ORIGINAL RESEARCH

International Journal of Surgery 9 (2011) 482–485

Contents lists available at ScienceDirect

International Journal of Surgery

journal homepage: www.theijs.com

Original Research

Gallbladder intestinal metaplasia in Pakistani patients with gallstones

Muhammad Rizwan Khan [a], Syed Ahsan Raza [a,b,*], Zubair Ahmad [c], Sana Naeem [a], Shahid Pervez [c], Anwar Ali Siddiqui [d], Mushtaq Ahmed [e], Rizwan Azami [a]

[a] Department of Surgery, Aga Khan University, Karachi, Pakistan
[b] Département de Médecine Sociale et Préventive, Université de Montréal, Montreal, Québec, Canada
[c] Department of Pathology & Microbiology, Aga Khan University, Karachi, Pakistan
[d] Department of Biological & Biomedical Sciences, Aga Khan University, Karachi, Pakistan
[e] Aga Khan University, East Africa, Nairobi, Kenya

ARTICLE INFO

Article history:
Received 22 March 2010
Received in revised form
26 June 2011
Accepted 1 July 2011
Available online 13 July 2011

Keywords:
Cancer
Gallbladder
Metaplasia
Gallstones

ABSTRACT

The gallbladder specimens of patients who underwent cholecystectomy for symptomatic gallstones between 2003 and 2005 were evaluated for the presence of Intestinal Metaplasia. (IM) and its risk factors. IM was positive in 39% of 293 patients tested, and in the comparative analysis of 114 metaplasia positive versus 179 negative patients, a high risk was found in patients who were 60 years or older [adjusted odds ratio (aOR) = 3.0, 95% confidence interval (CI): 1.5, 6.2]. Other factors with aOR greater than 1 were moderate to excessive use of chilies (1.8) and ethnic origin of North India (1.7). Screening method has yet to be devised for early detection of gallbladder cancer by identifying metaplastic lesions early in life. We believe that large geographic variation and lifestyle environmental factors associated with the development of gallbladder metaplasia and cancer mortality are concealed in our study that needs to be further explored.

1. Introduction

Evidence suggests that presence of stones in gallbladder is associated with a number of changes in the gallbladder epithelium that involves multistep progression from inflammation to glandular atrophy, metaplasia, dysplasia and ultimately to cancer.[1,2]

The inflammatory process initiated by the stones is aided by possible irritation factors in the diet and environment as well as by gene–environment interactions. The consequence of this inflammatory process is changes in cell differentiation thereby resulting in intestinal metaplasia (IM). Recent studies have suggested that these epithelial changes are distinct phases of epithelial differentiation leading to more severe cytological damage known as dysplasia, a lesion that ultimately precedes invasive gallbladder carcinoma and classified as mild, moderate and severe, the latter being termed as 'carcinoma in situ'.[3,4] The increasing frequency of these epithelial alterations has been reported with increasing age.[2,5] Identification of intestinal metaplasia which appear very early in the process of carcinogenesis in patients with gallstones is therefore important, so that early intervention can be made to prevent gallbladder cancer.[5] Studies have reported that approximately 15 years are required for progression from dysplasia to advanced carcinoma,[5,6] hence efforts should also be made to identify the risk factors for precursor lesions about 20 years before the appearance of cancer. Since IM has been suggested as a precursor lesion for gallbladder cancer,[7] we explored the presence of IM as well as sought to determine the risk factors for its presence in Pakistani patients with gallstones.

2. Materials and methods

The study was carried out prospectively at Aga Khan University Hospital from January 2003 to June 2005 and was approved by the Ethics Review Committee of the university (190-Sur/ERC-02). All patients themselves signed an informed consent form and were administered a questionnaire through an interview by trained medical personnel of the study. Patients admitted to surgical wards undergoing cholecystectomy for symptomatic gallstones and for complicated stone disease e.g., acute cholecystitis, acute pancreatitis and cholangitis were included in the study. Detailed personal and medical information that included demographic data, medical illnesses, family history of gallstones, smoking and history of enteric fever were collected. Ethnic origin was established by self reported confirmation of the interviewed person. Consumption of red chilies in daily diet was recorded on a scale of 1–4 categorized as no use, low, moderate and excessive use. Information on re-use of cooking oil was obtained if the patient had a history of using the same oil more than once for preparing subsequent meals. Information on both food item variables

* Correspondence. Département de Médecine Sociale et Préventive, Université de Montréal, 3875 rue St-Urbain, suite 305, Montreal, QC, Canada H2W1V1. Tel.: +1 514 890 8000x15904; fax: +1 514 412 7106. .

E-mail addresses: khan.rizwan@aku.edu (M. Rizwan Khan), syed.ahsan.raza@umontreal.ca (S. Ahsan Raza).

1743-9191/$ – see front matter © 2011 Surgical Associates Ltd. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.ijsu.2011.07.001

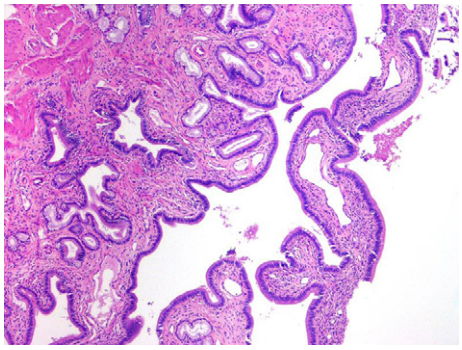

Fig. 1. Gallbladder mucosa without intestinal metaplasia (Hematoxylin and Eosin stain, magnification× 10).

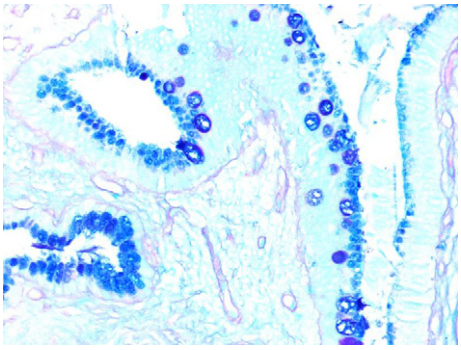

Fig. 3. Gallbladder mucosa with intestinal metaplasia showing goblet cells (Mucin stain, magnification× 10).

(use of chilies and re-use of cooking oil) was also confirmed through immediate family member of the relative at the time of interview. All patients were submitted to abdominal ultrasound to confirm the presence of gallstones before cholecystectomy. Ultrasound findings such as gallbladder wall thickness (≤3 mm as normal and >3 mm as thickened), number and size of stones were duly recorded.

Immediately after cholecystectomy, each gallbladder was opened and emptied. The fixed tissue specimens were divided into three sections corresponding to neck, body and fundus region of the gallbladder. Each region was longitudinally cut in 4 mm wide section, embedded in paraffin, cut and stained with hematoxylin and eosin for histological analysis (Figs. 1–3). Intestinal metaplasia was recognized morphologically by the presence of goblet cells, absorptive cells, and cells resembling colonocytes.

3. Statistical analysis

Statistical analysis was done by SPSS package (version 17.0, SPSS, Inc., Chicago, IL). Possible associations of covariables with IM in patients with gallstones were analyzed. The dependant variable was status of IM as positive or negative and independent variables were hypothesized risk factors in gallstone patients. We compared the patients' age categorized in 4 groups (<40, 40–49, 50–59 and ≥60 years), gender, ethnic origin, and body mass index (BMI) categorized according to WHO International classification,[8] family history of gallstones, smoking status, history of enteric fever, re-use of cooking oil, use of red chilies, gallbladder wall, stone size, and duration of symptoms. The odds ratios (OR) with 95% confidence intervals (95% CI) were computed to compare IM status as 'positive' or 'negative' for each hypothesized risk factor. *P* value of <0.25 on univariate analysis was used as a screening criterion for selection of variables to be included in final multivariate logistic regression model. This criterion was employed because the use of a more traditional *p* value of 0.05 on univariate analysis often fails to identify factors that are known to be important.[9]

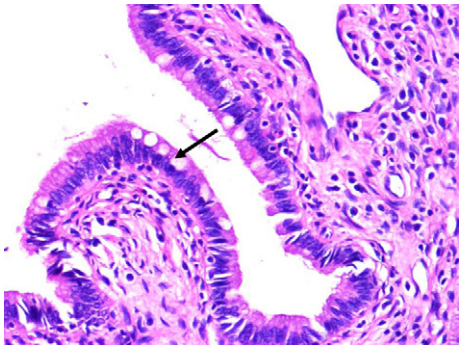

Fig. 2. Gallbladder mucosa with intestinal metaplasia showing goblet cells (Hematoxylin and Eosin stain, magnification× 20).

4. Results

Two hundred and ninety three patients were tested for IM; 114 were intestinal metaplasia positive (IM +ve) and 179 were intestinal metaplasia negative (IM −ve). Majority of patients recruited were females (73.4%) and mean age of study patients was 45.9 ± 14.0 years. Table 1 shows that patients with gallbladder IM were significantly older than those without metaplasia (mean age 48.3 ± 14.0 versus 44.4 ± 13.9 years, *p* value 0.02). For females, mean age at menarche in IM +ve patients was almost similar to IM −ve patients (12.8 ± 2.8 versus 13.3 ± 1.2 years). Majority of patients seen in our study were of North Indian origin (IM +ve: 68.4%, IM −ve: 53.6%).

The potential risk factors for IM were first explored through univariate analysis (Table 2). Most of the patients with gallstones (both IM + ve and IM −ve) were overall younger i.e., less than 40 years (109 out of 243), however older patients aged ≥ 60 years were more frequently IM +ve (27.2%) than IM −ve (15.1%). Patients who were 60 years or older were significantly associated with the presence of IM (OR = 2.3; 95% CI: 1.2, 4.5) as well as patients who had North Indian origin (OR = 1.9; 95%CI: 1.1, 3.1). Elevated OR and borderline trend toward significance was observed in patients who used moderate to high level of red chilies in their daily consumption of food (OR = 1.5; 95% CI: 0.9, 2.3).

Factors that were entered into multivariate logistic regression to assess their independent association with IM included 4 categories of age groups, moderate to excessive use of red chilies, ethnicity of North Indian origin and BMI (Table 3). All of these factors with the exception of BMI showed higher odds ratios of more than one

Table 1
Distribution of intestinal metaplasia positive (IM +ve) and negative (IM −ve) patients by gender, age and ethnic groups.

	% IM + ve (n = 114)	% IM −ve (n = 179)	*P* value
Gender			
Female	71.1	74.9	0.47
Mean age (years)	48.3 ± 14.0	44.4 ± 13.9	0.02
Mean age at menarche (years)	12.8 ± 2.8	13.3 ± 1.2	0.18
Ethnic groups			
North Indian[a]	68.4	53.6	0.01
Pukhtoon	8.8	3.9	
Gujrati	5.3	7.3	
Sindhi	5.3	6.1	
Punjabi	2.6	13.4	
Balochi	2.6	3.4	
Others[b]	7.0	12.3	

[a] Immigrants from Delhi, Uttar Pradesh and Bihar.
[b] Persians, Tajiks, Arabs.

Table 2
Univariate analysis of potential risk factors associated with the presence of intestinal metaplasia (IM) in patients with gallstones.

	% IM +ve ($n = 114$)	% IM −ve ($n = 179$)	OR (95% CI)	*P* value
Age (in years)				
<40	31.6	40.8	1.0	
40–49	23.7	24.6	1.2 (0.7, 2.3)	0.49
50–59	17.5	19.6	1.2 (0.6, 2.3)	0.67
≥60	27.2	15.1	2.3 (1.2, 4.5)	0.01
Ethnic origin				
Others	31.6	46.4	1.0	
Northern India	68.4	53.6	1.9 (1.1, 3.1)	0.01
Parity[a]				
≤5 pregnancies	73.1	79.1	1.0	
>5 pregnancies	26.9	20.9	1.3 (0.7, 2.8)	
BMI[b]				
<18.5	3.9	2.3	1.0	
18.5–24.9	33.0	29.7	0.7 (0.2, 2.8)	0.58
25.0–29.9	45.6	33.1	0.8 (0.2, 3.5)	0.79
≥30	17.5	34.9	0.3 (0.1, 1.3)	0.11
Family history of gallstones				
No	78.1	69.8	1.0	
Yes	21.9	30.2	0.7 (0.4, 1.1)	0.65
Smokers				
No	90.4	88.8	1.0	
Yes	9.6	11.2	0.8 (0.4, 1.8)	0.68
Enteric fever				
No	81.6	82.7	1.0	
Yes	18.4	17.3	1.1 (0.6, 2.0)	0.81
Re-use of cooking oil				
No	59.6	54.7	1.0	
Yes	40.4	45.3	0.8 (0.5, 1.3)	0.68
Use of chilies in food				
No-low	44.7	54.7	1.0	
Moderate-excessive	55.3	45.3	1.5 (0.9, 2.3)	0.09
Gallbladder wall[c]				
Normal	74.5	72.7	1.0	
Thick	25.5	27.3	0.9 (0.5, 1.6)	0.76
Number of gallstones[d]				
Single	24.2	20.1	1.0	
Multiple	75.8	79.9	0.8 (0.4, 1.4)	0.45
Stone size				
≤1 cm	32.3	42.2	1.0	
>1 cm	67.7	57.8	1.5 (0.8, 3.0)	0.20
Duration of symptoms				
≤1 year	70.3	69.6	1.0	
>1 year	29.7	30.4	1.0 (0.6, 1.7)	0.91

[a] (IM +ve = 67, IM −ve = 115).
[b] (IM +ve = 103, IM −ve = 172).
[c] (IM +ve = 94, IM −ve = 139).
[d] (IM +ve = 95, IM −ve = 154).

(Moderate to excessive use of chilies: 1.8 and ethnicity of North Indian origin: 1.7). Patients who were ≥60 years showed the highest adjusted odds ratio of 3.0 (95% CI: 1.5, 6.2) in the final model.

5. Discussion

In our study IM was positive in 114 patients (39%) of the total of 293 tested which in itself is an important finding. This study disclosed a relatively high frequency of IM in Pakistan in comparison to previous studies conducted in other countries e.g., Japan (30.6% of 1000 gallbladders), Canada (10.8% of 277 gallbladders) and United States (9.8% of 400 gallbladders).[3,10,11] More recently, a histological study from Brazil has reported IM in 73.8% of 80 gallbladders that were tested, with prevalence as high as 86% in patients younger than 40 years.[4] Our study has also demonstrated higher frequency of IM (31.6%) among patients younger than 40 years compared to other age groups (Table 2). Wide international variations in the prevalence of IM suggest that in addition to genetic factor, there could be an unknown carcinogenic factor originating from food in different countries. Like Brazilian study, a higher frequency in young patients indicate that external factors such as diet in Pakistan has changed over the last few decades with the evolution of histological alterations in gallbladder disease etiology.

Table 3
Multiple logistic regression analysis of factors associated with presence of gallbladder intestinal metaplasia in patients with symptomatic gallstones.

Factors	Adjusted OR (95% CI)[a]	*P* value
Age (in years)[b]		
40–49	1.4 (0.7, 2.8)	0.34
50–59	1.1 (0.5, 2.4)	0.78
≥ 60	3.0 (1.5, 6.2)	0.002
Moderate to excessive use of red chilies	1.8 (1.0, 3.0)	0.04
North Indian origin	1.7 (1.0, 2.8)	0.06

[a] Adjusted for BMI.
[b] Reference category: age < 40.

Multivariate analysis for comparison with metaplasia negative patients yielded age of 60 years or older, moderate to excessive use of red chilies in food and ethnicity of North Indian origin as factors with adjusted odds ratios of more than one. As in previous study of gastric metaplasia,[12] results from our study shows that age is an important factor for the development of IM in gallbladder. The odds of being 60 years or older among IM +ve gallstones patients is three times the odds among IM −ve patients, after controlling for the effect of other factors (Table 3). An interesting observation seen in our study is that more patients older than 60 years were IM +ve compared to 60 year old in IM −ve group. It should however be noted that a large number of IM +ve patients with gallstones are also younger than 40 (Table 2). Since gallbladder carcinogenesis involves multiple steps, and it may take decades to progress from metaplasia to invasive cancer, younger patients with gallstones remain at risk of developing gallbladder cancer.

Our finding of moderate to excessive use of red chilies in food (adjusted OR = 1.8; 95% CI: 1.0, 3.0, *p* value 0.04) confers with a case–control finding from Chile that has shown high risk of green and red chile pepper consumption for gallbladder cancer.[13] Research on animals has shown that one of the active ingredient of chili pepper, capsaicin, acts as carcinogen if taken in high amounts.[14] As suggested by the Chilean authors,[13] a possible explanation for predisposition to gallbladder cancer could be an association of capsaicin to an increase in bile concentration by an increase flow of water into mucosa resulting in concentration of carcinogen in the bile. In another study, high consumption of capsaicin (9–25 jalapeño peppers per day) was associated with gastric cancer.[15]

One of the finding in our study is ethnic origin of northern India that attained borderline significance in the final model (Adjusted OR = 1.7; 95%CI: 1.0, 2.8, *p* value 0.06). A worldwide meta-analysis on gallbladder cancer incidence had previously identified high-risk populations in northern India as well as in Karachi, Pakistan with a high female/male ratio and predominance of history of gallstones.[16] Karachi, the largest city of Pakistan is inhabited predominantly by an Urdu-speaking population comprising of North Indian origin that migrated in 1947 during creation of Pakistan mostly from Indian states of Delhi, Uttar Pradesh and Bihar. As seen in studies on gallbladder cancer from India, where there is a high preponderance among North Indians versus rest of the Indian

subcontinent,[17] we found a similar high proportion of North Indians positive with intestinal metaplasia in Karachi (Table 1) which lends credence to the fact that incidence of metaplasia might also parallel incidence of gallbladder cancer among this population. The ethnic variations in the distribution of gallbladder metaplasia suggest that there might be genetic and environmental influences on the development of gallbladder cancer in Indo-Pakistan region. These apparent ethnic differences and geographic predisposition in the incidence of metaplasia need to be explored through a nation-wide population based study along with concurrence of the incidence rates of gallstones and gallbladder cancer.

One of the limitations of our study was that the measurement assessment of chili consumption was not done through a proper dietary questionnaire. Pakistani diet is a complex mixture of spices and chilies that will need a proper dietary analysis through a structured dietary instrument. One such instrument is Food Frequency Questionnaire (FFQ) that aims at analyzing the daily diet over a period of 15 years.[13] The FFQ use however will entail its adoption according to local customs of food preparation in Pakistan. In spite of our inability to use proper dietary assessment tool, and smaller sample size compared to previous large scale studies,[18] we have shown that some trend of high odds with borderline significance exists with consumption of large amount of red chilies in home diet among Pakistani patients.

Other potential factors such as parity, BMI and enteric fever (Table 2) were examined in our study but no significant differences were seen when IM +ve were compared to IM −ve patients. Parity and BMI have appeared as risk factors for gallbladder cancer in older studies,[19–21] however consistent to relatively newer report from Chile,[13] women with more than 5 pregnancies and BMI of more than 25 were unrelated to IM in our study. Enteric fever is also a risk factor for gallbladder cancer[22] but was insignificant in our analysis and apossible explanation could be due to sub clinical and undiagnosed cases.[13]

In conclusion, we assessed a number of important risk factors associated with the development of premalignant gallbladder metaplasia, in patients with gallstones. Our data showed that ethnicity of North Indian origin is a risk factor for developing IM. Whereas screening and surveillance of subjects with premalignant lesions has shown to detect gastric cancer at an early stage through endoscopy,[23] screening methods are yet to be devised for early detection of gallbladder cancer by identifying metaplastic lesions early in life. We also believe that there are geographic variations and other lifestyle and environmental factors associated with gallbladder metaplasia and gallbladder cancer concealed in our study that are needed to be explored further.

Conflict of interest

None declared.

Ethical approval

Ethics Review Committee of Aga Khan University, Karachi, Pakistan (190-Sur/ERC-02).

Acknowledgments

This study was funded by Aga Khan University's Research Council Grant (AKURC#022020SURG) in Karachi, Pakistan. The work was undertaken under primary supervision of MR Khan, Assistant Professor in Department of Surgery of Aga Khan University. SA Raza was a research faculty in the Department of Surgery of Aga Khan University at the time the study was carried out and was involved in writing the manuscript and data-analysis with the assistance of all authors. Z Ahmad carried out testing on tissue specimen for gallbladder metaplasia. S Naeem was the research assistant and was responsible for the tissue storage and data management. R Azami was the principal investigator of the study. All authors including S Pervez, AA Siddiqui and M Ahmed have major contributions to the intellectual content and reviewed the final manuscript. Finally, the authors are grateful to all the cholecystectomy patients of Aga Khan University Hospital who agreed for testing on their removed tissue samples during the study.

References

1. Lazcano-Ponce EC, Miquel JF, Muñoz N, Herrero R, Ferrecio C, Wistub II , et al. Epidemiology and molecular pathology of gallbladder cancer. *CA Cancer J Clin* 2001;**51**:349–64.
2. Roa I, de Aretxabala X, Araya JC, Roa J. Preneoplastic lesions in gallbladder cancer. *J Surg Oncol* 2006;**93**:615–23.
3. Mukhopadhyay S, Landas SK. Putative precursors of gallbladder dysplasia: a review of 400 routinely resected specimens. *Arch Pathol Lab Med* 2005;**129**:386–90.
4. Eduardo J, Franco MIF, Suzuki RK, Taveres NM, Bromberg SH. Intestinal metaplasia in gallbladders: prevalence study. *Sao Paulo Med J* 2008;**126**:220–2.
5. Roa I, Araya JC, Villaseca M, De Aretxabala X, Riedemann P, Endoh K, et al. Preneoplastic lesions and gallbladder cancer: an estimate of the period required for progression. *Gastroenterology* 1996;**111**:232–6.
6. Roa I, Araya JC, Villaseca M, Roa J, de Aretxabala X, Ibacache G. Gallbladder cancer in high risk area:morphological features and spread patterns. *Hepatogastroenterology* 1999;**46**:1540–6.
7. Lewis JT, Talwalkar JA, Rosen CB, Smyrk TC, Abraham SC. Prevalence and risk factors for gallbladder neoplasia in patients with primary sclerosing cholangitis: evidence for a metaplasia-dysplasia-carcinoma sequence. *Am J Surg Pathol* 2007;**31**:907–13.
8. WHO. *Physical status: the use and interpretation of anthropometry*. Report of a WHO Expert Committee. WHO Technical Report Series 854. Geneva: World Health Organization. Available at, http://apps.who.int/bmi/index.jsp?introPage=intro_3.html; 1995.
9. Mickey RM, Greenland S. The impact of confounder selection criteria on effect estimation. *Am J Epidemiol* 1989;**129**:125–37.
10. Yamagiwa H, Tomiyama H. Intestinal metaplasia-dysplasia-carcinoma sequence of the gallbladder. *Acta Pathol Jpn* 1986;**36**:989–97.
11. Dowling GP, Kelly JK. The histogenesis of adenocarcinoma of the gallbladder. *Cancer* 1986;**58**:1702–8.
12. Leung WK, Ng EK, Chan WY, Auyeung AC, Chan KF, Lam CC, et al. Risk factors associated with the development of intestinal metaplasia in first-degree relatives of gastric cancer patients. *Cancer Epidemiol Biomarkers Prev* 2005;**14**:2982–6.
13. Serra I, Yamamoto M, Calvo A, Cavada G, Báez S, Endoh K, et al. Association of chili pepper consumption, low socioeconomic status and longstanding gallstones with gallbladder cancer in a Chilean population. *Int J Cancer* 2002;**102**:407–11.
14. Agarwal RC, Wiessler M, Hecker E, Bhide SV. Tumour-promoting effect of chili extract in Balb/c mice. *Int J Cancer* 1986;**38**:689–95.
15. López-Carrillo L, López-Cervantes M, Robles-Díaz G, Ramírez-Espitia A, Mohar-Betancourt A, Meneses-García A, et al. Capsaicin consumption, *Helicobacter pylori* positivity and gastric cancer in Mexico. *Int J Cancer* 2003;**106**:277–82.
16. Randi G, Franceschi S, La Vecchia C. Gallbladder cancer worldwide: geographical distribution and risk factors. *Int J Cancer* 2006;**118**:1591–602.
17. Misra S, Chaturvedi A, Misra NC, Sharma ID. Carcinoma of gallbladder. *Lancet Oncol* 2003;**4**:167–76.
18. Hsing AW, Gao YT, Han TQ, Rashid A, Sakoda LC, Wang BS, et al. Gallstones and the risk of biliary tract cancer: a population-based study in China. *Br J Cancer* 2007;**97**:1577–82.
19. Lambe M, Trichopoulos D, Hsieh C, Ekbom A, Pravia M. Parity and cancers of the gallbladder and the extra-hepatic bile ducts. *Int J Cancer* 1993;**54**:941–4.
20. Moerman CJ, Berns MPH, Bueno de Mesquita HB, Runia S. Reproductive history and cancer of biliary tract in women. *Int J Epidemiol* 1994;**23**:691–9.
21. Strom BL, Soloway RD, Rios Dalenz J, Rodriguez-Martinez HA, West SL, Kinman JL, et al. Risk factors for gallbladder cancer. An international collaborative case-control study. *Cancer* 1995;**76**:1747–87.
22. Dutta U, Garg PK, Kurmar R, Tandon RK. Typhoid carriers among patients with gallstones are at increased risk for carcinoma of the gallbladder. *Am J Gastroenterol* 2000;**95**:784–7.
23. Whiting JL, Sigurdsson A, Rowlands DC, Hallissey MT, Fielding JW. The long term results of endoscopic surveillance of premalignant gastric lesions. *Gut* 2002;**50**:378–81.