storage triacylglycerols is associated with fatty liver, insulin resistance and type II diabetes. In addition, hypertriglyceridemia may result in an elevation of circulating levels of C-reactive protein, a sensitive systemic marker of inflammation. This type of inflammatory marker may affect B-cell so reduce the secretion of insulin, resulted in metabolic syndrome which is associated with a systemic inflammatory response including hyperglycemia (31). Other studies found that the most widely available source of EPA and DHA is cold water oily fish such as salmon, herring, mackerel, anchovies and sardines. Oils from these fish have a profile of around seven times as much $n-3$ as $n-6$. Other oily fish such as tuna also contain $n-3$ in somewhat lesser amounts.

Consumers of oily fish should be aware of the potential presence of heavy metals and fat-soluble pollutants like polychlorinated biphenyls (PCBs) and dioxin which may accumulate up the Food chain. (32-33). Dioxins and dioxin-like PCBs (polychlorinated biphenyls) are very persistent chemicals that are widely present in the

environment and in foods at very low levels. They are found in all foods, but the highest levels are found in edible fish and other products with a high animal fat content. High levels of these pollutants have been linked to a number of health problems (34).

In regard to the blood electrolytes, Nigella Sativa was showed significant increased ($P<0.05$) in the level of sodium. The exact explanation is unknown. Sodium is a mineral element and plays an important part in the human body. It controls the volume of fluid in the body and helps maintain the acid-base level. About 40% of the body's sodium is contained in bone, some is found within organs and cells and the remaining 55% is in blood plasma and other fluids outside cells. Sodium is important in proper nerve conduction, the passage of various nutrients into cells, and the maintenance of blood pressure (35). Nigella Sativa may act to increase the production of aldosterone, this effect may increase sodium and water retention. In contrast, Khattab M and Nagi M, 2007 were disagreeing with our results. They found that NS counteract hypertension (36, 37).

However, some studies support our findings as they found that Black Cumin (Nigella Sativa) seed is a source of sodium. The main function of this element is to act considering needed cofactors guidance multitudinal enzyme functions (38) In contrast, Meral I and Kanter M, 2003 found that NS has no effect on sodium as it could not ameliorate CCl4-induced hypernateremia (39).

5. Conclusion

We can conclude from this study that both drugs have omega-3 fatty acid in their component; in addition NS has also omega-6 fatty acid. NS showed antidiabetic activity as it significantly reduced FBS. There is no change in lipid profiles of NS group in normal volunteers. Moreover, NS produced Transient elevation in sodium level with no effect on the other electrolytes.

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References

1. Bourgou S, Bettaieb I, Saidani M, Marzouk B. Fatty Acids, Essential Oil, And Phenolics Modifications of Black Cumin Fruit under NaCl Stress Conditions. *J. Agric. Food Chem.* 2010; *58*:12399–12406
2. Amin S, Mir SR, Kohli K, Ali B, Ali M. A study of the chemical composition of black cumin oil and its effect on penetration enhancement from transdermal formulations.Nat Prod Res. 2010; 24:1151-7.
3. http://news.bbc.co.uk/1/hi/health/1911312.stm (2010-01-06).
4. Keli, S.O., Feskens, E.J.; Kromhout, D. "Fish consumption and risk of stroke: The Zutphen Study". Stroke. 1994; 25: 328–32.
5. Gillum, R.F.; Mussolino, M.E.; Madans, J.H. "The relationship between fish consumption and stroke incidence: The NHANES I Epidemiologic Follow-up Study (National Health and Nutrition Examination Survey)". Arch Intern Med. 1996; 156: 537–42.
6. Iso, H.; Rexrode, K.M.; Stampfer, M.J.; Manson, J.E.; Colditz, G.A.; Speizer, F.E.; Hennekens, C.H.; Willett, W.C. "Intake of fish and omega-3 fatty acids and risk of stroke in women". JAMA .2001; 285: 304–12.
7. Holman RT. "The slow discovery of the importance of omega 3 essential fatty acids in human health". J. Nutr. 1998; 128 (2 Suppl): 427S–33S.
8. Augustsson K, Michaud D, Rimm E, Leitzmann M, Stampfer M et al,. "A prospective study of intake of fish and marine fatty acids and prostate cancer". Cancer Epidemiology, Biomarkers & Prevention .2003; 12:64–67.
9. De Deckere, E.A. "Possible beneficial effect of fish and fish *n*−3 polyunsaturated fatty acids in breast and colorectal cancer". Eur J Cancer Prev.1999; 8: 213–21.
10. Caygill, C.P.; Hill, M.J. "Fish, *n*−3 fatty acids and human colorectal and breast cancer mortality". Eur J Cancer Prev. 1995; 4: 329–32.
11. http://www.webmd.com/vitamins-supplements/ingredientmono-956-SHARK%20LIVER%20OIL.aspx?activeIngredientId=956& activeIngredientName=SHARK%20LIVER%20OIL (21-9-2011).
12. http://www.cantron.com/html/nutraceuticals/shark_liver.html (21-9-2011).
13. COBAS-Roche 400-Electrolyte analyzer.

14. Cohn JS, McNamara JR, Schaefer EJ. Lipoprotein cholesterol concentration in the plasma of human subjects as measured in the fed and fasted states. Clin Chem 1988;34:2456-2459.
15. Saguache H, lire T, Juicy , Ueno t, Chain T, Onekama k, okapi H. Homogenous assay for measuring low – density lipoprotein cholesterol in serum with tailback copolymer and a cyclodextrin sulfate. Clin Chem. 1998; 44: 522-31.
16. Breuer j .Report on the symposium Drug effects in clinical chemistry methods Eur j cling chem. Clint brioche 1996; 34:385-6.
17. COBAS-Roche 9180-Electrolyte analyzer.
18. Clavel s, Farout L, Briand M, Briand Y, Jouanel P. Effect of endurance training and/or fish oil supplemented diet on cytoplasmic fatty acid binding protein in rat skeletal muscles and heart. Eur J Appl Physiol. 2002; 8:193-201.
19. Kris-Etherton P, Harris W, Appel L. Fish consumption, fish oil, omega 3 fatty acids, and cardiovascular disease. Circulation. 2002; 106:2747-57.
20. Meddah B, Ducroc R, El Abbes Faouzi M, Eto B, Mahraoui L, Benhaddou-Andaloussi A, Martineau LC, Cherrah Y, Haddad PS. Nigella sativa inhibits intestinal glucose absorption and improves glucose tolerance in rats. J Ethnopharmacol. 2009;121:419-24.
21. Fararh, K., Atoji, Y., Shimizu, Y. and Takewaki, T. Insulinotropic properties of Nigella sativa oil in Streptozotocin plus Nicotinamide diabetic hamster. Research in Veterinary Science 2002, 73, 279-82.
22. Al-Hader A, Aqel M, Hassan Z. Hypoglycemic effects of the volatile oil of Nigella sativa seeds. Int J Pharmacol 1993;31:96-100.
23. Zubaida A. Hawsawi; Basil A. A;. Bamosa A O. Effect of *Nigella Sativa* (black seeds) and Thymoquinone on blood glucose in albino rats.*Annals of Saudi Medicine, vol 21,Nos 3-4* ,2001.
24. Houcher Z, Boudiaf K, Benboubetra M, Houcher B. Effects of Methanolic Extract and Commercial Oil of Nigella sativa L. on Blood Glucose and Antioxidant Capacity in Alloxan-Induced Diabetic Rats.Pteridine. 2007; 18: (8 – 18).
25. Schoub BD. The disease mechanisms of HIV in AIDS and HIV in perspective (A guide to understanding the virus and its consequences. Second edition Cambridge University Press. 1999 pp72-90.
26. Akutsu K, Tanaka Y, Hayakawa K: Occurrence of polybrominated diphenyl ethers and polychlorinated biphenyls in shark liver oil supplements. Food Addit Contam. 2006; 23:1323-29
27. Navarro-Garcia G., R. Pacheco-Aguilar B. Vallejo-Cordova J, Ramirez-Suarez C and Bolaños A. Lipid Composition of the Liver Oil of Shark Species from the Caribbean and Gulf of CaliFornia Waters. Centro de Investigacion en Alimentacion y Desarrollo Volume 13, Issue 5, 2000, Pages 791-8.
28. Athenstaedt, K. and Daum, G. The life cycle of neutral lipids: synthesis, storage and degradation. Cell. Mol. Life Sci. 2006; 63: 1355-69.
29. Coleman, R.A. and Lee, D.P. Enzymes of triacylglycerol synthesis and their regulation. Prog. Lipid Res. 2004; 43: 134-176.
30. Zechner, R., Kienesberger, P.C., Haemmerle, G., Zimmermann, R. and Lass, A. Adipose triglyceride lipase and the lipolytic catabolism of cellular fat stores. J. Lipid *Res*. 2009; 50: 3-21.
31. M Fröhlich, A Imhof, G Berg, W L Hutchinson, M B Pepys, H Boeing, R Muche, H Brenner, W KoenigAssociation between C-reactive protein and features of the metabolic syndrome: a population-based study. Diabetes Care, 2000; 23:1835-9.
32. Yokoyama, M; Origasa H, Matsuzaki M, Matsuzawa Y, Saito Y, Ishikawa Y, et al.,"Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis". Lancet .2007; 369: 1090–1098.
33. http://news.bbc.co.uk/1/hi/health/1911312.stm. (2010-01-06).
34. http://www.fda.gov/bbs/topics/news/2004/NEW01115.html (2011-8-12).
35. http://www.faqs.org/health/topics/4/Sodium imbalance.html#ixzz 0qF0LfmNz (2011-6-17)
36. http://www.diabeteshealth.com/solutions/Food/herbs/ (2011-9-18).
37. Khattab MM& Nagi M. Thymoquinone supplementation attenuates hypertension and renal damage in nitric oxide deficient hypertensive rats. Phytother Res. 2007; 2:410-4
38. http://hubpages.com/hub/habbatussauda. (2010-6-6).
39. Meral I& Kanter M. Effects of Nigella sativa L. and Urtica dioica L. on selected mineral status and hematological values in CCl4-treated rats. Biol Trace Elem Res. 2003; 96:263-70.

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ORIGINAL ARTICLE

Production of Exopolysacchardies by Probiotic Bacteria in the Presence of Bile Acids

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Abstract:

Introduction: Exopolysaccharides (EPS) are compounds that contain one or two types of monosaccharide. They are synthesized by some strains of bacteria during fermentation stage; they contribute to the improvement of health by improving the Rheological properties of fermented dairy products.

Objective: This study aimed to determine the ability of some bacteria to produce EPS in different Medias.

Methods: In this study, three strains of bacteria (*Bifidobacterium bifidus actiregulari***,** *lactobacillus delbrueckii subsp.bulgaricu* and *Streptococcus thermophilu*s were examined to evaluate their ability to grow and produce Exopolysaccharides in the presence of bile acids (Na-cholic and Na-dexoycholic Acid (50:50%)) in different concentrations.

Results: All tested bacterial strains were grown in the low and moderate concentrations of bile acids, while its growth was particularly inhibited with the presence of high concentration of bile acids. The results also show that all of the experiment strains produced different quantities of exopolysaccharides in different media cultures. The production of exopolysaccharides was ranged from 100 to 560 mg/l, and it was associated with bacterial growth.

Conclusion: Bacterial strains in this study have high ability to produce EPS in the presence of bile acids.

Key words: Exopolysaccharides, probiotic, *Bifidobacterium*, lactic acid bacteria, bile acids.

1. Introduction

A number of probiotic bacteria as, propionibacteria and bifidobacteria can synthesise Exopolysaccharides (EPS), which are excreted from the cell and which may or may not be loosely attached to the cell wall (1). There are two types of EPS; Homopolysaccharides composed of one type of monosaccharide, which is either D-glucose or D-fructose; these fructans and glucans have a common feature in being synthesized by extracellular transglycosylases (glycansucrases) using sucrose as the glycosyl donor (2). The heteropolysaccharides, which are most common among the yoghurt starter cultures, consist of a backbone of repeated subunits of three to eight monosaccharides. D-galactose, D-glucose and/or L-rhamnose are almost always present, but the ratios vary (3). The ability of lactic acid bacteria (LAB) to synthesize (EPS) is now regarded as particularly important because they enhance texture development in fermented dairy foods. Specifically, they can serve as natural thickeners, stabilizers, bodying agents, emulsifiers, gelling agents, and fat replacers (4,5).

EPS contribute in health improvement where it has prebiotic properties (6), immunostimulatory (7), anti-tumoral (8), and hypocholesterolemic effects (9). EPS that produced by lactobacillus kefiranofaciens which called (kefirian) had a hypoglycaemic effect in KKAy mice and could improve defecation in the levels of fecal moisture and wet weight of faeces in constipated SD rats induced by a low-fiber diet (10). Growth-associated EPS biosynthesis has been observed for several strains of LAB. In Streptococcus thermophilus LY03 and related strains, for example, there is a direct relationship between EPS yield and optimal growth conditions (11). The incubation temperatures result in higher EPS production, pH control generally enables higher cell counts and thus higher EPS levels, minerals or vitamins, will tend to increase bacterial populations, and thus EPS production (12). This study aimed to examine the ability of some strains of bacteria to produce EPS in the presence of deferent concentrations of bile acids.

2. Materials and Methods

Origin and maintenance of the bacterial strains

a) *Bifidobacterium bifidus Actiregularis* was isolated from DANONE ACTEVIA yoghurt (Danone Djurdjura Algeria) by using modified Columbia agar media (13) which was incubated anaerobically for 60 h at 37 °C.

b) *Lactobacillus delbrueckii subsp.bulgaricus* was isolated from SOUMMAM yoghurt (LAITERIE SOUMMAM RN 26.AKBOU-BEJAIA ,Algeria) using MRS agar (Fulka) (14) for 48h at 37 °C.

c) *Streptococcus thermophilus* was isolated from SOUMMAM yoghurt (LAITERIE SOUMMAM RN 26.AKBOU-BEJAIA, AlGERIE) using M17 agar (Difco) (15), by incubation for 24 h at 37 °C aerobically.

All strains were stored in the solid media at 4 °C for 2 weeks. And subcultured three times before use in broth MRS modified rogosa's 16). for *L. dellrueckii subsp . bulgaricus* and *B. bifidus Actiregularis* and in M17 broth for *S. thermophilus* using 1% inoculums and incubation for 24 h at 37 °C anaerobically for *L. delbrueckii* subsp. *bulgaricus* and *B. bifidus Actiregularis* and aerobically for *S. thermophilus* .

Bacterial growth and Bile salts Tolerance.

Growth rate of bacterial strains was measured in MRS and M17 broth containing different

concentration of bile salts (0, 0.3, 0.5, 0.6, and 0.9%) (Na-cholic and Na-dexoycholic Acid 50:50%; fulka). Other part of media (MRS and M17) and that of 0.5% of bile acids supplied with 100 g/l of cholesterol to examine cholesterol effects on bacterial growth. Freshly prepared cultures of experiment's strains were inoculated using 1% inocula in the previous medias and incubation at 37 °C for 72 h in anaerobic condition except for *S. thermophilus*, which was incubated under aerobic conditions. Optical densities of the cultures were measured spectrophotometrically at 620nm every two hours during the experiment period and compared within the different concentrations of bile salts for each bacterial culture used. All of the experiments were carried out in triplicate.

Measurement of EPS production

To determine the production of EPS by bacterial strains Lactose- MRS broth was prepared using Lactose in the place of Glucose (Because the amounts of EPS expressed as µg of glucose when it determinate) and supplemented with bile acid in (0,03,0.5,0.6 and 0.9%) of media. 9.9 ml of those media were inoculated with 1% of each test species and incubated at 37 °C for 24 h. EPS were separated by centrifugation for 10000 x g at 15 min. to eliminate bacteria from media according the method of Cerning et al. (17) with few modifications. Three volumes of cold ethanol were added to supernatant and stored overnight at 4 °C. Precipitated materials were collected by centrifugation (20 min at 5000 x g) and resuspended in demineralized water and mixed with three volumes of cold ethanol and centrifuged at 5000 x g for 20 min. The pellets were dried at 100 °C. The concentration of EPS produced by bacterial strains was determined by phenol-sulfuric method (18), with glucose as standard. The amounts of EPS expressed as µg of glucose / ml of the cultures. All of the experiments were carried out in triplicate.

3. Results

Bacterial growth and bile tolerance

Figures (1-3) Show growth of bacterial strains in MRS broth which was used as a control without bile source, And with the presence of several concentration of bile salts.

As we see in Figure 1 Growth of *B. bifidus Actiregularis* was optimum in MRS with absence of bile salts and in MRS-cholesterol, while it was minimum with at 0.3 and 0.9% of bile salts. The growth of *B. bifidus Actiregularis* was good in the presence of concentration of bile salts 0.5 and 0.6%.

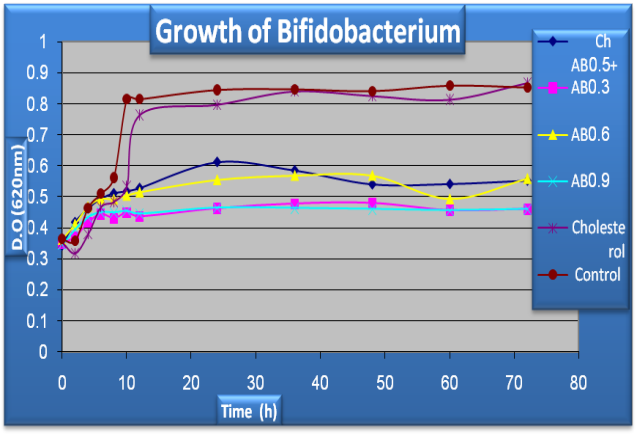

Figure 1. Growth of *B. bifidus Actiregularis* in deferent MRS in the presence of bile salts and cholesterol at 37C° for 72h

In Figure 2 Optical density arrives 1.6 for *L. delbrueckii* subsp. *bulgaricus* which grew in MRS with 0.3 and 0.6% of bile salts. And it was only 0.2 in the medias containing 0.9 %. And between 0.8 to 1 in the other smedias.

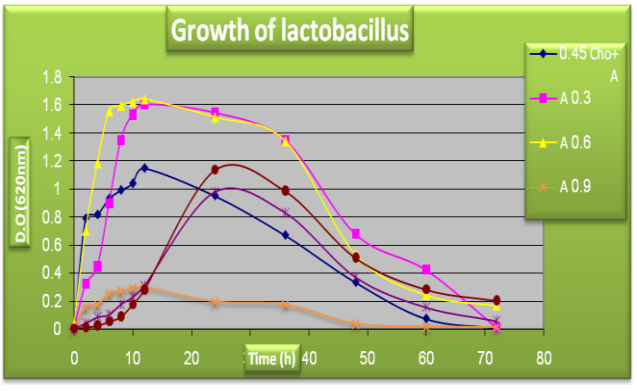

Figure 2. Growth of *L. delbrueckii subsp.bulgaricus* in deferent Medias of bile salts and cholesterol

S. thermophilus was growing with high quantity in MRS and MRS-cholesterol where the optical density was near to 1. But growing of *S. thermophilus* in the presence of bile salts was very weak specially with low concentration 0.3 and 0.6% .While in the presence of cholesterol and 0.5% of bile salt growth of *S. thermophilus* was good comparing with its growth in the other concentrations of bile salts where the $O.D_{620nm}$ arrives 0.500 at the end of incubation period (Figure 3).

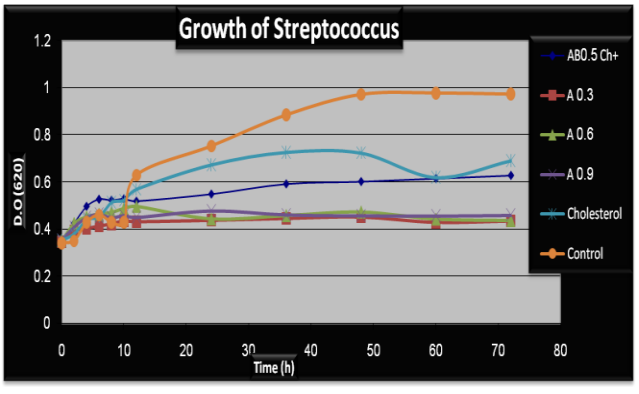

Figure 3. Growth of *S. thermophilus* in deferent Medias of bile salts and cholesterol

Production of Extracellular polysaccharides by bacterial strains

The ability of Extracellular polysaccharides production by bacterial strains in 24 h at 37°C in anaerobic conditions in L-MRS broth for *B. bifidus Actiregularis*, *L. delbrueckii* subsp. *bulgaricus*, and in L-MRS broth with aerobic conditions for *S. thermophillus* was measured. The results yielded variants quantities of EPS in the presence of several concentrations of bile acids by bacterial strains. The results show no significance when analyzed statistically by using ANOVA one way at the confidence level 0.05 between bacterial strains. However all bacterial strains reveal good ability to produce EPS in the presence of bile acids with various quantities.

The results of extracellular polysaccharides by *B. bifidus Actiregularis* are shown in figure 4 which shows difference in ability of *B. bifidus Actiregularis* to produce EPS in the presence of different concentrations of bile acids in media cultures used in the experiments whereas the production of EPS occurred in MRS broth only was used as control. The majority of EPS production was in MRS supplemented with 0.5 % and 0.3 % of bile acids which were 368 and 337 mg/l respectively, while it was few with the presence of 0.6% of bile acids and fewer with the 0.9%.

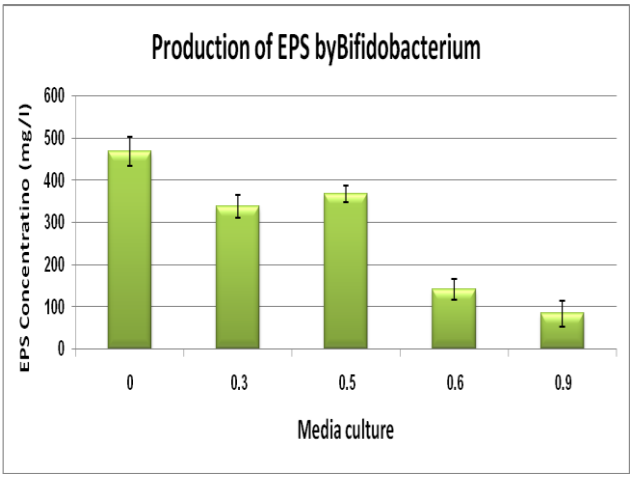

Figure 4. Production of Extracellular polysaccharides by *B. bifidus Actiregularis*

The ability to production of EPS by *L. delbrueckii* subsp. *bulgaricus* (figure 5) was better than that by *B. bifidus Actiregularis* in L-MRS broth containing 0.3 and 0.6% bile acid. It was approximately similar in the presence of 0.5% and 0.9% bile acids. But the quantity of EPS produced by *L. delbrueckii* subsp. *bulgaricus* with L-MRS broth only (control) was less than that with MRS broth plus 0.3% bile acids. Furthermore EPS produced by *L. delbrueckii* subsp. *bulgaricus* in L-MRS broth plus 0.5% was less than that with L-MRS broth plus 0.6% bile acids.

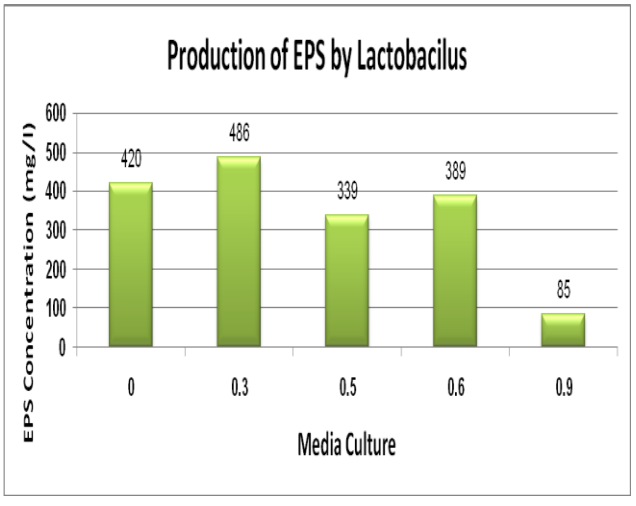

Figure 5. EPS production by *L. dellrueckii subsp bulgaricus*

The result of EPS production by *S. thermophillus* showed that the amount of EPS produced by *S. thermophillus* in the medium without bile acid was better than that with *B. bifidus Actiregularis* and *L. delbrueckii* subsp. *bulgaricus*. As in figure 6, the amount of EPS in the presence of 0.3 and 0.6% bile was more than that with 0.5% bile acid. While in MRS broth containing 0.9% bile acid, the ability of *S. thermophillus* to produce EPS was very feebly in comparing with that of the others medium used, and it was the minimum quantity of EPS produced between all bacterial strains in the experiment.

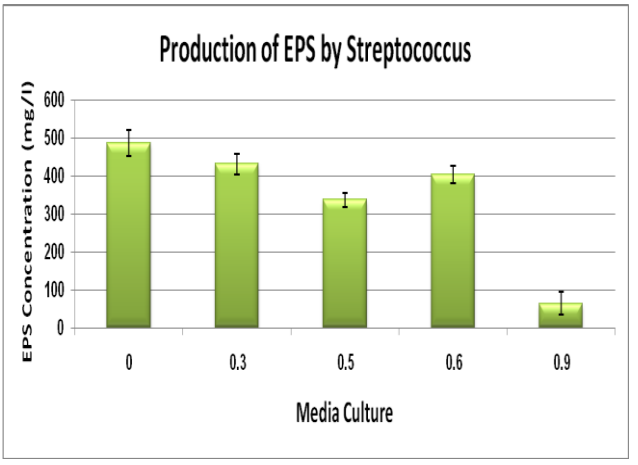

Figure 6. Production of EPS by *S. thermophilus*

4. Discussion

The results of our study strongly suggested that in the human intestine the populations of *lactobacilli* and *bifidobacterium* are controlled in part by the concentrations of free bile acids. Probiotic lactic acid bacteria accumulate Cholic Acid CA (or probably Dioxicholic Acid DCA as well) when their concentration is not so high with mild viability decrease. On the other hand, apparent proton conductance and severe membrane permeability disturbance can occur when CA or DCA concentrations in the human gut become higher than threshold value. And estimation from viability and membrane damage suggests a possibility that this compound causes considerable inhibition of bacterial growth in the human intestine (19). Survival at pH 3.0 for 2 h and in bile concentration of 1000 mg/L is considered optimum for acid and bile tolerance for probiotic strains (20).

Tannock et al. (21), and Yokota et al (22), reported that Deconjugated sodium taurocholate was reported to have such properties and therefore, have higher inhibitory effects against bacterial cells and suggest that under high concentration of conjugated bile, strains of *L. acidophilus* are likely

to survive best, whereas in presence of deconjugated bile, *L. casei* would survive best. Growth in the presence of oxgall showed that *L. casei* ASCC 1520, *L. casei* ASCC 290, and *L. casei* ATCC 15820 survived best, whereas *L. casei* ASCC 1521, *L. casei* ASCC 279, and *L. casei* ASCC 290 would be least bile tolerant (23).

In our study the result showed that high concentrations of bile acids (0.9%) had an inhibitory effect on all strains in the experiment. The result also suggested that there are inhibition occurred for *B. bifidus Actiregularis* at the low concentration of bile acid (0.3%), while the growth of all bacterial strains was good in the medium containing(0.5, 06%) of bile acids, except that of *S. thermophillus* which was inhibited in the presence of (0.6%) bile acid. We noted that the better growth of bacteria occurred in the presence of (0.5%) of bile acid and 100 g/l cholesterol, which suggests that these bacteria could grow well in the human intestine where the same concentration of bile acids is present. Moreover, cholesterol also stimulated the growth of bacteria. This may be due to using it by the bacteria in building its cell wall. This result was accepted with that of Kimoto .H et al (24), who focused on cholesterol removal by *lactococci* during growth and noted that the manner of cholesterol removal corresponded to the manner of its growth. These results indicated that cholesterol removal was due to bacterial growth in the broth containing cholesterol.

Pham et al. (25) reported that L. rhamnosus R produces EPS when cultivated on BMM supplemented with either glucose or lactose. When grown in batch cultures on lactose, the amount of EPS was comparable to that produced by glucose-grown cultures.

Cerning et al. (26) showed that for L. casei CG11, both the yield and the composition of the EPS produced were dependent on the carbon source present in the medium. For L. casei CG11, glucose was the most efficient carbon source for EPS production, whereas lactose was an inefficient carbon source.

Ginka et al (27), Reported that The L. bulgaricus HP1 strain had showed the highest exopolysaccharide activity. The streptococci tended to produce the most EPS per milliliter of culture; however, when compared on the basis of EPS amounts per 10^7 cfu, the lactobacilli produced the highest amounts per 10^7 cfu. *L. delbrueckii ssp. bulgaricus* strains (Lb-18, Lb-10442) and *S. thermophilus* (St-143) produced significantly larger amounts per 10^7 cfu than did other strains tested (28). *L. delbrueckii* subsp. *bulgaricus* NCFB 2772 produces EPS when they were grown in a defined medium supplemented with a carbohydrate source. The amount of the EPS produced was thought to be affected only by the growth temperature or the carbohydrate source (29).

In our study production of EPS was experimented by three bacterial strains in the presence of different concentrations of bile acids and lactose as source of carbon. Our result showed that all of strains were able to produce EPS in the presence of lactose with various quantities. The result was agreed with those of Pham et al. (25), and disagreed with that of Cerning et al. (26). The amount of EPS production by the strains of bacteria tested ranged from (100 to 560 mg/l). These quantities were in the same range obtained in the previous studies. The statistical analysis did not show significant difference ($P \leq 0.05$) between the amounts of EPS produced by bacterial strains. Our data showed significant difference between the production of EPS in the presence of different

concentration of bile acids, where the greater EPS production was 486 mg/l in L-MRS + 0.3% bile acid whereas the tiny amount of EPS produced was in the presence of 0.9% of bile acids. These results confirm the hypotheses which say that the EPS production is growth dependent.

5. Conclusion

All strains of bacteria studied in our work had survived in all bile acid concentrations to a variable extent. The optimal bacterial growth occurred in the moderate concentrations of bile acids (0.5 and 0.6%). The presence of cholesterol in the media stimulated the growth of bacteria for all strains while higher growth occurred in the broth containing in 100 mg/l. *L. delbrueckii* subsp. *bulgaricus* produced the largest quantity of EPS in MRS with 0.3% of bile acid. And *B. bifidus Actiregularis* produced the large concentration of EPS in MRS containing 0.5% bile acid and cholesterol. And *S. thermophilus* produced the large quantities of EPS in MRS with 0.3 and 0.6% of bile acids. The high concentrations of bile acid inhibited the production of EPS in parallel with bacterial growth.

References

1. Andrew L, Gu Y, and Valerie M. Biosynthesis, characterization, and design of bacterial exopolysaccharides from lactic acid bacteria, *Biotechnology Advances. 2001; 19:597-625.*
2. Monsan P, Bozonnet S, Albenne C, Joucla G, Willemot RM. and Remaud Simeon M. Homopolysaccharides from lactic acid bacteria. *International Dairy Journal,2001;11: 675 685.*
3. Uemura J, Itoh T, Kaneko T. and Noda K. Chemical characterization of exocellular polysaccharide from Lactobacillus delbrueckii subsp. bulgaricus OLL1073R 1. *Milchwissenschaft*, 1998*; 53: 443- 446.*
4. De Vuyst, L. and F.Vaningelgem. Developing new polysaccharides, 2003: 257-320. *In* B.M. McKenna *Texture in food, vol.2 Semisolid foods*. Wood head Publishing Ltd., Cambridge, U. K.
5. Ruas-Madiedo P. and de los Reyes-Gavilan, CG. Methods for the screening, isolation, and characterization of exopolysaccharides produced by lactic acid bacteria. *J. Dairy Sci.* 2005*; 88:843–856.*
6. Gibson GR. and Roberfroid, M. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *Journal of Nutrition*, 1995; 124, 1401–1412.
7. Hosono A, Lee J, Ametani A, Natsume M, Hirayama M, Adachi T. and Kaminogawa S. Characterisation of a water soluble polysaccharide fraction with immunopotentiating activity from *Bifi dobacterium adolescentis* M101-4. *Bioscience, Biotechnology and Biochemistry, 1997; 61, 312–316.*
8. Kitazawa H, Toba T, Kumano N, Adachi S. and Yamaguchi T. Antitumoral activity of slime forming encapsulated *Lactococcus lactis* ssp. *cremoris* isolated from Scandinavian ropy sour milk 'viili'. *Animal Science Technology, 1991; 62, 277–283.*
9. Nakajima H, Suzuki Y, Kaizu H. and Hirota, T. Cholesterol lowering activity in ropy fermented milk.*Journal of Food Science*, 1992*; 57, 1327–1329.*
10. Maeda H ,Zhu X and Mitsuoka T. Effects of an Exopolysaccharides (kefiran) from lactobacillus kefiranofaciens on Blood Gluocose In KKAy Mice and constipation in SD Rats induced by a low-fiber diet. *Biocscience Microflora*, 2004;*Vol.23(4), 149-153.*
11. De Vuyst L, Vanderveken FS, Van de Ven, and Degeest B. Production by and isolation of exopolysaccharides from *Streptococcus thermophilus* grown in milk medium and evidence for their growth-associated biosynthesis. *J.Appl. Microbiol.1998; 84:1059–1068.*
12. Robert EC. Wildman. Handbook of nutraceuticals and functional foods*. by Taylor & Francis Group, LLC. Boca Raton London New York* 2007.
13. Beerens H. A. elective and selective isolation medium for bifidobacterium spp. lett. *Appl.Microbiol, 1990; 11:155-157.*
14. De man jc, Rogosa W, scharpe ME. A medium from the cultivation of lactobacillus .*J.Appl.*Bacteriol.1960; 230:130-135.
15. Terzaghi BE and Sandine WE. Improved medium for lactic streptococcus and their bacteriophages. *Appl Environ Microbio*1975*; 29:807-813.*
16. Samona, A., and R. K. Robinson. Enumeration of bifidobacteria in dairy products*. 1. Soc. Dairy Techno.1991;44:64-66.*
17. Cerning J. Production of exopolysaccharides by lactic acid bacteria and dairy propionibacteria. *Le lait, 1995;75:463-472.*
18. Dubois MA, Gilles KA, Hamilton JK, Rebers PA, and Smith F. Colorimetric method for determination of sugar and related substances .*Anal.Chem.* 1956*; 28:350-356.*
19. Usman , H. A. Bile tolerance, taurocholate deconjugation, and binding of cholesterol by *Lactobacillus* gasseri strains. *J. Dairy Sci. 1999;82:243–248.*
20. Peter K, Koji K, Kanako M, Yokota A. Mechanism of Growth Inhibition by Free Bile Acids in Lactobacilli and Bifidobacteria. *Journal of bacteriology.* 2006*; 188:1979–1986.*
21. Tannock , G. W., G. M. Bateup, and H. F. Jenkinson. Effect of sodium taurocholate on the in-vitro growth of lactobacilli. *Microbiol. Ecology.* 1997; *33:163–167.*
22. Yokota, A., Veenstra M., Kurdi P., van Veen H. W., and Konings W. N. Cholate resistance in *Lactococcus lactis* is mediated by an ATP-dependent multispecific organic anion transporter. *J. Bacteriol. 2000; 182:5196–5201.*

23. Liong MT and Shah NP. Acid and Bile Tolerance and Cholesterol Removal Ability of Lactobacilli Strains. *J. Dairy Sci*. 2005*; 88:55–66.*
24. Kimoto H, Ohmomo S and Okamoto T. Cholesterol removal from media by lactococci. *J Dairy Sci 2002; 85: 3182-3188.*
25. Pham PL, Dupont I, Roy D, Lapointe G, Cering J. Production of Exopolysaccharide by Lactobacillus rhamnosus R and Analysis of Its Enzymatic Degradation during Prolonged Fermentation. Applied and environmental microbiology, 2000*: 2302–2310*.
26. Cerning J, Bouillanne C, Landon M, and Desmazeaud M. Isolation and characterization of exopolysaccharides from slime-forming mesophilic lactic acid bacteria. *J. Dairy Sci.1992; 75:692-699.*
27. Ginka IF, Emilina DS, Dora MB and Zhelyasko IS. Exopolysaccharides Produced by Lactic Acid Bacteria of Kefir Grains. Z. Naturforsch. 2002; 57c, 805.810.
28. Pigeon RM., Cuesta EP. and Gilliland S.E. Binding of Free Bile Acids by Cells of Yogurt Starter Culture Bacteria .J. Dairy Sci. 2002; 85:2705–2710.
29. Grobben GJ , Chin.JOE I, Kitzen VA, Boels IC, Boer F, Sikkema J, Smith MR. Enhancement of Exopolysaccharide Production by Lactobacillus delbrueckii subsp. bulgaricus NCFB 2772 with a Simplified Defined Medium .Applied and Environmental Microbiology, *1998; 1333–1337*.

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ORIGINAL ARTICLE

Management of Chest Injuries: A prospective study

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Abstract:

Objective: Chest injuries constitute a continuing challenge, relating to the trauma team or general surgeon, who practicing in a developing country. This study reviews the pattern and management of these injuries mainly by general surgeons.

Methods: A prospective review of 112 patients admitted into the surgical wards of the the AL-Gumhouri-Teaching Hospital Sana'a between March 2006 and August 2009.

Results: 112 patients with various forms of chest injuries were managed in the AL-Gumhouri Teaching Hospital Sana'a between March 2006 and August 2009. There were 100 (89.3%) males and 12 (10.7%) females.. The age ranged between 16-45 years, with a mean of 26.4 years. Most of the injuries, 68 (60.7%) were penetrating, 42 (37.5%) were due to gun shots and 26 (23.2%) from stab wounds. Blunt injuries constituted the remaining 44 (39.3%), 36 (32.1%) were due to road traffic accident and 8 (7.2%) fall from height. All patients had chest radiographs.The commonest abnormal findings were rib fractures 38 (33.9%), pneumothorax 36 (32.14%), haemothorax 30 (26.8%), haemopneumothorax 24 (21.43%). Most patients 80 (71.4%) required tube thoracostomy as definitive treatment. Ten patients (8.9%) required thoracotomy. The commonest extra-thoracic associated injuries were abdominal injuries in 12 (10.7%), head injuries 6 (5.4%), limb fractures 4 (3.6%), one (0.9%) vertebral bodies fracture. and eight (8.04%) had multiple injuries involving thorax with more than two body systems .The most encountered complications were posttraumatic infection. The overall mortality rate was (8.9%).

Conclusion: Penetrating injury of chest is the most common causes of chest injuries in adults in this study although blunt trauma is still common.The majority of the chest trauma are successfully managed by tube thoracostomy and supportive measures.

Key words: Chest injuries, penetrating trauma, thoracostomy.

1. Introduction

The most common life-threatening complications from both blunt and penetrating thoracic injury are hemothorax, pneumothorax, or a combination of both. Approximately 85% of these patients can be treated definitively with a chest tube (1). According to the United States National Trauma Data Bank, admissions due to trauma have steadily grown in the last decade (2,3) which is 12 per million population per day and 20 to 25% of deaths occurring due to trauma in the United States are due to thoracic injuries (4). The chest injuries are found to carry significant rates of morbidity and mortality, in particular when associated with other injuries including other bodily structures. Over the last century, there has been considerable improvement in traumatic care outcome, mainly due to availability of positive pressure ventilation, introduction of broad spectrum antibiotics, advent of radiological techniques, innovating of emergency thoracotomy and post-operative surgical intensive care units.

2. Materials and Methods

A prospective review of 112 patients admitted into the surgical department of the AL-Gumhouri - Teaching Hospital Sana'a between March 2006 to August 2009, with chest injuries was under taken. The hospital records were analyzed, assessing aspects of age, sex, types and mechanism of injury, associated injuries, management, complications, morbidity and mortality. The approach to diagnosis depended greatly on clinical signs, physical examination, abnormal chest radiographs and ultra-sonography, and investigating for other risky abdominal injuries.

3. Results

A total of 112 patients were studied for various chest injuries during fourty six months period from March 2006 to August 2009. The age of the patients ranged from 16 to 45 years with a mean age of 26.4 years. One hundred patients were males and only twelve were females (Table 1). Mode of injury is mentiond in Table 2. All the patients had chest radiographs carried out; the most common abnormal findings were rib fractures 38 (33.9%), pneumothorax 36 (32.14%), haemothorax 30 (26.8%) haemopneumothorax 24 (21.43%) (Table 3). As a whole (60.7%) of patients had penetrating chest injury as compared to (37.3%) who had blunt injuries.

Twenty two (19.6%) had chest wall injury (rib fracture, mild lung contusion) without hemothorax or pneumothorax and they were managed conservatively**.**

Chest intubation was required in 80 patients (71.4%) having hemothorax, pneumothorax and hemopneumothorax which included 56 patients who had penetrating injuries and 24 patients who had blunt chest trauma. There were 10 (8.9%) thoracotomies during the study period, 7 emergency thoracotomies, 4 (3.6%) were carried out or perfomed because initial blood drainage was >1500ml on chest intubation, 3 (2.7%) patients who had continuous bleeding even after placement of tube thoracostomy more than 200 ml/ hour for three consecutive hours. These cases were associated with homodynamic unstable tachycardia, hypotension and respiratory distress and bleeding from intercostal vessels, pulmonary parenchyma laceration and internal mammary artery was found on surgery. Three patients developed empyema as a complication of chest intubation and required decortication (Table 4).

The most common extra-thoracic associated injuries were abdominal injuries in 12 (10.7%), head injuries 6 (5.4%), limb fractures 4 (3.6%), one (0.9%) vertebral body fracture and eight (8.04%) had multiple injuries involving thorax with more than two body systems. Two patients with flail chest did well with chest intubation, oxygen and pain control, while one patient required ventilator support.

Nine (8.04%) patients had exploratory laparotomy. Splenectomy was carried out for three patients who had blunt thoraco-abdominal trauma. Four patinets had multiple perforations of the ileum and mesentery. They had resection of devitalized bowel and primary anastomoses along standard lines, and two stomach perforation they had debridement of wound edges and primary closure in layer. Four had diaphragmatic rupture, repaired through the abdomen.

Sixteen patients developed post operative complications of chest intubation, in which three had empyema, six had pneumonia, four had wound infection and three had atelectasis. In multiple traumas, two patients developed Acute Respiratory Distress Syndrome (ARDS) and four patients developed septicemia. Seven patients had respiratory tract infection during conservative treatment, in which three had lung contusion. Over all mortality was ten (8.9%) in which four had multiple trauma and three had associated neurosurgical trauma ,one with septicemia, one flail chest and one patients died following emergency thoracotomy.

Table 1. Age and sex distribution of 112 patients with chest injury

Age group	Sex		No (%)
	M	F	
16-25	41	6	47(42)
26- 35	36	4	40(35.7)
36-45	23	2	25(22.3)
Total	100	12	112(100)

Table 2. Mode of injuries of chest trauma

Type of trauma	No (%) n =112
Penetrating trauma	68 (60.7)
. Gunshot wound	42 (37.5)
. Stab wound	26 (23.2)
Blunt trauma	44 (39.3)
. Road traffic accident	36 (32.1)
. Fall from height	8 (7.2)
Total	112 (100)

Table 3. Types of injuries encountered in patients from chest trauma

Type of chest injury	No. of patients (n=112)	%
Rib fracture	38	33.9
Pneumothorax	36	32.14
Hemothorax	30	26.8
Hemopneumothorax	24	21.43
Lung contusion	5	4.5
flail chest	3	2.7
Diaphragmatic rupture	4	3.6
Multiple trauma/two system involvement	29	25.9

Table 4. Treatment required for patients suffering from chest trauma (n=112)

Treatment modality	N=112	%
No operative intervention	22	19.7
Tube thoracostomy	80	71.4
Thoracotomy	10	8.9

4. Discussion

Chest injuries are common reasons for emergency medical care. In civilian practice, chest trauma is commonly caused by road traffic accidents. With increasing urbanization, penetrating chest injuries are becoming more common in civilian practice (5). Most of the injuries are found among young adult males between the ages of 26 and 46 years, the very productive period of life. Eighety nine percent of patients were males are similar to other studies (2, 5,6). Of the 112 patients in this study, 68 (60.7%) had penetrating and 44 (39.3%) had blunt chest injury .While Farooq (7) shows the prevalence of blunt trauma and penetrating injuries as 44% and 56%, Hanif (8) shows these figures as 65% and 35% respectively. It supports the observation that incidence of penetrating trauma is rising with time because of gun shot injuries. The high incidence of gunshot wounds in this study can be explained by the fact that most people in our country own guns and use them at verity of occasions. Most of the wounds were caused by machine guns and stabbing wounds.

The majority of our patients 80 (71.5%), were successfully managed bychest tube, the site of intra-pleural drain was in the fifth intercostals space of midaxillary line and second inter-costal space midclavicular line, when only pneumothorax present, a large bore chest tube 36 franch was being used. The tube was fixed securely by a heavy suture which did not penetrate its own wall and is attached to an under water-seal drain, following chest intubations, the concerned patients need to be continuously monitored clinically as well as radiologically.The average duration of thoracostomy tube drainage was 10 days . Many western studies also suggest that observation or chest tube placement, an adequate volume replacement, occasional respiratory support and serial chest X-rays are the only treatment required in 80-85% of the patients (9, 10).

There were 10 (8.9%) thoracotomies during the study period,7 emergency thoracotomies ,4 patients (3.6%) were done because initial blood drainage was >1500ml on chest intubation, 3 patients (2.7%) patients who had continued to bleed even after placement of tube thoracostomy more than 200 ml/ hour for three consecutive hours, they were associated with homodynamic unstable tachycardia, hypotension and respiratory distress and bleeding from intercostals vessels, pulmonary parenchyma laceration and internal mammary artery was found on surgery, three patients developed empyema as a complication of chest intubation and required decortication. Stahel et al (11) have reported this in less than 10% in their study. It shows thoracotomy rate is more in penetrating injury than blunt trauma. Twenty two (19.6%) required a combination of wound excision, debridement, analgesics, antibiotics and tetanus prophylaxis. These groups were closely observed for any deterioration. Rib fracture were found in 44% of cases in the study by Farooq et al (7) while in our study the frequency of rib fracture was 34% .Extra thoracic associated injuries have been reported in the literature to be 50 to 70% (5-

12), yet their incidence was lower in our study showing 28.6%. The lower incidence of extra thoracic associated injuries in this study might be attributed to the lower incidence of blunt trauma, as the most encountered associated extra thoracic injuries.

Complications of the tube thoracostomy were found in 20% of cases .Our complications rate is comparable to other studies by Chan *et al* encountered 64 complications in 352 tube thoracostomies 18.2% complication rate (13). Three patients (3.8%) developed empyema as post operative complications of chest intubation and required decortications. This is in comparison to 3% by Helling and associates (14).

Over all mortality was ten (8.9%) in which four had multiple trauma and three had associated neurosurgical traum, one with septicemia, one flail chest and one patients deceased following emergency thoracotomy. Our mortality rate is comparable to other studies by Hanif et al (8) who have reported mortality rate of 7%. Hospital mortality rates for isolated chest injuries were reported to range from 4 to 8%, and increased to 13–15% when another organ system was involved and to 30–35% when more than one organ system was involved (15).

5. Conclusion

Penetrating injury of chest is the most common cause of chest injuries in adults in this study although blunt trauma is still common. The majority of the chest trauma are successfully managed by tube thoracostomy and supportive measures as well. Other organs or systems should be carefully assessed for associated injuries or damages.

References

1. Schwartzs principles of surgery. Trauma, Management of acute injuries; Text book of Surgery 8th Ed McGraw-Hill Companies 2005;6:157.
2. American College of Surgeons. National trauma data bank. Chicago: Ann Surg 2004;240:96-104.
3. Choi KC, Peek-Asa C, Lovell M, Tomer JC, Zwerling C, Kaeley GP. Complications after therapeutic trauma laparotomy. J Am Coll Surg 2005;201:546-53.
4. Locicerco J, Mattox KL. Epidemiology of chest trauma. Surg Clin North Am 1989;69:15-6.
5. Inci I, Ozçelik I, Tacyildiz O, Nizam N, Eren G, Ozen DB. Penetrating chest injuries: unusually high incidence of high velocity gunshot wounds in civilian practice. World J Surg 1998; 22: 438 – 42.
6. Vasquez TC, Castaneda E, Bazan N. Management of 240 cases of penetrating thoracic injuries. Injury 1997; 28: 45- 49.
7. Farooq U, Raza W, Zia N, Hanif M, Khan MM. Classification and management of chest trauma. J Coll Physicians Surg Pak 2006;16:101-3.
8. Hanif F, Mirza SM, Chaudhry AM. Re-appraisal of thoracic trauma. Pak J Surg 2000;16:25.
9. Bastos R, Baisden CE, Harker L, Calhoon JH. Penetrating thoracic trauma. Semin Thorac Cardiovasc Surg 2008;20:19-25.
10. Robison PD, Harman PK, Tinkle JK, Grover FL. Management of penetrating lung injuries in civilian practice. J Thorac Cardiovasc Surg 1988;95:184-90.
11. Stahel PF, Schneider P, Buhr HJ, Kruschewski M. Emergency management of thoracic trauma. Orthopade 2005;34:865-79.
12. Mansour MA, Moore EE, Moore FA, Read RR. Exigent Postinjury Thoracotomy: Analysis of blunt versus penetrating Trauma. Surg Gynecol Obstet 1992; 175:97 -101
13. Chan L, Reilly K, Henderson C, *et al*. Complication rates of tube thoracostomy. Am J Emerg Med 1997;15:368–70.
14. Helling TS, Gyles NR, Eisenstein CL, Soracco CA. Complications following blunt and penetrating injuries in 216 victims of chest trauma requiring tube thoracostomy. J Trauma 1989;29:1367-70.
15. Mayberry J.C., Trunkey D.D. The fractured rib in chest wall trauma. Chest Surg Clin North Am 1997;7:239-261.

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ORIGINAL ARTICLE

Practices and Beliefs of Yemeni Pediatricians Regarding Neonatal Hyperbilirubinemia

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Abstract:

Neonatal hyperbilirubinemia is a widespread and significant clinical condition amongst neonates worldwide

Methods: A specially designed questionnaire was distributed to a random sample of 200 pediatricians working in the main childhood hospitals in Sana'a.

Results: The pediatricians involved in the study stated high use (61.8%) of the cephalo-caudal progression as a method to evaluate jaundice in the newborn. Less proportion of pediatricians tested total serum bilirubin levels in jaundiced neonates ahead of their discharge from the hospital as compared to jaundiced neonates examined at the post-discharge visit (73.9% vs. 83.4%). The majority of pediatricians did not believe neonatal jaundice noted after discharge and gestational ages 37–38 weeks as being risk factor for the development of acute bilirubin encephalopathy. A considerable number of our pediatricians initiated phototherapy as well as exchange transfusion at lower levels of serum bilirubin than the recommended by the AAP guidelines at age of 24-48 hr, (66.7% and 57.6% respectively).

Conclusion: although Yemeni pediatricians in the study were liable to start up phototherapy and exchange blood transfusion at points lesser than those recommended, the study revealed an overall consistency of their practice with hyperbilirubinemia treatment guidelines of the American Academy of Pediatrics, 2004.

Key words: Neonatal hyperbilirubinemia, management, pediatricians.

1. Introduction

Neonatal hyperbilirubinemia is a widespread and significant clinical condition amongst neonates worldwide. By definition, it is the increase of a total serum bilirubin level above 5 mg/dl. This clinical condition comprises up to 75 percent of the total hospital readmissions in the early neonatal life (1).

Although the largest part of neonatal jaundice is benign, newborn infants are recommended to be watched to identify those who might build up severe hyperbilirubinemia and, in rare cases, acute bilirubin encephalopathy or kernicterus (2,23).

There is a preference to use the term "acute bilirubin encephalopathy" to portray the acute expressions of bilirubin toxicity seen in the first weeks of life and that the term "kernicterus" is held in reserve for the chronic and everlasting clinical sequelae of bilirubin toxicity (2,15).

It is accepted that the brain is matchless by having a blood brain barrier (BBB) that deliberates the equality between plasma and brain. In condition that the BBB is insulted, bilirubin shifts easily into the extra cellular space of the brain and, at higher bilirubin levels, will lead to instant large-scale neurotoxicity (3,4).

Newman and Maisels assumed in 1990 that, without hemolysis, the jaundiced infants would not be exposed to the damage of kernicterus as long as serum bilirubin levels remain well below 20 mg/dl (5). This conclusion had turned out to be the standard of care in the 1994 American Academy of Pediatrics (AAP) practice parameters (2). This practice guide for neonatal jaundice was concluded based on existing evidence that acute bilirubin encephalopathy was uncommon in healthy term infants with raised serum bilirubin level even beyond 30 mg/dl and on the notion that the jeopardy of intervention at a total serum bilirubin (TSB) levels of < 24 may exceed the risk of kernicterus (6).

A re-emergence of many cases of kernicterus during the precedent decade has lead to a second look of the 1994 AAP practice parameters and to the emergence of the 2004 AAP guidelines, which endorse deeper parent education and earlier pre-discharge and post-discharge screening for neonatal jaundice to prevent infants with jaundice from "falling through the cracks" (7).

The AAP 2004 guidelines supply a skeleton for the prevention and management of neonatal jaundice of 35 or more weeks of gestation. For each infant, it is recommend that clinicians encourage and support successful breastfeeding; carry out a systematic evaluation before discharge for the risk of dangerous hyperbilirubinemia; perform early follow-up based on the risk assessment; and when specified, manage with phototherapy or exchange transfusion to avoid the development of acute bilirubin encephalopathy and, consequently, kernicterus (7-9).

Studies of the reported cases of kernicterus propose that tracking the recommendations of the AAP guidelines by the pediatricians and health workers, would lead to a substantial prevention of kernicterus (10, 23).

In this study, we evaluated the Yemeni pediatricians' practices and beliefs regarding the management of neonatal hyperbilirubinemia and their conformity with the recommendations made by the American Academy of Pediatrics (AAP) 2004.

2. Materials and Methods

A survey questionnaire was prepared to be filled by a random sample of pediatricians from a list of the main childhood hospitals in Sana'a (Elsabeen Hospital, Eljomhoori hospital as well as a private health providing institution in Sana'a, the University of Science and Technology Hospital) in which a considerable number of neonatal incubators in the Neonatal Intensive Care Unit (NICU) were available. The instrument was distributed randomly to be filled by the pediatricians. The voluntary character of the study and its confidentiality were clarified to the participating pediatricians. The exclusion criteria were those pediatricians under training (residences), and pediatricians who had no practice in the neonatal units. The study was conducted during the period from August 2008 to January 2009.

The instrument:

A survey questionnaire that included 21 questions was prepared for the purpose of the study. The questions had tackled various features of the management of neonatal hyperbilirubinemia such as pre-discharge bilirubin testing, post discharge follow up, different diagnostic and treatment approaches used for the management of neonatal hyperbilirubinemia, and the public health significance of this condition. The physicians were also asked about their practice type, years of practice and the annual number of neonates seen in their practice.

Questions were formulated using a yes/no form, as well as a scale type layout that expressed the degree of the pediatrician's belief regarding certain procedure or fact (11,23). The instrument had also assessed the following risk factors: jaundice existing in the first 24 hours, jaundice noted at discharge, preceding siblings with jaundice, gestational age between 37 and 38 weeks, breast feeding, presence of bruising/cephalhematoma, Rh and ABO incompatibility. Moreover, other questions were structured to evaluate the decisions of the pediatricians regarding starting phototherapy or exchange transfusion according to the level of serum bilirubin at specific age per hours. We conducted a pilot study prior to the distribution of the questionnaire with six pediatricians and some modifications were made to make the questions more understandable.

Statistical Analysis

The statistical analysis was performed using SPSS to identify the significance of the observed differences in proportion (Chi-square test) and the continuous variables (analysis of variance).The assessment of the pediatrician's judgment regarding age-specific bilirubin levels at which decisions of phototherapy and exchange transfusion was taken depending on the AAP guidelines in 2004.

3. Results

The response rate was 85%. Among the respondents (10%) were pediatricians under training and were excluded from the statistical analysis. The larger proportion of pediatricians (60.1%) were board certified, (10.5%) were MD certified. Eight percent of the respondents were practicing in private sector, the remaining were in community hospitals (Figure1). The years of work of the participating pediatricians ranged from one year to 23 years with a mean of 10.7 year. The number of neonates seen annually by the pediatricians ranged from 30 to 400 neonates with a mean of 142.9.

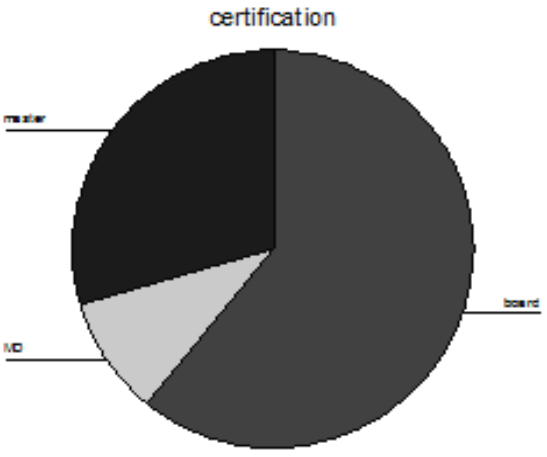

Figure 1. Pediatricians according to their certifications

The proportion of pediatricians who checked TSB levels for all neonates prior to their discharge from the hospital was (73.9%) while those who checked it in the visits after discharge represented 83.4% (Table 1). Pediatricians in the private practice showed more TSB testing activity prior to the neonate's discharge as compared to pediatricians in the public practice although the difference was not statistically significant. Most of the participating pediatricians (80%) recognized post discharge follow up of the jaundiced neonates as a valuable procedure.

Table 1. Pediatricians who check TSB at the first visit after discharge

	Percent	Frequency
yes	83.4	128
No	16.6	25
Total	100.0	153

In conditions where the infant's mother had called the doctor for advice regarding the baby's jaundice, the majority of pediatricians asked the mother to bring the infant to the office and only (11.8%) had referred the infant directly to a laboratory in order to obtain a TSB serum level. Only15.5% of the pediatricians recommended the mother to stop breastfeeding during neonatal hyperbilirubinemia as a procedure that lowers the level of bilirubin (Figure 2).

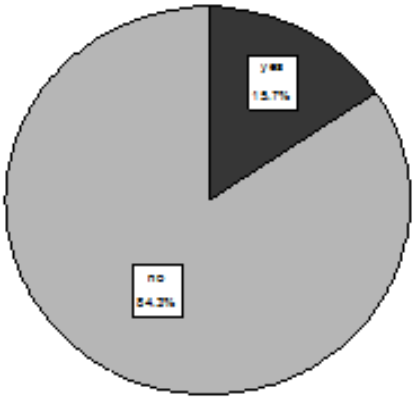

Figure 2. Pediatricians' practice regarding stopping breast feeding according to their certifications

A large proportion of the respondents (61.8%) used the cephalo-caudal progression to estimate the severity of jaundice (sometimes) in their practice (Table 2).

Table 2. Using cephalo-caudal progression by pediatricians

	Percent	Frequency
always	32.9	50
sometimes	61.8	95
never	5.3	8
Total	100.0	153

About (66.7%) of the pediatricians used phototherapy at lower levels of serum bilirubin than the recommended by the AAP guidelines at age of 24-48 hr which is ≥ 15 mg/dl (Table 3). Initiation of phototherapy at levels lower than that advised by AAP at age 49-72hr (≥18 mg/dl), was practiced by (58.6%) of pediatricians.

Exchange transfusions at serum TSB levels lower than that recommended by the AAP guidelines at the age of 24–48 hr (≥20 mg/dl), 49–72 hr (≥25 mg/dl), and >72 hours (≥25 mg/dl) were reported by 57.6%, 59.2%, and 14.5% of pediatricians respectively. The mean level of serum bilirubin at which the pediatricians initiate phototherapy at the age of 24- 48 hrs was lower in the private hospital (13.5mg/dl) compared with (14.6 mg/dl) in community hospitals although the difference was not statistically significant at P<0.05. While the difference was statistically significant regarding initiation of blood transfusion at the age of 24-48 hrs (16.25mg/dl) compared with the community pediatricians (20.4 mg/dl) (Table 3).

Table 3. Management practices among pediatricians according to the site of work

	Type of practice	Mean	Std. Deviation	Std. Error	Sig. (2-tailed)
Level at which phototherapy was initiated	Community hospital	14.6477	2.21821	.23646	.280
	Private hospital	13.5000	1.73205	.86603	
Level at which exchange transfusion was initiated	Community hospital	20.4091	3.73747	.39842	*.000
	Private hospital	16.2500	.95743	.47871	

* Statistically significant at P<0.05

The majority of pediatricians (93.5%) trusted "to a very high or moderate degree" that jaundice existing in the first 24 hours as well as Rh /ABO incompatibility were noteworthy risk factors. More than 65% of the pediatricians declared that they do not consider gestational age between 37–38 weeks as well as jaundice appearing following release from the hospital as risky for the consequences of neonatal jaundice.

A large section of pediatricians (60.8%) believed that a serum TSB more than 20 mg/dl was a significant risk factor for the development of kernicterus, and a higher proportion of pediatricians (86.8%) regarded bilirubin >30 mg/dl as a risk factor for kernicterus. More than (68%) of pediatricians believed kernicterus as an important public health problem (Table 4).

Table 4. Belief of pediatricians regarding kernicterus as a public health

	Percent	Frequency
To a small degree	19.2	29
To a moderate degree	22.0	35
To a very high degree	58.8	89
Total	100.0	153

4. Discussion

The results of this study showed a general consistency of the beliefs and practices of Yemeni pediatricians in Sana'a with the 2004 AAP recommendations for the management of neonatal hyperbilirubinemia (2).

Routine pre-discharge bilirubin test for jaundiced neonates was not regarded as an important step by most of our pediatricians. Ignoring such a step may play a part in the development of the complications of neonatal jaundice especially in our country where early postnatal discharge (before 72 hrs of age) is the rule, both in public and private practice (12). A claim is present to make pre-discharge bilirubin check as a routine (11). A study was done by Suresh and Clark and they concluded that setting pre-discharge bilirubin test for all neonate as a custom is a worthy, although costly procedure (13,14). It was demonstrated by Bhutani et.al, in their surveillance that this practice had lowered the

occurrence of dangerous hyperbilirubinemia (serum bilirubin>25mg/dl) to 1 in 15000 newborns compared with the definite incidence of 1 in 625 (16). Concerning the decision of initiating phototherapy in neonates, a bulky fraction of the pediatricians in this study reported lower serum TSB levels than those recommended by the AAP. Similar trend had been reported by Gartner et.al, in his survey in 1992 (13). Although the strategy of early intervention with phototherapy at low TSB levels had accomplished a significant reduction of serum bilirubin especially during the first 48 hours of life, the clinical advantage was not proved (17). Nevertheless, such a practice by the pediatricians may indicate their realization of this dangerous sign and its serious consequences.

Greater part of the respondents used cephalo-caudal progression of jaundice as a rapid method of assessment for the severity of jaundice despite the imprecision of this procedure especially in those infants with dark skin. Riskin et al. concluded the unreliability of this method of assessment because it may underestimate existing jaundice leading to erroneous decisions (18).

Most of the pediatricians in our study did not counsel the mother of a jaundiced infant to discontinue breast feeding. This corresponds well with the universal recommendations concerning management of neonatal jaundice despite plenty reports of the well-built involvement of breast-feeding as a risk for neonatal jaundice (7). Proposed mechanisms for these reports include reduced caloric and fluid intake, inhibition of hepatic excretion of bilirubin, and increased enterohepatic circulation which may lead, uncommonly, to kernicterus (19). However, initiating ample breast feeding early in life (8 – 12 times a day) and reducing artificial feeds will result in regular bowel motions and reduced enterohepatic circulation ,and hence, less bilirubin concentrations (20,21).

Most pediatricians in our study reported jaundice within the first 24 hours of life as well as Rh and ABO incompatibility as significant hazardous factors for severe hyperbilirubinemia which corresponds well with the guidelines of the AAP, 2004 (7). However, most pediatricians in our study did not report small gestational age, bruising and cephalhematoma as significant risk factors for the development of severe hyperbilirubinemia which opposes many observations and guidelines (7,22).

5. Conclusion

In bringing to a close, the study had revealed an overall consistency of the practice of our pediatricians with 2004 AAP guidelines. The tendency of our pediatricians to commence phototherapy and exchange transfusion at TSB levels underneath those recommended by the AAP is apparent and understandable as a reflection of their awareness of the importance of preventing severe hyperbilirubinemia and its related harmful complications.

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References

1. Koosha A, Rafizadeh B. Evaluation of neonatal indirect hyperbilirubinaemia at Zanjan Province of Iran in 2001-2003. Singapore Med J. 2007; 48:424-8.
2. American Academy of Pediatrics, Provisional Committee for Quality Improvement and Subcommittee on Hyperbilirubinemia. Practice parameter: management of hyperbilirubinemia in the healthy term newborn. Pediatrics. 1994; 94:558-62.
3. Maisels MJ, Newman TB. Kernicterus in otherwise healthy, breast-fed term newborns. Pediatrics. 1995; 96:730-3.
4. Richard PW,Charles E.A, Vinod KB.Toward Understanding Kernicterus: A Challenge to Improve the Management of Jaundiced Newborns. Pediatrics.2006; 117: 474-85.
5. Newman TB, Maisels MJ. Does hyperbilirubinemia damage the brain of full-term infants? Clin Perinatol. 1990; 17:331-8.
6. American Academy of Pediatrics, Provisional Committee for Quality Improvement and Subcommittee on Hyperbilirubinemia. Practice parameter: management of hyperbilirubinemia in the healthy term infant published correction appears in Pediatrics. 1995; 95:458-61.
7. American Academy of Pediatrics, Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics. 2004; 114:297-316.
8. Ahlfors CE, Wennberg RP. Bilirubin-albumin binding and neonatal jaundice. Semin Perinatol. 2004; 28:334-9.
9. American Academy of Pediatrics Subcommittee on Neonatal Hyperbilirubinemia. Neonatal jaundice and kernicterus. Pediatrics. 2001; 108:763-5.
10. Ebbesen F. Recurrence of kernicterus in term and near-term infants in Denmark. Acta Paediatr. 2000; 89:1213-7.
11. Petrone E, Mansi G, Tosco A, Capasso L, Migliaro F, Umbaldo A et al., Early hospital discharge of the healthy term neonate: the Italian perspective. Minerva Pediatr. 2008; 60:273-6.
12. Straczek H, Vieux R, Hubert C, Miton A, Hascoet JM Early discharge from maternity hospital: what problems to prevent? Arch Pediatr 2008; 15:1076-82.
13. Gartner LM, Herrarias CT, Sebring RH. Practice patterns in neonatal hyperbilirubinemia. Pediatrics. 1998; 101:25–31.
14. Suresh GK, Clark RE. Cost-effectiveness of strategies that are intended to prevent kernicterus in newborn infants. Pediatrics. 2004; 114:917-24.
15. Hosseinpour SS, Gharehbaghi MM. Exchange transfusion in severe hyperbilirubinemia: an experience in northwest Iran. Turk J Pediatr. 2010; 52:367-71.
16. Bhutani VK, Johnson LH, Schwoebel A, Gennaro S. A systems approach for neonatal hyperbilirubinemia in term and near-term newborns. J Obstet Gynecol Neonatal Nurs. 2006; 35:444-55.
17. Yaseen H, Khalaf M, Rashid N, Darwich M. Does prophylactic phototherapy prevent hyperbilirubinemia in neonates with ABO incompatibility and positive Coombs' test? J Perinatol. 2005; 25:590-4.
18. Riskin A, Tamir A, Kugelman A, Hemo M, Bader D Is visual assessment of jaundice reliable as a screening tool to detect significant neonatal hyperbilirubinemia? J Pediatr. 2008; 152:782-7.
19. Gourley GR Breast-feeding, neonatal jaundice and kernicterus. Semin Neonatol. 2002; 7:135-41.
20. Semmekrot BA, de Vries MC, Gerrits GP, van Wieringen PM. Optimal breastfeeding to prevent hyperbilirubinaemia in healthy, term newborns. Ned Tijdschr Geneeskd. 2004; 9148:2016-9.
21. Kuzniewicz MW, Escobar GJ, Wi S, Liljestrand P, McCulloch C, Newman TB Risk factors for severe hyperbilirubinemia among infants with borderline bilirubin levels: a nested case-control study. J Pediatr. 2008; 153:234-40.
22. Manning D, Todd P, Maxwell M, Jane M. Prospective surveillance study of severe hyperbilirubinaemia in the newborn in the UK and Ireland. Arch Dis Child Fetal Neonatal. 2007; 92: 342-6.
23. Petrova A, Mehta R, Birchwood G, Ostfeld B, Hegyi T. Management of neonatal hyperbilirubinemia: Pediatricians' practices and educational needs. BMC Pediatr. 2006; 6:2431- 6.