Garyounis Medical Journal

Official Journal of The Medical Faculties of Garyounis University

P.O. Box 5718

Benghazi
Great Socialist Peoples' Libyan Arab Jamahiriya

The Garyounis Medical Journal is for the time being a half-yearly publication dealing with medical and para-medical sciences. Each volume consists of two parts (published January and July).

Price	Local	Foreign
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Garyounis Medical Journal (GMJ)

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Acknowledgements

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More than six authors:

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2. *Organization as author*

The Cardiac Society of Australia and New Zealand. Clinical exercise stress testing. Safety and performance guidelines. Med J Aust 1996; 164: 282-4.

3. *No author given*

Cancer in South Africa [editorial]. S Afr Med J 1994;84:15.

4. Article not in English

Ryder TE, Haukeland EA, Solhaug JH. Bilateral infrapatellar seneruptur hostidligere frisk kvinne. Tidsskr Nor Laegeforen 1996;116:41-2.

N.B. an English translation should be submitted.

5. *Volume with supplement*

Shen HM, Zhang QF. Risk assessment of nickel carcinogenicity and occupational lung cancer. Environ Health Perspect 1994;102 Suppl 1:275-82.

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Enzensberger W, Fischer PA. Metronome in Parkinson's disease [letter]. Lancet 1996;347:1337.

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4. *Conference proceedings*
   Kimura J, Shibasaki H, editors. Recent advances in clinical neurophysiology. Proceedings of the 10th International Congress of EMG and Clinical Neurophysiology; 1995 Oct 15-19; Kyoto, Japan. Amsterdam: Elsevier: 1996.

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   Kaplan SJ. Post-hospital home health care: the elderly's access and utilization [dissertation]. St. Louis (MO): Washington Univ.; 1995.

6. *Dictionary and similar references*
   Stedman's medical dictionary. 26th ed. Baltimore: Williams & Wilkins; 1995. Apraxia; p. 119-20.

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β-blocker in the Treatment of Heart Failure: From Scientific Evidences to Clinical Practice

(Review Article)

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The burden of heart failure

Heart failure (HF) is a major public health problem. It is the only cardiovascular disease that increase in prevalence in the recent years and it has been projected that HF will be a major cause of morbidity and mortality in the future [1-3].

Heart failure is a frequent disease with an incidence of about 0.3-2% per year among those below the age of 65 years and rising up to 3-13% among those above the age of 65 years [3].

In Europe it had been estimated that there are 5-7 million HF patients at present [4, 5]. The incidence of HF is about 2-3 per 1000 new cases per year in Europe [6, 7]. In USA 400.000 new HF cases are diagnosed per year (a new case every 1-3 minutes) [8, 9].

Heart failure is a debilitating disease causing frequent hospital admission and it is the principle discharge diagnosis in patients above the age of 65 [4, 10]. Heart failure have a very poor prognosis with a mortality

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rate of about 10% per year for mild and moderate HF, rising up to 50%/year for severe cases [11, 12]

Comparing its prognosis with some common malignant diseases, HF has been considered to be one of the most killing diseases (table 1)

Table1: Comparing survival rate of heart failure with some common malignant diseases.

	1 year	2year	3year
Breast cancer	88%	80%	72%
Prostate cancer	75%	64%	55%
Colon cancer	56%	48%	42%
Heart Failure	67%	41%	25%

In order to improve the prognosis of patients with this debilitating and killing disease we need to translate scientific evidences into clinical practice by applying recent evidence-based knowledge, recommendations and guidelines.

Role of adrenergic system in heart failure:

Heart failure is the end stage of a continuum leading from risk factors to ventricular dysfunction.

Excess adrenergic system activation plays an important role in the pathogenesis and progression of heart failure. Adrenergic hormones act directly on the heart through different adrenergic receptors causing various effects (table 2)[13]. Excess long standing adrenergic activation leads to myocardial remodeling, myocyte hypertrophy, abnormal contractile function and myocyte apoptosis ending into cardiac dysfunction and progressive heart failure[14, 15]. On the other hand HF will strongly stimulate the secretion of more adrenergic hormones leading to a vicious circle and worsening HF. In a study of patients with asymptomatic left ventricular dysfunction (NYHA class I), Benedict CR and coworkers [16]demonstrated that plasma level of noradrenaline was the most important

Table 2a. Effects of adrenergic hormones on the heart and the mediated receptors

Response	Receptors
Cardiac myocte growth	β1, β2, α1
Positive inotropic effect	β1, β2, (α1)
Positive chronotropic effect	β1, β2
Myocyte toxicity	β1, β2
Myocyte apoptosis	β1, β2

predictor for mortality (Odds ratio "OR" =2.59), hospitalization or death from HF (OR=2.55) and for the development of symptomatic heart failure (OR=1.88). Moreover, in follow-up studies of patients with symptomatic heart failure, high plasma level of norepinephrin was a strong predictor for mortality in these patients [17, 18].

Accordingly, it has been proposed that blocking the adrenergic receptors (e.g. by β-blocker) might be useful in the management of heart failure via severeal mechanisms: 1. Anti-ischemic effect through reduction of heart rate, blood pressure and myocardial oxygen demand [13, 16-18]. 2. Anti-arrhythmic effect, and also decrease the incidence of sudden death, especially after a myocardial infarction [19]. 3. Prevention of the progression of myocardial dysfunction and remodeling, and even causes regression of remodeling, through their direct effect on myocytes [20]. The Australian-New Zealand Heart Failure Research Collaborative Group [21] performed echocardiography on 123 patients before randomization and at six and 12 months after therapy with a nonselective β-blocker (carvedilol). Compared to placebo, carvedilol reduced left ventricular volumes, increased left ventricular ejection fraction, and prevented left ventricular dilatation. 4. Improvement in renal blood flow and hence increase in diuresis and decrease in fluid retention[22]. 5. Lowering of the level of endothelin, another potent vasoconstrictor that is present in increased concentrations in CHF[23]. Furthermore, the degree of reduction in endothelin levels may correlate with the functional and hemodynamic improvement in these patients.

β-blockers were previously considered to be contraindicated in HF because of their short-term adverse effects. However, studies in

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Sweden in the 1970s [24,25] raised the possibility that long-term therapy of HF patients with these agents might reduce symptoms, improve left ventricular function, increase functional capacity and, more importantly reduce mortality to a significant degree.

β-blockers used in large heart failure trial:

Carvedilol is a non-selective β-blocker and α1-blocker[26]. It is a vasodilator and has ancillary properties including antioxidant effect, improving metabolic parameters, enhancing left ventricle mass regression and reduce remodelling (table 2a)[20, 27].

Metoprolol and Bisoprolol are cardio-selective β1-blocker. They have no intrinsic sympathetic activity. There is no evidence to suggest that cardio-selectivity is associated with more clinical benefits. Furthermore, it seems that the broader the adrenergic blocker the better the cardiac and the metabolic effects and therefore, regression of remodelling and improvement of heart failure.

Table 2b: Pharmacological differences within β-blocker drugs evaluated in large heart failure trials

	β1 Blocker	β2 Blocker	α1 Blocker	ISA	Ancillary effect*
Carvedilol	++	++	++	-	++
Metoprolol	+++	-	-	-	-
Bisoprolol	+++	-	-	-	-
Bucindolol	++	++	-	+	-

*anti-oxidant, anti-endothelin, anti-proliferative, ISA= intrinsic sympathatic activity.

Clinical trials of β--blocker in heart failure:

β-blockers have now been evaluated in more than 20 published placebo-controlled clinical trials[28-36]. All trials enrolled patients with systolic left ventricular dysfunction (ejection fraction less than 45%) who had already been treated with diuretics and an ACE inhibitor, with or without digitalis. These trials recruited many types of patients, including women and the elderly, as well as patients with a wide range of causes and severity of left ventricular dysfunction. Patients with preserved systolic function, low heart rates (less than 65 beats per min),

or low systolic blood pressure (less than 85 mm Hg) were either not recruited or represented a small proportion of the patients participating in these studies.

Major β-blocker clinical trials:

Summary of the major β-blocker trials in heart failure is shown in table 3.

US Carvedilol program trial[29-32] Is a combination of three individual carvedilol studies; which include the Prospective Randomized Evaluation of Carvedilol on Symptoms and Exercise study (PRECISE), the US Carvedilol Heart Failure Study, and the Multicenter Oral Carvedilol Heart Failure Assessment study (MOCHA). The US carvedilol trial was a double-blind controlled study enrolled 1094 patients with mild, moderate and severee HF (NYHA class II-IV). Patients were maintained on digoxin, diuretics, and ACE inhibitors and then randomized to treatment with carvedilol or placebo.

The improvement in mortality in patients treated with carvedilol was so large that the study was terminated prematurely after 25 months of enrolment and when the following were observed:

- A reduction in total mortality (3.2% Vs 7.8%, P= 0.001). This represented a 65% decrease in the risk of death, which was similar regardless of age, sex, HF etiology, ejection fraction, exercise tolerance, systolic blood pressure or heart rate.
- A reduction in death due to progressive heart failure (0.7% Vs 3.3%; P<0.001) and sudden death (1.7 Vs 3.8 percent; P< 0.001).
- A 27 % reduction in the need for hospitalization for any cause and a 38% reduction in hospitalisation for heart failure.
- A 38% increase in event-free survival, i.e. need for hospitalization or death (15.8% Vs 24.6 %, P=0.001).

CIBIS I & II trials [33, 34] (Cardiac Insufficiency Bisoprolol Study I & II): The relationship between the hemodynamic effects of β-blockers and survival was reported by the CIBIS I investigators. Among 557 patients, Bisoprolol significantly reduced heart rate compared to placebo at two months, and the change in heart rate over time had the highest predictive value for survival. At five months, left ventricular fractional shortening significantly increased in the bisoprolol group compared to placebo and changes in this parameter also correlated with improved survival. Thus, β-blocker-induced cardiac effects observed within a

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few months of therapy might identify patients who will have improved long-term survival. Although the CIBIS I trial[33] showed a 20 percent reduction in mortality with bisoprolol compared with placebo, this was not significant because of the small number of patients and events.

CIBIS II[34] randomized 2647 patients with class III or IV HF and an ejection fraction <35 %. After an average follow up of 1.4 years, the trial was prematurely terminated when the following were observed:

- A reduction in the total all-cause mortality with Bisoprolol compared to placebo (11.8% Vs 17.3%); the estimated annual mortality was reduced by 32% with Bisoprolol (8.8% Vs 13.2%, hazard ratio 0.66, P<0.0001).
- The reduction in mortality was independent of severity or cause of HF.
- Sudden cardiac death was reduced by 44% with Bisoprolol (3.6% versus 6.3%, P<0.001).
- Bisoprolol therapy resulted in a 15% reduction in hospital admissions for any cause and a 30 percent reduction in admissions for HF (P<0.0001).

MERIT-HF trial [35]: (Metoprolol Randomized Intervention Trial in HF)

The study randomized 4000 patients with NYHA class III to IV HF who were receiving digoxin, an ACE inhibitor, and a diuretic to extended-release Metoprolol (100 to 200 mg/day) or placebo. This study was prematurely terminated when a 35% reduction in all-cause mortality and all-cause hospitalization were observed in the Metoprolol group at 12 months (P<0.006).

All these studies included small numbers of patients with severe heart failure (NYHA class IV) and a strong evidence of benefit in this subgroup was difficult to establish which highlights the need for large study including this group of patients

COPERNICUS (Carvedilol Prospective Randomized Cumulative Survival) [36]:

The objective of this study was to show the effect of Carvedilol on patients with severe HF (NYHA class IV). It was a randomized double-blind placebo controlled study enrolling 2289 patients with severe HF NYHA IV (EF<25%) to placebo or Carvedilol (initial 3.125mg bid, doubling the dose every 2 weeks until target dose of 25 mg bid was reached, patients received highest tolerated dose). The trial was also prematurely terminated because of:

- Highly significant reduction in all cause mortality (35% reduction, p<0.0001) which was consistent in all patients

subgroups (elderly, women, EF<20%, all aetiology subgroups).

- A reduction in combined death or hospitalization for any reason by 24% (P<0.0001).
- A reduction in combined death or hospitalization for cardiovascular diseases by 27% (P<0.0001).
- A reduction of combined death or hospitalization for heart failure by 31% (P<0.0001).

COPERNICUS established the efficacy of carvedilol in severee heart failure and extended the benefits of this drug; first observed in patients with mild and moderate symptoms; to those with advanced disease. However COPERNICUS does not settle the question as to whether all other β-blockers (other than carvidelol) are effective in patients with severee heart failure which remain to be determined.

This collective experience indicates that long-term treatment with beta-blockers can lessen the symptoms of HF, improve the clinical status of patients, enhance the overall sense of well-being, reduce the risk of death and the combined risk of death or hospitalisation[29-42]. These benefits of beta-blockers were seen in patients with or without coronary artery disease and in patients with or without diabetes. The favourable effects of beta-blockers were also observed in patients already taking ACE inhibitors, which suggests that combined block of two neuro-hormonal systems can produce additive effects.

Clinical implication of β-blocker in heart failure

Translation of this scientific evidence into clinical practice is important to help our patients with HF in order to improve their quality of life and reduce their mortality.

1. Which heart failure patients are candidates for treatment with beta-blockers?

Scientific evidences[29-42] and recent recommendations [43] suggest that β-blockers should be given to all stable patients with NYHA class II, III & IV HF due to systolic left ventricular dysfunction unless contraindicated or patients who can not tolerate the drug. Patients with HF who are hemodynamically unstable or who have acute HF should not receive beta-blockers until being stabilized. Treatments with β-blocker should be given concomitantly with other medications proven to be beneficial for HF such as ACE inhibitors, diuretics, spironolactone and in some cases digoxine.

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Treatment with β-blocker should not be delayed till patient is found to be resistant to treatment with other drugs.

2. Which β-blocker to use?

Carvedilol, Metoprolol and Bisoprolol are the only β-blockers tested in large clinical trials and they are the only drugs recommended for treatment of HF to date. There was a need for large comparison studies to compare the differences between these drugs in the treatment of patients with HF. The ongoing COMET[44, 45] study (Carvedilol or Metoprolol European Trial) was designed to establish the relative benefits of Carvedilol or Metoprolol on survival of patients with different classes of HF. The results of this trial is expected in the year 2003.

3. Doses and Up-titration:

In order to have the benefit of β-blockade the effective doses used in major trials should be the target doses. There is no evidence of decreased mortality with lower doses. Table 4 illustrates the effective starting and target dose of recommended drugs. Treatment with β-blocker should be initiated at a very low dose, followed by gradual increments if lower doses have been well-tolerated [46]. Patients should be monitored closely for changes in vital signs and symptoms during this up-titration period. In addition, because initiation of therapy with a beta-blocker can cause fluid retention [47, 48], concomitant administration of diuretics may be beneficial if signs of fluid retention had developed. Planned increments in the dose of a β-blocker should be delayed until any side effects that observed with lower doses have disappeared. Using such a cautious approach, most patients (approximately 85%) enrolled in clinical trials with beta-blockers were able to tolerate short- and long-term treatment with these drugs and achieved the maximum planned trial dose [49].

4. Complications of Therapy with β-blockers and their management

According to major clinical trials, β-blockers are usually well tolerated and their side effects are rare[49]. *(I) Non cardiac adverse effects:* include the adverse effects on the lung, peripheral blood vessels, glucose tolerance and central nervous system [50]. *(II) Cardiac adverse effects:* they include sinus bradycardia, complete heart block and worsening of heart failure. Despite this

concern, only a minority of patients with stable HF deteriorates after the initiation of β- blocker therapy. Worsening HF was observed in less than 6 % of patients who were treated with Carvedilol[51]. The aim should be to continue with β-blocker and to look for and treat other common treatable reasons and precipitating factors for worsening HF. Abrupt withdrawal of treatment with β-blocker can lead to clinical deterioration and should be avoided.

I. Worsening HF without disease progression: Reasons:

1. Poor compliance: in patients who stop or reduce the treatment and those not sticking to dietary advice.
2. Inappropriate treatment e.g. NSAID or other drugs that cause fluid retention.
3. Co- morbid event such as infection, uncontrolled diabetes mellitus, hypertension or arrhythmia such as atrial fibrillation.

All these should be looked for and treated accordingly. Patient education to prevent recurrence is important. The aim is to continue β-blockers if patient is hemodynamically stable or no significant evidence of disease progression is found..

The dose of β-blocker could be reduced or temporary withdrawn if there is evidences of hypoperfusion such as hypo. ion or renal dysfunction and then to be re-uptitrate β-blocker after stabilization.

II. Worsening HF with disease progression

The continuation of B-blockers depends on the clinical status of the patient, whether he has mild or severee low output state.

1. Disease progression with mild output state: treat the precipitating factors if correctable e.g. AF (rate control, cardioversion), infection, ischemia (drugs, revascularization) and uncontroled diabetes mellitus. The aim is to continue with β-blocker, possibly lower dose may be needed, then re-uptitrate after stabilization.

2- Disease progression with evidence of severe low output state: unstable patient with hypotension, increased blood urea and creatinine or evidence of hepatic dysfunction.

ACE inhibitors may be discontinued to reduce progression of renal failure. If there are no immediate correctable precipitating factors, discontinue β-blocker completely and consider inotropic therapy

and other supportive management. After patient stabilization, try re-uptitrate β-blocker and may consider switching to Carvedilol. Inability to reintroduce β-blocker indicates poor outcome.

Summary

There is a strong evidence based research data supporting the benefits of β-blockers in the treatment of patients with HF. Over 13,000 HF patients evaluated in around 29 placebo-controlled clinical trials, all showed a consistent improvement in cardiac function, symptoms and clinical status, decrease in all-cause mortality by 30–35% ($p<0.0001$) and a consistent decrease in combined risk of death and hospitalisation by 25–30% ($p<0.0001$).

Although it is estimated that 85% of HF patients would benefit from β-blockers, only few patients (in some places about 5%) are actually receiving these medications. A strong offer is needed from all physicians treating such patients to apply recent evidence based recommendations and guidelines in order to improve their patients life quality and prognosis.

Table 3: Major β-blocker trials in Heart failure (Adopted from reference 52)

Study	Drug	Patients (NYHA class)	N		Follow-up (months)	Death(%)		Risk Change %	P
			Placebo	β-blocker		Placebo	β-blocker		
US Carvedilol	Carvedilol	**II, III**, *IV*	398	696	6.5	31(7.8)	22(3.2)	-65	< 0.0001
CIBIS-II	Bisoprolol	**III**, *IV*	1320	1327	16	228(17)	156(12)	-32	< 0.0001
MERIT-HF	Metoprolol	**II, III**, *IV*	2001	1990	12	217(10.8)	145(7.3)	-35	0.0062
COPERNICUS	Carvedilol	**IV**	1133	1156	15-45	190(8.8)	130(11)	-35	0.00014

Table 4: β-blockade dose titration scheme

Drug	First dose {mg}	Titration Scheme {mg}									Target dose {mg}
		Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7	Wk8-11	Wk12-13	
Carvedilol	3.125	6.25		12.5		25		50			50
Metoprolol	12.5	25	50		75		100	150	200		200
Bisoprolol	1.25		2.5	3.75	5				7.5	10	10

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REFERENCES

1.O'Connell JB, Bristow M. Economic impact of heart failure in the United States: time for a different approach. J Heart Lung Transplant 1993;13:S107-S112.

2. Kannel WB, Belanger AJ. Epidemiology of heart failure. Am Heart J 1991;121:951-7.

3. Kannel WB. Epidemiology and prevention of cardiac failure: Framingham Study insights.
Eur Heart J 1987;8(suppl F):23-6.

4. Measuring the burden of cardiovascular diseases in Europe: steps toward establishing complete data. The Hauge. The Netherlands Heart Foundation, 1995

5. Parameshawar J, Poole Wilson PA, Sutton GC. Heart failure in a district general hospital. J Roy Coll Phys Lond 1992;26:139-42

6. Cowie MR, Wood DA, Coats A, et al. Incidence and etiology of heart failure in the general population. Heart 1997; 77(suppl.):P7

7. Mair FS, Crowley TS, Bundred PE. Prevalence, aetiology and management of heart failure in general practice. Br J Gen Pract 1996;42:287-9.

8. Massie BM, Shah NB. Evolving trends in the epidemiologic factors of heart failure: rationale for preventive strategies and comprehensive disease management. Am Heart J 1997;133:703-12.

9. Rodeheffer RJ, Jacobsen SJ, Gersh BJ, et al. The incidence and prevalence of congestive heart failure in Rochester, Minnesota. Mayo Clin Proc 1993;68:1143-50

10. Haldeman GA, Croft JB, Giles WH, Rashidee A. Hospitalization of patients with heart failure: National Hospital Discharge Survey, 1985 to 1995. Am Heart J 1999;137:352-60.

11. McKee PA, Castelli WP, McNamara PM, Kannel WB. The natural history of congestive heart failure: The Framingham Heart Study. N Engl J Med 1971;285: 1441-46

12.Ho KKL, Anderson KM, Kannel WB, Grossman W, Levy D. Survival after the onset of congestive heart failure in Framingham Heart Study Subjects. Circulation 1993;88:107-15

13. Bristow MR, Hershberger RE, Prt JD, etal. B-Adrenergic pathway in nonfaling and failing human ventricular myocardium. Circulation 1990;82(Suppl I):I-12-25

14. Mann, DL, Kent, RL, Parsons, B, et al. Adrenergic effects on the biology of the adult mammalian cardiocyte. Circulation 1992; 85:790.

15. Communal C, Singh K, Pimentel DR, Colucci WS. Norepinephrine stimulates apoptosis in adult rat ventricular myocytes by activation of the beta-adrenergic pathway. Circulation 1998;98:1329-34.

16. Benedict CR, Weiner DH, Johanstone DE, Bourassa MG, et al. Comperative neurohrmonal responses in patients with preserved and impaired left ventricular ejection fraction: results of the studies of the left ventricular dysfunction (SOLVD) registery. The SOLVD investigators. J Am Coll Cardiol 1993; 22(Suppl 4A):146A-153A.

17. Francis GS, Cohn JN, Johanson G, Rector TS, Goldmnan S, Simon A. Plasma norepinephrine, plasma renin activity and congestive heart failure. Relation to survival and the effects of therapy in V-HeFT-II. The V-HeFT cooperative studies group. Circulation 1993;87(Suppl 6) VI40-48.

18. Cohn, JN, Levine, B, Olivari, MT, et al. Plasma norepinephrine as a guide to prognosis in patients

with chronic congestive heart failure. N Engl J Med 1984; 311:819.

19. Billman, GE, Castillo, LC, Hensley. J, et al. ß2-adrenergic receptor antagonists protect against ventricular fibrillation: In vivo and in vitro evidence for enhanced sensitivity to ß2-adrenergic stimulation in animals susceptible to sudden death. Circulation 1997; 96:1914.

20. Lowes, BD, Gill, EA, Abraham, WT, et al. Effects of carvedilol on left ventricular mass, chamber geometry, and mitral regurgitation in chronic heart failure. Am J Cardiol 1999; 83:1201.

21. Australia/New Zealand Heart Failure Research Collaborative Group. Randomised, placebo-controlled trial of carvedilol in patients with congestive heart failure due to ischaemic heart disease. Lancet 1997; 349:375.

22. Epstein SE, Braunwald E. The effect of beta adrenergic blockade on patterns of urinary sodium excretion: studies in normal subjects and in patients with heart disease. Ann Intern Med 1966;65:20-7.

23. Krum, H, Gu, A, Wilshire-Clement, M, et al. Changes in plasma endothelin-1 levels reflect clinical response to ß-blockade in chronic heart failure. Am Heart J 1996; 131:337.

24. Waagstein, F, Hjalmarson, A, Varnauskas E, et al. Effect of chronic beta-adrenergic receptor blockade in congestive cardiomyopathy. Br Heart J 1975; 37:1022.

25. Swedberg, K, Waagstein, F, Hjalmarson, A, Wallentin, I. Prolongation of survival in congestive cardiomyopathy by beta receptor blockade. Lancet 1979; 1:1374.

26. Frishman WH, nalamati J. Carvedilol: A new alpha-and beta-adrenergic blocker for heart failure and hypertension. N Engl J Med 1997.

27. Yue TL, Lysko PG, Barone FC, Gu JL, et al Carvidolol; a new anti-hypertensive with unique antioxident activity:potential role in cerebroprotection. Ann NY Acad Sci 1994; 738:230-242.

28. Lechat P, Packer M, Chalon S, Cucherat M, Arab T, Boissel JP. Clinical effects of beta-adrenergic blockade in chronic heart failure: a meta-analysis of double-blind, placebo-controlled, randomized trials. Circulation 1998;98:1184-91.

29. Packer M, Bristow MR, Cohn JN, et al. The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. U.S. Carvedilol Heart Failure Study Group. N Engl J Med 1996;334:1349-55.

30. Packer, M, Colucci, WS, Sackner-Bernstein, JD, et al. Double-blind, placebo-controlled study of the effects of carvedilol in patients with moderate to severe heart failure. The PRECISE trial. Circulation 1996; 94:2793

31. Packer M, Coats AJ, Fowler MB, et al. Effect of carvedilol on survival in severee chronic heart failure. N Engl J Med 2001;344:1651-8.

32. Colucci WS, Packer M, Bristow MR, et al. Carvedilol inhibits clinical progression in patients with mild symptoms of heart failure. US Carvedilol Heart Failure Study Group. Circulation 1996;94:2800-6.

33. Lechat, P, Escolano, S, Golmard, JL, et al. Prognostic value of bisoprolol-induced hemodynamic effects in heart failure during the Cardiac

Insufficiency Bisoprolol study (CIBIS). Circulation 1997; 96:2197.

34. CIBIS-II Investigators and Committees. The Cardiac Insufficiency Bisoprolol Study II (CIBIS-II): A randomised trial. Lancet 1999; 353:9.

35. Hjalmarson A, Goldstein S, Fagerberg B, et al. Effects of controlled-release metoprolol on total mortality, hospitalization, and well-being in patients with heart failure. The Metoprolol CR/XL randomized International Trial in Congestive Heart Failure (MERIT-HF). JAMA 2000;283:1295-302

36. Packer M, Fowler M, Rouleau J, et al. COPERNICUS (Carvedilol Prospective Randomized Cumulative Survival Trial). A multicenter randomized double-blind, placebo-controlled study to determine the effect of carvidolo on mortality in severe congestive heart failure. Cardiovasc Drugs Ther 1999;13:24

37. Bristow, MR, Gilbert, EM, Abraham, WT, et al. Carvedilol produces dose-related improvements in left ventricular function and survival in subjects with chronic heart failure. Circulation 1996; 94:2807.

38. Fisher ML, Gottlieb SS, Plotnick GD, et al. Beneficial effects of metoprolol in heart failure associated with coronary artery disease: a randomized trial. J Am Coll Cardiol 1994;23:943-50.

39. Metra M, Nardi M, Giubbini R, Dei CL. Effects of short- and long-term carvedilol administration on rest and exercise hemodynamic variables, exercise capacity and clinical conditions in patients with idiopathic dilated cardiomyopathy. J Am Coll Cardiol 1994;24:1678-87.

40. Olsen SL, Gilbert EM, Renlund DG, Taylor DO, Yanowitz FD, Bristow MR. Carvedilol improves left ventricular function and symptoms in chronic heart failure: a double-blind randomized study. J Am Coll Cardiol 1995;25:1225-31.

41. Krum H, Sackner-Bernstein JD, Goldsmith RL, et al. Double-blind, placebo-controlled study of the long-term efficacy of carvedilol in patients with severee chronic heart failure. Circulation 1995;92:1499-506.

42. Waagstein F, Bristow MR, Swedberg K, et al. Beneficial effects of metoprolol in idiopathic dilated cardiomyopathy. Metoprolol in Dilated Cardiomyopathy (MDC) Trial Study Group. Lancet 1993;342:1441-6.

43. AHA/ACC Guidelines for evaluation and management of chronic heart failure in the adulth 2001 American heart association web site, www.AHA.org.

44.Poole-Wilson P, Remme WJ. COMMET A multicenter randomised double-blind study to compare the effect of carvidolol and metoprolol on mortlaity and morbidity in patients with moderate or severe congestive heart failure (NYHA class II-IV). Cardiovasc Drugs Ther 1999;13:24

45. Poole-Wilson PA, Celand JG, Dilenarde A, Komajda M, Metra M. Rationale and design of the Carvedilol or metoprolo European trial in patient with chronic heart failure (COMET). Eur J Heart fail. 2002;4(3):321-9.

46. Eichhorn, EJ, Bristow, MR. Practical guidelines for initiation of beta-adrenergic blockade in patients with chronic heart failure. Am J Cardiol 1997; 79:794.

47. Epstein SE, Braunwald E. The effect of beta adrenergic blockade on patterns of urinary sodium excretion: studies in normal subjects and in patients with heart disease. Ann Intern Med 1966;65:20-7.

48. Weil JV, Chidsey CA. Plasma volume expansion resulting from interference with adrenergic function in normal man. Circulation 1968;37:54-61.

Garyounis Medical Journal. Vol.20 No.1,pp.1-16.

49. Macdonald, PS, Keogh, AM, Aboyoun, CL, et al. Tolerability and efficacy of carvedilol in patients with New York Heart Association class IV heart failure. J Am Coll Cardiol 1999; 33:924.

50. Gaffney TE, Braunwald E. Importance of the adrenergic nervous system in the support of circulatory function in patients with congestive heart failure. Am J Med 2000;34:320-4.

51. Waagstein F, Caidahl K, Wallentin I, Bergh CH, Hjalmarson A. Long-term beta-blockade in dilated cardiomyopathy: effects of short- and long-term metoprolol treatment followed by withdrawal and readministration of metoprolol. Circulation 1989;80:551-63.

Breast cancer: the nature of the genetic defect.

(Review Article)

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Introduction:

Breast cancer is now one of the commonly encountered human malignancies representing a considerable share of all incident malignancies in Libya.

Currently about one million ladies worldwide are diagnosed with breast cancer every year[1].An increasing number of investigators have concentrated on studying the genetic basis of this disease because of the magnitude of the public health problem and the need to reduce the impact of the disease on the affected patients.

The aim of this review is to describe the susceptibility genes for inherited breast cancer and characterize some genes which are somatically altered in sporadic breast cancer and the biochemical derangements that follow these alterations.

Interactions between genetic and non-genetic factors lead to the complexity and heterogeneity of breast cancer disease. It has long been recognized that a positive family history of breast cancer is a significant risk factor. In a study done by Williams and Anderson in 1984 on 200 Danish pedigrees which involved the examination of the segregation pattern of breast cancer in families, a model for an autosomal dominant breast cancer susceptibility gene was first provided[2]. Subsequently Newman and colleagues provided evidence for autosomal transmission in high- risk families in support for the

model proposed by Williams and Anderson[3].

The isolation of the first breast cancer susceptibility gene (BRCA1) in 1994 by Miki and colleagues provided a proof of correctness of speculations made by previous researchers[4]. A second breast cancer susceptibility gene (BRCA2) has been identified by Wooster and colleagues in 1995[5]. A third breast cancer susceptibility gene (BRCA3) is currently being actively investigated.

BRCA1 gene

The BRCA1 gene has been mapped to chromosome 17q21 using genetic linkage analysis[6]. BRCA1 gene is composed of 24 exons which cover approximately 100kb of genomic sequence. Most of these exons have the size of 100-500 bp except exon 11which has a size of around 3500 bp and constitutes about 60% of the coding region of the gene. There exists only one copy of the BRCA1 gene in the human genome, based on the detection of a single band after Southern blotting of human genomic DNA. However, Northern blotting with a BRCA1 fragment as a probe revealed the presence of several splice variants with no known functional significance[7].

A sequence of 1345 bp of genomic DNA has been found proximal to the BRCA1 transcription start site[8]. This 1345 bp region contained the promoter for the BRCA1 gene and contains a binding element for transcription factors such as P53.

The BRCA1 gene has been found to be responsive for estrogen and progesterone hormones as evidenced by the increased BRCA1 RNA levels and proteins levels in both human cell lines and animal models[9,10]. The BRCA1 protein has been shown to be located in the nucleus as evidenced by the presence of a nuclear localization signal in exon 11 of the gene using monoclonal antibody-based immunofluorescent studies[11]. It has been demonstrated that BRCA1 may act as a tumour suppressor when its decreased expression has been shown to accelerate growth in sporadic breast cancer[12]. Moreover, the use of anti-sense RNA to BRCA1 gene has been shown to significantly increase the rate of growth in non-tumourigenic NIH-3T3 cell lines[13].

A further support to the hypothesis that BRCA1 functions as a tumour suppressor was provided by the demonstration by Holt and his colleagues that the development of MCF-7 tumors in nude mice was inhibited in the presence of wild type BRCA1 but not the mutant BRCA1[14]. It has been suggested that BRCA1 may play a role in the regulation of apoptosis in serum-starved cells[14]. Gudas and his colleagues demonstrated that BRCA1 RNA levels increased in parallel with cyclin-A RNA levels in response to treatment of breast cancer cell lines with estrogen hormone, suggesting a connection between BRCA1 and cell cycle regulation[9]. Subsequently, BRCA1 was shown to be bound and phosphorylated by cyclin D, A and cyclin-dependent kinases 2 and that BRCA1 RNA levels were highest in growth factor-treated breast cancer cell lines and lowest after growth factor withdrawal[15]. It has been shown by Vaughan and colleagues that BRCA1 RNA and protein levels were highest particularly at the G1/S boundary suggesting a possible role of BRCA1 in cell cycle checkpoint control[16].

BRCA1 may play a role in cell proliferation in breast epithelial tissues as suggested by increased BRCA1 RNA levels in developing embryonic tissues and lactating mammary glands in experiments carried out by Marquis and his colleagues[10].

BRCA2 gene

The existence of at least one additional cancer susceptibility gene, in addition to BRCA1, was suspected when 22 families with early onset breast cancer were analyzed for BRCA1 mutations using linkage analysis and only in a small percentage of them the cancer was associated with germline mutations in BRCA1 gene[17].

In subsequent studies, a linkage between polymorphic genetic markers on chromosome 13q12-13 and early onset cancer phenotype was identified and this lead to the localization of the second breast cancer susceptibility gene BRCA2 to chromosome 13q12-13. Mutations in BRCA2 have been

found to carry a lifetime breast cancer risk of 85% by Wooster and his colleagues[5]. Wooster and his colleagues published the complete cDNA sequence of BRCA2[18,19]. Mutations in BRCA2 have been shown to span the entire coding region of the gene and most of them are the truncating type except for one deletion mutation in which 126 bp region is deleted from exon 23 without causing frame-shift but the resulting protein is dysfunctional[20].

Sporadic breast cancer:

A great majority of breast cancer does not arise due to mutations in breast cancer susceptibility genes. This group of non-inherited breast cancer shows a fundamental molecular genetic difference from the inherited one. The genetic instability, which is inherent in cancer cells result from genetic alterations such as mutations, deletions, and amplifications. Tumour suppressor genes undergo two types of inactivating mutations at different stages of tumorigenesis. One type of inactivating mutation is germ-line mutation occurring in one allele in all cells as suggested by the two hit hypothesis of Knudson[21]. The second type of inactivating mutation occurs as a somatic event in the wild-type allele of mutated tumour suppressor gene. This phenomenon is known as loss of heterozygosity and it makes the development of cancer a much more common event than is seen in individuals born without germ-line mutations in tumour suppressor genes. The type of inactivating mutations in the wild type alleles of tumour suppressor genes that result in loss of heterozygosity is a physical deletion of large regions of chromosome.

Growth factor receptor genes:

The growth factor receptor genes comprise a family of genes that share an extensive homology. They encode membrane proteins with tyrosine kinase activity. The epidermal growth factor receptor (EGFR) gene is the most extensively studied member of this family of protoncogenes and is known to be expressed in over 40% of breast cancers[22,23]. Overexpression of EGFR, erbB2, and fibroblast growth factor receptor (FGFR) genes at mRNA levels have been shown to cause aberration in signal transduction pathway and deregulation of cellular proliferation[22,24,25].

Intracellular signaling:

Malignant transformation results mainly from over expression of mediators of intracellular signaling. Mutations in H-ras oncogenes occur in less than 10% of breast cancers and are associated with increased expression of mediators of intracellular signaling such as receptor tyrosine kinase[26].

Cell cycle regulation:

Derangement's in the protein mechanism that normally regulate the cell cycle greatly contribute to cancer development[27]. The P53 gene encodes a protein with both a DNA-binding and a transcriptional activation domain. It has been established that P53 plays a central role in regulating progression through the cell cycle. Mutations in P53 have been associated with increased risk of breast cancer[28]. About 15-45% of cases of breast cancer specimens studied showed mutations in P53 gene[29]. The retinoblastoma gene (RB) gene encodes a phosphoprotein involved with the regulation of cell cycle progression. Structural rearrangement and inactivation of RB protein have been observed in breast cancer[30]. The evidence for the role of RB protein in breast cancer was provided by Wang and his colleagues when they demonstrated that wild type RB gene introduced into breast cancer cells with RB gene mutation reduced tumourigenicity in experimental animals[31]. Cyclins and cyclin-dependent kinases (CDK) accumulate at different stages of the cell cycle. Activation of CDK occur on binding with specific cyclins and this in turn phosphorylates and activates downstream target proteins which are required to drive the cell into the next stage of the cell cycle[32]. Overproduction of cyclins particularly D1 and CDK has been observed in 45% of breast cancer in a study carried out by Buckley and his colleagues[33]. Transforming growth factor β(TGF-β) is a growth inhibitory protein that is believed to exert its action by inhibiting progression of the cell cycle. TGF-B binds to a cell surface TGF receptor resulting in down-regulation of cyclins and CDK expression and

therefore accumulation of hypophosphorylated RB protein resulting in cell cycle arrest at the G/S border[34]. Apoptosis is a genetically programmed energy-requiring process of cell death that is triggered by several factors including DNA damage, withdrawal of growth factors and expression of P53 gene products. The bc-12 gene encodes a protein which functions to inhibit apoptosis in a variety of tissues. Joensu and his colleagues found that bc-12 is over expressed in 45% of breast cancers[35]. Increased bc-12 levels has not been shown to be due to any genetic alterations but it could be hormone-dependent as high levels have been observed in cancers with estrogen receptors[36].

Conclusion:

Advances in molecular genetic have provided information that allows the understanding of breast cancer susceptibility in both its inherited and sporadic forms. This information would in the future allow estimation of the risk of developing breast cancer. Information gathered on the molecular characteristics of breast cancer is expected to benefit patients and eventually prevent the development of the disease.

The understanding of the role of growth factors receptors and their biologic and immunologic behavior would allow the development of specific vaccines.

Work is currently under way in many laboratories to try and develop ErbB2-based vaccines aimed at developing antibody-mediated anti-tumour effects[37].

References:

1. Kelsey JL, Horn-Ross PL: Breast cancer: Magnitude of the problem and descriptive epidemiology. Epidemiol Rev 1993; 15:7-16.
2. Williams WR, Anderson DE, Genetic epidemiology of breast cancer. Segregation and analysis of 200 Danish pedigrees. Genet Epidemiol 1984; 1:7-20.
3. Newman B, Austin MA, Lee MA, King MC: Inheritance of breast cancer. Evidence of autosomal dominant transmission in high risk families. Proc Natl Acad Sci USA 1988; 85: 3044.
4. Miki Y, Swensew J, Shattuck - Eidens D, Futreal A, Harshman K, Tavtigian S, et al: A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 1994; 266: 66-71.
5. Wooster R, Bignell G, Lancaster J, et al. Identification of the breast cancer

susceptibility gene BRCA2. Nature 1995;378: 789-792.

6. Simard J, Feunteun J, Lenior G, Tonin P, Normand T, et al: Genetic mapping of the breast-ovarian cancer syndrome to a small interval of chromosome 127q12-21: Exclusion of the candidate genes EDH1 7B2 and RARA. Human Molecular genet 1993;2: 1193-1199.

7. Wilson CA, et al: Deferential subcellular localization, expression and biological toxicity of BRCA1 and the splice variant BRCA1-delta 11b. Oncogene 1997;14: 1-16.

8. Brown MA, XU CF, Nicoli H, Griffiths B, Chambers JA, Black D, Solomon E: the 5' end of the BRCA1 gene lies within a duplicated region of human chromosome 17q21. Cancer Res 1996;12: 2507-2513.

9. Gudas JM, Ngyyen H, Li T, Cowan KH: Hormone- dependent regulation of BCRA1 in human cancer cells. Cancer Res 1995;55: 4561-4565.

10. Marquis St, Rajan JV, Wynshaw-Boris A et al. The development pattern of BCRA1 expression and modulation by ovarian hormones implies a role in differentiation of the breast and other tissues. Nat Genet 1995; 11:17-26.

11. Thakur S, Zhang HB, Peng Y, et al. Localization of BRCA1 and a splice variant identifies the neclear localization signal. Mol Cell Biol 1997; 17: 444-452.

12. Thompson ME, Jensen RA, Obermiller PS, et al. Decreased expression of BRCA1 accelerates growth and is often present during sporadic breast cancer progression. Nat Genet 1995;9: 444-450.

13. Rao VN, Shao N, Ahmad M et al. Antisense RNA to the putative tumour suppressor gene BRCA1 transforms mouse fibroblasts. Oncogene 1996;12: 523-528.

14. Holt Jt, Thompson ME, Szabo C, et al. Growth retardation and tumour inhibition by BRCA1. Nat Genet 1996;12: 298-302.

15. Gudas JM, Li T, Nguyen H, Jensen D, Rauscher FJ III, Cowan KH: Cell cycle regulation of BCRA1 mRNA in human breast epithelial cells. Cell growth Diff 1996; 7: 717.

16. Vaughan JP, David PL, Jarobe MD, et al. BRCA1A expression is induced before DNA synthesis in both normal and tumour-derived breast cells. Cell Growth Diff 1996;17: 711-715.

17. Stratton MR, Ford D, Neuhausen S, et al. Familial male breast cancer is not linked to the BRCA1 locus chromosome 17q. Nat Genet 1994; 7: 103-107.

18. Wooster R, Neuhausen S, Mangion J, Quirk Y, Ford D, Collins N et al: Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. Science 1994; 265: 2088-2090.

19. Tavtigian SV, Simard J, Romens J, Couch F, Shttuck-Eiedens D, Neuhausen S, et al: The complete BRCA2 gene and mutations in chromosome 13q-linked kindreds. Nat Genet 1996; 12: 333-337.

20. Couch FJ, Farid LM, DeShano ML, Tavtigian SV, Clazone KA, Campeau L, Peng Y, et al: BRCA2 germline mutations in male breast cancer cases and breast cancer families. Nat Genet 1996; 13: 123-125.

21. Knudson AG: Mutations and cancer: Statistical study of retinoblastoma. Prc Natl Sci UAS 1971;68: 820-823.

22. Slamon Dj, Godolphin W, Jones LA, et al: Studies of the Her-2/neu protooncogene in human breast and ovarian cancer. Science 1989; 244: 707-712.

23. Clark GM, McGuire WL: Follow-up study of Her-2/neuamplification in primary breast cancer. Cancer Res 1991; 51: 944-948.

24. Bacus SS, Zelnick CR, Plowman g, Yarden Y: Expression of erb.-2 family of growth factors receptors and their ligands in breast cancer. Am J Clin Pathol 1994; 102: S13-24.

25. Penault-Llorca F, Bertucci F, Adelaide J, et al: Expression of FGF and FGFR genes in human breast cancer. Intl J Cancer 1995;61: 170-176.

26. Conway K, Edmiston S, Fried DB, Hulka BS, Garret PA, Liu ET: Ha-ras rare alleles in breast cancer susceptibility. Breast Cancer Res Treat 1995; 35: 97-104.

27. Marx J: How cells cycle towards cancer. Science 1994; 263: 319-321.

28. Malkin D, LiFP, Strong LC, Fraumeni JF, Nelson CE, Kim HD, et al: Germ line P53 mutations in familial syndrome of breast cancer, sarcoma and other neoplasms. Science 1990; 250: 1233-1238.

29. Saitoh S, Cunningham J, De vries EM, McGovern RM, Sachroeder JJ, Hartman A, et al: P53 gene mutation in breast cancer in mid-western US women: Null as missense- type mutations are associated with poor prognosis. Oncogene 1994;9: 2869-2875.

30. T'ang A, varley JM, Chakraborty S, Murphree AL, Fung YK: Structural rearrangement of the retinoblastoma gene in human breast carcinoma. Science 1988;242:263-266.

31. Wang NP, To H, Lee WH, Lee EY: Tumour suppressor activity in of Rb and P53 genes in human breast carcinoma cells. Oncogene 1993; 8: 279-288.

32. Hunter T, Pines J: Cyclins and cancer: II cyclin D and CDK inhibitors come of age. Cell 1994;79: 573-82.

33. Buckley MF, Sweeney KJF, Hamilton JA, Sini RL, Manning DL, Nicholson R, DeFazio A, et al: Expression and

amplification of cyclin genes in human breast cancer. Oncogene 1993; 8:2127-33.

34. Alexandro MG, Moses HL: Transforming growth factors b and cell cycle regulation. Cancer Res 1995;55: 1452-7.

35. Joensuu H, Pylkanen L, Toikkanen S: bcl-2 protein expression and long term survival in breast cancer. Am J Pathol 1992; 145: 1191.

36. Johnston SRD, McLennan KA, Sacks NPM, Salter J, et al: Modulation of Bc12 and Ki67 expression in estrogen receptor human breast cancer by tamoxifen. Eur J Cancer 1994; 30A: 1663.

37. Wei W, Piechocki M, Pilon S, and Kelly C: Developments in erbB-2DNA vaccines. Biotech. Lab International. January-February: 13, 2000.

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Etiology of Childhood Diarrhoea in Benghazi-Libya

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Abstract

Objectives: To determine the causative agents of diarrhea among children in Benghazi.**Materials&Methods**: Three hundred and fifty six children with acute diarrhea and 100 control children were enrolled in this study at AL-Fateh Children's Hospital, Benghazi, Libya. Standard laboratory techniques were used for the detection of enteropathogens and for antibiotic susceptibility testing.**Results**: Of the patients examined, 145 (40.7%) were found to be infected compared to two (2%) control subjects infected with *Shigella sonnei.* A single enteric pathogen was detected in 85.5% of patients, while multiple pathogens were seen in 14.5%. The prevalence of enteropathogens was as follows: rotavirus (24%), diarrheagenic *Escherichia coli* (by serotyping, 8.5%), salmonella (7.3%), shigella (4.8%) and campylobacter (2.4%). Dehydration was evident in 79% of patients, with 7% affected by severe dehydration. Vomiting and fever were seen in 94% and 75% of patients respectively. Diarrheagenic *E. coli,* salmonella and shigella were highly resistant to most of the antibiotics tested. Although, rotavirus and diarrheagenic *E. coli* were among the main agents causing diarrhea in Libyan children. However, they are not screened for during routine stool examination in public health laboratories. **Conclusion**: these results highlight the importance of modifying the current techniques that are used in the diagnosis of diarrheal disease in our hospitals.

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Introduction:

Diarrhea is a major cause of morbidity and mortality among children in tropical developing countries. 1 Recent studies have estimated that diarrhea is the second leading cause of death in children worldwide accounting for 2.4 to 3.3 million deaths each year.[1,2]

Although gastroenteritis is recognized as a serious public health problem in Libya, but no comprehensive investigations have been carried out so far and there is only limited information on the distribution of some enteric pathogens associated with childhood diarrhea.[3,4] Therefore, this investigation was designed to determine the etiological agents responsible for diarrhea in children in Benghazi, Libya.

Materials and Methods:

The study population included 356 children suffering from acute diarrhea and another 100 children without diarrhea (control group). All patients were treated at AL-Fateh Children's Hospital, Benghazi-Libya (AFCH). The study covered a period of ten months from August 1996 to May 1997. Acute diarrhea was defined as the passage of three or more loose stools or one loose stool combined with fever, vomiting or abdominal pain for less than 24 hrs. The control group consisted of non hospitalized healthy children attending kindergarten. Clinical and demographic data in addition to feeding and medical histories were systematically collected from patients and controls. Children with a history of antibiotic use or diarrhea within the preceding one month were excluded from the study. One stool sample or a rectal swab when deemed suitable was collected from each individual and sent to the microbiology laboratory at AFCH. In order to reduce the chance of hospital acquired infection; all stool samples were collected within 24 hrs of admission to hospital and processed within 3 hrs of collection.

Laboratory Examination:

Stool and rectal swab smears were stained with 1% basic fuchsin and examined microscopically for the

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presence of campylobacter. Occult blood was detected using hemo FEC-kit (Boehringer Mannheim, Company, France). A monoclonal antibody sensitized latex reagent (Slidex Rota-Kit 2, BioMerieux, France) was used for the detection of rotavirus.

In order to isolate enteropathogens, all specimens were plated directly onto the following media: MacConkey agar, Salmonella Shigella (SSA) agar, Thiosulfate-Citrate-Bile Salts-Sucrose agar (TCBS); enriched into Selenite-F-Broth, Alkaline Peptone Water and incubated at 37°C in air for 18-24 hrs. Skirrow's agar plates (Oxoid, Ltd., Basingstoke, England) incubated under microaerophilic conditions were used for the isolation of campylobacter at 42°C for >48 hrs. All isolates were identified by standard methods.[5] Serotyping of salmonella and shigella was performed by slide agglutination test (Difco-Laboratories, USA). Pathogenic *E. coli* were serotyped by the Eurobio Laboratories kit (France) using slide and tube agglutination tests. Antimicrobial susceptibility testing was performed using a disc diffusion technique in accordance with the NCCLS (M100-S8) regulation.[6]

Clinical and numerical data were analyzed using Chi-square (Computer package SPSS version 6.1).

Results:

Of the 356 patients with diarrhea 221(62%) were boys and 135(38%) were girls with a male to female ratio of 1.9:1. Their ages ranged from 1.0 month to 11 years (mean 13.5 months). Approximately, 75% of the positive

cases were recorded among those aged between 1 to 12 months old. The average birth body weight of the patients was 3171g for males and 3086g for females. Most of the infected children (i.e. those with a positive microbiological yield) had a birth body weight between 1500-2500g (P=0.013).

Etiological Agents:

Isolation of an enteric pathogen from patients was possible in 145 (40.7%), of whom 95(65.5%) were males and 50 (34.5%) were females with no significant differences. Overall, 166 different enteric pathogens were detected among these patients. A single pathogen was identified in 85.5% of patients, while the remaining yielded multiple pathogens. Rotavirus plus

diarrheagenic *E. coli* was the most prevalent combination being seen in 38% of cases with mixed infection. All of the enteric agents were recovered throughout the study period with the exception of rotavirus infection, which showed a peak incidence in the cooler months of September to December (P= 0.043). Rotavirus was the most prevalent pathogen in 85(24%) children followed by diarrheagenic *E. coli* (8.5%); non-typhoidal salmonella (7.3%); shigella (4.8%) and campylobacter (2.2%). In comparison, All control subjects were free from enteric bacteria with the exception of two case infected by *Shigella sonnei* (2%). Vibrios, Aeromonas and Yersinia spp. were not detected among any of the two groups studied.

Table 1 shows the distribution of the 166 enteropathogens identified among different age groups of patients. Seventy five percent of the isolates were recovered from children aged 1 to 12 months old. In general, it is clearly demonstrated that younger children (1-18 months old) are more likely to harbor enteropathogens in comparison with older children. Eighty five (51.2%) of these pathogens were rotavirus; of which more than, 90% were seen in those aged 1 to 18 months old. Diarrheagenic *E. coli* was the second most commonly detected pathogen during this study with 30(18%) isolates. Of these, ETEC was detected in 16 cases, whereas, the remaining 14 isolates were EPEC. Serotypes O6, O111, O127a, O166, and O126 accounted for 23 isolates (77%).

Four serogroups of salmonella (A, B, C and F) were detected; with serogroup A being the most commonly recovered group (58%) followed by serogroups C, F and B in a descending order of frequency. Shigella was isolated from older patients aged 4 months to 11 years (mean 3 yrs). Of the 17 isolated shigella spp., 7(41%) were *S. flexneri*; 6(35%) *S. sonnei*; 3(17.7%) *S. dysenteriae* and one isolate as *S. boydii*. More than 50% of patients infected with shigella showed the presence of mucus with or without blood in their stools. Campylobacter spp. was the least frequent 8 (2.2%)

bacterium to be detected as a cause of diarrhea. As in other cases, patients infected with campylobacter presented with vomiting and fever. All exclusively breast fed children were free from campylobacter infection.

As shown in Tables 2 and 3, the majority of infected children experienced vomiting and fevers which were recorded in 94% and 75% of cases respectively. There was a clear association between diarrhea and feeding practices. For instance, about 50% of children with diarrhea were bottle-fed compared with 14% and 11% among exclusively breast fed babies and those on a combination of bottle and breast feeding respectively.

Approximately 80% were dehydrated at the time of admission with 7% suffering from severe dehydration. Rotavirus infections were frequently associated with watery diarrhea and vomiting but not significantly more than that of infections caused by other agents (Table 2). Occult blood was recorded in approximately 37% of infected children.

Antimicrobial Susceptibility:

Antimicrobial resistance as determined by the disc diffusion method revealed that the vast majority of isolated enteric bacteria were highly resistant to most of the antibiotics tested in this study. In comparison, the highest rate of resistance was for salmonella followed by diarrheagenic *E. coli* and Shigella spp. Over 80% of salmonella and diarrheagenic *E. coli* isolates were resistant to ampicillin and >50% were resistant to amoxicillin-clavulanic acid, carbenicillin, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole. Shigella on the other hand, showed a reduced level of resistance towards these antibiotics. Nonetheless, more than 50% of shigella isolates were also resistant to ampicillin, cefoxitin, and trimethoprim-sulfamethoxazole (Table 4).

Discussion:

To our knowledge, this is one of the very few systematic studies that have tackled the role of various enteropathogens in the etiology of childhood diarrhea in Libya. The results have clearly demonstrated that pathogens such as: rotavirus, diarrheagenic *E. coli,* shigella and campylobacter are important etiological agents of community

acquired childhood diarrheal disease that required hospitalization.

Rotavirus was the leading cause with a detection rate of 24%. This is similar to some reports in industrialized countries [7] and some developing countries [8,9] and also consistent with some previous local studies conducted in Benghazi and also in Tripoli in the western part of Libya.[4, 10] A range of 13-55% was also reported among children in a number of different countries in Africa [11]; rotavirus being responsible for about one-fourth of diarrhea cases identified in both hospitalized and outpatients. Surprisingly, rotavirus was not detected among our control subjects and this may be explained by the fact that older children comprised the control group (mean 26.4 months) compared to the patients (13.4 months). Most of rotavirus infections occurred among children less than 18 months of age with a maximum incidence in infants 1-12 months old. This is consistent with a number of hospital based studies carried out in developed and developing countries alike.[12,13]

The findings of diarrheagenic *E. coli* (ETEC & EPEC) as the second most commonly identifiable cause of diarrhea in children in this study is in agreement with previous reported studies worldwide.[14-17] However, further identification of *E.coli* as to its pathogenic mechanisms is required to clarify this situation. The isolation of non-typhoidal salmonella from diarrheal illnesses in Libyan children has been well documented in previous studies.[3,10,18] Salmonellae were responsible for 7.3% of diarrhea episodes in the present study. Similar incidence figures were reported in some parts of the world such as Hong Kong and Saudi Arabia.[19,20] Nonetheless, lower values have also been reported in Brazil, USA, Canada and Korea.[15,21,22] Although, the annual peak incidence of salmonella gastroenteritis is usually in warm months, however, this was not seen in our study as infection by salmonella occurred throughout the study period.

As shigella and campylobacter infections tended to be a serious problem in developing countries[23], they were not a major problem in this

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As shigella and campylobacter infections tended to be a serious problem in developing countries[23], they were not a major problem in this

part of Libya. Shigella spp. was detected in 4.8% of patients and in 2% of the controls without significant differences between the two groups. Similar detection rates were reported in China and Jordan[19,24], however, in neighboring Egypt, a rate of 2.2% was reported.[14] On the other hand, campylobacter was only seen in 2.3% of Libyan children. This is similar to some figures reported among Arab children.[24,25] However, higher detection rates have been reported in other countries, 4.3% in Egypt[14] and 26% in Bangladesh.[26] All of our patients infected by campylobacter were on artificial milk, which may point to the route of infection in this subgroup of patients (Table 3).

In this study salmonella spp. showed the highest rate of antibiotic resistance with 84% of isolates being resistant to ampicillin. Rates of resistance exceeding 50% were also recorded for amoxicillin-clavulanic acid; chloramphenicol and co-trimoxazole.

Table 1: Distribution of enteropathogens among different age groups of patients

	Enteropathogens isolated from 145 Patients No. (%)					
Age (month)	Salmonella	Rotavirus	Shigella	*E.coli*	Campylobacter	Overall (%)
1-6	18 (69.2)	28 (33)	3 (17.6)	17 (56.7)	6 (75)	72 (43.4)
7-12	6 (23.1)	32 (37.6)	6 (35.3)	7 (23.3)	1 (12.5)	52 (31.3)
13-18	0 (0.0)	17 (20)	0 (0.0)	2 (6.7)	1 (12.5)	20 (12.1)
19-24	0 (0.0)	5 (5.9)	2 (11.8)	3 (10.0)	0 (0.0)	10 (6.0)
25-30	0 (0.0)	0 (0.0)	1 (5.8)	0 (0.0)	0 (0.0)	1(0.6)
31-36	0 (0.0)	1 (1.2)	0 (0.0)	1 (3.3)	0 (0.0)	2 (1.2)
>37	2 (7.7)	2 (2.4)	5 (29.4)	0 (0.0)	0 (0.0)	9 (5.4)
Total (%) 166		26 (15.6)	85 (51.2)	17 (10.2)	30 (18.0)	8 (4.8)

Table 2: Frequency distribution of clinical features in relation to different enteropathogens.

Enteropathogens	Clinical Features (% of patients)				
	Vomiting	Fever	Tenesmus	Severe dehydration	Cough
Rotavirus	97	71	21	06	30
E. coli	93	67	17	07	30
Salmonella spp.	89	85	31	12	39
Shigella spp.	88	94	12	06	18
Campylobacter spp.	100	88	0	13	25

Table 3: Distribution of enteropathogens in relation to feeding practices.

Enteropathogens	% Cases with diarrhea			
	Bottle	Breast	Breast & Bottle	Weaning
Rotavirus	46	15	11	28
E. coli	63	13	10	13
Salmonella spp.	61	12	12	15
Shigella spp.	23	18	12	47
Campylobacter spp.	63	0	12	25

Table 4: Antimicrobial resistance of isolated enteropathogens.

Antimicrobial	% Resistance		
	E. coli (n=30)	Salmonella spp. (n=26)	Shigella spp. (n=17)
Ampicillin	80	84	53
Cefoxitin	NT*	84	53
Amoxicillin-clavulanic acid	54	76	40
Tetracycline	63	68	47
Carbenicillin	67	64	40
Cefotaxime	37	60	20
Chloramphenicol	54	65	20
Trimethoprim-sulfamethoxazole	67	52	60
Kanamycin	40	48	20
Gentamicin	33	44	20
Nalidixic acid	03	12	07

*NT= Not tested.

Comparable results were reported for salmonella isolated from children with diarrhea in Tripoli area.[27] Similarly, 80% of diarrheagenic *E. coli* were resistant to ampicillin. Shigella spp. on the other hand, showed a lesser degree of resistance to all of the antibiotics tested in this study, with the exception of trimethoprim-sulfamethoxazole

where 60% of shigellae were resistant to this drug (Table 4). The high resistance rate by these enteropathogens could either be explained by the misuse of antibiotics in the Libyan medical community or to a transfer of genes coding for resistance against these antibiotics, but most probably as a result from a combination of both.

In conclusion, the results of the current study complement the findings of previous investigations. The age of patients, the feeding practices and the birth body weights all contributed to diarrheas in our children. Encouraging exclusive breast-feeding, hand-washing, personal hygiene and development of vaccine against the most common agents of diarrhea may prove to be beneficial in reducing the incidence of these potentially serious infections in young infants and children in Libya. The high rate of antimicrobial resistance needs to be given a serious consideration, since it is likely to have significant clinical implications in patient management, particularly in case of extra-intestinal infections by these microorganisms.

References:

1- Snyder JD, Merinos MH. The magnitude of the global problem of acute diarrhoeal disease: A review of active surveillance data. Bull WHO 1982; 60:605-613.

2- Murray CJ, Lopez AD. Global mortality, disability and the contribution of risk factors: global burden of disease study. Lancet 1997; 349:1436-1442.

3- EL-Nageh MM. Salmonellae isolation from human feces in Tripoli, Libya. Trans Royal SocTrop Med Hyg 1988; 82:324-326.

4- Filipovic D. Acute infectious diarrhea in infants. Garyounis Med J 1980; 3:135-138.

5- WHO. Manual for laboratory investigations of acute enteric infection. CDD/83 Review 1, 1987.

6- National Committee for Clinical Laboratory Standards. Performance standards for antimcicrobial disk susceptibility tests: eighth information supplement. NCCLS document M100S8. Villanova, PA: NCCLS, 1998.

7- Kotloff KI, Wasserman SS, Steciak JY, et al. Acute diarrhea in Baltimore children attending an outpatient clinic. Paed Infect Dis J 1988; 7:753-759.

8- Echeverria P, Taylor DN, Lexsomboon U, et al. Case-control study of endemic diarrhea disease in Thai children. J infect Dis 1989; 159: 543-548.

9- Khalil K, Lindblom GB, Mazhar K, Khan SR, Kajiser B. Early child health in Lahore, Pakistan: VIII Microbiology. Akta Paed 1993; 390: 87-94.

10- Ghenghesh KS, Abeid SS, Bara F, Bukris B. Etiology of childhood diarrhoea in Tripoli-Libya. Jamahiriya Med J 2001; 1:23-29.

11- Cunliffe NA, Kilgore PE, Bresee JS, et al. Epidemiology of Rotavirus diarrhea in Africa:

a review to assess the need for rotavirus immunization. Bull WHO 1998; 76: 525-537.

12- Kapikian AZ, Kim HW, Wyatt RG, et al. Human Reovirus-like agent as the major pathogen associated with "winter" gastroenteritis in hospitalized infants and young children. N Eng J Med 1976; 294: 965-972.

13- EL-Assouli SM, Banjar ZM, Mohamed KA, Zamakhchari FT. Rotavirus infection in children in Saudi Arabia. Am J Trop Med Hyg 1992; 3:272-277.

14- Abou-Attta HNE, Elassal HM, Afify MS. Etiology of acute diarrhea in children: Diarrheagenic Escherichia coli and other infective agents. Egypt J Med Microbiol 1995; 4:187-192.

15- Regua Mangia AH, Duarte AN, Duarte R, Silva LA, Bravo VLR, Leal MC. Etiology of acute diarrhea in hospitalized children in Rio de Janeiro city-Brazil. J Trop Ped 1993; 39: 365-367.

16- Kain KC, Barteluk RL, Kelly MT, et al. Etiology of childhood diarrhea in Beijing China. J Clin Microbiol 1991; 29: 90-95.

17-Ogunsanya TI, Rotimi VO, Adenuga A. A study of the etiological agents of childhood diarrhea in Lagos, Nigeria. J Med Microbiol 1994; 40:10-14.

18- Taher IAA, Tobgi RS. Emergence of multiple-drug resistant salmonella isolated from neonates in Benghazi, Libya. J Bahrain Med Soc 1997; 9:165-167.

19- Ling JM, Cheng AF. Infectious diarrhea in Hong Kong. J Trop Med Hyg 1993; 96: 107-112.

20- Zaman R. Campylobacter enteritis in Saudi Arabia. Epidemiol Infect 1992; 108:51-58.

21- Todd E. Epidemiology of food borne illness: North America. Lancet 1990; 336:788-790.

22- Kim KH, Shu IS, Kim JM, Kim CW, Cho YA. Etiology of childhood diarrhea in Korea. J Clin Miorobiol 1989; 27: 1192-1196.

23-Ebrahim GJ. Shigellosis. J Trop Ped 1991; 37: 98-99.

24- Youssef M, Shurman A, Bougnoux M, Rawashdeh M, Bretagne S, Strockbine N. Bacterial, viral and parasitic enteric pathogens associated with acute diarrhea in hospitalized children from northern Jordan. FEMS Immunol Med Microbiol 2000; 28:257-263.

25- Banajeh SM, Ba-Oun NH, Al-Sanabani RM. Bacterial aetiology and antimicrobial resistance of childhood diarrhoea in Yemen. J Trop Ped 2001; 47:301-303.

26- Hoque SS, Faruque ASG, Mahalanabis D, Hasnat A. Infectious agents causing acute diarrhea in infants and young children in Bangladesh and their public health implications. J Trop Ped 1994; 40:351-354.

27- El-Ghodban A, Ghenghesh KS, Marialigeti K, Abeid S. Serotypes, virulence factors, antibiotic sensitivity, beta-lactamase activity and plasmid analysis of Salmonella from children with diarrhea in Tripoli (Libya). Acta Microbiol Immunol Hungarica 2002; 49: 433-444.

Chromosomal localization of Human Microsomal Epoxide Hydrolase gene

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الملخص

الابوكسيد هيدروليز هو من ضمن الخمائر المسؤلة عن تمثيل المادة المسببة للسرطان المعروفة بالبنزبيرين. يقوم الابوكسيد هيدروليز بتحويل الأكسيدات النشطة الى مواد ثنائية الكحول، وهذا يعتبر عملية تنشيط لهذه لمواد وكذلك طريقة للتخلص من لسموم لمسببة للسرطان. في دراسات سابقة تم تعيين موضع مورثة الابوكسيد هيدروليز على الجسم الصبغي رقم (6) ولكن تقارير تابعة تناقضت معها وتم على ضونها تعيين موضع المورثة على الجسم الصبغي رقم (1) ولهذا كان من الضروري حسم هذا الاختلاف باستخدام طرق اكثر دقة منها فصل وتحليل الحامض النووي من خلايا هجينة فقدت اعدادا عشوائية من الأجسام الصبغية واستخدام سلسلة تفاعلات البلمرة. ولقد اكدت هذه الدراسة ان مورثة الابوكسيد هيدروليز يقع على الجسم الصبغي رقم (1).

Abstract

Objectives :Epoxide hydrolase is an enzyme involved in the metabolism of the carcinogen benzo[a] pyrene. It converts reactive arene oxides into dihydrodiols. This can be considered either as a part of an activation process or as a part of a detoxification process with respect to benzo[a]pyrene metabolism. Initially the human microsomal epoxide hydrolase gene was mapped to chromosome (6) and then a contradicting report mapped it to chromosome (1). Thus it was deemed necessary to confirm its chromosome location. **Materials and methods:** Primers were produced which could amplify a region of the human gene and were then used to test a series of hybrid clones for the presence or absence of the gene. The data obtained was correlated with the presence or absence of each human chromosome **Results and conclusion:** it was found that the human microsomal epoxide hydrolase gene segregated with chromosome (1) markers confirming one of the previous assignments.

Key Words: Epoxide Hydrolase; Hybrid cells; PCR.

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INTRODUCTION

The human microsomal epoxide hydrolase gene was originally assigned to chromosome 6 by Brown and Chalmers[1] in a study based upon indirect immunological evidence. In this study, Brown and Chalmers measured the microsomal epoxide hydrolase activity in human x mouse hybrid cell clones prepared by fusing human cells expressing 6-7 times the activity of the mouse cells. They raised rabbit antihuman and antimouse antisera against the purified microsomal epoxide hydrolase enzyme and used those antisera to discriminate between human and mouse enzymes. They isolated 25 human x mouse hybrid cell clones and tested them for their human chromosome contents using starch gel electrophoresis and then examined them for the expression of the human microsomal epoxide hydrolase enzyme activity. Four of them expressed the highest EH levels of all the cell lines tested and four only expressed levels similar to the mouse parent cells. The human cells exhibited EH activity 6-8 fold higher than the mouse cells bunon of the hybrids expressed more than 2-3 times the level of mouse parents. They found that the antihuman antiserum specifically inhibited 90% of the human enzyme in extracts after overnight incubations and examined both the parent and the hybrid cells with the antisera and were able to correlate the absence of the enzyme activity with the absence of human chromosome 6. Four other hybrid cell clones they examined expressed a lower human enzyme activity and correlated that with the specific retention of human chromosome 19. They concluded that the human gene for the microsomal epoxide hydrolase enzyme activity may be on chromosome 6 and that a possible regulatory element lies on chromosome 19.

In a contrasting report Skoda and co-workers[2] mapped the human microsmal epoxide hydrolase gene to chromosome 1. In their study they used Southern blot analysis of *Eco*R1-digested DNAs isolated from human x rodent somatic cell hybrids segregating human chromosomes. Using a full-length cDNA insert probe, they

detected four hybridizing bands in *Eco*R1 digests of human placental DNA and distinguished them from similar rodent sequences. They found that the four hybridizing bands were either all present or all absent in any human x rodent DNA indicating that all those sequences were present on a single human chromosome. They examined a series of human x rodent somatic cell hybrids and assigned all four hybridizing sequences to the long arm of human chromosome 1 linked to the *arg* protooncogene, peptidase C gene and metallothionine pseudogenes previously assigned to the long arm of human chromosome 1.

The above mentioned studies presented two different assignments of the human microsomal epoxide hydrolase gene. It was decided that a method for directly testing cells for the presence or absence of the gene needed to be employed to determine the correct assignment of the human microsomal epoxide hydrolase gene. PCR was used to analyse human x rodent hybrid cells for the presence or absence of the human microsomal epoxide hydrolase gene.

Materials and Methods

Human A549 cells deficient in hypoxanthine phosphoribsoyltransferase and mouse B82 cells deficient in thymidine kinase were grown separately in D5 medium until they are cofluent.

Human A549 cells were fused to mouse B82 cells to obtain human x mouse cell hybrid cell clones which were subsequently grown in a HAT medium to select against non-fused human and mouse cells. Parent and hybrid clones were grown up in large quantities and cell pellets were frozen prior to analysis. The human chromosome content of the hybrid cells were determined by analysing cell pellet homogenates for the presence of specific isoenzymes previously assigned to each huuman chromosome. Total genomic DNA was isolated from human A459 cells and B82 cells and human x mouse somatic cell hybrids using the chloroform-phenol extraction and used in the PCR reactions to detect the human epoxide hydrolase gene as described below.

The nucleotide sequence of the human microsomal epoxide hydrolase

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cDNA was obtained from GenBank/EMBL Data Bank with accession number Jo3518.

RESULTS

Because the sequence of the mouse microsomal epoxide hydrolase gene was not available for identifying regions of difference between the human and mouse sequences and which would allow the design of PCR primers that amplify fragments that can be used to test for the human gene in human x mouse somatic cell hybrids, it was decided to design a series of PCR primers based on the available cDNA sequence. Initially, a pair of primers named EH1 and EH2 were designed to span a region of 920-1330 bp in the cDNA sequence but these failed to produce a PCR product using either mouse or human DNA under a range of conditions. A second set of primers named EH1 and EH3 were designed to span a region of 920-1115 bp in the cDNA sequence but they also failed to produce a PCR product under a range of conditions.

Primers EH3 and EH4 were designed to span a region of 990-1525 bp and these gave a PCR product of the size 535 bp with either mouse or human DNA. It was decided to find out if the mouse and human products differed in restriction pattern as the cDNA sequence of the gene shows an EcoR1 restriction site which would cut the 535 bp PCR product into 180 bp and 355 bp pieces. PCR products from mouse B82 cells and human A549 cells were incubated with EcoR1 restriction enzyme for one hour as described in the Methods section. Both the incubations resulted in the production of the same fragments which did not help in the process of testing the somatic cell hybrids.

A fourth set of primers was now tested. PCR oligonucleotide primers EH2 and EH3 were used to span a fragment of 990-1330 and after initial tests to optimize conditions they were found to amplify a fragment of the size 340bp from the human microsomal epoxide hydrolase gene and a fragment of size 500bp from the mouse microsomal epoxide hydrolase gene under the same PCR conditions which were:

Denaturation at 95C for one minute.

Annealing at 60C for 1.5 minute.

Extension at 70C for two minutes for 30 cycles.

This pair of primers was used to test human x mouse somatic cell hybrid cell clones for the presence of the human microsomal epoxide hydrolase gene. The human x mouse hybrid cell clones examined consisted of 16 primary clones and 10 subclones.

The presence or absence of the human microsomal epoxide hydrolase gene was correlated with the presence or absence of each human chromosome in the group of human x mouse somatic cell hybrids. Table 1 shows the pattern of segrgation of the human microsomai epoxide hydrolase gene with enzyme markers for human chromosomes. Discordance was indicated by the presence of the gene when the chromosome was absent (+/-) or absence of the gene when the chromosome was present (-/+). The results showed that the human microsomal epoxide hydrolase gene segregated with the genes for peptidase C and adenylate kinase 2 which has been previously assigned to human chromosome 1.

Two points concerning these results suggested further experiments needed to be carried out:

1) The results obtained were identical to those for thr segregation of the cytochrome *P450 4A1* gene in a previous publication. If the EH gene was located on human chromosome 1, this would be expected since the same hybrid cell DNA samples were used for both experiments. Hybrids did not have to be regrown and reanalysed. This may have led to further chromosome loss.

2) In view of the contradicting reports which gave different assignments of the EH gene we wished to ensure the results were reliable. It was decided to further confirm this assignment by testing a number of hybrid subclones. One hybrid clone (AB11)that was positive for human EH gene was subloned as described in Methods.

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Table 1: Segregation of the EH gene in human x mouse hybrid clones

Chromosome	Enzyme	EH/enzyme			
		No of hybrid clones:			
		+/+	+/-	-/+	-/-
X	Glucose-6-phosphate dehydrogenase	3	1	2	6
1	Peptidase C	5	0	0	9
1	Adenylate kinase-2	5	0	0	9
2	Malate dehydrogenase	4	0	4	4
4	Phospho glucomutase	4	2	1	7
5	Hexosaminidase B	2	2	2	6
6	Malic enzyme 1	3	2	2	6
7	β-glucuronidase	4	1	3	5
8	Glutathione reductase	3	2	1	7
9	Adenylate kinase-1	3	3	2	8
10	Glutamate oxaloacetate transaminase	2	4	3	5
11	Lactate dehydrogenase A	1	3	5	3
12	Lactate dehydrogenase B	2	2	3	6
13	Esterase D	4	0	3	7
14	Nucleoside phosphorylase	1	4	4	6
15	Hexosaminidase A	2	2	3	8
16	adenosine phosphoribosyl transferase	2	3	4	5
17	Thymidine kinase	5	0	11	0
18	Peptidase A	1	3	2	7
19	Glucose phosphate isomerase	3	2	2	8
20	Adenosine deaminase	1	4	5	4
21	Superoxide dismutase	2	3	3	7

The presence or absence of the gene is marked by + or - respectively. Chromosomes 3 and 22 were not tested.

Table 2: Segregation of human microsomal epoxide hydrolase gene(EH) in somatic cell hybrid AB11 subclones. The presence or absence of the gene is marked by + or - respectively.

Chromosome	PCR marker	EH/Marker			
		(+/+)	(+/-)	(+/-)	(-/-)
1	P4501A1	14	0	1	11
6	Oestrogen receptor gene	1	3	13	7
15	α-actin gene (cardiac)	3	5	12	6

Individual subclones were isolated, grown up and tested for the EH gene and for selected chromosome markers. Some of the subclones no longer expressed the EH gene i.e. they must have lost the human chromosome containing the EH gene which was originally present in the parent hybrid clone. The pattern of segregation of the EH gene with respect to selected chromosome markers was investigated. The results showed that the human EH gene again segregated with the markers for chromosome 1 as seen in Table 2. This makes the assignment reliable since the loss of chromosome 1 from some of the hybrid subclones tested was associated with the loss of the EH gene.

These results clearly demonstrate that the human microsomal epoxide hydrolase gene was present when human chromosome 1 was present and was absent when the chromosome was absent. All other chromosomes segregated in a discordant manner with respect to the EH gene. It is of interest that one hybrid clone was positive for the EH gene but the markers for chromosome 1 were absent. This could reflect the fact that a fragment of the chromosome is missing. Clones such as this can be used in experiments to sublocalizethe position of the gene on a chromosome.

DISCUSSION

In this work the human microsomal epoxide hydrolase gene was unequivocally localized on chromosome 1 using a combination of somatic cell hybridization and PCR.

Brown and Chalmers[1] made a tentative assignment of the human microsomal epoxide hydrolase gene to chromosome 6 and they based their results upon indirect immunological evidence for the lack of expression of the human enzyme in a series of human x mouse hybrid cell lines, all of which were similar only in lacking

human chromosome 6. However, here PCR analysis of somatic cell hybrids has provided unequivocal evidence for the localization of the human microsomal epoxide hydrolase structural gene on chromosome 1. The discrepancy between this result and those of Brown and Chalmers could be explained by the possibility of the presence of nonsyntenic species specific regulatory genes on chromosome 6 or the formation of hybrid enzyme molecules with altered immunological reactivity which could make the hybrid cell clone negative for the enzyme on immunological testing.

These results are consistent with the assignment by Simmons and co-workers[3] of the mouse microsomal epoxide hydrolase gene which has been assigned to mouse chromosome 1, which is homologous to parts of human chromosome 1, based both on Southern analyses of mouse x hamster hybrid cell DNA with a cloned rodent epoxide hydrolase cDNA probe and the use of enzyme activity polymorphism for linkage analysis in recombinant inbred mice.

Moreover, these results are consistent with the assignment of the human microsomal epoxide hydrolase gene to chromosome 1 by Skoda and coworkers using Southern blot hybridization techniques[2].

REFERENCES

1) Brown, S., and Chalmers, D.E. Microsomal epoxide hydrolase activity in x mouse hybrid cells. Biochem Biophys Res Comm 1986; 137: 775-780.

2) Skoda, RC, Demierre, A, McBaide, O, Gonzalez, F, and Meyer,U, Human microsomal xenobiotic epoxide hydrolase. Complementary DNA sequence, complementary DNA-directed expression in COS-1 cells and chromosomal localization. J Biol Chem 1988; 26:1549-1554.

3) Simmons, D.L., Lalley, P.A., and Kasper, C.B., Chromosomal assignments of genes coding for components of the mixed-function oxidase system in mice. Genetic localization of the cytochrome P-450PCN and P-450PB gene families and the NADPH-cytochrome P-450 oxidoreductase and epoxide hydratase genes. J Biol Chem 1985;260:515-521.

Clinical Characteristics of Geographic Stomatitis in 145 Libyans

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ملخص

يعتبر التهاب الفم الجغرافي حالة حميدة شائعة نسبيا، تظهر عادة على ظهر وجوانب اللسان، ومن النادر ظهورها خارجه. الهدف من هذه الدراسة هو استكشاف بعض الجوانب الإكلينيكية لهذه الحالة في مجموعة من المرضى الليبيين مع التركيز بشكل خاص على أسلوب تشخيص وعلاج الحالات المصحوبة بالألم وكذلك ارتباطاتها بالحالة الصحية العامة وتواجدها مع حالات اللسان المرضية الأخرى .اشتملت هذه الدراسة على عدد 145 مريضا ليبيا لديهم هذه الاذيات على اللسان كما ظهرت هذه الاذيات أيضا على الغشاء المخاطي المبطن للشفتين وجوانب الفم عند مريض واحد. وجدت تشققات باللسان عند 72 مريضا، أما أعراض الآلام التي تشبه الحرقان الخفيف مع قليل من عدم الارتياح بالفم فقد اشتكى منها 13 مريضا.

لوحظت هذه الحالة عند المرضى من مختلف الأعمار، حيث تمتع أغلبهم بصحة جيدة باستثناء 3 منهم لديهم داء السكري2 لديهم ارتفاع ضغط الدم و 3 لديهم فقر دم.مزمن أما الأمراض الجلدية المصاحبة لهذه الحالة فاشتملت على ثلاثة مرضى يعانون من اكزيما الجلد المزمنة واثنان من التهابات الجلد الذهنية. من جهة أخرى فإن مصاحبة اللسان الجغرافي لمرض الصدفية فلم تلاحظ إلا في مريض واحد فقط في هذه الدراسة. أما الحالات اللسانية المصاحبة فاشتملت على كبر اللسان عند مريضين اثنين و التصاق راس اللسان بقاع الفم عند مريضين آخرين. كذلك تم في هذه الدراسة تسجيل تسع حالات للسان الجغرافي ظهرت بشكل عائلي.

تتوافق نتائج هذه الدراسة عن الخصائص العامة لالتهاب الفم الجغرافي مع النتائج المستخلصة من الدراسات العالمية الأخرى وتسلط بعض الضوء على احتمال الظهور العائلي لها والذي بدوره قد يحتاج إلى دراسات جينية.

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Abstract

Objectives: Geographic stomatitis is a relatively common benign condition of the oral mucosa that mainly involves the dorsum and lateral borders of the tongue and rarely extraglossaly. The aim of this work is to elucidate some of the clinical aspects of this condition in a cohort of Libyan patients, with special regard to diagnosis and management of symptomatic cases as well as systemic disease association and co-existence of other tongue conditions. **Patients & Methods:** The study comprised 145 Libyans examined prospectively for evidence of oral pathology. **Results & Methods:** Fissured tongue was detected in 72 patients (49.6%), whereas oral discomfort and mild burning symptoms reported by 13 patients (8.9%). The condition was seen in patients from all age groups, most of them were in good general health except for 3 with diabetes mellitus, 2 had hypertension and 3 with chronic anemia. Cutaneous diseases included 3 patients with eczema and 2 with "seborrheic dermatitis. On the other hand, the alleged association with psoriasis was seen in one patient only in this study. Associating tongue conditions included 2 patients with macroglossia and another two with ankyloglossia. Familial occurrences were reported in nine occasions. **Conclusions:**These findings agree with worldwide reports about the general characteristics of this condition, and shed some light on its possible familial inclination, that needs further genetic studies.

Keywords: Geographic stomatitis, Benign migratory glossitis, Libya epidemiology, psoriasis.

Introduction

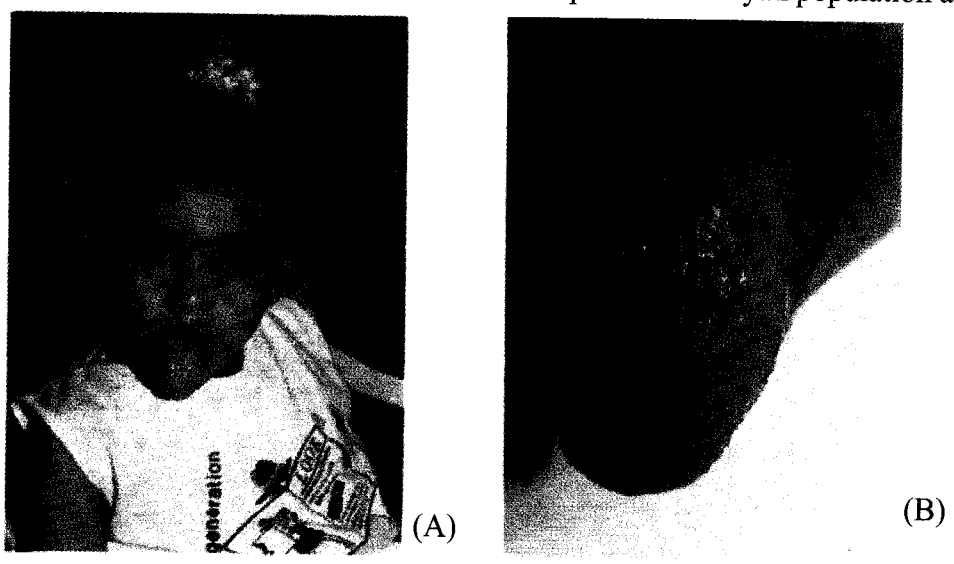
"Geographic stomatitis" is a term used to describe benign lesions frequently seen on oral mucosa. Its prevalence ranges from 1-3% in the general population[1-3]. Lesions are seen mainly on the dorsum and lateral borders of the tongue and sometimes, albeit rarity, on other intraoral mucosal sites particularly on the buccal and labial mucosa, floor of the mouth, lip, gingiva and uvula [4-6]. Many synonyms have been used to describe this condition especially when occurring on other mucosal sites such as erythema circinata migrans, annulus migrans, exfoliation areata linguae et mucosae oris and migratory stomatitis [7,8]. The recent increased frequency of reports of this condition indicates growing awareness among clinicians about this rather innocuous lesion [5,9,10].

Although the etiology of this condition is unknown, contributory factors include chronic anemia, insulin dependent diabetes mellitus [11] and emotional stress. The reported

association with fissured tongue in about 40 to 50% of the patients [12,13] may have some genetic linkage [7,14,15]. Nonetheless, Geographic stomatitis was thought to be linked to psoriasis as similar tongue lesions have accompanied cutaneous postular psoriasis [16,17]. The diagnosis of oral psoriasis should be applied only if oral lesions were accompanied with dermatological lesions of psoriasis [18].

The clinical presentation of geographic stomatitis lesions had been described by many clinicians as flattened, circinate, erythematous areas surrounded by flattened or slightly raised keratotic white border that vary in size from few millimeters to several centimeters in diameter [1,12,19]. The condition seems to have slight female predilection [20] without any clear racial inclination [21].

Generally, geographic stomatitis is asymptomatic. In few occasions, patients complain of tongue soreness and intolerance to acidic or hot spicy food [22]. Lesions may persist for long time or even for life without the need for any active treatment. Though various aspects of this condition were previously investigated in different populations worldwide. To the best of our knowledge this is the first report from Libyan population and



(A) (B)

Figure- (A &B): Clinical presentation of geographic stomatitis in a Libyan female child

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aimed to highlight the main clinical features of this condition in substantial number of patients.

Patients and methods:

Among consecutive patients screened during the year 2002 at the academic department of Oral Medicine, Oral Pathology, Diagnosis and Radiology, faculty of dentistry, Garyounis University, in the city of Benghazi Libya, patients were enrolled for this study that comprised 145 Libyans with "geographic stomatitis" living in the same district except for 3 people who came from nearby vicinity. Fifty patients (34.5%) were males and 95 (65.5%) were females, their age ranged from 2 to 67 years (median age=22 years). Most patients were seeking treatment for dental caries and periodontal diseases and only five of them came because of oral discomfort and tongue sensitivity especially during drinking acidic or spicy food. The same specialist clinicians collected data in pre-designed clinical audit to obtain general information regarding patient's general health, oral complaints, and the presence of any other tongue or skin conditions. Other questions asked regarding geographic oral lesions included lesion locations, distribution, extent, and associating pain symptoms. All data collected were subjected to statistical analysis using (SPSS ver-10.1) package.

Results

All patients included in this study have geographic tongue lesions; one patient had in addition further lesions on his buccal and alveolar mucosae. The majority of patients were not even aware of the presence of such lesions. The condition was seen in all age groups as indicated in (Table 1). 13 (8.9%) patients reported mild burning symptoms and oral discomfort; nine of them have fissured tongue. There is no relation between pain symptoms and the age or gender of the patient or the lesion location (at the time of examination). One patient only has deep concern about the nature of his condition and cancerphopia.

Fissured tongue was detected in 72 (49.6%) patients as indicated in (Table 2). There were no significant general medical problems in almost all the included patients apart from three with type II diabetes mellitus (Fig. 3), three with chronic anemia, two with hypertension and one with bronchial asthma. Dermatological conditions included one patient with psoriasis,

three with eczema and two with seborrheic dermatitis (Table 3). Oral mucous membrane lesions seen in this group included aphthous ulcers in 2 patients, angular cheilitis in 2 patients, whereas tongue anomalies included macroglossia in 2 patients and ankyloglossia in another two. On nine occasions a possible family history was found, in one occasion there was a case of 8 members of the same family including mother and her 4 daughters and 3 sons having identical tongue lesions, another case of 3 sisters and their father having the same lesions, and a male patient along with his 4 sisters had geographic and fissured tongue and neither of their parents having the lesions (Table 4).

Figure-: Geographic tongue lesions in a patient with type II diabetes mellitus

Discussion:

Various synonyms have been used to describe the amazing geographic oral lesions [7], however, few studies have looked at the extra-glossal geographic lesions as a separate clinical entity from those cases with geographic lesions on the tongue [6]. On the other hand, there was substantial evidence of the similarity of both conditions supported by the fact that in almost all reported cases of extra-glossal geographic lesions there had been a tongue involvement [4]. ical features of both conditions are similar [23]. Thus some investigators advised discarding the misleading term "geographic tongue" with the simpler

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and more appropriate term "geographic stomatitis" [5,7,8].

Geographic stomatitis can be seen at any age (Table 1), and its main intraoral site is the tongue. However, it may be seen, albeit much less frequently, on other sites of oral mucosa. According to the clinical classification suggested by Hume [7], all our cases are of (type I) except for one case that was (type II). This finding is in consistence with all previous studies. There is a likely genetically determined association between geographic and fissured tongue [24,25]. As suggested by some investigators geographic tongue may eventually ends into fissured tongue [7,26]. In our study 72 patients from both sexes had fissured tongue. The tongue fissures may be contributory in producing pain symptoms, as it had been associated with 84% of symptomatic lesions.

Although the incidence of geographic stomatitis is cited at about 1-3% of the general population [1-3,20,27], its exact etiology is still unclear. Many factors were proposed as a possible cause included anemia, gastrointestinal disorders [28], infection [12], psychological disturbances [29], emotional stress [30] and menstruation [31]. In this study few patients have systemic diseases such as iron deficiency anemia in 3 female patients, hypertension in 2 and asthma in 1 patient only. Similarly few patients have skin diseases, such as eczema in 3 patients, seborrhoeic dermatitis in 2 and psoriasis in 1 patient (Table 3). The proposed possible link between geographic tongue lesions and type-I diabetes mellitus [11] was not possible to evaluate in our study because none of our patients had type-I diabetes mellitus.

The diagnosis of geographic stomatitis is usually clinical, aided by the lack of symptoms, shape of the lesion, pattern of migration, and the chronicity of lesions [6]. Biopsy would be helpful in establishing diagnosis in difficult cases. In this study all cases were diagnosed on clinical grounds. The differential diagnosis included lichen planus, atrophic candidiasis, psoriasis, Reiter syndrome, and lupus erythematosus [32]. Generally the condition tends to be persistent [29], but it may disappear completely as reported by Prinz [28].

Geographic stomatitis was included with psoriatic diatheses such

as Reiter's syndrome, impetigo herpatiformis, acrodermatitis continua and postular psoriasis because of their histological similarities [26]. Furthermore, some researchers suggested that geographic stomatitis is an oral manifestation of psoriasis [33]. According to the current view based upon the findings in the literature, there is insufficient evidence to consider geographic stomatitis an oral manifestation of psoriasis [25], and the diagnosis of oral psoriasis should be applied only if such oral lesions were accompanied with dermatological lesions of psoriasis [18,34].

Evidence exists that genetic factors may play a role in the etiology of this condition, as genetic and familial occurrences were reported by many investigators [1,26]. Similarly, HLA antigen studies showed an increased incidence of HLA-B15, DR7, DR5 and DRw6 [17,35,36]. In the last few years the familial occurrence of geographic tongue was reported in 6- and 4-year-old sisters whose father had fissured tongue, without a family history suggestive of psoriasis[35]. In our study 9 occasions of familial occurrence involving either of the parents is reported (Table 4); there was a case of three females and their father having geographic, non-fissured tongue, another case of a mother and her 4 daughters and 3 sons, and another case of male patient with his 4 sisters having the lesions, but none of the parents had it. This is clearly indicating the possibility of the familial occurrences of geographic stomatitis, and probably incomplete pentrance. However, because most of the cases have no family history, it seems that genetic predisposition may not be a key factor.

Fortunately, geographic oral lesions seldom need any sort of treatment, except for few cases with mild soreness and sometimes intolerance to hot or spicy food, seldom the condition become severe enough to disturb the daily activity of the patient [22]. Patient reassurance is all that is needed in most cases [37]. One patient in our series reported benefiting

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from a course of broad-spectrum antibiotic prescribed for something else. However in all cases the reassurance about the benign nature of their condition, and in some of them palliative treatment with antiseptic mouthwashes and meticulous tongue brushing seemed to be effective. Nevertheless, the general dental practitioner is advised to become familiar with the salient features of this condition through prompt recognition and correct diagnosis based upon the history and clinical examination findings that will usually mitigate the need for biopsy.

In conclusion, the general features of geographic stomatitis in Libyan patients are similar to those of other worldwide populations with clear female predilection, possible familial occurrences, mostly asymptomatic with few systemic disease associations.

Table 1: Age distribution of the study group

Age (years)	Frequency	Percent	Cumulative
02 – 09	41	28.3%	28.2%
10 – 19	15	10.3%	38.6%
20 – 29	50	34.5%	73.1%
30 – 39	24	16.6%	89.6%
40 – 49	7	4.8%	94.4%
50 – 59	4	2.8%	97.2%
60 and above	4	2.8%	100.0%
Total	145	100.0%	100.0%

Figure 3: Age groups of the patients

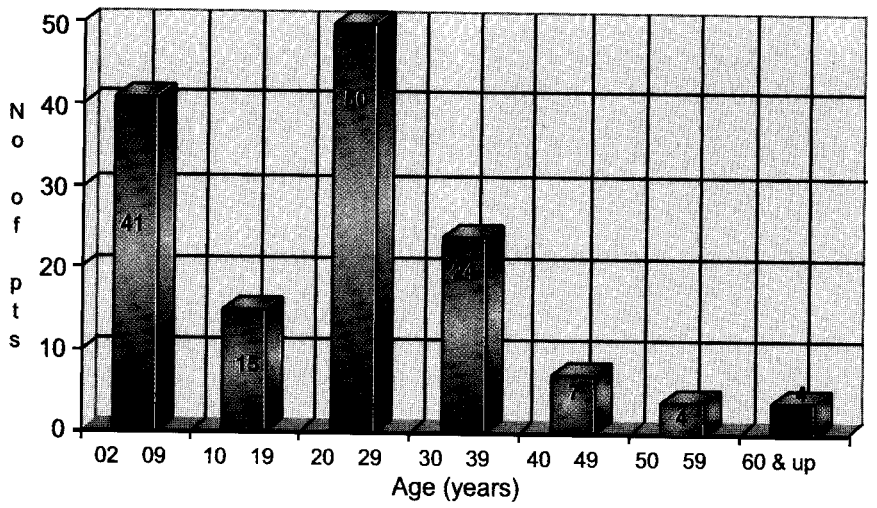

Table 2: Clinical presentation and associating oral mucosal conditions

Feature	Frequency	Percent
Fissured tongue	72	49.6%
Mild burning symptoms	13	8.9%
Positive family history	9	6.2%
Macroglossia	2	1.4%
Angular cheilitis	2	1.4%
Aphthous ulceration	2	1.4%
Ankyloglossia	2	1.4%

Table 3 : Associated systemic and cutaneous conditions

Condition	Frequency	Percent
Diabetes mellitus	3	2.1%
Eczema	3	2.1%
Anemia	3	2.1%
Hypertension	2	1.4%
Seborrhoeic dermatitis	2	1.4%
Asthma	1	0.7%
Hypotension	1	0.7%
Psoriasis	1	0.7%

Table 4: Cases of familial occurrence of geographic tongue lesions

Case No.	Gender of the affected patient	Relation
Case 1	Male	Father
Case 2	Female	Father
Case 3	Female	Father and 1 sister
Case 4	Female	Sisters
Case 5	Male	4 sisters
Case 6	Female	Sister
Case 7	Female	Sister
Case 8	Female	3 sons and 4 daughters
Case 9	Female	Mother and son

Acknowledgements

We are grateful to Dr Hamid Alorfi, dean of the dental faculty and Dr. Ali Al-Murtadi for their support. We would like to thank Mrs. Hammala Eljerby, the librarian and Mr. Ahmed Elmajperi from the audiovisual department for their assistance.

References:

1- Redman RS. Prevalence of geographic tongue, fissured tongue, rhomboid glossitis and hairy tongue among 3611 Minnesota schoolchildren. Oral Surg Oral Med Oral Pathol 1970;**30**:390-5.

2- Hietanen J, Salo OP, Kanerva L, Juvakoski T. Study of the oral mucosa in 200 consecutive patients with

psoriasis. Scand J Dent Res 1984;**92**:50-4.

3- Dombi C, Czegledy A. Incidence of tongue diseases based on epidemiologic studies (review of the literature). Fogorv Sz 1992;**85**:335-41.

4- Cooke BE. Erythema migrans affecting oral mucosa. Oral Surg Oral Med Oral Pathol 1955;**8**:164-7.

5- Kuffer R. Exfoliatio areata linguae et mucosae oris. Rev Stomatol 1971;**72**:109-19.

6- Brooks JK, Balciunas BA. Geographic stomatitis: review of the literature and report of five cases. J Am Dent Assoc 1987;**115**:421-4.

7- Hume WJ. Geographic stomatitis: a critical review. J Dent 1975;**3**:25-43.

8- Zhu J, Kaminski MJ, Pulitzer DR, Hu J, Thomas HF. Psoriasis: pathophysiology and oral manifestations. Oral Dis 1996;**2**:135-44.

9- Drezner DA, Schaffer SR. Geographic tongue. Otolaryngol Head Neck Surg 1997;**117**:291.

10- Younai FS, Phelan JA. Oral mucositis with features of psoriasis: report of a case and review of the literature. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1997;**84**:61-7.

11- Wysocki GP, Daley TD. Benign migratory glossitis in patients with juvenile diabetes. Oral Surg Oral Med Oral Pathol 1987;**63**:68-70.

12- Cooke BE. Median rhomboid glossitis and benign migratory glossitis. Oral Surg Oral Med Oral Pathol 1962;**112**:389-93.

13- Mani NJ. Occurrence of fissured tongue, geographic tongue and filiform atrophy among dental patients in Saudi Arabia. Ann Dent 1985;**44**:14-6.

14- Donelli RA. Geographic stomatitis (tongue and mucosae). Journal of Clinical Stomatol Conf 1964;**5**:22.

15- Kullaa Mikkonen A. Familial study of fissured tongue. Scand J Dent Res 1988;**96**:366-75.

16- Dawson BE. Microscopic appearance of geographic tongue. Br J Dermatol 1969;**81**:827-8.

17- Pogrel MA, Cram D. Intraoral findings in patients with psoriasis with a special reference to ectopic geographic tongue (erythema circinata). Oral Surg Oral Med Oral Pathol 1988;**64**:184-189.

18- Tholen S, Lubach D. Changes in the oral mucosa in psoriasis pustulosa generalisata. Hautarzt 1987;**38**:419-21.

19- Farman AG, Gould AR. Case No. 21: red lesions with serpiginous white margins. Ky Dent J 1987;**39**:36-7.

20- Halperin V, Kolas S, Jefferis KR. The occurrence of Fordyce spots, benign migratory glossitis, median rhomboid glossitis, and fissured tongue in 2478 dental patients. Oral Surg Oral Med Oral Pathol 1953;**6**:1077.

21- Richardson ER. Incidence of geographic tongue and median rhomboid glossitis in 3319 Negro college students. Oral Surg Oral Med Oral Pathol 1968;**26**:623-5.

22- Sigal MJ, Mock D. Symptomatic benign migratory glossitis: report of two cases and literature review. Pediatr Dent 1992;**14**:392-6.

23 McCarthy PL, Shklar G. Lesions on the tongue. In: Diseases of Oral Mucosa. Philadelphia Lea & Febiger, 2nd ed. 1980;82-93.

24- Eidelman E, Chosack, Cohen T. Scrotal tongue and geographic tongue: polygenic and associated traits. Oral Surg Oral Med Oral Pathol 1976;**42**:591-6.

25- van der Wal WA, van Dijk E, van der Waal I. Geographic stomatitis and psoriasis. Int J Oral Maxillofac Surg 1988;**17**:106-9.

26- Dawson TAJ, Pielou WD. Geographic tongue in three generations. Br J Dermatol 1967;**79**:678-81.

27- Meskin LH, Redman RS, Gorlin RJ. Incidence of geographic tongue among 3668 students at the university of Mennesota. Journal of Dental Research 1963;**42**:895.

28- Prinz H. Wandering rash of the tongue (geographical tongue). Dent Cosmos 1927;**69**:272-5.

29- Monica WS. geographic tongue. Dent Radiogr Photogr 1967;**40**:14-5.

30- Redman RS. Psychological component in the aetiology of geographic tongue. J Dent Res 1966;**45**:1403-8.

31- Waltimo J. Geographic tongue during a year of oral contraceptive cycles. Br Dent J 1991;**171**:94-6.

32- Fotiou G, Laskaris G. Reiter's syndrome oral manifestations. Hell Stomatol Chron 1988;148-51.

33- Morris LF, Phillips CM, Binnie WH, Sander HM, Silverman AK, Menter MA. Oral lesions in patients with psoriasis: a controlled study. Cutis 1992;**49**:339-44.

34- Weathers DR, baker G, Archard HO. Psoriasiform lesions of the oral mucosa with emphasis on "ectopic geographic tongue". Oral Surg Oral Med Oral Pathol 1974;**37**:872-88.

35- Kuramoto Y, Tadaki T, Hatchome N, Tagami H. Geographic tongue in two siblings. Dermatologica 1987;**174**:298-302.

36- Fenerli A, Papanicolaou S, Papanicolaou M, Laskaris G. Histocompatibility antigens and geographic tongue. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1993;**76**:476-9.

37- Powell FC. Glossodynia and other disorders of the tongue. Dermatol Clin 1987;**5**:687-93.

Favism in AL-Fateh Children Hospital : A Case Series

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Abstract

Objectives: to outline the characteristics of favism in Al-Fateh Children Hospital and compare them with those reported elsewhere. **Setting:** Al-Fateh Children Hospital, Benghazi, Libya. **Materials and Methods:** Patients presenting with features of favism were investigated and their demographic features, mode of presentation, severity and the kind of management were analysed. **Study period:** 6 months(from 1st December 2001 to 31st May 2002). **Results:** 18 patients had favism crisis during the study period. Male to female ratio was 2.6:1. Most of the cases were between 1-3 years of age. 88.9% were incident patients, and only 11% of them were born to Libyan mothers. The commonest trigger of the hemolysis in our series was cooked fava beans (55.55%). One patient had it by fava beans pollen inhalation and another one through breast milk. All patients had pallor, jaundice, and discolored urine and no patient had signs of heart failure or shock. Hepatomegaly and splenomegaly were present in 22.2% and 16.67% of patients respectively. Hemoglobin values were lower than 7 gm/dl in 66.67% of patients, reticulocyte counts ranged from 3 to 15% and unconjugated bilirubin values ranged from 2.5 to 16.5 mg/dl. Dye decolorization screening test was applied for 14 patients 2 to 3 weeks after recovery. Not surprisingly, all were labeled as normal (non-deficient of G6PD). **Conclusion:** Our findings were generally comparable to those reported elsewhere. It seems that the carrier rate of the mutated gene in Libyan mothers is lower than that reported in nearby countries. Red blood cell-G6PD could be estimated confidently by spectrophotometric methods while patients are in a steady-state (6-8 weeks after recovery).

Key words: Favism, glucose-6-phosphate dehydrogenase deficiency, hemolytic anemia, G6PD screening tests, Benghazi, Libya.

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Introduction

The glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common inherited metabolic human red cell enzymopathy, frequently occurs in certain Mediterranean countries, American and African blacks, and among Orientals[1]. Red blood cell-G6PD has high rate of variation, resulting from point mutations with in the coding region of the G6PD gene and individual variants are characteristically found in certain geographic areas[2,3]. Three clinical syndromes associated with G6PD deficiency have been described; the most classic manifestation is acute hemolytic anemia in children. Another syndrome of great clinical and public health importance is neonatal jaundice. Chronic non-spherocytic hemolytic anemia is a much more rare manifestation of G6PD deficiency and a life long hemolytic process[4,5,6]. Because the metabolic role of G6PD in red blood cells is primarily related to its reductive potential, the threat to G6PD deficient red cells is of oxidative damage, hence the majority of children with G6PD deficiency are clinically and hematologically normal most of the time, and this can be designated as a steady-state condition. They develop hemolysis as a result of challenge by exogenous oxidative agent and this situation has been best described after ingestion of fava beans (Favism) which is a prototypical example of acute hemolytic anemia associated with G6PD deficiency[7].

Extensive studies on the components of fava beans responsible for hemolysis led to the identification of vicine and convicine, two β-glycosides having as aglycones the substituted pyrimidines divicine and isouramil[8], these compounds in the course of their auto-oxidation produce free radicals, which in turn oxidize glutathione (GSH) to GSSG, activating the chain reaction which leads to coarse precipitates of denatured hemoglobin causing irreversible damage to red cell membranes and their lysis[9].

Fava beans are not the only exogenous agents that can cause this manifestation; numerous drugs have been reported as potentially dangerous in G6PD deficient individuals. They have the ability to stimulate the

pentose phosphate pathway in red blood cells[10], and they are able to oxidize nicotinamide adenine dinucleotide phosphate (NADPH) which is closely related to the metabolism of glutathione [7,10].

Patients and Methods

A prospective study was carried out in Al-Fateh Children Hospital, Benghazi, in the period from 1st December 2001 to 31st May 2002. Patients who admitted with acute hemolysis after exposure to fava beans were analyzed and followed.

Demographic features, quantity and type of ingested fava beans, duration between ingestion of fava beans and onset of symptoms, history of previous acute hemolytic illness especially during neonatal period and detailed family history were recorded.

Clinical manifestations including pallor, jaundice, fever, GIT symptoms, change of urine color, hepatomegaly, splenomegaly, signs of heart failure, were recorded at time of admission and after recovery of acute hemolysis. Hemoglobin values, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), reticulocyte counts, bilirubin values (conjugated and unconjugated), serum glutamic-oxalacetic transaminase (AST), serum glutamic-pyruvic transaminase (ALT), and renal function tests were estimated initially and after recovery.

G6PD activity was estimated 2 to 3 weeks after acute hemolysis recovery, using dye decolorization test. Confirmation of G6PD deficiency by enzymatic quantification using spectrophotometry was not available.

Results

In the period from 1st December 2001 to 31st May 2002, 18 patients with acute hemolysis following exposure to fava beens were admitted to Al-Fateh Children Hospital. Sixteen patients were incident patients while 2 had suffered favism crisis. Age range was from 8 months to 9 years with peak age from 1 to 3 years. Thirteen patients were

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males and 5 were females with M : F ratio of 2.6:1, 60% of females had fathers with history of favism. Two out of 18 patients (11.1%) were born to Libyan parents. 7 out of 18 patients (38.9%) were born to mothers, the remaining 50% were from other nationalities including Egyptian (16.7%), Palestinian (16.7%) as well as other nationalities (see figure). Family history of favism was found in 50% of patients. Five out of 18 patients (27.8%) had history of neonatal jaundice which was considered as physiological jaundice. One of 5 patients needed blood exchange, the other 4 responded to phototherapy.

Analysis of the ways of exposure to fava beans revealed that ingestion of cooked fava beans was the most common trigger (10 patients, 55.55%), followed by fresh (raw) fava beans in 6 patients (33.33%), then inhalation of fava beans pollen and ingestion of breast milk in one each. Frozen, dried, and canned fava beans were not consumed by any patient. The time elapsed between exposure to fava beans and onset of symptoms ranged from 24 to 72 hours, with majority from 24 to 48 hours (88.88%). Quantity of fava beans ingested could not be exactly determined by patients or their care takers.

Analysis of clinical manifestations revealed that 50% of patients had history of pallor and 83.33% had history of jaundice while on clinical examination 100% of patients had both pallor and jaundice. GIT symptoms were as follows: 44.4% had vomiting, 22.2% epigastric pain and 5.55% diarrhea. Regarding the change of urine color 88.89% noted tea-coloured urine, and during hospitalization all patients had discolored urine. 22.2% had low grade fever. 11.1% of patients were dehydrated at time of admission but signs of heart failure or shock were not detected in any patient. Hepatomegaly and splenomegaly were present in 22.2% and 16.67% of patients respectively.

Twelve patients (66.67%) had severe hemolysis with hemoglobin values between 3 and 6.8 gm/dl and reticulocytosis of 10 to 15% and serum bilirubin from 7.5 to 16.5 mg/dl. They required blood transfusions once for each.

The remaining 6 patients had hemoglobin values between 7.2 and 9 gm/dl, their reticulocytosis between 3 to 8%, and bilirubin values between 2.5 and 5 mg/dl. They were treated with intravenous fluids. MCV and MCH were low in 38.9% of patients, all of them had normal AST, ALT, and renal function tests. All patients were discharged in good condition. Fourteen patients (77.8%) were followed for 2 to 3 weeks after recovery for G6PD screening test. The other 4 patients didn't show up. The G6PD screening test of the 14 patients was normal.

Discussion

Although favism occurs at any age, children are more susceptible than adults[4]. The present study showed that the peak age was 1 to 3 years. This finding contrasts with the finding of a study carried out in Sardinia by Meloni T. et al.(1983) where the highest incidence occurred in children aged between 2 and 6 years[11]. Eighty nine percent of our patients were incident patients, this finding can be partially explained by the observation of Meloni T.[12] that a dramatic reduction in the incidence of favism occurred by adopting two preventive measures; first screening of all newborns for G6PD deficiency and then education to give up eating fava beans to halt future favism crises .

Male to female ratio in this study was 2.6:1. Many studies[7,11] found that males are more affected by favism, because G6PD deficiency is an X-linked disease[7]. However, an increase in the proportion of females is noted by many studies[7,12]. This is attributed to failure of the screening method used to pick out many heterozygote females[12] .

Fifty percent of our patients had family history of favism. 60% of the females had paternal history of favism, the other 40% did not. Several causes for this negative family history have been put forward; among these is the fact that in adults the ingestion of fava beans does not trigger favism crisis in

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more than 25% of cases, and even in the same person favism may occur in one occasion but not in another[13,14]. Other factors may be involved in pathogenesis of favism such as the impaired liver capacity of favic subjects[15,16] and the amount of fava beans ingested in relation to body mass[7].

Analysis of the nationality revealed that 50% of patients were Libyans, however only 11.1% were born to Libyan mothers. Although G6PD deficiency is rare in original Libyan population[3] , the number of cases could be rising in the Libyan population as a whole. Shibuya A. et al.[17] observed a rise in the number of cases in the original Japanese population in which G6PD deficiency is known to be rare due to coupling with Chinese mothers.

Our data clearly point out that ingestion of cooked fava beans is the commonest trigger of favism (55.55%), followed by fresh (raw) fava beans (33.33%). This finding is quite different from the usual observation that fresh fava beans are the major cause of favism, as described by Meloni T. et al.[11] , who 94.4% of their patients were due to fresh fava beans. As mentioned by Luzzatto L[7], fresh fava beans are more likely to cause favism than the cooked, because the glycoside content is a function of the maturity of the beans with the young small fresh beans being much richer than other forms. However, high incidence from dried fava beans was observed by kattamis C. et al[18]. which was not found in our patients. Unlike Meloni T. et al.[11] who observed 3 cases of favism after ingestion of frozen fava beans, none of ours was exposed to this kind of beans. This discrepancy may be related to different use of fava beans types in each community[18], with a greater use of the cooked beans in our community.

Inhaling the pollen of fava beans plant can also induce hemolysis in favic individuals. One of our patients had this way of exposure to fava beans, and it has been described by Schiliro G. et al[19].

Another patient was exposed to fava beans through breast milk. This has been described by Kaplan M. et al.[20] in 2 G6PD deficient infants whose mothers had recently eaten fava beans. Several authors described the same[19,21].

In our survey, no patient had favism in the neonatal period. Indeed, it is extremely rare but had been reported by Corchia C., et al.[22] in a G6PD-deficient newborn whose mother had a fava beans meal 5 days before delivery. This infant had been described as having favism in utero. On the other hand, 27.77% of our patients had history of neonatal jaundice which was passed as physiological in all of them. Many authors found that G6PD deficient newborns have higher incidence of neonatal unconjugated hyperbilirubinemia compared to normal newborns, with marked exaggeration of physiological jaundice[23,24]. This is a different entity from favism, and is not of hemolytic but of hepatic origin, explained by many authors by the fact that favic subjects had impaired liver capacity in the glucuronization with varying degrees[15,16,25,26,27].

This study showed that the time elapsed between the exposure to fava beans and the appearance of symptoms was 24 to 72 hours, with the majority (88.88%) from 24 to 48 hours; in agreement with findings of Meloni T. et al.[11] and Luzzatto L.[7]. However, 11.1% of patients had onset of symptoms after 72 hours; this delayed onset had been also described[7].

Regarding the clinical presentation of favism in our study, pallor and jaundice were evident in all patients, although discolored urine was noted by 88.89% of mothers. During hospitalization all patients had urine discoloration, less than half of patients complained of vomiting and only one third had lethargy. These findings were a little bit different from those of Meloni T. et al[11], who found that pallor, urine discoloration, lethargy and vomiting were the most evident signs, while jaundice was not always present. Low grade fever was measured in 22.2% of patients, it is similar to the observation of Luzzatto L.[7]. Hypovolemic shock and heart failure were not detected in our patients.

Despite that the anemia described with favism is almost always normocytic

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normochromic in type[7], 38.9% of our favic children had microcytic hypochromic morphology. This is probably due to pre-existing iron deficiency or other hematologic abnormalities. No attempt was made to elaborate on this in our patients. All of our patients had normal values of AST and ALT, which is in disagreement with the finding of Meloni T. et al.[11], who confirmed that the favic patients with higher bilirubin values had higher ALT levels and normal AST. It also disagree with Kattamis C. et al.[28] who found higher levels of both AST and ALT during favic crisis.The increase in these enzymatic activities is related to transient cholestasis[29], which may have not occurred in our patients. Renal failure was not detected in any patient which is similar to observation of Luzzatto L.[7] who pointed out that renal failure in favic children is rare even with high hemoglobinemia.

Blood transfusion was indicated for patients whose hemoglobin values were less than 7 gm/. The guide lines for blood transfusion for favic children were delineated by Luzzatto L[30] as follows: any patient with hemoglobin value less than 7 gm/dl or with hemoglobin value less than 9 gm/dl in presence of persistent hemolysis (urine discoloration and hemoglobinuria) need to be transfused.

Diagnosis of favism in this study was made on the basis of history of fava beans exposure followed by the characteristic clinical picture (anemia, unconjugated hyperbilirubinemia and urine discoloration), although with this history and clinical picture the diagnosis is almost always made quite confidently as mentioned by Luzzatto L.[7], the final diagnosis must rely on the direct demonstration of decreased activity of G6PD in red blood cells[7,31]. Dye decolorization test which is one of most popular screening tests[32] was used in this study. Not surprisingly, this screening test labeled all our patients as normal (non-deficient of G6PD) even in presence of the characteristic history and clinical features. We explained this on the basis of statements by Bienzleu et al[33]; a number of potential pitfalls and sources of error may occur, amongst them that screening tests can not identify heterozygote females, and it is adequate only for diagnosis in patients who are in the steady state but not for

patients in post-hemolytic period in which the elder more enzyme deficient red cells are removed from the circulation and are replaced by young cells which are rich in G6PD. The level of the enzyme begins to increase toward normal and gives misdiagnosis of deficient patients as normal, and this period may be as long as 3 to 6 weeks. Another confusing factor may be the admixture of G6PD-normal red blood cells if patient had been transfused. Luzzatto L[7] gives several ways to circumvent these problems; a G6PD level in the low-normal range in the presence of reticulocytosis is always suggestive of a deficient patient.It is better that a blood sample during hemolysis or recovery period is kept in store for a few weeks, when the situation will be evolving toward the steady state, and repeat test will prove wither the patient is indeed G6PD deficient. Ideally every patient in favism crisis should have his red blood cell-G6PD be estimated by spectrophotometric assay while he is in a steady-state (6 to 8 weeks after recovery).

References:

1. Scriver CR. Glucose -6- phosphate dehydrogenase deficiency. In: Scriver CR. (ed): The metabolic and molecular bases of inherited disease. 7th ed. McGraw-Hill: 1995; 3367-98.
2. Luzzatto L, Battistuzzi G. Glucose-6-phosphate dehydrogenase. Adv Hum Genet J. 1985; 14:217-220.
3. WHO Working Group. Glucose -6-phosphate dehydrogenase deficiency. Bull WHO. 1989; 67:601-610.
4. Beutler E. Glucose -6- phosphate dehydrogenase deficiency. In : Beutler E. (ed): Hemolytic anemia in disorders of red cell metabolism. New York: plenum Medical Book; 1978:23-167.
5. Dacie JV. Hereditary enzyme deficiency haemolytic anaemias: deficiency of glucose -6- phosphate dehydrogenase. In : Dacie JV. (ed): Haemolytic anaemias. 3rd ed. London: Churchill Livingstone; 1985: 364 – 418.
6. Luzzatto L. Inherited haemolytic anaemias. In: Hoffbrand AH, Lewis SW. (eds): Postgraduate haematology. 3rd ed. London: Heinemann; 1989: 146 – 182.
7. Luzzatto L. Glucose -6- phosphate dehydrogenase deficiency and hemolytic anemia. In : Nathan D G, Orkin SH. (eds): Hematology of infancy and

childhood. 5th ed. Philadelphia: WB Saunders; 1998 : 704 – 726.

8. Chevion M, Navok T. The chemistry of favism – inducing compounds : the properties of isouramil and divicine and their reaction with glutathione. Eur J Biochem. 1982; 127:405-409.
9. Winterbourn C, Cowden WB, Sutton HC. Auto – oxidation of dialuric acid, divicine and isouramil: superoxide dependent and independent mechanisms. Biochem Pharmacol J. 1989; 38:611-615.
10. Szeinbreg A, Marks PA. Substances stimulating glucose catabolism by the oxidative reactions of the pentose phosphate pathway in human erythrocytes. J Clin Invest. 1961; 40:914-916.
11. Meloni T, Forteleoni G, Dore A, Cutillo S. Favism and hemolytic anemia in glucose -6- phosphate dehydrogenase – deficient subjects in north Sardinia. Acta Haematol J. 1983; 70:83-90.
12. Meloni T, Forteleoni G, Meloni G F. Marked decline of favism after neonatal glucose-6-phosphate dehydrogenase screening and health education. Acta Haematol J. 1992; 87:29-34.
13. Arese P, De Flora A. Pathophysiology of hemolysis in glucose -6-phosphate dehydrogenase deficiency. Semin Hematol. 1990; 27:1-3.
14. Stamatoyannopoulos G, Fraser GR., Motulski AG, Fessas P, Akrivakis A, Papayannopoulos T. On the familial predisposition to favism. Am J hum Genet. 1966; 18:253-263.
15. Cassimos C, Malaka–Zafiriu K, Tsiures J, Danielides B. Variations in salicylamide glucuronide formation in normal and in G6PD deficient children. J Pediat. 1974; 84:110-111.
16. Cutillo S, Costa S, Vintuleddu MC, Meloni T. Salicylamide– glucuronide formation in children with favism and in their parents. Acta Haematol J. 1976; 44: 296 – 299.
17. Shibuya A, Hirono A, Ishii S, Fujii H., Miwa S. Hemolytic crisis after excessive ingesion of fava beans in a male infant with G6PD canton. Int J Hematol. 1991; 70 (4) : 233-235.
18. Kattamis CA, Kyriazokou M, Chaidas S. Favism. Clinical and biochemical data. J Med Genet. 1969; 6:34-41.
19. Schiliro C, Russo A, Curreri R, Marino S, Sciotto A, Russo G. Glucose-6-phosphate dehydrogenase deficiency in Sicily. Incidence, biochemical characteristics and clinical implications. Clin Genet J. 1979; 15: 183 – 188.
20. Kaplan M, . Vreman HJ, Hammerman C, Schimmel MS, Abrahamov A, Stevenson DK. Favism by proxy in nursing glucose -6-phosphate dehydrogenase – deficient neonates. J Perinatol. 1998; 18 (6 Pt 1): 477 – 479.
21. Kattamis C. Favism in breast–fed infants. Arch Dis Childh. 1971; 46: 741-743.
22. Corchia C, Balata A. Favism in a female newborn infant whose mother ingested fava beans before delivery. J Pediatrics. 1995;127: 807-809.

23. Bienzle U, Effiong CE, Luzzatto L. Erythrocyte glucose-6-phosphate dehydrogenase deficiency and neonatal jaundice. Acta Paediatr Scand. 1976; 65:701-705.

24. Tanphaichitr VS, Pung-amritt P, Yodthong S, Soongswang J, Mahasandana C, Suvatte V. Glucose-6-phosphate dehydrogenase deficiency in the newborn, its prevalence and relation to neonatal jaundice. Southeast Asian J Trop Med Pub Health. 1995; 26(1):137-141.

25. Meloni T, Cagnazzo G. Phenobarbital for prevention of hyperbilirubinemia in glucose-6-phosphate dehydrogenase deficient newborn infants. J Pediatr. 1973; 82:1048-1051.

26. Kaplan M, Hammerman C. Severe neonatal hyperbilirubinemia: a potential complication of glucose -6-phosphate dehydrogenase deficiency. Clin Perinatol J. 1998; 25 : 575 – 590.

27. Kaplan M, Muraca M, Hammerman C, Vilei MT, Leiter C, Rudensky B, et al. Bilirubin conjugation, reflected by conjugated bilirubin fractions, in G6PD deficient neonates, a determining factor in the pathogenesis of hyperbilirubinemia. J pediatrics. 1998; 102 (3) : 37-42.

28. Kattamis C, Karambula K, Ioannidou V, Hatzikou V. Jaundice and bilirubin levels in Greek children with favism. Arch Dis child. 1976; 51:233 – 235.

29. Meloni T, Pilo C, Gallisai D, Dore A. Serum glutamic oxalacetic transaminase, glutamic pyruvic transaminase, gamma – glutmyl transpeptidase and glutamic dehydrogenase levels in favism. Acta Haemat. 1979; 62:71-73.

30. Luzzatto L, Meloni T. Hemolytic anemia due to glucose-6-phosphate dehydrogenase deficiency. In : Brain MC, Carbone PP. (eds) : Current therapy in hematology-oncology. Toronto: BC Decker; 1985 : 21 – 24.

31. Brewer GJ, Tarlov AR, Alving AS. The methemoglobin reduction test: a new simple in vitro test for identifying primaquine sensitivity. Bull WHO 1960; 22:633 – 640.

32. Motulsky AG, Campbell JM. Population genetics of glucose-6-phosphate dehydrogenase deficiency of the red cell. In: Blumbergs BS. (ed): Proceedings of conference on genetic polymorphisms and geographic variations in disease. New York: Grune and Stratton; 1961: 159 – 180.

33. Bienzle U, Sodeinde O. G6PD deficiency and sickle cell anemia. Blood. 1975; 46 : 591-593.

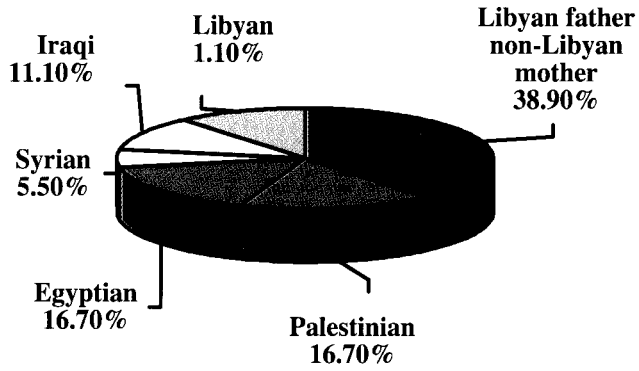

Figure : Nationality distribution of favism

Editorial Notes

Classification of G6PD variants – WHO has classified the different G6PD variants according to the magnitude of the enzyme deficiency and the severity of hemolysis. Classes IV and V are of no clinical significance.

- **Class I**: severe enzyme deficiency (less than 10 percent of normal) and have chronic hemolytic anemia
- **Class II** : severe enzyme deficiency, but there is usually only intermittent hemolysis
- **Class III** : moderate enzyme deficiency (10 to 60 percent of normal) with intermittent hemolysis usually associated with infection or drugs
- **Class IV** : no enzyme deficiency or hemolysis
- **Class V** : increased enzyme activity.

Prevalence of hepatitis B and / or C in Benghazi adult renal units .

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Abstract

Aim : monitoring the trend of seropositivity for HbsAg and HCV antibodies in our hemodialysis patients over a 4 year period. **Setting**: Adult renal units of Benghazi (7th April and 7th Oct. Hospitals) . **Materials and Methods**: It was carried out in 285 end stage renal disease patients, on heamodialysis. Third generation enzyme linked immunosorbent assays for HCV ab and HBsAg were used. 72 dialysis unit health care workers were used as a control. **Results**: None of our staff members tested positive. In ESRD patients, the overall HBs Ag positivity showed a statistically significant decrease over the last four years from 21.4 % in 1998 to 12.3 % in this report ($p< 0.05$). This was accompanied by slight decrease in total viral hepatitis seropositivity from 88 % to 77 %. There was no significant change in our HCV ab positivity rate (75%) when compared with our previous report in 1998 (78%). The new change in prevalence rates is mainly attributed to a reduction HbsAg positivity as a result of successful HBV vaccination program in ESRD patients. As to date,there is no effective HCV vaccine available. **Conclusion**: we emphasize the value of use of erythropioetin ,instead of blood transfusions, in treatment of ESRD anaemia,together with use of recently available virus screening tests in addition to the widely known HD universal percautions(UP).The above preventive measures have been worldwide reported to decrease viral hepatitis prevalence rates including HCV in USA and Europe.

Keywords : HBV,HCV , dialysis units,Benghazi – Libya

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Introduction

The risk of viral hepatitis is greatly increased in,ESRD patients on maintenance HD program especially,who are in need for frequent blood transfusions,as a treatment for their chronic renal failure anaemia[1-3],when there is shortage of erythropoietin supply in renal units, or sometimes it is needed in emergency life saving surgical operations. Many ESRD patients gave history of frequent minor and major surgical procedures, locally or abroad, as repeated trials of kidney transplant[4].From above predisposing factors as well as other welf known worldwide reported causes[5-6],it is expected to have a high prevalence rates of hepatitis B and / or C in dialysis patients especially, if already known HD universal precautions are neglected. These viral hepatitis infected patients may eventually suffer from more serious complications e.g chronic hepatitis, liver cirrhosis and/or hepatocellular carcinoma[7-9]and/or renal involvement such as protenuria,haematuria ,nephrotic or nephritic syndrome,memembranous glomerulonephritis (GN),membranoproliferative GN renal insufficiency,vasculitis and mixed cryoglobulinaemia in HCV[10-11] .In regard of above viral hepatitis serious complications,it is important to implement strict regulations aiming to control cross infection in renal units[12-14].

Material and methods

Seventh of October hospital renal unit ESRD patients (n = 32), staff (n=20), 7th of April hospital renal unit ESRD patients (n=253) and staff (n=52) sera were screened for HBs Ag and HCV ab using ElISA third generation tests (table1). Serologically positive and negative patients are dialysed in separate labeled rooms, equipped with dedicated HD machines. The HD sessions were performed by well trained HD nurses for each patient group. Disposable HD kits as blood line sets, syringes, needles and filters are used for each patient's HD session. HD machines are

Table (1) The ordered ELISA tests :

Ordered test	Company	Principle of the ELISA test
HBS Ag	Hepanostike HbsAg uniform II organon teknika	Qualitatively detects HBSAg subtypes ad. And ay
HCV – ab	HBI , HCV , EIV 4.0 united biochemical Inc. USA	Synthetic peptides which corrospond to highly antigenic segments of HCV core antigens (NS_3 , NS_4 , NS_5) that detects HCV antibody

sterelized by circulating hot water (80 C°) for 45 minutes after each patient's HD session. It is also subjected to chemical disinfection twice per week . ELISA tests are monthly performed to all registerd ESRD patients, and routinely done before the start of HD session for any newly diagnosed acute or chronic renal failure patient, as well as for already enrolled ERSD patients when subjected to surgical procedures or in case of having HD sessions outside the original HD unit.

Results:

Analysis of dialysis units records of 285 ESRD patients and 72 staff members working in Benghazi adult renal units showed the following results: patient HD sessions ranged from 2 – 4 (average 3) per week, each session lasted 3 – 4 hours. All patients were on HD for a mean duration of 72.5 months (range 3– 184). The mean patients age was 34.6 years (range 16 –75) and for staff members was 22 years (range 18 - 34). 219 ESRD patients (77 %) were found to have positive ELISA results for viral hepatitis (table3). All staff personnel were hepatitis negative.Table (2) shows the studied ESRD patients and staff ELISA results.Table (3) compares our figures with those reported in 1998[15].

Table (2) ELISA test results in Benghazi – HD adult renal units year (2002)

ELISA test results	HD patients (n. 285)	DUHCW staff (n. 72)	Total (n. 357)
Positive HBs Ag	8 (2.8 %)	-	8 (2.2 %)
Positive HC Ab	184 (65%)	-	184 (51.5 %)
+ve both HBs Ag + HCV	27 (9.5%)	-	27 (7.6%)
Total negative results	66 (23 %)	72 (100 %)	138 (39 %)

n= total number of patients or staff .

Table (3) Summary of ELISA tests results of two studies in years (1998 and 2002) in Benghazi adult renal units

ELISA Test results	1998 (n. 201 HD pts)	2002 (n. 285 HD pts)
Positive HBs Ag	20 (10%)	8 (2.8%)
Positive HC Ab	133 (66.2%)	184 (65 %)
+ve both HBs Ag + HCV	23 (11.4%)	27 (9.5%)
Dual infection cases	176 (88 %)	219 (77%)
Total negative patients	25 (12 %)	66 (23%)

Table (4) Summary of HBV screeing tests H D patients in Benghazi renal units over the last 24 years

Authors	No. of H. D pts studied	Frequency of		
		Total HBs Ag	HBs Ab	Others
Prasad et al * (1978)	77	5.19 %	24.7 %	ND
El – Arabi et al** (1994)	143	13 %	19 %	18 % anti Hbc
Zaied (et al)** (1998)	201	21.4 %	ND	Dual B + C = 11.4%
Zaied / Shibani ** (2002)	285	12.3 %	ND	Dual B+ C = 9.5%

* = ELISA 1st generation. ** = ELISA 3rd generation. ND= not done

Table (5) Comparison of HCV Ab prevalence rates in different renal units

Country	Prevalence	Year	Ref.no.
Benghazi – Libya	78% 75%	1998 2002	15 (The present report)
Kuwait	71%	1994	24
* Saudi Arabia			
Najaran	51%	1997	25
Multicenters study	68%	1993	26
Western province	72%	1995	27
Al- Hasa	44%	2002	28
U.S.A	0.4% - 15%	1999 2002	20 29

Discussion :

None of our health workers tested positive. This is in fact an expected result attributed to our strictly applied rules of regular HBV and HCV screening . ELISA positive cases are not allowed to work in dialysis unit.The prevalence of HbsAg positivity in HD patients in this study has significantly decreased from 21.4 % in 1998 to 12.3 % in this report ($p < 0.05$) during last four years[15]. This is probably attributed to the following factors: successful HBV vaccination program[16] in community population,and ESRD patients, use of recombinant human erythropoietin[17-18]to cut short frequency of blood transfusion. This later policy allowed us to stop completely blood transfusions in young ESRD patients and to minimize it in elderly patient as it is considered the safest treatment for chronic renal failure associted anaemia [17]. The application of HD universal precuations (UP) in dialysis units[12-14] should have a role as well. The above mentioned factors also resulted in slight decrease in dual HBV and HCVinfection prevalence rate from 11.4% in 1998 to 9.5% in 2002 . Our study did not show any statistically significant change in HCV ab prevalence rate (tables3 –5) over the last 4 years[15,22,23] in Benghazi dialysis units unlike that of HbsAg. Although the figure quoted for HBsAg in our study is still higher than that reported in the general Libyan population and healthy blood donors[19] , we have to stress the importance of applying preventive measures such as : regular application of dialysis UP in renal units, doubling HBV vaccine dose in ESRD patients and to check its protective effect on regular periods; by measuring HBs antibody titre; with clear instructions to give a booster dose if Hbs antibody is less than 10 international units as recommended by WHO Global advisory group[16]. The recent slight improvement in dual HBV and HCV infection rate compared to that reported in 1998[15] is a result of genuine decrease in HBs Ag infection rate. On the other hand, HCV ab result in this study did not show any significant change from what was reported in 1998[15]. Concerning HCV ab prevalence rate, among HD Libyan patients there were only few local reports. It varied from 20.5 %[30] to 78%[15].The statistically

insignificant recent decrease in HCV ab prevalence rate in this study could be explained to some extent by reduction in frequency of blood transfusion. Worldwide, the reported HCVab prevalence rate is variable [24-29] . This variation was attributed to many factors. These include : non availability of vaccine against HCV, the direct positive reported relationship between HCV infection and longer duration of HD treatment and increased number of blood units transfused to HD patient[1-3] .Other possibly related factors include possible spread of HCV infection through dialysis membranes[13], increased numbers of minor and major surgical procedures related to ESRD such as vascular access problems, kidney transplantation[4] and lastly possibility of nosocomial trasmission in dialysis unit[12].The total negative ELISA results for both HbsAg and HCV ab in our study were noticed in 27% of ESRD patients. It could be a true negative result, if we can safely rule out other misleading factors such as the window period. Lastly, it is worth to mention that hepatitis infected ESRD patients, whether symtomatic or not should be thoroughly investigated from time to time for possibility of the well known hepatitis B and C complications on hepatic and / or renal functions and the effect on the patient's life expectancy[30-32] . The benefit of early start of antiviral therapy is to abolish or decrease virus replication before embarking on renal transplantation[32-36].

Conclusion

In ESRD patients on H.D HBs Ag prevalence has significantly decreased over the last four years, due to successful HBV vaccination program. It was accompanied with a decrease in dual infection rate, while HCV ab positivity rate did not show any significant change. The value of using erythropoitin, HD universal precuations together with early start of a well planned antiviral as needed, will certainly decrease viral hepatitis spread and its serious complications.

References:

1-Knudesen F ,Wantzin P , Ramussen K. etal .Hepatitis C in dialysis patient. relationship to blood trasfusion ,dialysis and liver disease. Kidney Int 1993 ;43:1356-1366.

2-Seeff L B , Hollinger . B . Alter . HJ. et al . Long term mortality and morbidity of transfusion associated non – B and type C hepatitis : a National Heart , Lung and Blood Institute collaborative study. Hepatology 2001; 33 : 455 – 463 .

3-Tong MJ, El – Farra N, Reikes AR, Et al . Clinical outcomes after transfusion associated hepatitis C. N Engl J Med 1995; 332 : 1463 – 1466

4-Conway M, Cutterall A P , Brown EA , et al : Prevalence of antibodies to hepatitis C in dialysis patient and transplant recipients with possible route of transmission. Nephro Dial Transplant 1992;7: 122 – 1229

5- Strickland GT. Hepatitis C in developing countries. Post Grad Doctor 2001; 16: 375 – 378.

6- Mele A, Spada E, Sagliocca L, et al . Risk of parenterally transmitted hepatitis following exposure to surgery or other invasive procedures: results from the hepatitis surveillance system in Italy. J Hepatol 2001;35: 284- 289.

7-Sherlock S.Viruses and hepatocellular carcinoma. Gut 1994; 35:828–832.

8- Al – Karawi MA , Shariq. S , Mohamed AE et al : Hepatitis C virus infection in chronic liver disease and hepatocallular carcinoma in Saudi Arabia. J Gastrontrol and Hepatol 1992;7 : 237 –239.

9- Kalamani E , Trichopoulos D , Tznonou A , et al :Hepatitis B and C virus and their interaction in the origin of hepatocellular carcinoma. JAMA 1991; 265: 1974-1976

10- Altraif I, Abdula AL-Sebagel M , etal:Hepatitis C associated glomerulonephritis. Am J Nephrol 1995; 15:407-410.

11- Tarantino A, Campise M, Banfi G, et al . Long – term predictors of survival in essential mixed cryoglobulinemic glomerulonephritis. Kidney Int 1995;47 : 618 – 623

12- Jose M, Sanchez-tapias: Nosocomial transmission of hepatitis C virus. J Hepatol 1999;31:107-112.

13-Valtuille R, Fernandez JL, Berridi J, et al . Evidence of hepatitis C passage accross dialysis membrane (1998) Nephron 1998; 80 :194-196.

14-Mac-Donald M, Crofts N, Kaldor J :Transmisson of hepatitis C virus: rates, routes ,and cofactors .Epidemol Rev 1996; 18:137-148

15- Zaied AF,Bugaigis HMT,AL-Sharaki YA et al :Prevalence of hepatitis B and/or C in haemdialysis patients in Benghazi renal units (1998) :4th Jamahiriya medical scienecs congress, Benghazi 1-4/11.

16- WHO expanded programme on Immunization. Global advisory group.Weekly Epidomiol Record 1992; 3: 11-16.

17-Mann J : What are the short term and long term consequences of anaemia in chronic renal failure ? Nephrol Dial Transplant 1999; 14: 29-36.

18- Macdoughall L: Poor response to erythropoietin: practical guidelines on investigations and management. Nephrol Dial Trasplant 1995; 10:607 -614.

19- Daw M A ,Siala IM ,Warfalli MM et al . Prevalence of HbsAg among Libyan population ,a comparative study. Ann.Saudi J 2001; 21: 130 -132.

20- Thomas S, Hall A, Newell ML-Dore GJ , et al : PCR as a marker of infectivity in people with hepatitis C. B M J 1998; 316: 553 –554.

21- Alter M J,Kruszon – Moran D, Nainan O , et al . The prevalence of hepatitisC virus in the United States. N Engl J Med 1999; 341: 556 – 562.

22- Prasad PN ,Pathuk SK :Studies on hepatitis B surface antigen and antibody in hospital population

Garyounis Med J 1978; 1:15 –19.

23- El-Arabi MB,Tobogi RS,Taher I AA. et al :Hepatitis B markers in Libya haemdialysis patients. Abstract of Jamahiriya Second conference on medical science ,Benghazi 1944 ,page 60.

24- El-Reshaid K:Renal replacement therapy in Kwait. Saudi J Kidney Dis Transplant 1994; 5:493-497.

25- Kumar R:Hepatitis C virus infection among haemodialysis patients in Nigeran Region of Saudi

Arabia . Saudi J Kidney Dis Transplant 1997;2 :134-137.

26- Huraib S,Al-Rasheed R,Aldress A etal :The prevalence and risk factors for hepatitis C in Saudia Arabia. Saudi Kidney Dis Transplant Bull 1993;4:74.

27-Shaheen FAM,Huraib SO, AlRasheed R et al:Prevalence of hepatitis C antibodies in the Western

province of Saudi Arabia . Saudi J Kidney Dis Trasplant 1995; 6 :136-139.

28- Anil K.Saxena,Panthora BR,Naguib M, et al:Prevalence of hepatitis C antibodies among haemodialysis patients in Al-Hasa region of Saudi Arabia. Saudi J Kidney Dis Trasplant 200; 12:

562-565.

29-Levy J,Morgan J,Brown E (eds). Viral infection:HBV and HCV in Oxford handbook of dialysis 2002. Oxford Univerity Press. 496-497.

30-Daw MA,Elkaber MA,Drah A M etal. Prevalence of hepatitisC virus antibodies among different population vary in relative and attributable risk. Saudi Med.Journal 2002; 23:447-451.

31- Foster GR, Goldin RD, Thomas HC. Chronic hepatitis C virus infection causes a significant reduction in quality of life in the absence of cirrhosis. Hepatology 1998; 1: 209 –212.

32- Rodger AJ, Jolley D, Thompson SC et al . The impact of diagnosis of hepatitis C virus on quality of life . Hepatology 1999; 30 : 1299 – 1301.

33-Di Bisceglie AM ,MC Hutchison J , Rice C :New therapeutic stratiges for hepatits . Hepatology 2002 ;35 :224-231.

34-Johnson RJ, Gretch DR, Couser WG. Et al . Hepatitis C virus infection responsive to interferon alfa. Am J Kidney Dis 1995; 25:67 – 69.

35-Leigh JP, Bowlus CL, Lesitkow BN et al . Costs of hepatitis C. Arch Intern Med 2001; 161: 2231 – 2217.

36- Mailk AH, Lee WM: Chronic hepatitis B virus infection : treatment strategies for the next millenium . Ann Inter Med 2000; 132:723–731.

ARYL HYDROCARBON (Ah) RECEPTOR GENE EXPRESSION

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Abstract

Objectives: A gene had been identified as being necessary for the induced activity of aryl hydrocarbon hydroxylase(AHH) in human cells. This enzyme, which contains the cytochrome P450 1A1, is the primary activation enzyme responsible for the metabolism of carcinogens such as benzo[a]pyrene. Its level of activity has been associated with the risk of lung cancer in cigarette smokers. A gene necessary for AHH activity in hybrid cells had previously been located on human chromosome 2. The nature of this gene was unclear when the structural gene for cytochrome P4501A1 was subsequently located on human chromosome 15. RAG cells were known not to express any detectable levels of basal or inducible AHH activity. **Aim**: It was decided to test whether the gene on human chromosome 2 could be the Ah receptor gene which is necessary for the induced AHH activity in cells. It was also decided to test mouse RAG cells for the mouse Ah receptor gene and its activity. **Materials & Methods**: Primers were designed which could detect the human receptor gene in human x mouse hybrids and a number of clones were examined. RAG cell mRNA was isolated and the combination of reverse transcription and PCR was then used to test for expression of the gene in induced and uninduced RAG cells. **Results:** Correlation of the segregation of the receptor gene with human chromosome markers did show that it segregated with human chromosome 2. Moreover, when a different set of hybrid cells were tested it was found that in a primary hybrid clone containing the complete human chromosome 2 the receptor gene was present but a hybrid subclone which had previously been shown to lack the p terminus of human chromosome 2 lacked the Ah receptor gene. **Conclusion**: we can conclude that the Ah receptor gene is located at the p terminus of human chromosome 2. It has also been shown that there is a low level of expression in RAG cells with and without induction.

Key words: Aryl hydrocarbon receptor, PCR, Benzo[a]pyrene, A549 cells, RAG cells.

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Garyounis Medical Journal. Vol.20 No.1, pp.79-88.

INTRODUCTION

The cellular response to xenobiotics, in the form of aryl hydrocarbon hydroxylase induction, has been found to be mediated by an aryl hydrocarbon receptor protein[1]. Aryl hydrocarbon receptor protein is a 200-277 kDa soluble cytoplasmic protein found in tissues of several mammalian species[2] . It is known to bind only exogenous ligands such as 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) and to a lesser extent some halogenated , polycyclic aromatic hydrocarbons[3]. It has been shown that the receptor mediates the biochemical response to TCDD by finding that the occupied receptor binds specific DNA sequences upstream of the cytochrome P450 gene, *CYP1A1*[4]. When in the cytoplasm, the unoccupied receptor is associated with the heat shock protein (HSP90) and when it binds the ligand it dissociates from HSP90 and is translocated into the nucleus to enhance the transcription of *CYP1A1* gene through interaction with the xenobiotic responsive element (XRE), a cis-acting DNA element[5]. The receptor has been identified in the hepatic cytosol but in very low concentrations and the active protein was not purified because of loss of ligand binding activity during the purification process. Recently, the ligand binding subunit has been purified from mouse liver[6]. A cDNA clone has been isolated for this ligand binding subunit and it encodes a polypeptide exhibiting the Helix-Loop-Helix motif seen in some DNA binding proteins including aryl hydrocarbon nuclear translocator (Arnt), a protein proposed to be involved in the process of translocation of AH receptor from cytoplasm to nucleus [7].

It has been presumed that the AH receptor is a member of a superfamily of proteins which includes the steroid hormone, the thyroid hormone, vitamin D and retinoic acid receptors [8]. The AH receptor and the steroid hormone receptor share some similarities which include their binding of low molecular weight hydrophobic ligands and having similar ligand binding kinetics but they differ in that there is no known endogenous

ligand for the AH receptor and that the AH receptor shows a remarkable structural diversity in different species and different strains within the same species[9].

Chromosomal localisation of the regulatory and structural genes of the AHH enzyme activity is essential for a clear understanding of the mechanism of gene expression and enzyme induction. The regulatory and structural genes are not necessarily situated on the same chromosome. For example, a regulatory gene controlling murine AHH induction has been localised to the distal part of chromosome 17. [10]

Brown *et al.*[1] examined the linkage between AHH and 22 human isozymes; they found that the expression of AHH activity correlated with the expression of isocitrate dehydrogenase and malate dehydrogenase, which had been previously mapped to human chromosome 2. These results suggested that the gene for a gene product, necessary for AHH induction, was located on human chromosome 2. Analysis of hybrid cells prepared from a balanced translocation carrier [46 XX t (2:12) (q31-q24)] for segregation of chromosome 2 markers resulted in the assignment of this gene to the 2q31→2pter region[11]. On the other hand studies have indicated that the cytochrome P450 1A1 gene, a part of the AHH enzyme, lies on human chromsome 15q22→qter region[12]. Thus the nature of the gene on chromosome 2 remained unclear. There are two other genes responsible for the AHH activity namely the AH receptor and the AH receptor nuclear translocator that allows the AH receptor to interact with nuclear chromatin and the chromosomal position of these two genes is still unknown. In this study, We concentrated on defining the position of the AH receptor gene using a combination of somatic cell hybridization techniques and PCR.

Material and methods

Human lung type 2 pneumocytes (A549) deficient in hypoxanthine

phosphoribosyltransferase, and mouse B82 cells, an L cell subclone deficient in thymidine kinase, were used to prepare hybrid cells. Parent and hybrid cells were grown up in large quantities and cell pellets were frozen prior to analysis. The human chromosome content of the hybrid cells were determined by analysing cell pellet homogenates for the presence of specific isoenzymes previously assigned to each human chromosome. The cell pellets were also used isolate high-molecular-mass DNA from parent and hybrid cells.

Human A549 cells and mouse B82 cells were grown in D5 medium.Human A549 cells were fused to mouse B82 cells to obtain human x mouse cell hybrids which were subsequently grown in clones in a HAT medium to select against non-fused human and mouse cells.Total genomic DNA was isolated from human A459 cells and B82 cells and human x mouse somatic cell hybrids using the chloroform-phenol extraction and used in the PCR reactions.

RESULTS

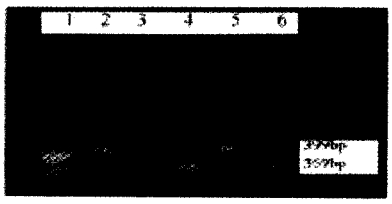
Total genomic DNA was extracted from mouse B82 cells, human A549 and human x mouse somatic cell hybrids. The mouse Ah receptor cDNA sequence was used to design a pair of PCR primers to test somatic cell hybrid clones for the presence or absence of the human and mouse AH receptor gene. When DNA prepared from human A549 cells was tested with these primers, a product of 369bp was obtained indicating that the primers were spanning a fragment corresponding to the published amino acid sequence of the human Ah receptor protein[13]. When DNA prepared from mouse Bcells was tested with the same pair of primers under the same conditions which were:

1) 94°C denaturation for one minute,
2) 55°C annealing for 90 seconds and
3) 72°C extension for 2 minutes for a total of thirty cycles and it gave aof 399bp size which was consistent with the size of the fragment flanked by the pair of primers on the published mouse cDNA sequence[7]. The difference in the size of the PCR products in the mouse and human that were obtained using the same set of primers must be due to a non-conserved region of the gene but this was very helpful in distinguishing between a mouse and a

human PCR product in DNA samples from human x mouse somatic cell hybrids. When aliquots from the human and mouse PCR products were mixed and loaded on a 2% (w/v) agarose gel and viewed under ultraviolet light after staining with ethidium bromide, it was possible to differentiate between them on the basis of size difference. The separation observed was small and thus it was decided to run the gels for a longer period when examining the hybrid clones.

It was then decided to use the PCR primers to test human x mouse hybrid clones with known human chromosome content and look at the pattern of segregation of the Ah receptor gene fragment.

The panel of human-mouse somatic cell hybrids used in previous experiments were thawed out and grown up in large quantity. Homogenates were tested for human chromosome complement using the starch gel electrophoresis and staining for enzyme markers. DNA was also isolated from the same hybrid cell samples and used in the PCR reactions. Several primary human x mouse hybrid cell clones were tested for the presence or absence of the Ah gene and the pattern of segregation with human chromsome markers was determined. An example of the results obtained from the PCR analysis is shown in Figure.



Agarose gel electrophoresis
Analysis of 20 l of PCR products of the Ah receptor
gene using primers AH5 and Ah6.
Lane 1: Hybrid cell clone positive for human Ah gene.
Lane 2: Hybrid cell clone negative for human Ah gene But with the 399bp mouse product.
Lane 4: Human product using human genomic DNA.
Lane 5: Mouse product using mouse genomic DNA

It is clear from the picture that the conditions employed for the PCR allowed for easy identification of positive and negative hybrid clones. Hybrids were scored plus or minus according to the presence or absence of the human PCR product. The data from all the hybrid cells was collected and examined (Table 1). The Ah receptor gene appeared to segregate with the human gene for malate dehydrogenase previously assigned to chromosome 2. Other chromosomes tested segregated independently from the human Ah gene.

Brown and co-workers[1] prepared a panel of mouse x human somatic cell hybrids and tested them for AHH and MDH enzyme activities as well as for their human chromosome contents using starch gel electrophoresis. Both MDH and AHH were shown to segregate with enzyme markers for human chromosome 2. When a hybrid cell clone expressing AHH and MDH enzyme activities named RKO20, was subcloned, one of its subclones named RKO20S3 showed absence of the AHH and MDH enzyme activities but it was still positive for isocitrate dehydrogenase, another human chromosome 2 enzyme marker. Chromosome analysis of this RKO20S3 subclone revealed a deletion in the short arm of chromosome 2. This deleted segment of the human chromosome 2 has been shown to carry the genes required for the AHH and MDH enzyme activities and the loss of the enzyme activities was explained on the basis of the deletion of the 2p25 region of human chromosome 2 from this subclone. Subsequently, Nebbali [14] mapped the MDH gene to the tip of the short arm of chromosome 2p25 using PCR.

We have tested both the intact hybrid cell clone RKO20 and the hybrid cell clone RKO20S3 which has the deletion in the short arm of chromosome 2 for the human Ah receptor gene using PCR primers. After PCR analysis on agarose gels, the369bp and the 399bp products were detected for the positive hybrid clone RKO20 which has the intact human chromosome 2 but only the 399bp product corresponding to the mouse gene was detected in the hybrid cell clone RKO20S3 containing the 2p25 deletion. Thus not only can it be concluded that the gene for the human

AH receptor is located on human chromosome but it can be sulocalised to the 2p25 region of chromosome 2.

Ah Receptor Gene Expression in Mouse RAG cells:

Mouse (BALB/cd) 8-azaguanine resistant RAG cells, an established heteroploid cell line derived from a renal adenocarcinoma [15] and known to be deficient in AHH activity [16] were grown in tissue culture and the cells were then harvested for DNA isolation using a rapid phenol extraction method.

RAG cells were tested for the presence of the Ah receptor gene using PCR amplification of a segment of the gene flanked by a pair of primers termed Ah5 and Ah6 as described above.

The Ah receptor gene was found to be present in RAG cells inspite of the fact that RAG cells have been shown by Brown and co-workers not to express basal or benzanthracene induced AHH enzyme activity.

In order to determine the nature of the defect in RAG cells which made them deficient in AHH enzyme activity, an experiment was designed to study the expression of the Ah receptor gene.

RAG cells were incubated with benzanthracene for 2, 4, 6, and 8 hours and then, after aliquotes were taken for a Lowry protein assay, total cellular RNA was isolated from each tissue culture plate using the rapid two step phenol extraction method of RNA isolation. Levels of total cellular RNA were measured spectrophotometrically and there was no difference between controls, and cells incubated for 2 hours, 4 hours, 6 hours, and 8 hours with benzanthracene.

cDNA synthesis was performed as described previously. PCR was performed using PCR primers in tubes containing 2µl aliquots of cDNA. PCR products were loaded on to 2% agarose gels and the intensities of the bands were compared by examining the ethidium bromide-stained gels under ultraviolet light. There was no

discernable difference in the intensity of the bands between the 0, 2, 4 and 8 hour samples suggesting that Ah receptor gene expression in RAG cells is not affected by incubation of the cells with the AHH enzyme inducer benzanthracene.

DISCUSSION

Responsiveness to TCDD, in the form of AHH induction has been thought to be coded by the Ah locus. The Ah locus is composed of several genes that could be linked. Hildebrand and co-workers have assigned the human *CYP1A1* gene to 15q22-qter region of chromosome 15 [12].

Brown and co-workers correlated a gene necessary for AHH activity in hybrid cells with the expression of malate dehydrogenase and isocitrate dehydrogenase which have both been mapped to chromosome 2 [1].

Ocraft and co-workers analysed a hybrid cell clone with a balanced translocation 46XX t (2:12)(q31-q24) and assigned this AHH gene to 2q31→pter region of chromosome 2 [11]. Susequently, Nebbali [14] mapped MDH and this AHH gene to the tip of the short arm of chromosome 2 using PCR.

The nature of the gene on chromosome 2 responsible for AHH activity was the target of this current investigation. Ema and co-workers isolated c-DNA clones encoding the murone Ah receptor gene. The deduced amino acid sequence of the human AH receptor gene was 72% homologous to the mouse sequence .

This describes the first evidence that the gene originally described by Brown *et al.* is the Ah receptor gene. It can also be concluded that this gene is located in the 2p25 region of chromosome 2.

The experiments performed on the RAG cells are of interest. Despite their inability to express basal or inducible AHH activity and inducible Ah receptor, do have the Ah receptor structural gene. This gene is expressed in uninduced cells. It can be concluded that either the receptor mRNA or the protein must be non-functional. A similar situation was described for a particular strain of uninducible mice studied in 1978 by Nebert *et al.* In this work D2 mice were injected with methylcholanthrene but no induction of AHH activity could be detected.

It will be of interest to design primers for the murine cyp 1a1 gene and test whether this gene is being expressed in the RAG cells but in inactive form.

Future work will also concentrate on studying the location of the aryl hydrocarbon nuclear translocator and its association with expression of aryl hydrocarbon activity in human cells.

REFERENCES

(1) Brown, S., Wiebel, F.J., Gelboin, H.V., Minna, J.D. Assignment of a locus required for expression of flavoprotein-linked monoxygenase to human chromosome 2. Proc Natl Acad Sci USA 1976; **73**: 4628-4632.

(2) Henry, E.C., Rucci, G., Gasi, T.A. Characterization of multiple forms of the Ah receptor: comparison of species and tissues. Biochemistry 1989; **28**, 6430-6440.

(3) Gillner, M., Bergman, J., Cambilla, C., Gustaffson, J.A. Interactions of rutaecarpine alkaloids with specific binding sites for 2,3.7,8itetrachlorodibenzo-p-dioxin in rat liver. Carcinogenesis 1989; **10**: 651-654.

(4) Neohold LA, Shirayoshi Y, Ozato K , Jones JF , Nebert DW : Regulation of mouse CYP1A1 gene expression by dioxin: requirement of two cis-acting elements during induction. Mol Cell Biol 1989; 9: 2378-2386.

(5) Whitlock JP, 1991, Ann Rev Pharmacol, 39, 13-19.

(6) Bradfield, C.A., Glover, E., Poland, A. Purification and N-terminal amino acid sequence of the Ah receptor from the C57BL/6J mouse. Mol Pharm 1991; 39: 13-19.

(7) Ema, E., Sogawa, K., and Watanabe, N., (1992), Biochem. Biophys. Res. Comm., 184(1), 246-253.

(8) Evans, R.M. 1991, Science, 240, 889-895.

(9) Poland A, Glover E, Bradford CA. Characterization of polyclonal antibodies to the Ah receptor prepared by immunization with a synthetic peptide hapten. Mol Phrmacol 1991;39: 20-26.

(10) Legraverand, C., Karenlampi, S., and Bigelow, S., (1984), Genetics, 107, 447-461.

(11) Ocraft, K.R., Muskett, J.M.& Brown, S. Localization of the human aryl hydrocarbon hydroxylase gene to the 2q31----2pter region of chromosome 2. Ann Hum Genet 1985; 49: 237-239.

(12) Hildebrand, C.E., Gonzalez, F.J., Mcbride, O.W. and Nebert, D.W. Assignment of the human 2,3,7,8-tetrachlorodibenzo-p-dioxin-inducible cytochrome P1-450 gene to chromosome 15. Nucleic Acids Res 1985; 13: 2009-2016.

(13) Itoh, S. and Kamataki, T. Human Ah receptor cDNA: analysis for highly conserved sequences. Nucleic Acids Res 1993; **21**: 3578-3581.

(14) Nebbali M, 1992 PhD thesis, Dept Biochemistry, University of Nottingham.

(15) Felluga, B., Claude A. and Merna, E. Electron microscope observations on virus particles associated with a transplantable renal adenocarcinoma in BALB-cf-Cd mice J Natl Cancer Inst 1969; 43: 319-324.

(16) Brown, S, and Chalmers, DE. Microsomal epoxide hydrolase activity in human x mouse hybrid cells. Biochem Biophys Res Comm 1986; 37: 775-780.

Table: Segregation of the EH gene in human x mouse hybrid clones

Chromosome	Enzyme	EH/enzyme			
		No of hybrid clones:			
		+/+	+/-	-/+	-/-
X	Glucose-6-phosphate dehydrogenase	3	1	2	6
1	Peptidase C	5	0	0	9
1	Adenylate kinase-2	5	0	0	9
2	Malate dehydrogenase	4	0	4	4
4	Phospho glucomutase	4	2	1	7
5	Hexosaminidase B	2	2	2	6
6	Malic enzyme 1	3	2	2	6
7	β-glucuronidase	4	1	3	5
8	Glutathione reductase	3	2	1	7
9	Adenylate kinase-1	3	3	2	8
10	Glutamate oxaloacetate transaminase	2	4	3	5
11	Lactate dehydrogenase A	1	3	5	3
12	Lactate dehydrogenase B	2	2	3	6
13	Esterase D	4	0	3	7
14	Nucleoside phosphorylase	1	4	4	6
15	Hexosaminidase A	2	2	3	8
16	adenosine phosphoribosyl transferase	2	3	4	5
17	Thymidine kinase	5	0	11	0
18	Peptidase A	1	3	2	7
19	Glucose phosphate isomerase	3	2	2	8
20	Adenosine deaminase	1	4	5	4
21	Superoxide dismutase	2	3	3	7

The presence or absence of the gene is marked by + or - respectively. Chromosomes 3 and 22 were not tested

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High Antimicrobial-Resistance Rates of *Escherichia coli* from Urine Specimens in Tripoli - Libya

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Abstract

Objectives: To determine the resistance rates of *Escherichia coli* from urine specimens collected from patients with urinary tract infections (UTI) in Tripoli. **Materials and Methods:**Included in the study 2209 mid-stream urine specimens. Isolation, identification and susceptibility testing of *E. coli* were carried out using standard bacteriological procedures. **Results:** *E. coli* was detected in significant numbers in 538 (24%) specimens. Of these 74% were resistant to ampicillin, 49% to cephaloridine, 25% to nitrofurantoin, 49% to tetracycline, and 45% to trimethoprim-sulphamethoxazole. **Conclusion:** The findings of the present work demonstrates clearly that the problem of antibiotic resistance among uropathogens in Libya is a very serious one and needs to be addressed urgently by the health authorities and the medical community.

Key word: *E.Coli,* antimicrobial resistance, Urine, Tripoli, Libya

Introduction:

Escherichia coli, a member of the family *Enterobacteriaceae*, is the leading cause of urinary tract infections (UTI) in man. These infections represent a major health problem with an estimated 10-20% of women are affected at some point of their life time[1].

On the other hand UTI accounts for less than 0.1% in healthy males[2]. Antimicrobials are the major tool in the management of UTIs. However, increased antimicrobial resistance among the causative agents makes

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such management not an easy job in clinical practice[3-5].

Data regarding the resistance rates of uropathogens on the national level are few and in Tripoli are lacking. The aim of the present work to report on antimicrobial-resistance rates of *E. coli* from urine specimens submitted to the bacteriology laboratory in the Centre of Plastic Surgery and Burns, Tripoli, to serve as a basis for comparison with future studies in Libya and elsewhere.

Materials and Methods:

Specimens:

From April 1994 to March 1995, 2209 mid-stream urine specimens from patients with UTI were examined. Most (>96%) of the specimens were from outpatients. The age of the individuals ranged from 3 months to 75 years.

Bacteriology:

Urine specimens were plated on McConkey or CLED agar (both from Oxoid, UK) using sterile calibrated loops. After incubation at 37°C for 18-24 hours, plates with $>10^5$ colony forming units (cfu) were considered significant. Urine Cultures with 3 different colonies or more were discarded. Isolated organisms were identified using standard bacteriological procedures[6] and the API 20E System (bioMerieux, France). Antibiotic susceptibility testing was performed using the disc diffusion method[7]

Results:

E. coli was detected in 538 (24%) urine specimens. Other bacteria detected included *Staphylococcus* spp. in 8%, *Proteus* spp. in 4%, *Klebsiella* spp. in 2%, *Pseudomonas* spp. in 1% and beta-haemolytic streptococci in 0.3% (Table 1). Antibacterial susceptibility testing of 538 *E. coli* strains resulted in 74% being resistant to ampicillin, 31% to cephalexin, 49% to cephaloridine, 29% to chloramphenicol, 7% to gentamicin, 33% to kanamycin, 11% to nalidixic acid, 25% to nitrofurantoin, 49% to tetracycline, and 45% to trimethoprim-sulphamethoxazole (TMB-SMZ). (Table 2).

Table 1. Bacteria detected in 2209 urine specimens from patients with UTI in Tripoli

Bacteria	No.(%) Positive
Escherichia coli	538 (24)
Staphylococcus spp.	176 (8)
Proteus spp.	85(4)
Klebsiella spp	49(2)
Pseudomonas spp	27 (1)
β-haemolytic streptococci	6 (0.3)
Total	881

Table 2. Antibiotic-resistance of E. coli from UTI in Tripoli.

Antibiotic	Resistance No.(%)
Ampicillin	398(74)
Cephalexin	166(31)
Cephaloridine	264(49)
Chloramphenicol	156(29)
Gentamicin	38(7)
Kanamycin	178(33)
Nalidixic acid	59(11)
Nitrofurantoin	135(25)
Tetracycline	264(49)
TMB-SMZ	242(45)

Discussion:

In a recent work by Tobgi et al[8], the antibiotic sensitivity of Gram-negative bacterial isolates from urine specimens, representing both hospitalized and out-patients, was investigated.

They reported that of the 148 *E. coli* isolated from outpatients 75% of them being resistant to ampicillin, 78% to cephaloridine, 45% to chloramphenicol, 18% to gentamicin, 82% to tetracycline, and 81% to TMP-SMZ. Although, the rate of resistance to ampicillin is nearly similar to ours (74%), the rates reported by the above mentioned investigators to other antimicrobials are higher than those reported by the present study. Of the antimicrobials tested, in the present investigation, nalidixic acid and nitrofurantoin appears to be the most effective against *E. coli* examined. Although, Tobgi et al[8] reported similar findings their *E. coli* resistance-rates to both drugs were lower than ours (10% and 7% respectively). These differences in the resistance rates may be due to availability of antibiotics and their usage in a practice population. Studies have shown that among isolates from practices with high usage of an antibiotic, rates of resistance to that antibiotic tended to be high, and

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usage correlated significantly with resistance between practice population units[9]

There is a growing concern on the increase of antibiotic resistance among bacteria including the uropathogens in Europe, United States and Canada. However, the rates of resistance among such pathogens in those countries still low[2,10,11] compared to the rates found in our study and that of Benghazi. This can be shown clearly by a very recently published study by Karlowsky et al[11] on the trends in antimicrobial resistance among urinary tract infection isolates of *E. coli* from female outpatients in the United States. Analyzing susceptibility testing data from The Surveillance Network (TSN) Database-USA from 1995 to 2001, these workers found that resistance rates among *E. coli* isolates to ampicillin (range, 36.0 to 37.4% per year), TMP-SMZ (range, 14.8 to 17.0%), ciprofloxacin (range, 0.7 to 2.5%), and nitrofurantoin (range, 0.4 to 0.8%) varied only slightly over the 7-year period.

The findings of the present work and that of Benghazi demonstrates clearly that the problem of antibiotic resistance among uropathogens in Libya is a very serious one and needs to be addressed urgently by the health authorities and the medical community. Furthermore, alternatives to the commonly used antimicrobials (particularly TMP-SMZ) for the treatment of UTI needs to be considered in Libya.

References:

1. Johnson JR, Stamm WE. Urinary tract infections in women: diagnosis and treatment. *Ann Intern Med 1989*; 111: 906-117.

2. Blondeau JM, Yaschuk Y, Suter M, Vaughan D. The Canadian Antimicrobial Study Group. In-vitro susceptibility of 1982 respiratory tract pathogens and 1921 urinary tract pathogens against 19 antimicrobial agents: a Canadian multicentre study. *J Antimicrob Chemother 1999*; 43 (suppl): 3-23.

3. Gupta K, Scholes D and Stamm WE. Increasing prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in women. *JAMA 1999*; 281: 736-738.

4. Kunin CM Resistance to antimicrobial agents-a world calamity. *Ann Intern Med 1993*; 118: 557-561.

5. Weidermann B and Zuhlsdorf MT. Resistance development to to fluoroquinolones in Europe. *Am J Med 1989*; 87 (Suppl 5A): 9S-11S.

6. Collee JG., Duguid JP., Fraser AG. and Marmion BP. *Practical Medical Microbiology.*

13th ed. Churchill Livingstone, Edinburgh;1989.

7. Bauer AW, Kirby WMM, Sherris IC, Turck M. Antibiotic susceptibility testing by a standard single disk method. Am J Clin Pathol 1966; 45: 493-496.

8. Tobgi RS, Taher IAA, Ali MA. Antibiotic susceptibility of uropathogens in Benghazi, Libya. *JMJ 2001*; 1(2): 46-49.

9. Howard AJ, Magee JT, Fitzgerald KA, Dunstan FDJ. Factors associated with antibiotic resistance in coliform organisms from community urinary tract infection in Wales. *J Antimicrob Chemother 2001*; 47; 305-313.

10. Barrett SP, Savage MA, Rebec MP, Guyot A, Andrews N, Shrimpton SB. Antibiotic sensitivity of bacteria associated with community acquired urinary tract infection in Britain. *J Antimicrob Chemother 1999*; 44: 359-365.

11. Karlowsky JA, Kelly LJ, Thornsberry C, Jones ME, Sahm DF. Trends in antimicrobial resistance among urinary tract infection isolates of *Escherichia coli* from female outpatients in the United States. *Antimicrob Agents Chemother 2002*; 46: 2540-2545.

Cleft lip and palate: A review and experience with 623 cases

Abdul Rahman El Gallal, Mohamed Sanusi, Adel El Batrawi, MohiEdyn El Shokri

الملخص

أهداف البحث: تحليل حالات انشقاق الشفة و شراع الحنك و اللهاة التي استقبلناهم خلال 11 عام وذلك فيما يتعلق بتصنيف الشق، الجنس، المشاكل الصحية المصاحبة، عوامل الخطر المسببة، نوع الجراحة و تكرارها والمضاعفات الناجمة.

مكان البحث: وحدة جراحة التجميل بمستشفى الجلاء، بنغازي.

المرضى / طرق البحث: تم تحليل البيانات المأخوذة من ملفات المستشفى لعدد 623 حالة. استعرضت نتائج التقييم السريرى وقدمت الجوانب الطبية و النقاط المتعلقة وتمت مناقشتها بإيجاز.تم أيضا استعراض 592 حالة أجريت لها جراحة مع مراجعة المضاعفات المبكرة و المتبقية للجراحة، و قورنت نتائجنا أيضا بدراسات مشابه.

النتائج: يشكل نوع الشق أمام القاطع نسبة 59.87 % من حالات، و تسود الإناث الحالات ذات الشق خلف القاطع بينما أظهرت الذكور الغلبة في الحالات الأخرى من ذوى الشق أمام القاطع و خلال القاطع.من المجموع الكلى للحالات، مثلت الحالات التي تجاوزت العامين من العمر نسبة 11.88 %، و 8.35 % من الحالات لها ترافق بتشوهات خلقية أخرى، و 9.3 % قدمت تاريخ عائلي ذات علاقة، بينما عوامل الخطر البيئية ظهرت في 23.76 % من الحالات. 727 عملية جراحية أولية أجريت لتقويم و بناء 592 حالة، 270 منها أجريت من أجل 135 حالة من ذوات الشق خلال القاطع. إصلاح الشفة بطريقة تدوير وتقديم سدالة سجل لصالح 312 حالة، بينما إصلاح الشفة باستعمال السدالة المثلثة و أسلوب إلصاق الشفة سجل لصالح 134 و 47 حالة على التوالي. إصلاح اللهاة من داخلها كان الأسلوب الرئيسي المتبع وقد سجل لصالح 123 من مجموع 225 حالة.

Abstract

Objectives: To analyse cleft lip and palate patients whom we have seen over a period of 11 years, regarding type of cleft, sex of patient, associated problems, predisposing risk factors, methods and frequency of surgery performed and complications encountered. **Setting**: Plastic surgery unit at Aljala Hospital, Benghazi. **Patients & Methods:** The data from hospital files of 623 cases were analysed, the results of our clinical assessment were demonstrated, and the clinical profiles and other related points are presented and briefly discussed. Also, 592 operated cases were reviewed and particular reference is paid to early and residual postoperative complications encountered.Our results were also compared with similar studies. Results: The preincisive variety comprised 59.87 % of cleft cases. Females predominated the post-incisive variety while trans-incisive and pre-incisive varieties have shown male predominance. Out of all cases, 11.88 % were over 2 years of age, 8.35 % revealed other associated deformities, and 9.3 % had a family history of cleft deformity, while environmental risk factors

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accounted for 23.76 %. A total of 727 primary procedures were carried out to reconstruct 592 patients, out of these 270 were performed for 135 trans-incisive cleft cases. Rotation advancement flap repair accounted for 312 lip repair, while triangular flap repair and lip adhesion accounted for 134 and 47 repairs respectively. Intravelar veloplasty was the main adopted palatal repair and accounted for 123 out of 225 cases. **Conclusion:** The need for additional research in this area is unequivocal. We must increase our efforts to obtain a sound cleft lip and palate epidemiology. Furthermore, to specify outcome predictors to select the most suitable method for the initial repair so to minimise the need for secondary procedures.

Key Words: Cleft lip; Cleft palate; Cleft lip and palate; Rotation advancement flap; Triangular flap repair; Lip adhesion; Intravelar veloplasty.

Introduction:

Patients with clefts of the lip and palate present a number of problems associated with speech and hearing during early childhood, dental anomalies and malocclusion in the developing dentition, and secondary facial deformities and emotional upset in adolescence, and they are at risk for learning disability and low school achievement. Our total number of patients with such deformity was 623, of them 592 patients were operated and have had their primary repair in form of single or multi stage reconstruction at Aljala Hospital of Benghazi over a period of 11 years (Jan.1990 – Dec. 2000). Throughout the stated period, we have adapted different kinds of repair for both lip and palate cases. Herein we attempt to analyze the clinical findings, aiming to identify the pattern of this deformity in our cases and to evaluate the results of our management in respect to the existence of great personal variation and late presentation of some cases, with special reference to types of repair performed.

Methods and Patients:

The medical case records of 623 patients who had been admitted to our side with such deformity during the stated period were reviewed

thoroughly. Personal and medical data were collected, and special attention was given to the presence of any associated medical conditions or concomitant deformities, and also to the existence of any significant points in family or prenatal history. Excluding those who have had permanent contraindications for surgery or been repaired elsewhere, and those who were admitted for secondary reconstructive procedures, we counted 592 operated patients. Their preoperative requirements, which include being of good health without any evidence of either infection or poor nutrition, were fulfilled for all of our patients. The anterior palate repair (including lip repair) for most of our patients was carried out at about age of 7 months, while that for posterior palate at about age of 16 months.

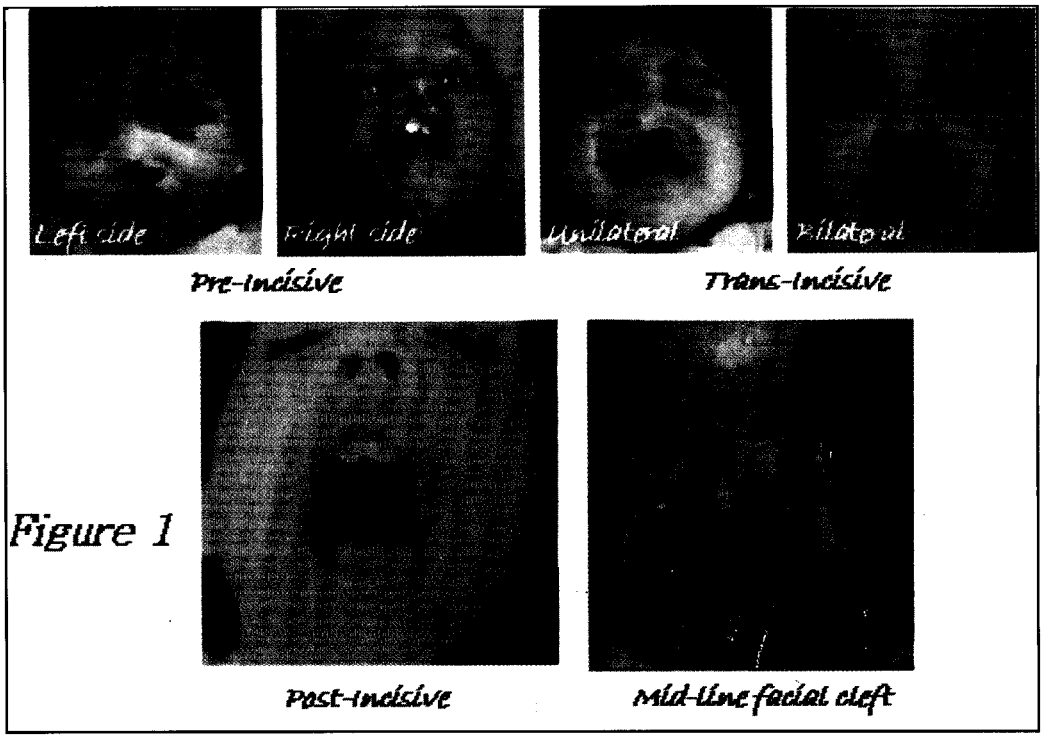

Figure 1

In relation to incisive foramen, patients were grouped according to their deformity into pre-incisive, trans-incisive, and post-incisive groups *(Figures 1)*. Choice of repair was individualized, and prophylactic ampiclox was given infrequently. All operated cases were kept in hospital for 3 to 10 days, and have been followed up for a minimum of 6 months. Early local post-operative complications and the residual defects, which do not

include those due to hypoplasia, shortage of tissues, or severe skeletal distortion, were analysed in relation to the different types of repair.

Results:

The frequency of various types of cleft lip and palate that we have seen over the period of study is presented in (***Table I***) which also illustrates their distribution according to the involved side and sex of the patient. The pre-incisive clefts was the most common variety, being observed in 59.87 % (n = 373) of our patients, while the post-incisive was the least frequent variety only 14.93% (n = 93). Excluding patients with bilateral trans-incisive type of cleft, all pre- and trans-incisive varieties were predominated by males while the post-incisive one had slight female predominance. Left side clefts were found more frequent than right side or bilateral ones. Only 17 patients (2.7 %) were found to be non-Libyan.

The age of our patients ranged from 2 days to 41 years, 74 patients (11.88%) were over 2 years, of these 56 patients (8.98 %) belonged to post-incisive group that includes submucous cleft variety. Although facial disfigurement, feeding difficulties,

Table I
The frequency of various types of cleft deformity

Sex / *Type of cleft*	*Male*	*Female*	*Total*
Pre-incisive	***210***	***163***	***373***
Left	121	94	215
Right	73	58	131
Bilateral	16	11	27
Median	0	0	0
Trans-incisive	***84***	***73***	***157***
Left	41	29	70
Right	14	9	23
Bilateral	29	33	62
Median	0	02	02
Post-incisive	***39***	***54***	***93***
Soft	13	19	32
Soft & Hard	19	31	50
Submucosal	07	04	11
Total	***333***	***290***	***623***

nasal regurgitation, and speech impairment were the main presenting features, some of our cases had additional symptoms related to co-existing medical diseases or complications like otitis media, malnutrition, and respiratory infections. Other associated congenital deformities were seen in 52 patients (8.35%), of them 27 (4.3%) were considered to be syndromal (***Table II***), and they were relatively more frequent.

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Figure 2: Member of Family with cleft lip

in patients who had transincisive type of clefts 3.85% (n = 24) than in those with postincisive 2.57% (n = 16) or preincisive ones 1.93% (n = 12). In 58 cases (9.3%) there was a definite familial history of cleft deformity (***Figure 2***), 31 of them belonged to the post-incisive group, while parental consanguinity was found in 96 cases (15.41 %). Maternal exposure to other risk factors during the first 3 months of gestation was obtained in 148 cases (23.76 %). 68 mothers were over 40 years of age (***Table III***). Out of the total 623 patients, 31 have had no surgery in our unit either because they have got a major cardiac illness or

Table II: ***Concomitant Deformities***

Concomitant Deformities	*No.*
Syndromic	*27*
Trisomy 21	16
	04
Pierre Robin Sequence	07
Asyndromic	*25*
V.S.D.	03
Hydrocephalus	03
Hypospadias	02
Polydactyl	02
	01
Spina bifida	01
C.D.H.	01
Ectopia Vesica	01
Club foot	01
Mandibular lip pits	08
Multiple Deformities	02
Total	*52*

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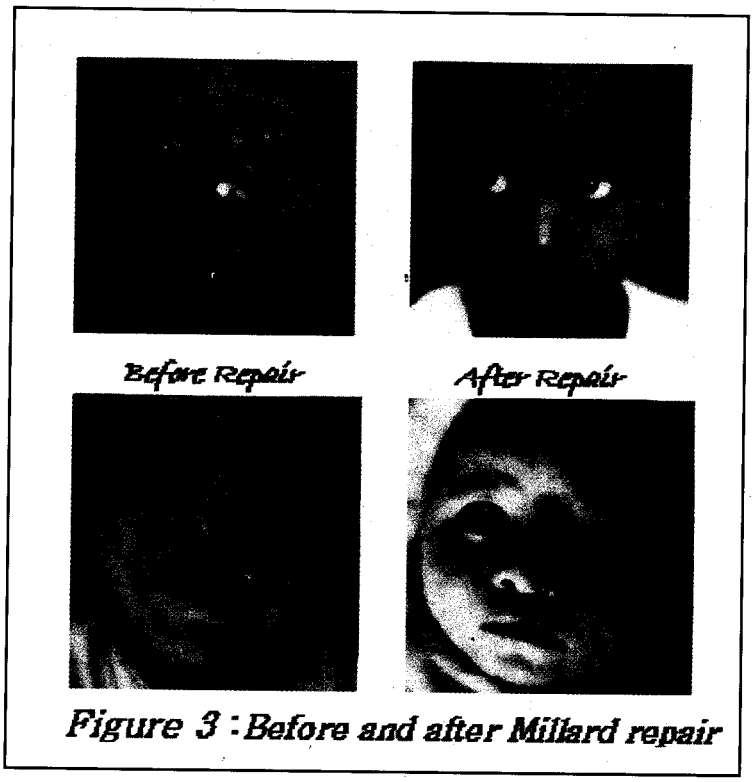

Figure 3 : Before and after Millard repair

severe mental retardation, or have left to get operated elsewhere. 727 primary procedures were carried out for the remaining 592 patients (***Table IV***). Repair of cleft lip with or without anterior palate repair (502 repairs) represent (69%) of the total cleft surgery, while the remaining 225 repairs (31%) were for cleft of posterior palate. 135 patients with transincisive cleft have had their repair in 2 stages (270 procedures). There were no perioperative deaths among our patients. Rotation – advancement flap repair (Millard) [1] was the most common one performed (***Figure 3***), and it was accounting for 312 repairs out of the total primary cleft lip surgery (62.15%), while triangular flap

Table III : Risk Factors Encountered

Maternal History	*No.*
Environmental Risk Factor	*148*
Diabetes (on Insulin)	22
Diabetes (on tablets)	07
Threatened Abortion	23
Hypertension	18
Epilepsy	07
Febrile illness	11
Psychic or Mental stress	40
Drugs:	20
Steroids	*7*
Anti-epileptics	*7*
Voltaren (Diclofenac sodium)	*3*
Librax (chlordiazepoxide)	*1*
Phenergan (promethazine HCl)	*1*
Buscopan (Hyoscine butylbromide)	*1*
Old mother (over 40 years)	*68*

repair (Tennison–Randall)[2,3,4] and lip adhesion accounted for 143 repairs (28.48%) and 47 repairs (9.36%) respectively (***Figure 4, 5, 6***). Two of our female patients presented with mid-line facial cleft (***Figure 1***) and one of them had her repair in our unit (***Figure 7***). The frequency of early and residual local complications in our cases is detailed in (***Table V***).

Frequency of different palatal repairs and distributions of their postoperative complications are shown in (***Table VI***). In the first 3 years, VonLangenbeck and Wardill repairs [5, 6] were frequently performed, 43 and 38 patients out of 225 patients underwent previous repairs respectively. Intravelar veloplasty [7] was adopted for many years and it is accounting for 123

Figure 4: Before and after Tennison–Randall lip repair

patients ,while Furlow double Z plasty [8] was reserved mainly for short soft palate with narrow cleft or submucous one (21 patients).

Discussion:

With this in-born defect, patient develops crucial problems with feeding, phonation, growth and development of affected and allied soft and hard tissue structures. This in turn results in deformity and asymmetry, which is going to affect functional requirements as well as aesthetic outlook. To our knowledge, the data about the cleft lip is still rather limited

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as there is no national registry for congenital anomalies. Our study was to make a preliminary estimate of the

Table IV: *Frequency of Surgery among different groups of operated patients*

	Pre-incisive	*Trans-incisive*	*Post-incisive*	*Total*
Referred patients	***373***	***157***	***93***	***623***
Bilateral	27	62		
Unilateral	346	94		
Operated patients	***367***	***135***	***90***	***592***
Bilateral	23	55		
Unilateral	344	80		
Frequency of surgery	***367***	***270***	***90***	***727***
Bilateral	23	110		
Unilateral	344	160		

Table V: Frequency of different Methods used for lip repair & their complications

Methods of repair	***Millard*** *312* (62.15%)		***Tennison*** *143* (28.48%)		***Lip adhesion*** *47* (9.36%)		***Total*** *502* (100%)
	Bilat. *21*	***Unilat.*** *291*	***Bilat.*** *13*	***Unilat.*** *130*	***Bilat.*** *44*	***Unilat.*** *3*	
Local Complications							
Early							
Infection	0	6	0	4	0	0	10
Haematoma	1	0	0	2	0	0	3
Ischaemia (*necrosis*)	3	0	2	0	0	0	5
W. Dehiscence	4	8	2	6	0	0	20
Residual Defect	**9**	**14**	**4**	**17**	**8**	**1**	**53**

Table VI: *Frequency of different palatal repair & their complications*

Methods of repair	***I.V.Veloplasty***	***Furlow.Plasty***	***Von Langenbeck***	***Weardill-Kilner***	*Total* 255
Complications	*123*	*21*	*43*	*38*	
Early					
Infection	*2*	*0*	*1*	*0*	*03*
Haematoma	*0*	*0*	*1*	*0*	*01*
Ischaemia(necrosi)	*3*	*2*	*4*	*3*	*12*
W. Dehiscence	*5*	*2*	*6*	*3*	*16*
Air way obstruction	*2*	*0*	*3*	*2*	*07*
Haemorrhage	*1*	*1*	*3*	*4*	*09*
Residual Defect					
Fistula	*5*	*2*	*6*	*2*	*15*
V.P.I.*	*7*	*2*	*10*	*8*	*27*

**V.P.I: Velopharyngeal Incompetence.*

frequency of different cleft deformities and also to review our management and evaluate the outcome.

Among our patients, the most frequent type of cleft deformity was found to be the unilateral pre-incisive one and within this group the left side was more commonly affected, followed by bilateral clefts. And overall, males had a higher incidence of cleft deformities than females, though for isolated clefts females predominated both the post-incisive cleft palate group and the bilateral trans-incisive subgroup. These findings were nearly consistent with what has been reported by others.[9-16] Consequences of delay in presentation and getting early medical advice in such cases have been highlighted in previous studies[15, 17]. In the present one, 11.88% of our patients (n = 74) presented after 2 years of age. The reason for this is partially because most of these cases have had posterior (soft palate) clefts that include submucous variety which are very easy to pass unnoticed by the parents in the first few years, and to some extent due to our poor primary health care service that should screen all newborns for common congenital anomalies. In addition to the typical presenting symptoms and those related to otitis media and respiratory complications. 52 patients (8.3%) had associated congenital anomalies and 27 (4.33%) of them were considered to be syndromal. This figure is considerably lower than that reported from European, Scandinavian, Middle Eastern, and Far Eastern countries [9, 13- 15, 18-20]. In our study, these associated congenital anomalies were most common among the transincisive group, similar findings were also observed by Lilius,[14] though Milan et al [13] found them higher among the post incisive one.

Family history of non syndromal cleft deformities was positive in 58 of our patients (9.3%), and this is relatively low figure when compared with figures of 26.8% and 18.8% from Saudi Arabia and Malaysia respectively.[15, 20] On the other hand and like others, our results show that parental consanguinity, age of the mother, and exposure at early pregnancy to environmental risk factors are important predisposing factors[10,13,18,21-26].Although more reports

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are appearing from centres where early palate repair is performed [27], neonatal reconstruction is still not universally accepted, as early surgery for cleft palate is known to have an effect on growth and development of the mid-face[28–30], hence controversy still exists regarding the optimal timing and surgical technique for primary repair, and treatment protocols vary considerably. On the other hand late repair of the alveolus and anterior hard palate subjects the patient to the problems of anterior palate fistula for extended periods of time, therefore we have utilised a single combined procedure performed when the child is about 8 months of age that completely closes the anterior hard palate and alveolus (using vomer and lateral nasal mucosal turnover flaps) along with the cleft lip. Techniques for lip repair (cheiloplasty) continue to evolve as plastic surgeons strive to achieve the most natural lip, and it is always a challenging task with no single technique satisfactory for all types of cleft deformity. Hence and for many years a wide variety of surgical techniques has been described, and out of them we have had experience with the (Millard) Rotation-advancement flap repair (62.15%), (Tennison-Randall) triangular flap repair (28.48%), and lip adhesion (9.36%). When we look at the outcome of our lip repair, Millard repair (291 unilateral & 21 bilateral), Tennison-Randall repair (130 unilateral & 13 bilateral), and adhesion (44 unilateral & 3 bilateral), one could not find reasonable link between early local complications and types of lip repair. However, we could notice that, out of 20 patients who had wound dehiscence, 18 were just consequences of other early post operative complications like wound infection, haematoma, and ischemia, while the remaining 2 were caused by direct trauma. Different known residual complications like horizontal and vertical lip defects, whistling deformity, orbicularis bulge, malalignment, and nasal stenosis, have been observed distributed in 53 patients (10.55%). Furthermore, differences in their distribution among various lip repairs were clearly illustrated; they were relatively less frequent with Millard type of repair (23 per 312). The

learning curve of palatal surgery is long, and as no single repair would have been adequate for all varieties of clefts. Therefore, right through the studied period, four popular procedures that are currently used in many centres for closing the posterior cleft palate have utilised. Our criteria for choosing the suitable palatoplasty for given patient are based mainly on the type of cleft and personal preference. Though, Intravelar veloplasty created particularly favorable conditions for

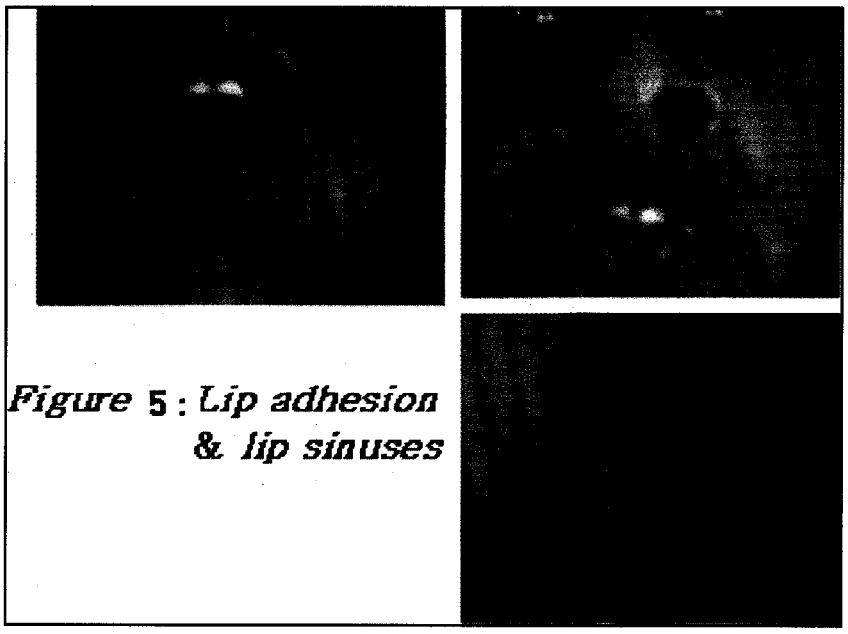

Figure 5 : Lip adhesion & lip sinuses

postoperative haemorrhage among our cases (7 out of 9 cases). Residual palatal fistula accounted for 15 out of 225 palatal repairs, 8 of them occurred in those who underwent VonLangenbeck

good velar function with relatively low complication. Other than airway obstruction and secondary haemorrhage the nature of early local postoperative complications and their relations to different types of palatal repair do not differ much from those of cheiloplasty. Marginal ischaemia or necrosis was relatively more frequent with VonLagenbeck and Wardill types of repair having ratio of 4 per 43 and 3 per 38 respectively. These repairs were also responsible for high proportion of.

and Wardill repairs. All fistulae were consequences of wound dehiscence resulting from poor wound healing,

tension, haematoma, or poor multi-layer repair, 4 fistulae were anteriorly located and 11 were at the junction between hard and soft palate. Most of these residual fistulae underwent successful closure at minimum of eight months post repair. Evidence of velopharyngeal incompetence was found in 27 patients, 18 of them underwent either VonLengenbeck or Wardill type of repair.Overall, when one considers the wide varieties and complex nature of cleft lip and palate deformity and the related surgical difficulties, the major complications encountered do not appear to be excessive and are within the acceptable range and the main ones like residual

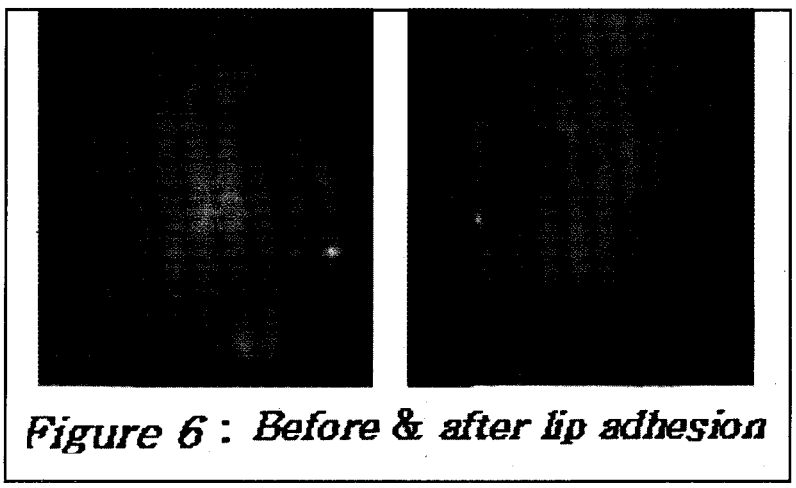

Figure 6 : Before & after lip adhesion

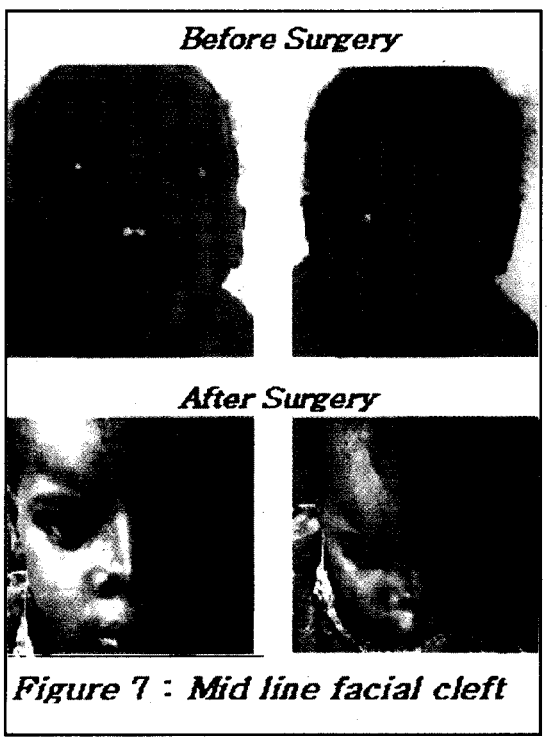

Figure 7 : Mid line facial cleft

fistulae compare favorable with what has been reported by others. 31 Although the majority of our patients have achieved excellent aesthetic results and good velar function, we believe that a much longer follow up is necessary in order to assess our protocol in management of such problem

References:

1- Millard D. R.. A primary camouflage of the unilateral harelip. In: Transactions of the International Society of Plastic Surgery, First Congress, 1955. Baltimore, The Williams & Wilkins Co, p 160-165. 1957.

2- Tennison CW.. The repair of the unilateral cleft lip by the stencil method. Plast. Recontr. Surg. 9:115-120,1952.

3- Randall P.. A triangular flap operation for the primary repair of unilateral clefts of the lip. Plast. Recontr. Surg. 23:331-347, 1959.

4- Randall P.. A lip adhesion operation in cleft lip surgery. Plast. Recontr. Surg. 35: 371, 1965.

5- Langenbeck B. Von. Operation der angeborenen totalen spaltung des harten gaumens nach einer neuen methode. Deutsche klinik, 8, 231. Reprinted in English in plastic and Reconstructive Surgery, 49: 323, 1972.

6- Wardill W. E. M.. The technique of operation for cleft palate. Br. J. Surg. 25: 117-119, 1937.

7- Kriens O. B.. Fundamental anatomic findings for an intravelar veloplasty. Cleft palate Journal, 7: 27, 1970.

8- Furlow LT.. Cleft palate repair by double opposing Z-plasty. Plast. Recontr. Surg. 78:724-733, 1986.

9- Hagberg C., Larson O., Milerad J.. Incidence of cleft lip and palate and risks of additional malformations. Cleft Palate Craniofac. J. 1998 Jan; 35(1): 40-45

10- Chuangsuwanich A., Aojanepong C., Muangsombut S., Tongpiew P.. Epidemiology of cleft lip and palate in Thailand. Ann-Plast-Surg. 1998 Jul; 41(1): 7-10

11- Das SK., Runnels RS Jr., Smith JC., Cohly HH.. Epidemiology of cleft lip and cleft palate in Mississippi. South. Med. J. 1995 Apr; 88(4): 437-442.

12- Gregg T., Boyd D., Richardson A.. The incidence of cleft lip and palate in Northern Ireland from 1980-1990. Br. J. Orthod. 1994 Nov; 21(4): 387-392.

13- Milan M., Astolfi G., Volpato S., et al. 766 cases of oral cleft in Italy. Data from Emilia Romagna (IMER) and northeast Italy (NEI) registers. Eur. J. Epidemiol. 1994 Jun; 10(3): 317-324

14- Lilius GP.. Clefts with associated anomalies and syndromes in Finland. Scand-J-Plast. Reconstr. Surg. Hand Surg. 1992; 26(2): 185-196

15- Kumar P., Hussain MT., Cardoso E., et al. Facial clefts in Saudi Arabia: an epidemiologic analysis in 179 patients. Plast. Reconstr. Surg. 1991 Dec; 88(6): 955-958

16- Shaw GM., Croen LA., Curry CJ.. Isolated oral cleft malformations: associations with maternal and infant characteristics in a

California population. Teratology. 1991 Mar; 43(3): 225-228

17- Philip Kuo-Ting Chen, Jorie Wu, Kai-Fong Hung, et al.. Surgical Correction of submucous Cleft palate with Furlow Palatoplasty. Plast. Reconstr. Surg. 1996; 97(6): 1136-46

18- Stoll C., Alembik Y., Dott B., Roth MP.. Epidemiological and genetic study in 207 cases of oral clefts in Alsace, north-eastern France. J. Med. Genet. 1991 May; 28(5): 325-329

19- Murray JC., Daack Hirsch S., Buetow KH., et al. Clinical and epidemiologic studies of cleft lip and palate in the Philippines. Cleft Palate Craniofac. J. 1997 Jan; 34(1): 7-10

20- Boo NY., Arshad AR.. A study of cleft lip and palate in neonates born in a large Malaysian maternity hospital over a 2-year period. Singapore Med. J. 1990 Feb; 31(1): 59-62

21- Taher AA. Cleft lip and palate in Tehran. Cleft Palate Craniofac. J. 1992 Jan; 29(1): 15-16

22- Shaw GM., Croen LA., Curry CJ.. Isolated oral cleft malformations: associations with maternal and infant characteristics in a California population. Teratology. 1991 Mar; 43(3): 225-228

23- Slavkin HC.. Incidence of cleft lips, palates rising. J. Am. Dent. Assoc. 1992 Nov; 123(11): 61-65

24- Peterka M., Tvrdek M., Likovsky Z., et al. Maternal hyperthermia and infection as one of possible causes of orofacial clefts. Acta. Chir. Plast. 1994; 36(4): 114-118

25- Loffredo L. De. C., de Souza JM., Yunes J., et al. Cleft lip and palate: case control study Rev. Saude. Publica. 1994 Jun; 28(3): 213-7

26- King PB., Lie RT., Irgens LM.. Spina bifida and cleft lip among newborns of Norwegian women with epilepsy: changes related to the use of anticonvulsants. Am. J. Public Health. 1996 Oct; 86(10): 1454-6

27- Denk MJ., Magee WP Jr.. Cleft palate closure in the neonate: preliminary report.Cleft Palate Craniofac. J. 1996 Jan; 33(1): 57-61

28- Capelozza Filho L, Normando AD., da Silva Filho OG.. Isolated influences of lip and palate surgery on facial growth: comparison of operated and unoperated male adults with UCLP. Cleft Palate Craniofac. J. 1996 Jan; 33(1): 51-56

29- Adcock S., Markus AF..Mid-facial growth following functional cleft surgery. Br. J. Oral Maxillofac. Surg. 1997 Feb; 35(1): 1-5.

30- Han BJ., Suzuki A., Tashiro H.. Longitudinal study of craniofacial growth in subjects with cleft lip and palate: from cheiloplasty to 8 years of age Cleft Palate Craniofac. J. 1995 Mar; 32(2):156-166

31- Lehman JA. Jr., Douglas BK., Ho WC., Husami TW.. One-stage closure of the entire primary palate. Plast. Reconstr. Surg. 1990 Oct; 86(4): 675-681.

Probability of malignancy in the solitary pulmonary nodule

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Abstract

Objectives: Solitary pulmonary nodules have always represented a challenge in determining their nature. Our aim was to determine what variables predict malignant outcome in such nodules. **Materials & methods**: In a prospective cohort study, we followed up 102 patients with solitary pulmonary nodule looking for factors likely to be associated with a poor outcome. **Results and conclusions**:Three clinical characteristics; age, sex and cigarette smoking, and three radiological characteristic of the nodule; diameter, irregular border, and the presence of certain patterns of calcification, were found to be independent predictors of malignancy.

The location of the SPN and presence of respiratory symptoms did not have any significant influence.

Key words: Solitary pulmonary nodule, Solitary pulmonary lesion, Coin lesions, Lung tumors.

Introduction:

A solitary pulmonary nodule (SPN) is defined as a single, discrete intrapulmonary radiographic density less than 4 cm in diameter surrounded by aerated lung and not associated with mediastinal lymphadenopathy, atelectasis, or pneumonitis. [1,2]

A diverse number of malignant and benign processes may manifest as a solitary pulmonary nodule on a chest roentgenogram with out any symptoms the management of these "coin lesions" is still controversial. Some patients with SPN have potentially curable malignancies that, ideally, should be resected without delay while others have benign nodules and should not be exposed to the risk of surgery and invasive procedures. [3,4] The major question following the detection of a

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solitary pulmonary nodule is whether the lesion represents a benign or a malignant process. Once the probability of malignancy is defined for a particular nodule, decision analysis can be used to determine the preferred approach to initial management. [2,3,5] In this report, based on the analysis of our own case, we determine what clinical and radiological factors predict malignant outcome of SPNs.

Materials & Methods:

Patients

Patients with SPNs referred for bronchoscopy in the period from January 1994 to August 1998 that fulfilled the above definition of SPN, and had negative bronchoscopic examination were enrolled in the study. Cases with clinical or radiographic evidence of metastatic disease and those who had recent primary malignancy outside the chest were excluded. Initially 147 patients were considered eligible for enrollment. Data for final analysis were available for 102 patients. Forty- five patients were lost for follow up.

Statistical analysis:

Measures of comparative risk (relative risk and Odds ratio) are calculated from two-way tables (2x2 tables).[6]

Results:

Data were available for final calculation at the end of follow - up for 102 patients with solitary pulmonary nodules; 72 patients (70.6%) were males and 30 patients (29.4%) were females. The mean age was 61.0 years (range 32-81). Most patients were a symptomatic at the time of radiographic detection of the SPN. Respiratory symptoms (cough, chest pain and hemoptysis) were present in only 16 patients (15.7%). A final diagnosis was established by thoractomy in 44 patients, transthorasic needle aspiration in 46 patients, transbronchial needle aspiration in 7 patients, thoracoscopy in 3 patients and video-assisted thoracoscopic (VATS) biopsy in 2 patients. Malignant nature of the SPN was demonstrated in 48

patients (47.1%), while 54 patients (52.9%) had benign nodules (Table1). The most frequent malignancy was squamous cell carcinoma, which was diagnosed in 19 patients (18.6%). Adenocarcinoma was diagnosed in 14 patients (13.7%). On the other hand tuberculosis was the most common benign condition (14.7%) followed by non-specific granulomas (10.8%). Benign tumors collectively accounted for 21 nodules, i.e. 20.6%. The group designated "others" in the table represent a group of 6 patients who had the following rare conditions: rheumatoid nodule, fibroma, histoplasmosis, bronchogenic cyst, echinococcus granulosus, and lipoid pneumonia each represented by only one case. The likelihood of malignant outcome was found to be dependent on three epidemiological variables; age, sex, and smoking history, and three radiological variable; size and borders of the nodule and the presence of calcification. Table 2 demonstrates malignant and benign outcome in relation to these predictive factors. Location of the pulmonary nodules and the presence of symptoms did not have any influence on the final outcome. The rate of growth of the lesion could not be assessed because old chest films were unavailable for comparison in most patients.

(30.6%0 had malignant nodules. On the other hand, in -patients aged 50 years or more 73 of the nodules (56.06%) were malignant. The calculated relative risk (RR) was 1.83 and odds ratio (OR) was 2.90. The odds malignancy were almost three times higher in- patients above the age of 50 years (95% CI OR) = 0.009 to 7.133).

Gender: Forty males (55.6%) had malignant nodules as compared to only eight females (26.7%). The RR was 2.08 and OR was 3.44, (95% CL OR = 0.495 to 4.733). The odds of malignant outcome were almost three and half times greater in males than females. The influence of smoking on these results could be substantial since most males were smokers as compared to very few females, (88.9% vs 6.6%).

Smoking: Seventy-six patients (74.5%) of the whole group were smokers and 29 (24.8%) were nonsmokers. Ex-smokers accounted only for 6.9%. Malignant outcome was recorded in 48 smokers and 7

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nonsmokers (63.1% and 24.1% respectively). The RR was 2.07 and OR was 3.53 (95%cl OR = 0.629 to 4.620). When calculations were repeated for heavy smokers versus nonsmokers, the RR was 3.0 and the OR was 8.17, in other words smokers were 2.9 times, and heavy smokers were 8.17 times more likely to have malignant outcome than non-smokers.

Size of the nodule: The mean nodule size was 2.72cm. Table 2&3 show the relation of the size of SPNs to the final outcome. Nodules 2.5 cm or more in diameter were found in 64 patients (62.7%) while 38 patients (37.3%) had nodules less than 2.5cm in diameter. In the first group, 38 patients (59.4%) had malignant SPNs as compared to only 10 patients (26.3%) in the second group. The RR was 2.26 and the OR was 4.09 (95%CI_{OR} = 1.061 to 5.784). The odds of malignancy in the first group patients were four times greater. In other words, large nodule size was associated with higher likelihood of malignancy.

Borders: Irregular outline of the nodule was observed in 46 patients (45.1%). Out of these 36 patients (78.3%) proved to have malignant nodules as compared to 12 out of 56 (21.4%) with smooth well-defined borders. The RR was 3.66 and OR was 13.2

(95% CI_{OR} = 11.4 to 24.1). It may be noted from table 3 that certain types of border irregularities were exclusively associated with malignant lesions. All SPNs with Scalloped outline i.e. positive Rigler's sign (10 cases) and all those with corona radiate borders turned out to be malignant. In addition, most nodules with spiculated borders and satellite lesions were malignant.

Calcification: Eccentric, amorphous, and reticular calcification patterns were seen exclusively with malignant lesions while diffuse, target, lamellate, and popcorn calcifications were exclusively found in benign nodules (table 3). However, the vast majority of nodules (72.6%) did not show any calcification. For that reason proper

calculation of relative risk indices was impossible.

Discussion:

Malignant outcome was recorded in 47.1% of our patients. The percentage of SPNs that are due to malignant disease varies considerable among studies depending on how patients are selected. Recent studies that included computerized tomography (CT) as a standard preoperative test have reported malignancy in 45% to 90% of resected SPNs [2,5,7,8].We have demonstrated that the probability of a solitary nodule being malignant rises with increasing patient age: The odds of malignancy were 3 times higher above the age of 50. Many studies have shown that the chance that a SPN is malignant increases with age. For instance, in men over age 50, 50% to 65% of SPNs are malignant. [1,5] In contrast, the risk of a malignant SPN among nonsmokers under age 35 is sufficiently low to justify observation alone with serial chest radiography in many cases.[9] Nevertheless, age alone is not infallible evidence against malignancy. Indeed, studies have shown that up to one third of SPNs in patients under age 50 ultimately prove to be malignant. [7,9] Due to the strong association of cigarette smoking with primary lung cancer, the possibility of lung cancer is a concern when a solitary nodule is found in a patient with a history of smoking. [2,3,5] in our patients, it was evident that smoking history increases the likelihood of malignancy. In addition, it was demonstrated that heavy-smokers had significantly higher odds of malignant outcome as compared to smokers.

Our finding that the likelihood of malignancy is much higher in males than females with SPNs could be biased by the effect of smoking differences since much smaller percentage of females smoked as compared to males. In our patients, the effect of sex could not be separated with confidence from that of smoking.

We have also demonstrated that the probability of malignancy in an SPN is much higher when its size is larger than 2.5cm and when its borders are irregular, notched or spiculated. It is widely accepted that larger SPNs are more likely to be malignant, however, smaller size does not mean an SPN is benign. Up to 42% of nodules smaller than 2cm and 15% of nodules smaller

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than 1cm subsequently proves to be malignant. [10,11] Likewise, many authors agree on the association of irregularities of the outline of pulmonary nodules with increased likelihood of malignancy. [2,5,11] Nevertheless, well-demarkated borders should not be taken as unequivocal indicator of benignity.

Benign SPNs are more likely than malignant SPNs to show calcification on chest radiographs. Calcification in SPNs can follow several characteristic patterns. Some patterns of calcification have a very high predictive value for benign nature of the SPN. [5,12,13,14]

In our group calcification was seen in only 28 patients (27.5%). That is why proper calculation of the comparative risk indices was not possible. However, certain patterns of calcifications were noted for their association with benign lesions, these include; diffuse, central (bull's eye), laminated and popcorn calcification. On the other hand, eccentric, amorphous and reticular patterns of calcification were associated with malignant outcome. According to Khan et al [15] and Munden et al [16] the presence of a benign pattern of calcification virtually establishes benignity but the presence of calcification in other patterns is not helpful, as only less than 10% of malignant SPNs may show evidence of calcification. It could be concluded from our results and review of literature that the probability of malignancy in a solitary pulmonary nodule is higher with increasing patient's age, history of smoking, increasing size of the nodule, irregular edge, and the presence of certain patterns of calcification. No single ideal approach to the management of patients with an SPN has been developed. [2,5,8,12] as a general rule, when clinical and radiological features strongly suggest a benign SPN, no further intervention is warranted. [5,8] these patients may be followed prospectively with serial chest radiography for further confirmation of benignity. On the other hand, patients in whom the likelihood of malignancy

is very high may undergo surgery with curative intent without further diagnostic testing. [7,16] Still, in a significant proportion of patients, the initial clinical and radiological evaluation fails to confirm if the SPN is benign or malignant. There is no consensus as to what constitutes the best approach to patients with indeterminate SPNs. [21,22]

Management options include: [23]

1- Biopsy of the SPNs; percutaneous, transbronchial or via vido-assisted thoracoscopy.
2- Prospective observation, and
3- Referral to a thoracic surgeon for definitive resection.

Quantitative approaches have been developed to aid the clinician in the decision-making process. These approaches involve estimating the likelihood of malignancy and then using decision analysis for determining the optimal strategy in a given case. Cumming, et al [14] recommended the use of Bayes' theorem to estimate the likelihood of malignancy in SPNs based upon clinical findings. According to this method, a series of likelihood ratios (LR) are used, with each LR representing the ratio of the prevalence of a specific finding among all subjects with a malignant nodule to the prevalence of that finding among all patients with a benign nodule.

The predictive factors used in the calculation included the probability of malignancy within the population of solitary pulmonary nodules (i.e., the prior odds), size of the nodule, patient age, smoking history, contour characteristics of the nodule, and calcification. Subsequent studies expanded upon the list of likelihood ratios to include history of extrathoracic malignancy, the location of the nodule (i.e., whether it is located in an upper lobe), and growth rate.[11, 16,17,21,22,23] A large number of studies have shown that 2-[F-18] fluoro-2-deoxy-D-glucose-positron emission tomography (FDG-PET) can identify malignant SPNs with a 93 % to 97% sensitivity and 80 % to 100% specificity. 26,28,31 As a single test it was a better predictor of malignancy in solitary pulmonary nodules than the standard criteria using Bayesian analysis.[26,28,31] This technology remains expensive and is not readily

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available in many medical centers.[21, 24,26,28]

Conclusions:

1. The likelihood of malignancy in solitary pulmonary nodule increases with age, smoking history, larger nodule size, ill-defined borders, male gender and the presence of certain calcification patterns. It is not influenced by the location of the SPN or the presence of respiratory symptoms.
2. These characteristics, when used separately, can distinguish unequivocally those patients who have benign versus malignant nodules in only a small percentage of cases.
3. The use of Bayesian analysis may improve the accuracy of estimating the likelihood of malignancy for individual patients with solitary pulmonary nodules.
4. Positron emission tomography (FDG-PET) can identify malignant SPNs with a high sensitivity and specificity but this technology is expensive and is not readily available.

Table 1. Relative frequency of different histopathologic diagnosis.

Malignant	Number	%	Benign	Number	%
Squamous carcinoma	19	18.6	Tuberculosis	15	14.7
Adenocarcinoma	14	13.7	Granuloma	11	10.8
Large cell carcinoma	5	4.9	Carcinoid	09	08.8
Alveolar cell carcinoma	3	2.9	Adenoma	07	06.9
Small cell carcinoma	3	2.9	Hamartoma	04	03.9
Undifferentiated	4	3.9	All benign tumors	21	20.6
			AV-malformation	02	1.96
			Others	06	05.9
Total	**48**	**47.1**		**54**	**52.9**

Table 2. Factors influencing the probability of malignancy in SPN.

Risk factor	Malignant	Benign	Total	RR*	OR**	95% CI_{OR} [$]
Smoking						
Smoker	48	28	76	2.07	**3.53**	0.63 to 4.62
Nonsmoker	7	22	29			
Age:						
>50 years.	37	29	66	1.83	**2.90**	0.009 to 7.13
<50 years.	11	25	36			
Sex						
Males	40	32	72	2.08	**3.44**	0.50 to 4.73
Females	8	22	30			
Diameter						
2.5cm or more	38	26	64	2.26	**4.09**	1.06 to 5.78
< 2.5 cm	10	28	38			
Border						
Irregular	36	10	46	3.66	**13.2**	11.39 to 24.13
Well-defined	12	44	56			

*Relative risk ** odds ratio [$]95% confidence interval of the odd ratio.

Table 3. Nodule characteristics in relation to malignant outcome.

Characteristics	All patients N= 102	Malignant N= 48	Percent
Border (outline)			
Spiculated	26	19	73.07
Scalloped	10	10	100.0
Corona radiata	04	04	100.0
Satellite	04	03	75.0
Tails	02	0	00.0
Irregular (total)	46	36	78.26
Smooth	56	12	21.43
Size:			
1.5cm or less	21	05	23.8
1.6-2.5	25	07	28.0
2.6-3.5	37	22	59.5
3.6 or more	19	14	73.7
Calcification:			
No calcification	74	31	41.9
Eccentric	07	07	100.0
Amorphus	06	06	100.0
Reticular	02	02	100.0
Diffuse	06	0	00.0
Target	03	0	00.0
Lamellated	01	0	00.0
Popcorn	01	0	00.0

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References:

1- Toome H, Delphendhal A, et al. The Coin lesion of the lung. A review of 955 resected coin lesions. Cancer 1983; 51:534-537.

2- Libby DM, Henschke CL, Yankelevitz DF. The solitary pulmonary nodule: updated 1995. Am J Med 1995; 99:491-496.

3- Lillington, GA, Caskey, CL. Evaluation and management of solitary and multiple pulmonary nodules. Clin Chest Med 1993; 14:111-119.

4- Chechani V. Bronchoscopic diagnosis of solitary pulmonary nodules and lung masses in the absence of endobronchial abnormality. Chest 1996; 109:620-625.

5- Ost, D., Fein, A. Evaluation and management of the solitary pulmonary nodule. Am J Respir Crit Care Med 2000; 162: 782-787.

6- Knapp RG, Miller MC. (editors). Clinical epidemiology and biostatistics, first edition, Williams & Wilkins, Baltimore, Maryland. 1992.

7- Rubins, JB, Rubins, HB. Temopral trends in the prevelance of malignancy in resected solitary pulmonary lesions. Chest 1996; 109:100.

8- Midthun, DE, Swensen, SJ, Jett, JR, A pproach to the solitary pulmonary nodule [abstract]. Mayo Clin Proc 1993; 68: 378.

9- Bourke, W, Milstein, D, Giura, R, et al. Lung cancer in young adults. Chest 1992; 102: 17-23.

10- Zerhoni EA, Stitik FP, Siegelman SS, et al. CT of the pulmonary nodule: A cooperative study. Radiology 1986; 160: 319-327.

11- Swensen SJ; Silverstein MD; Ilstrup DM; Schleck CD; Edell ES. The probability of malignancy in solitary pulmonary nodules. Application to small radiologically indeterminate nodule. Arch Intern Med 1997: 28; 157(8): 849.

12- Caskey CL, Templeton PA, Zerhouni EA. Current evaluation of solitary pulmonary nodule. Rad Clin North Am 1990; 28; 511-520.

13- Cummings, SR, Lillington, GA, Richard, RJ. Estimating the probability of malignancy in solitary pulmonary nodules: a Bayesian approach. Am Rev Respir Dis 1986; 134: 449.

14- Bhalla, M, Shepard, JA, Nakamura, k, et al. Dual kV CT to detect calcification in solitary pulmonary nodule [abstract]. J comput Assist Tomogr 1995; 19, 44-47.

15- Khan, A, Herman, PG, Vorwerk, P, et al. Solitary pulmonary nodules: comparison and classification with standard, thin section, and reference phantom CT. Radiology 1991; 179, 477-481.

16- Munden, RF, Pugatch, RD, Liptay, MJ, et al. Small pulmonary lesion detected at CT: clinical importance. Radiology 1997; 202, 105-110.

17- Swensen SJ; Silverstein MD; Edell ES, Trastek VF; Aughenbaugh GL; llstrup DM; Schleck CD. Solitary pulmonary nodules: clinical prediction model versus physician [abstract]. Mayo Clin Proc 1999; 74 (4): 319-29.

18- Zwirewich CV, Vedal S, Miller RR, Muller NL. Solitary pulmonary nodule: High resolution CT and radiologic-pathologic correlation. Radiology 1991; 179: 469-476.

19- Kui M, Templeton PA, White CS, Cai ZL, Bai XY, Cai YQ. Evaluation of air bronchogram sign on CT in solitary pulmonary lesion [abstract]. J Comput Assist Tomogr 1996; 20: 983-986.

20- Baldwin DR, Eaton, T, Kolbe, J, Christmas, T, Milne, D, Mercer, J, Steel, E, Garrett, J, Wilsher, ML, Wells, AU. Management of solitary pulmonary nodules: how do thoracic computed tomography and guided fine needle biopsy influence clinical decisions? Thorax 2002; 57:817-822.

21- Goldberg-Kahn, B, Healy, JC, Bishop, JW, The cost of diagnosis: a comparison of four different strategies in the workup of solitary radiographic lung lesions. Chest 1997; 11, 870-876.

22- Erasmus, J.J., McAdmas, H.P., Connolly, J.E. Solitary Pulmonary Nodules: part II. Evaluation of the indeterminate nodule. Radiographics 2000; 20: 59-66.

23- Liptay M.J. Solitary pulmonary nodule: Treatment Options. Chest 1999; 116: 517S-518.

24- Matthies A, Hickeson M, Cuchiara A, Alavi A. Dual time point 18F-FDG PET for the evaluation of pulmonary nodules. J Nucl Med 2002; 43(7): 871-5.

25- Baldwin DR, Eaton T, Kolbe J, et al. M management of solitary pulmonary nodules: how do thoracic computed tomography and guided fine needle biopsy influence clinical decisions? Thorax 2002; 57 (9): 817-22.

26- Czernin, J., Phelps, M.E. Positron Emission Tomography Scanning: Current and future application. Annu Rev Medicine 2002 53:89-112.

27- Lillington, GA. Management of solitary pulmonary nodules: how to decide when resection is required. Postgrad Med 1997; 101, 145-150.

28- Dewan, NS, Shehan, CJ, Reeb, SD, et al. Likelihood of malignancy in a solitary pulmonary nodule: comparison of Bayesian analysis and results of FDG-PET scan. Chest 1997; 12, 416-422.

29- Bernard A and the thorax Group. Resection of pulmonary nodules using video assisted thoracic surgery. Ann Thorac Surg 1996; 61:202-205.

30- Mitruka S, et al. Diagnosing the indeterminate pulmonary nodule: Percutaneous versus thoracoscopy. Surgery 1995; 118: 676-684.

Garyounis Medical Journal. Vol.20 No.1, pp.108-119.

31- Gupta NC, et al. Probability of malignancy in solitary pulmonary nodules using fluorine- 18FDG and PET. J Nucl Med 1996; 37: 943-948.

32- Siegelman SS, Khouri NF, Leo FP, Fishman EK, Braverman RM, Zerhouni EA. Solitary pulmonary nodules: CT assessment radiology 1986; 160: 307 -312.

33- Santaambrogio L, Nosotti M, Bellaviti N, Mezzetti M. Video thoracoscopy versus thoracotomy for the diagnosis of the indeterminate solitary nodule. Ann Thorac. Surg. 1995; 868-871.

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Prevalence of Ribavirin Induced Anaemia in Patients Treated for Chronic HCV Infection

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Abstract

Objectives: to determine the frequency of ribavirin induced anaemia in patients with chronic hepatitis C under treatment with ribavirin. Setting: Viral hepatitis clinic, Jamahiriya hospital, Benghazi libya. **Material & Methods**: 64 patients (36 males and 28 females) under follow up in our viral Hepatitis clinic and receiving combination therapy (interferon α2b / Ribavirin) for chronic HCV infection (Dose ranging from 800mg to 1200mg) were watched for the development of anaemia as a side effect of Ribavirin. Hemoglobin level was measured before and during first 18 weeks of combination therapy. **Results:** Ten patients developed significant anaemia (6.4%) (8 females and 2 males) and Hb < 10 gm/dl which was dose dependent and required Dose reduction up to 400mg . Nine patients (8 female and 1 male) (90%) responded to dose reduction. One patient showed no response and discovered to be megaloblastic anaemia. **Conclusion**: Dose reduction is the preferable mode of treatment of ribavirin induces anaemia. In addition to dose modification, other treatment modalities have been tried. Only recombinant human erythropoietin-alfa has shown promising result.

Key words : HCV, Ribavirin, Hemolytic anemia.

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Introduction :

Chronic hepatitis C infection affecs at least 170 million world wide and it is the leading cause of chronic liver disease , liver cirrhosis and hepatocellular carcinoma world wide. These facts underscore the need for the development of effective therapies for this disease[1,2]. Interferon α2a and 2b was licensed for use in United States and Europe in 1991 and the results of many randomised studies indicate that interferon alone can induce sustained clinical response only in 15% of patients. Subsequently, several studies demonstrated that the addition of ribavirin to interferon therapy is associated with higher rates of sustained virological, biochemical and histological response and this regimen was approved in 1998[3,4]. Ribavirin is a synthetic nucleoside analogue with broad spectrum of antiviral activity that was used initially in 1970 for treatment of respiratory syncytial virus infection. The putative antiviral mechanisms of action of Ribavirin are unknown but may include inhibition of RNA- dependent RNA polymerase , depletion of intracellular GTP pools and immunomedulatory action that altering the balance between proinflammatory (Th1–like) and anti inflammatory(Th2-like)cytokines[5-8]. The major side effect of ribavirin is the occurrence of reversible non immune hemolytic anaemia in substantial proportion of treated patients which may lead to reduction in the dose of ribavirin and discontinuation of therapy in a significant number of them which leading to reduction in the response rate. In addition, hemolysis increases the rate of hepatic iron deposition which lead to further liver damage and interfere with the action of interferon[9,10]. Almost all patients who receive ribavirin develop some degree of non-immune hemolytic anaemia but 7-10 % of them develop a significant decrease in their haemoglobin level during first 24 weeks of treatment.The fall in haemoglobin starts between 2nd and 3rd week and reaches a nadir between 6th and 8th week of treatment[5,6]. A higher drop in hemoglobin is seen in patients with higher pretreatment hemoglobin level and lower pretreatment platelet count.The correlation between the pretreatment hemoglobin level and hemoglobin after starting treatment seems logical and can be explained by the

fact that the hemolysis associated with Ribavirin breaks down a certain proportion of the available erythrocyte pool. Patients with lower pretreatment platelet counts had significantly higher drop in haemoglobin level than those with with higher counts. This can be explained by early liver cirrhosis or due to interferon treatment in the induction phase which cause thombocytopnia and anemia[11]. The underlying mechanism of ribavirin-associated anaemia is unknown, but oxidative damage of the erythrocyte membrane has been implied , and as a nucleoside analogue, it is also linked to mitochondrial toxicity[12]. Dose reduction of ribavirin to 600 mg and in some instances down to 400 mg/day is recommended for patients whose haemoglobin values fall to less than 10 gm/dl, this dose reduction usually leads to an increase of haemoglobin between 1-1.5gm/dl which get stabilized throughout the course of treatment[13].

Patients and methods

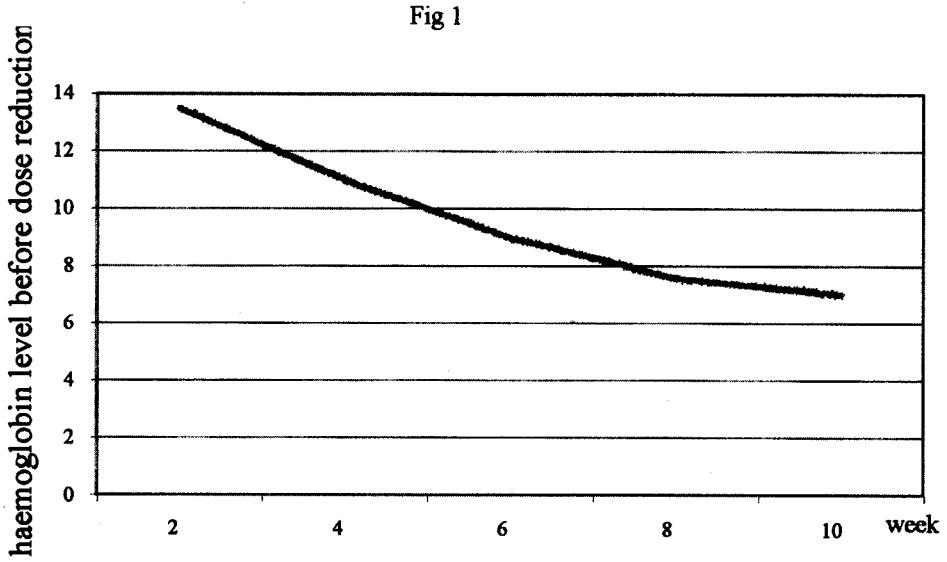
A total of 64 patients (36 males and 28 females) were followed up in the viral hepatitis clinic over a period of 12 months and meeting the criteria for treatment of chronic hepatitis C virus infection by combination therapy were enrolled in the study. The treatment regimen consisted of interferon α2b three mega units subcutanously three times weekly and ribavirin in a dose ranging from 800-1200 mg/day according to body weight . The median age of the cohort was 45 years(range from 35-55),and median body weight was 73kg.

Haemoglobin level measured weekly from the start of treatment until 18th week using an automated hematological analyzer. Anemia was defined as haemoglobin level less than 12.5 g/dl for male patients and less than 11 g/dl for female patients. In addition, measurement of liver enzymes AST+ALT was carried out monthly. Hepatitis C virus RNA level was asseyed before, during and after completion of treatment. In addition pretreatment platelet count (median 210,000+_ 60.000.) was calculated as independent risk factor for the development of anaemia.

Results:

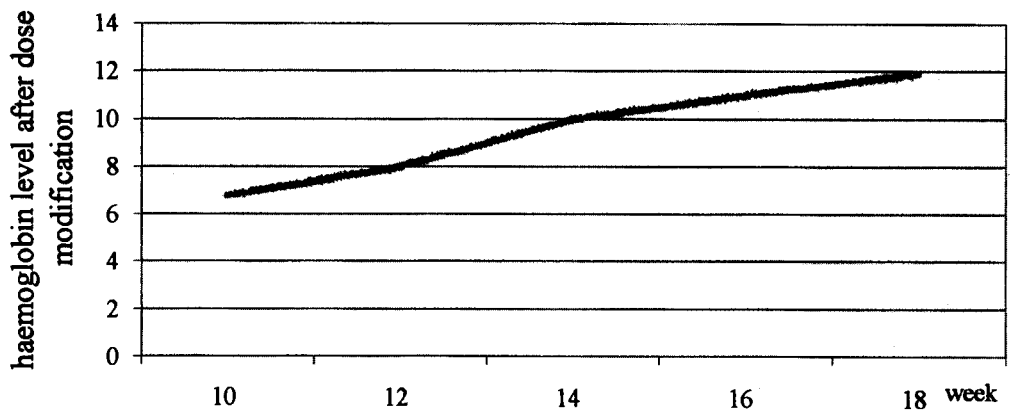
Base line median haemoglobin level was 13.0 g/dl (range 11.5 –14.2 g/dl) and nadir haemoglobin level was 10.2 g/dl (range 8.4 –11.6 g /dl) (Fig. 1). Ten patients (6.4%, 8 female and 2 male) showed a significant reduction in their haemoglobin level (less than 10gm/dl), nine of them responded well to dose reduction by improvement of their Hb level (90%).Hb

Fig 1



duration of treatment (dose of ribavirin range from 800 - 1200 mg/ day) Total No of patient whose haemoglobin level less than 10 gm/dl (10 patients)

Fig 2



Ribavirin dose reduction from 800 - up to 400 mg
Total No of patient that need dose modification and respond (9)

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level increased from 1 to 1.5 gm and stabilized throught out the course of the treatmen (see fig.2).

Discussion :

Treatmen with ribavirin , an oral nucleoside analogue, frequently induces hemolytic anaemia . Anaemia is a frequent finding, occurring in 25- 45 % of patients . Little is known about the risk factors for hemolytic anaemia in patients treated for chronic hepatitis C.In our study we followed 64 patients 36 male and 28 female for the development of anaemia over 18 weeks . Ten patients developed significant drop in their haemoglobin needing dose reduction. Nine patients showed a favourable response (90%). One male patient showed no response. Ribavirin is not the only medication causing anaemia in patients treated for chronic hepatitis C. Interferon also can induce anaemia through different mechanisms. The response to dose reduction makes anaemia most likely due to ribavirin. We compared our results with others which concluded that careful haemoglobin measurment during first 18 weeks of treatment and dose adjustment is the preferable mode of treatment of ribavirin induced anaemia. In one study, recombinant human erythropoietin alfa was used to prevent this type of anaemia with promizing results. Dose reduction is not necessary in those responding to erythropoietin therapy with a consequent improvement in response rate[12]. In anthor study folic acid supplementation did not prevent Ribavirin-induced anaemia[14]. In another approch the addition of vitamin C (1000mg) and vitamin E (800 IU) daily appeared to reduce the severity of anaemia during first weeks of treatment but not to the same level [15]. Hemolysis is typically an important issue in patients with preexisting anaemia , renal insufficiency or coronary artery disease. In addition, chronic hemolysis induced by prolonged therapy with Ribavirin can lead to increased deposition of iron in the liver[16.17]. In one report for example, an average rate of hepatic iron accumulation in six patients who had recieved ribavirin for 6 to 12 months was 1500 μg / year which might exacerbate underlying liver fibrosis and interfer with the action of interferon α in those patients[16].

References :

1.Alter M J, Kruszon-Moran D, Nainan O V , et al , The prevalence of hepatitis Cvirus infection in the untied states, 1988. N Engl J Med 1999 : 341 : 556-62.

2.Deter K M , Belle S H, Lombardero M. Liver transplantation for chronic viral hepatitis. Viral Hepatitis Rev 1996 ; 2:219-28.

3. Hoofnagle J H, Mullen K D, Jones D B, et al . Treatment of chronic non-A,non-B hepatitis with recombinant human alpha interferon. A preliminary report. N Engl J Med 1986; 315 :1575-8.

4. Carithers R L , Emerson S S. Therapy of hepatitisC: meta - analysis of interferon alfa-2b trials. Hepatology 1997; 25 (suppl 1) ; 835-85.

5)Hulthgren C, Milich D R , Weiland O, Sallberg M. The antiviral compound ribavirin modulates the hepatitis B and C virus – spesific immune responses. J Gen Virol 1998; 79: 2381-91 .

6)Bodenheimer H C, Lindsay K L, Davis G L, Lewis J H, Thung S N, Seeff L B. Tolerance and efficacy of oral ribavirine treatment of chronic hepatitis C : multicenter trials . Hepatology 1997 ; 26 :473 – 75.

7)Di Bisceglie A M , Conjeevaram H S, Fried M W, et al. Ribavirin as therapy for chronic hepatitis C :a randomized , double – blind , placebo controlled trial . Ann Inter Med 1995 ; 123 : 897 – 903.

8)Ning Q, Brown D, Parodo J, et al. Ribavirin inhibits viral – induced macrophage prouction of TNF, IL-1 procoagulant fg12 prothrombinase . J Immunol 1998; 160:3487

9) MacHutchison J G , Gordon S C, Schiff E R, et al. Interferon alfa-2b alone or in combination with Ribavirin as initial treatment for chronic hepatitis C. N Eng J Med 1998; 339:1485-92.

10) Poynard T, Marcelin P, Lee S S, et al. Randomised trial of interferon alpha- 2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha – 2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus . Lancet 1998; 352:1426 –32.

11)Van vlierberghe H, Delanghe J R, Devos M, Leroud Q. Factors influencing Ribavirin-induced hemolysis. J Hepatology 2001; 34:911-916.

12)Anna E G, Patricia L . Treatment of ribavirin/ interferon – induced anaemia with erythropoietin in patient with hepatitis C . Hepatology 2002; 25(5):1-3.

13)Hoofnagle J H, Lau D, Conjeevaram H, Kleiner D, Di Bisceglie A M . Prolonged therapy of chronic hepatitis C with ribavirin . J Viral Hepatitis 1996 ; 3 : 247 – 52.

14) Gonzalez H, Rios M E, Torres E H, et al. Folic acid supplementation does not prevent ribavirin induced anaemia. Digestive disease week, Orlando, Fla, 1999.

15)Brass C A, Picken E. Do antioxidant amleiorate ribavirin – related anaemia in HCV patients. Digestive disease week, Orlando, Fla. 1999.

16)Di Bisceglie A M , Bacon B R, Kleiner D E, Hoofnagle J H. Increase in hepatic iron stores following prolonged therapy with ribavirin in patients with chronic hepatitis C . J Hepatol 1994;21: 1109-12.

17)Reichard O. Schvatez R. Weiland O. Therapy of hepatitis C: Alpha interferon and Ribavirin . Hepatology 1997;26 : 1085.

Churg -Strauss syndrome : Early diagnosis and management may save the patient's life: A Case report

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Introduction

Churg Strauss syndrome (CSS) or allergic granalomatous angiitis (AGA), is a rare syndrome that affects small to median– sized arteries and veins described by Churg and Strauss in 1951 [1] .The American College of Rheumatology (ACR) has proposed 6 criteria for CSS diagnosis [2,3] . The presence of 4 or more yeilds a sensitivity of 85 % and specificity of 99.7% . It is grouped as systemic necrotizing vasculitic disorder associated with severe bronchial asthma, marked peripheral and/or tissue eosinophilic infiltrations. It is classified as a variant of polyarteritis nodosa (PAN) with common pulmonary involvement that is lacking in the classical PAN. If the clinical manifestations of both vasculopathies are present in the same patient, it is known as overlap syndrome [3,4] . CSS diagnosis is usually late or missed, to endanger the patient's life, especially if it is of limited type [5,6] and not associated with helpful diagnosic clinical clues such as, bronchial asthma, skin eruptions or eosinophilia. The histopatholigical evidence of vasculitis from tissue specimen of affected organs or skin lesions will certainly confirm diagnosis. CSS gastro–intestinal changes or clinical involvement[7,8] are common (31%), but poorly reported worldwide. We

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therefore describe a patient clinically suspected and treated as CSS. Two days after admission to intensive care unit (ICU), she suffered complete acute small intestinal obstruction ..Later on, histological examination of resected ileum at emergency operation confirmed CSS diagnosis . The early start of high does steroid therapy with or without cytotoxic should be encouraged due to rapid CSS clinical response this kind of therapy and the real chance of inducing sustained remission as shown in this case report .

Case report

A 38 year old Libyan housewife,referred with non responding attack of late onset bronchial asthma associated with skin lesions. General and systemic examination was normal except of the presence of polymorphic reddish multiple sized macular skin lesions of both lower limbs (fig. 1), tender ischemic dry gangrenous lesions of palmar surface tips of right little and ring fingers (fig.2), hyperinflated chest with generalised rhonchi and scattered crepitations.The preliminary investingations were normal apart from elevated erythrocyte sedmintation rate (ESR), C-reactive protein (CRP), anaemia , leucocytosis and eosinophilia (15%). Chest radiograph showed bilateral,peripheral localized patchy pulmonary opacities. She was treated with steroids on clinical suspicion of Churg Strauss syndrome. Two days later she complained of epigastric pain and increasing vomiting. On examination she appeared unwell dehydrated,cyanotic,dyspneic and pyrexial,with epigastric tenderness,distended abdomin expiratory rhonchi and coarse crepitations all over the chest. She was transferred to medical I.C.U. for close management. Urgent plain abdominal X–rays showed multiple fluid levels.Intravenous methylprednisolone pulse therapy for 3 days together with heparin infusion according to patient's APTT levels were added and observed for possible surgical intervention. On the same day evening, she became more sick with clinical signs of complete intestinal obstruction.That necessitated an emergency exploratory laparotomy and resulted in resection of 25 cms long gangrenous part of ileum

proximal to ileocaecal valve. Characteristic histological evidence of vasculitis from ileal biopsy confirmed Churg Strauss diagnosis (fig. 3, 4). She made a good recovery and oral therapy was re-started which included a tapered dose of perdnisolone, azathioprine, bronchodilators, and **aspirin**. Tip of 5th finger was surgically amputated with debridement of the 4th right finger (fig .2). She was eventually discharged on the above treatment and advised for monthly routine investigations, for early detection of disease activity, drug side effects and any new complaints. Two years later while, she was on 10mg maintenance prednisolone therapy and salbutamol inhaler, she became pregnant and delivered normally, at full term a healthy baby girl with no complications. Mother and daughter are enjoying good health.The patient is still under regular check ups over the last 4 years.

Discussion

In systemic vasculitis inflammation of blood vessel walls lead to impairement of blood flow,ischemia,variable organ damage and various associated systemic disturbances.Although CSS is an uncommon disorder and it is now being recognised with increasing clinical frequency. The Americen College of Rheumatology (ACR) has proposed 6 critenia[2,3] for CSS diagnosis .The presence of 4 or more yeields a sensitivity of 85 % and specificity of 99.7 %. These criteria are(1) bronchlial asthma (2) eosinophilia (> 10 %) in peripheral blood (3)paranasal sinusitis(4) pulmonay infiltrates (may be transient)(5) histological proof of vasculitis with extravascular eosinophils and (6) Mononuritis multiplex or polyneurpathy. Our patient presented with vasculitic skin manifestations on both lower limbs and palmer surface of right hand little and ring fingers, recurrent non-responding attacks of bronchial asthma, complicated later on with acute complete small intestinal obstruction which was confirmed by preliminary investigations and

emergency exploratory lapaorotomy findings,that resulted in rescetion of scattered ischemic gangrenous areas of 25 cms long of ileum proximal to ileocaecal valve, later on it showed histological evidence of CSS vasculitis. One gram. I.V methyl prednisolone therapy for 3 successive days together with I.V heparin were added to patients' treatment regimen. Our aim was to decrease risk of CSS vasculitic inflamatory processes and to avoid the need to surgical intervention. Unfortunately this was not successful. It is probably a result of the already organised vasculitic changes[9], thromboembolus formation or both in the ileum blood vessels, which is an expected CSS complication. The relationship between the thrombus formation and CSS is unclear. A hypercoagulability state during vasculitis inflammatory process may have played a part in its pathogenesis[10].The presence of gasterointestinal, myocardial, renal or central nervous system involvement was reported to be associted with poor clinical outcome due to major life threatening complications [7]. CSS gastrointestinal involvement has been reported to occur in 31 % of patients. It included GI bleeding, vasculitis causing bowel ischemia, perforation, gastroenteritis, appendicitis and pancreatitis[7,8,10-12].The sudden onset of acute intestinal obstruction in this patient indicated an aggressive CSS course, that needed early mangament with high dose steroids to avoid the need for emergency surgical intervention as a life saving procedure . Histological diagnostic proof from involved organ(s) during surgery is important, for accurate diagnosis and for future decisions regarding drug treatment. In this patient, azothioprine was used with steroid ,after emergency surgery for its cytotoxic and steroid sparing effects. It was stopped 6 months later as the patient's general condition improved. That has also encouraged us to taper steroids to reach a maintenance dose of 10 mg/day. The patient is now enjoying a sound clinical status four years from her acute presentation. Other reported lines of treatment of CSS include I.V immune globulin, alpha interferon and plasma exchange. Elevated levels of eosinophil cationic protein (ECP) soluble interleukin –2 receptor (SIL-

2R) and soluble thrombomodulin (STM) indicate endothelial damage and are markers for disease activity. ECP levels are also used to monitor the response to drug treatment [13].

Conclusion

The presence of peripheral eosinophilia in a patient with late onset bronchial asthma and/or transient pulmonary infiltrates may be a hint for other symptoms and signs of CSS. Early diagnosis and treatment of systemic necrotizing vasulitis with high dose steroids with or without cytotoxic drugs, may be a life saving measure.This early clinical decision is important and should be encouraged due to its rapid and dramatic positive effect and the real possibility of inducing of sustained remission.We believe that keeping a high index of suspicion is needed to pick up cases at an early stage.

REFRENCES

1-Churg J , Strauss L :Allergic granulomatosis , allergic angiitis , and periarteritis nodosa Am J Pathol 1951; 27 - 277

2-Masi AT , Hunder GG, Lie JT : The American College of Rheumatology 1990 Criteria for the classification of Churg – Strauss Syndrome (allergic granulomatosis and angiitis)Artheritis Rheum 1990; 33:1094 –100.

3- Hunder GG : Vasculitis diagnosis and therapy. Am J Med 1996 100 (supp A) 37 - 45

4-Lhote F, Guillevin L : Polyarteritis nodosa , microscopic polyangiitis and Churg – Strauss Syndrome:Clinical aspects and treatment. Rheum Dis Clin North Am 1995; 21: 911-47.

5-Eustace JA , Nasdasdy T , Choi M : Disease of the muoth . The Churg Strauss Syndrome. Am Soc Nephrol (1999) Sep , 10 (9) 2048 - 55

6-Yamashita Y, Yorika N , Taniguchi Y etal : Non asthmatic case of Churg –Strauss Syndrome with rapidly progressive glomerulonephritis . Intern Med 1998; 37: 561 – 3.

7-Guillervin L , Cohen P , Gayraud M etal : Churg – Strauss Syndrome Clinical study and long term follow up of 96 patients . Medicine Baltimore . (1999) Jan (780) 26 – 37.

8- Leen EJ , Rees PJ , Sanderson JD etal : A case of Churg –Strauss Syndrome : Tissue diagnosis established by sigmoidoscoic rectal biopsy Gut (1996) Feb 28 (20) 299 - 301

9- Lie JT :Histopathologic specificity of systemic vasculitis . Rheum Dis Clin North Am . (1995) Nov , 21 (4) : 883 – 909.

10- Ames PR , Roes L , Lupli S etal : Thrombosis in Churg- Strauss Syndrome

Garyounis Medical Journal. Vol.20 No.1, pp. 127–132.

Beyond vasculitis Br .J Rheumatol . (1996) 35 (11) 1181 - 3

11- Sharma MC , Safaya R , Sidhu BS : Perforation of small intestine caused by Churg-Strauss Syndrome J. Clin . Gastroentrol (1996)Oct.23(3) 232 – 5.

12- Kaneti T , Kawashima A , Hayano T etal : Churg–Strauss Syndrome presenting with ileus caused by ischemic ileal ulcer J Gastroentrol (1988) Feb 33 (1) 112 - 6

13- Memain N , De BM , Guillevin Letal : Delayed relapse of Churg –Strauss Syndrome manifesting as colon ulcer with mucosal granuloma 3 cases (2002) J. Rheumatol Feb 29 (2) 388 – 9.

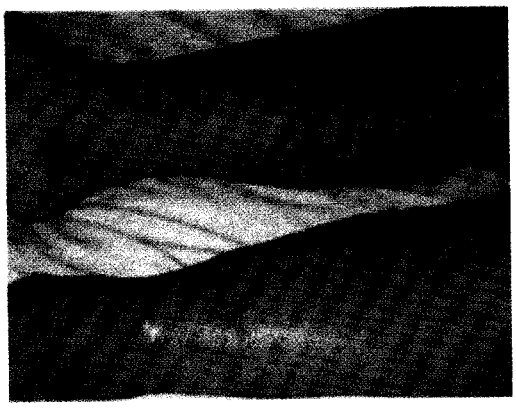


Fig.(1) : Multiple polymorphic macular skin lesions of both lower limbbelow knees.

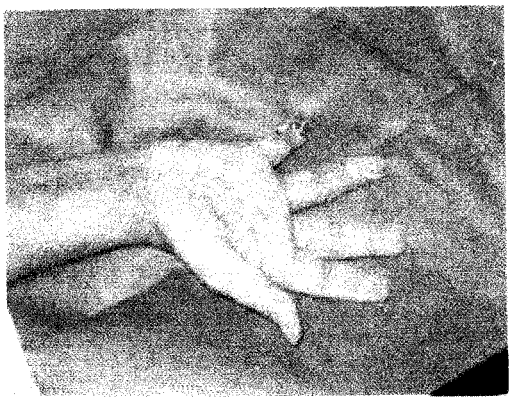
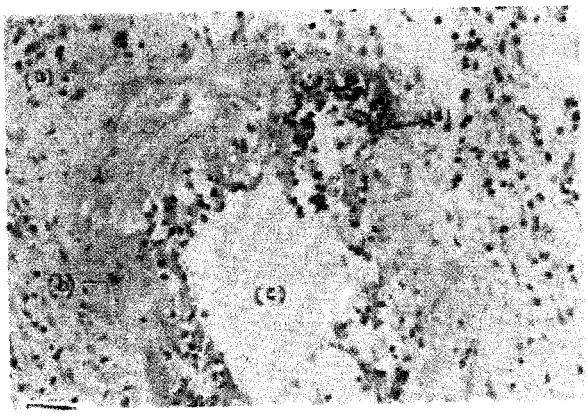
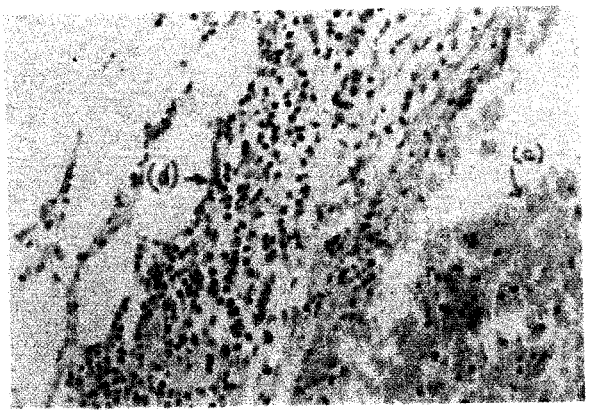


Fig.2: Ischemic vasculitis and dry gangrenous lesions on palmarsurface on tips of right 5th and 4th fingers ,after surgical intervention.





Figures (3-4) :Histological findings of ileal biopsy after emergency exploratory laporatomy , showing (a) mesenteric wall (b) a section in blood vessel (c) with organised haemorrhagic infraction at its center associated with (d) extensive vasculitis and eosinphilic cell infiltrations which are characterstic findings in Churg Strauss vasculitis .

مجـلـــــــة

قـــاريونـــس الطبيـــــــة

المجلة الرسمية للكليات الطبية-جامعة قاريونس

ص . ب 5718 بنغازى

الجماهيرية العربية الليبية الشعبية الاشتراكية العظمى

مجلة قاريونس الطبية تصدر موقتا بشكل نصف سنوي وتتناول العلوم الطبية . مجلد يحوى عددين احدهما فى يناير والآخر فى يوليو

السعر	العملة المحلية	العملة الاجنبية مع رسوم البريد
اشتراك سنوى	16 دينار ليبى	12 دولار
نسخة واحدة	08 دينار ليبى	6 دولار
25 مستخلص من البحث	20 دينار ليبى	15 دولار

تنبيـــه :

- لا تمثل المقالات التى تنشر فى هذه المجلة وجهة نظر الكلية ولا هيئة التحرير ويعتبر الكاتب وحده المسئول عما فيها من آراء وعن صحة ماجاء فى المقالات.
- يرجى من الكاتب قراءة التعليمات والارشادات المطبوعة فى هـــذه المجـــلة وينصح الراغبين فى نشر إنتاجهم بقراءة الارشادات المطبوعة للمؤلفين والتقيد بها .