The role of the cytokines and cell-adhesion molecules on the immunopathology of acute appendicitis

Mahmood Y. Hachim, MBChB, MSc, *Abdul-Razak H. Ahmed,* MBChB, PhD.

ABSTRACT

Objectives: To study the local expression of the proinflammatory cytokine such as interferon gamma and anti-inflammatory cytokine like interleukin-10 (IL-10) and their role in cell adhesion molecules (CAM) expression on the surface of endothelial cell at the site of inflammation in acute appendicitis. The local expression of these cytokines and CAM was correlated with clinical findings to shed light on their role in the pathogenesis of acute appendicitis.

Methods: Thirty-five patients with acute appendicitis and 6 apparently normal appendices were removed incidentally from individuals presented with problems other than appendicitis, were included in this prospective study. They were attendant of the emergency room in Al-Khadhumiyah Teaching Hospital in Baghdad, from October 2003 to September 2004. Cell adhesion molecules (intracellular adhesion molecule-1 [ICAM-l], ICAM-3 and vascular cell adhesion molecule-1 [VCAM-1]) were detected by immunohistochemistry while IL-10 and interferon gamma were detected by in situ hybridization. The specimens were classified into 5 groups; early acute appendicitis, phlegmonous appendicitis, ulcero-phlegmonousappendicitis, and gangrenous appendicitis, and the fifth group included specimens that showed no histopathological changes, defined as histologically normal appendix.

Results: Intracellular adhesion molecule-1, VCAM-I, IL-10 and interferon gamma were expressed weakly in the control group, while ICAM-3 was not detected in the control group. The average score for ICAM-I, VCAM-1 and the percentage of cells expressing IL-l0 and interferon gamma were significantly higher in the patient groups when compared with the control group. Intracellular adhesion molecule-3 was expressed in the patient group.

Conclusions: The kinetics of CAM expression were tightly correlated to the balance between IL-10 and interferon gamma especially after 12.5 hours from the first symptoms experienced by the patients. The interferon gamma was the main player and the most significant factor that leads to shifting of cases towards gangrenous appendicitis.

Saudi Med J 2006; Vol. 27 (12): 1815-1821

The acute inflammation of vermiform appendix (an important component of the mammalian mucosal immune system) is the most common cause of abdominal pain;[1] it is exceeded only by trauma as a cause of abdominal surgical emergencies. Current estimates that approximately 7% of the population will develop appendicitis at some time during their lives.[2] It is often considered a disease of youth, however, approximately 7% of the patients were under 5 years old and 5% were over 60.[3] The early misconceptions (the appendix has often seen as a nuisance rather than important part of human

From the Department of Medical Microbiology, College of Medicine, Al-Nahrain University, Baghdad, *Iraq*.

Received 25th March 2006. Accepted for publication in final form 12th August 2006.

Address correspondence and reprint request to: Dr. Mahmood Y. Hachim, Department of Medical Microbiology, College of Medicine, Al-Nahrain University, Baghdad, *Iraq*. Tel. +964 7901323533. E-mail: chimeric20032000@yahoo.com

anatomy) have led to the indiscriminate removal of the appendix from the body were corrected by recent identification of the importance of the appendix, so the removal of the appendix should be reevaluated also.[4] Proper study of the immunopathology of the disease is fundamental not only to understand the cellular and molecular mechanisms controlling the adhesion and trans-endothelial migration of the leukocytes and lymphocytes, but also to offer a new interesting aspect in the investigation, prognosis, and even treatment of the acute appendicitis. The important aspect of studying acute appendicitis is the leukocytes extravasations, which control the recruitment of inflammatory cells to the site of infection. Recruitment is mediated by cell adhesion molecules (CAM) that are induced on the surface of the local blood vessels of the endothelium.[5] The balance between the pro-inflammatory and anti-inflammatory molecules will determine the severity, extent and the outcome of the mucosal inflammation.[6] For that, we designed this study to understand some aspects of the immunopathology of acute appendicitis through the study of the local expression of the proinflammatory cytokine such as interferon gamma and anti-inflammatory cytokine like interleukin-10 (IL-10) and we investigated their role in CAM expression on the surface of endothelial cell at the site of inflammation in acute appendicitis. The local expression of these cytokines and CAM was correlated with clinical findings to shed light on their role in the pathogenesis of acute appendicitis. In this study, we try to investigate the pathogenesis of acute appendicitis in vivo, to avoid the validity problems which is associated with in vitro studies like the problem of metabolites accumulation in culture media which could influence the sensitivity of endothelial cells and in prospective study to correlate the results with a proper clinical data, in addition we studied the expression of Intercellular adhesion molecule (ICAM)-3 for the first time.

Methods. Thirty-five patients with acute appendicitis, 16 females and 19 males, with an age range of 9-45 years, were included in this prospective study. They were attendant of the emergency room in Al-Khadhumiyah Teaching Hospital in Baghdad, from October 2003 to September 2004. The diagnosis of acute appendicitis was based on clinical history and physical examination, and confirmed by the histopathological examination after appendicectomy. Baseline data about the patients were obtained from routine history and clinical examination in addition to relevant medical and drug history.

Six apparently normal appendices were removed incidentally from individuals presented with road traffic accident (n=2), bullet injury (n=1) and from patients with colon resections due to malignant or inflammatory disease such as ulcerative colitis or Crohn's disease (n=3). Surgical biopsies of excised appendix were made from patients and controls. They were formalin fixed and paraffin embedded. Tissue blocks were sectioned (4 μm thickness). One section was stained with hematoxyline and eosin (H&E), 3 sections were used in immunohistochemical assessment of CAM and 2 sections were used in insitu hybridization detection of cytokines mRNA.Immunohistochemical detection of CAMs (vascular cell adhesion molecule-1 [VCAM-1] Intracellular adhesion molecule-1 [ICAM-I] and Intracellular adhesion molecule-3 [ICAM-3]) by immunoperoxidase immunostaining technique was carried out according to the manufacturer instructions with some modifications as follows: (i) slides preparation and pre-staining management: formalin fixed paraffin embedded tissue section on positive charged slides placed in a 60°C hot-air oven over night and slides were cleared in 2 changes of xylene for 5 minutes each, excess liquid was shacked off and were rehydrated in 2 changes of absolute ethyl alcohol for 3 minutes each, excess liquid were shacked off and slides were placed in fresh 95% ethyl-alcohol for 3 minutes, excess liquid were shacked off and slides were placed in fresh 70% ethyl alcohol for 3 minutes then slides rinse in gently running tap water for 30 seconds and placed in PBS wash bath for further rehydration (3 minutes at room temperature). Slides then were immersed in retrieval solution and placed in autoclave at 121°C for 15 minutes, and then sections were cooled at room temperature for 20 minutes. The slides were rinsed in buffer and bathing 3 minutes in buffer, then excess of buffer was tapped and wiped around sections by gauze and make circle around the tissue by PAP-pen. Slides were placed on a flat level surface, then enough drops of 3% hydrogen peroxide were dropped to cover the whole section and incubated for 5 minutes at room temperature then rinsed with PBS from a wash bottle, then slides placed in PBS wash bath for 2 minutes excess buffer were taped and wiped around sections. Enough power block reagents were applied for 5 minutes and excess blocking reagent were tapped but not washed. (ii) Staining procedure: Primary antibodies (monoclonal antibodies against CAM, Dako) were diluted (1/50) in a diluent, and diluent alone was used as negative control. Then, 100 μL were applied to appropriate slides to cover the section, and then the slides were left in humid chamber and kept in refrigerator at 4°C overnight. Then the slides were rinsed gently with PBS from wash bottle then slides

were placed in PBS wash bath for 3 minutes, and then wiped around sections. Enough solution of 1/20 diluted biotinylated anti-mouse Ab in Streptavidin-peroxidase diluent were applied to cover the sections and distributed evenly over the tissue section, slides then placed in humidified chamber for 20 minutes at 37°C and washed in buffer and bathed in PBS for 2 minutes. The excess around the section was wiped. Then enough solution of 1/20 diluted streptavidin conjugated peroxidase were applied to cover the tissue section and slides were placed in humidified chamber for 20 minutes at 37C° then washed in PBS and bathed in PBS for 2 minutes then wiped around the sections. Enough drops of freshly prepared DAB working solution were applied to cover the section at room temperature for 5-10 minutes or until the color was observed, the reaction was terminated by rinsing gently with distilled water from a washing bottle. The slides were placed in a bath of Mayer's hematoxylin for one minute at room temperature. Then the slides were rinsed gently with distilled water from a wash bottle then rinsed under gently running tap water for 5 minutes. The slides were dehydrated by passing in 80% afterwards in 90% then to absolute ethyl alcohol containing staining jar subsequently, 3 minutes in each, then to xylol containing jar for 5 minutes. Finally, sections were mounted with DPX mounting medium, covered with coverslip. Then slides were examined under light microscope. (iii) Immunohistological analysis: For the evaluation of the CAM expression, a semiquantitative evaluation system was used to register the staining intensity and the numbers of positive cells. Therefore, the expression was classified according to Bittinger et al[7] (**Table 1**). That the definition of stain reaction as negative, weakly positive, mildly positive, moderately positive and strong positive was based according to the magnification power used when the positive reaction was detectable (**Table 2**).

In-situ hybridization detection of the IL-10 and INF-gamma mRNA. By using biotinylated long cDNA probe together with Maxim's in situ hybridization (ISH)-detection kit. In situ hybridization was carried out according to the manufacturer instructions with some modifications as following: All reagents used during hybridization and detection were warmed to room temperature before use. Tissue sections were deparaffinize and dehydrated as in immunohistochemistry (IHC). Then deproteinized by allowed to dry completely by incubating them at 37°C for 5 minutes. To each tissue section, 2 drops of freshly diluted 1X proteinase K were added. Then slides were incubated at 37°C for 10 minutes. Then tissue sections required to be dehydrated, thus slides were dipped in distilled water jar for one minute, then dipped in 70% ethanol jar, for 5 minutes, and then slides were dipped in 95% ethanol jar for another 5 minutes, finally slides were dipped in absolute ethanol jar, for 3 minutes, twice. At the end of that process slides were dried by incubating them at 37°C for 5 minutes.

Hybridization and detection. One drop of the working DNA probe/hybridization solution was added on the tissue section. Then a cover slip was put over each slide. Trapping any air bubbles was avoided. Then the slides with cover slips were placed in an oven at 95°C for 8-10 minutes (to denature the DNA), the slides were placed in humid chamber and incubated at 37°C for 2 hours, to allow hybridization of the probe with the target nucleic acid. Then, 2 drops of RNase-A (l5 μg/ml) were placed on tissue section. Then slides were incubated in humid chamber at 37°C for 30 minutes. Slides were soaked in protein block buffer at 37°C until cover slips fall off. The slides were allowed to remain in the buffer for 30 minutes after the cover slips were removed then they were washed again with protein block at 37°C for 30 minutes, twice. Excess buffer was carefully wiped from around the tissue section. And 2 drops of linker-1 were placed on tissue section then were placed in humid chamber at 37°C for 40 minutes. Excess buffer was carefully wiped off from around the tissue section and 2 drops of linker-2 were put on tissue section, then they were placed in humid chamber and incubated at 37°C for

Table 1 - Cell adhesion molecules expression grading according to Bittinger et al.[7]

Grading	Staining reaction
Grade 0	Negative reaction
Grade 1	Homogenously weak or focally mild (<30% of cells)
Grade 2	Homogenously mild or focally moderate (<30% of cells)
Grade 3	Homogenously moderate or focally strong (<30% of cells)
Grade 4	Strong in more than 30% of cells

Table 2 - Definition of stain reaction according to the magnification power.

Stain reaction	Magnification
Negative	No staining detectable
Weakly positive	At x 25 to 40 magnification detectable
Mildly positive	At x10 magnification weakly detectable
Moderately positive	At x10 magnification clearly detectable
Strong positive	Prominent at x 10 magnification

20 minutes. Excess buffer was carefully wiped off from around the tissue section. Two drops of the conjugate were applied on the tissue section and slides were placed in humid chamber and incubated at 37°C for 20 minutes. Excess reagent was tapped then the slides were rinsed in detergent wash buffer for 5 minutes. Excess buffer was carefully wiped off from around the tissue section. Two drops of substrate were placed on tissue section and incubated at room temperature for about 10 minutes, or until color development is complete. A blue colored precipitate was formed at the site of the probe in positive cells. Color appeared after 3-5 minutes, usually reaching sufficient development after 10 minutes. Excess reagent was taped off and slides were rinsed in 2 changes of distilled water. Slides were counterstained using nuclear fast red. Prior to application of a mounting medium, the sections were dehydrated by sequentially dipping the slides in graded alcohols, once in 95% then twice in absolute ethanol, and after that in xylene. The sections were mounted with a permanent mounting medium and examined under light microscope. Each run of ISH assay should include positive (the probe is complementary to human genomic DNA) and negative control (such as plant genomic DNA-probe). Positive cells were counted in 5 different locations in each layer of tissue, in each location 100 nucleated cells were counted and the average percentage of positive cells will give the final score.

Statistical analysis. Comparison between CAM and cytokine expression in control and patients groups was studied statistically by student t-test, while between groups was made by ANOVA test. Other variables were studied using the Chi-square test. The probability-value that was considered as a significant was 0.05. The software used was Excel package from Microsoft Corporation (XP series).

Results. ***Histopathological classification.*** The specimens were classified into 4 groups according to the Aschoff's classification of acute appendicitis. They were 1) early acute appendicitis (EAA) (n=8), 2) Phlegmonous appendicitis (PHA) (n=10), 3) ulcero-phlegmonous appendicitis (AUP) (n=6), 4) gangrenous appendicitis (GAA) (n=5) and 5) the group which include the specimens that showed no histopathological changes, this group was defined as histologically normal appendix (HNA) (n=6).

Results of immunohistochemistry detection of CAM. Intracellular adhesion molecule-1, VCAM-1 were homogenously and weakly expressed in the control group, while ICAM-3 was not detected in any of the control group. The average score for ICAM-l was 2.37 and VCAM-1 expression was 2.48 in all patient groups. The highest expression of ICAM-1 and VCAM-1 was in ulcero-phlegmonous appendicitis group (score 3.3 for ICAM-1 and 3.3 for VCAM-1) and the lowest expression of those CAMs was in gangrenous appendicitis group (score 1.4 for ICAM.1 and 1.4 for VCAM-1). The average expression of ICAM-1 and VCAM-1 was significantly higher in the patient group ($p<0.05$) than that of the control group. The average expression of both CAM was significantly higher in the patient group ($p<0.05$) in both cases than that of the control group. Whereas, the average score for ICAM-3 expression was 1.51 in all patients. The highest expression was observed in phlegmonous appendicitis group (2.9) and the lowest was seen in the HNA, phlegmonous and gangrenous groups.

Results of in-situ hybridization detection of the cytokines mRNA. The percentage of cells expressing mRNA of IL-10 and INF-gamma among nucleated cells of the control were on average (17.5% and 6.3%), while the average percentage of cell expressing mRNA were 21% and 27% in patients group. The difference in the mean number of cells expressing these cytokines between control

Table 3 - Average scoring for ICAM-1, ICAM-3, VCAM-1 and percentage for IL-10 and INF-gamma in patients groups.

Groups	ICAM-1	ICAM-3	VCAM	INF-gamma	IL-10
Control	0.3	0.0	1.2	6.3	17.5
Patients	2.37	1.5	2.48	27.07	21.02
Histologically normal appendicitis	1.5	1	2.66	9.66	29.16
Early Acute Appendicitis	2.75	2	2.25	34.25	21.57
Phlegmonous Appendicitis	3.7	2.9	3.5	39	24.2
Ulcero-phlegmonous Appendicitis	3.3	1	3.3	29	17.83
Gangrenous appendicitis	1.4	1	1.4	37.4	15.6

ICAM - intercellular adhesion molecule, VCAM - vascular cell adhesion molecule, IL - interleukin, INF-gamma - interferon gamma.

Table 4 - The correlation among CAM-expression, the number of the cells that expressing cytokines mRNA and the clinical and histopathological finding.

Parameters	Age	M:F ratio	S to D	Dx to Sx	total	Histo	INF-gamma	IL-10	ICAM-1	ICAM-3	VCAM
Age	1										
M:F	-0.73141	1									
S to D	-0.62555	0.591893	1								
Dx to Sx	-0.3677	0.676816	0.729692	1							
Total	-0.58063	0.657044	0.974632	0.864219	1						
HISTO	-0.43504	0.404237	0.766772	0.87017	0.849006	1					
INF-gamma	0.191786	-0.04783	0.058962	0.589152	0.236224	0.666204	1				
IL-10	0.32493	-0.37752	-0.82274	-0.88764	-0.8959	-0.97257	-0.60868	1			
ICAM-1	0.511576	-0.86128	-0.36901	-0.32239	-0.37704	0.057788	0.464943	-0.01777	1		
ICAM-3	0.471188	-0.54746	-0.76675	-0.31529	-0.66737	-0.18481	0.533163	0.313492	0.675227	1	
VCAM	0.219972	-0.82388	-0.38961	-0.70335	-0.51689	-0.26878	-0.10663	0.332769	0.796828	0.436094	1

CAM - cell adhesion molecules, M - male, F - female, S to D- time from first symptom until diagnosis, Dx to Sx- time from diagnosis to surgery, Histo - histopathological stage ICAM - intercellular adhesion molecule, VCAM - vascular cell adhesion molecule, IL - interleukin, INF-gamma - interferon gamma.

and patients group and among the different groups of patients was statistically significant ($p<0.05$). The results of immunohistochemistry for CAMs and in situ hybridization for cytokines are summarized in **Table 3**.

The results of correlation among the study variables. A negative correlation was observed between the age of the patient and the male to female ratio, the duration between first symptom until surgery, histopathological stage with correlation of r= -0.73, r= -0.58, r= -0.44. And a positive correlation was observed between the age of the patient and the ICAM-1 (r=0.51) and ICAM-3 expression (r=0.47). There was a positive correlation between the duration and the histopathological stage (r=0.84), INF-gamma expression (r=0.23) and a negative correlation with IL-10 expression (r= -0.89), ICAM-3 (r= -0.66), VCAM-l (r= -0.51) and ICAM-1 expression (r= -0.37). The histopathological stage had a positive correlation with the duration of symptoms (r=0.5), percentage of cells expressing INF-gamma (r=0.67), and had a negative correlation with percentage of cells expressing IL-10 (r= -0.97). The correlation among CAM-expression, the number of the cells that expressing cytokines mRNA and the clinical and histopathological finding were summarized in **Table 4**. There was a significant correlation between ICAM-l, ICAM-3 and VCAM-1 expression (r=0.68, r=0.8). Thus, whenever the ICAM-l is increased, ICAM-3 and VCAM expression will increase. There was a weak positive correlation between ICAM-3 and VCAM-1 expression (r=0.44). The IL-10 expression was inversely correlated with expression of INF-gamma (r=- 0.61) and there were no significant correlation with CAM expression. Interferon-gamma had a moderate correlation with ICAM-3 expression (r=0.53), weak correlation with ICAM-1 (r=0.46), and no clear correlation with VCAM expression.

Discussion. Leukocytes-endothelial cell adhesion has been implicated in the pathogenesis of a variety of diseases and recognition that leukocytes must firmly adhere to vascular endothelial cells in order to mediate the organ dysfunction and tissue injury associated with these diseases has resulted in an intensive effort to define the factors that modulate this cell-cell interaction. Data derived from both in vitro and in vivo models of leukocyte-endothelial cell adhesion have revealed the relative contributions of different leukocyte and endothelial CAMs to adhesion responses elicited by various inflammatory stimuli and these studies also led to appreciation of the cellular and molecular loci that can be targeted to interfere with leukocyte-endothelial cell adhesion.[8] Such knowledge could be a fundamental development of novel forms of therapy for intestinal inflammation,[5] this may include acute appendicitis. In this study, we analyzed the differential expression of CAM and we tried to investigate the role of cytokines on their expression, and we try to correlate the level of their expression with different clinical and histomorphological aspects. The results of this study revealed a negative correlation between the age of the patients and the histological stage of appendicitis, thus, the younger age patient will have more advanced stage of appendicitis with a shift from early acute appendicitis to gangrenous

one. This finding supported by a negative correlation between the age of the patient and the total time since first symptom experienced until the time of surgery and these data agree with previous study carried out by Pieper et al.[9] They stated that the younger the children, the more likely a delay in diagnosis happen. The histomorphological distribution of the patient in this study was in agreement with Bittinger et al,[7] except that phlegmonous appendicitis was more common among our patients instead of the early acute appendicitis, this could be due to early diagnosis as a result of availability of CT-scan and MRI as a diagnostic tools in that study. Unfortunately, there was no histologically normal appendix in that study. The histologically normal appendix patients group in our study was found to involve those patients whom presented with symptoms and signs of acute appendicitis and their clinical features disappear after surgery, the appendices were macroscopically and microscopically by H&E stained histopathology normal, but at the molecular level revealed a higher expression of CAM and cytokines.

E-selectin and VCAM-l expression by endothelial cells was described by Rice et al,[8] but without correlation with clinical data, while Bittinger et al,[7] was the first who correlate CAM-expression with different histopathological stage of appendicitis (except the HNA). Our findings confirmed the feasibility of performing immunoperoxidase immunostaining of CAM on paraffin embedded tissue sections. This could be helpful in expanding the diagnostic role of CAM analysis in other inflammation conditions and in archival material analysis, that was considered normal histopathologically. One of the important finding in our study is that the demonstration of visible mucosal lesion is not necessary to be the initial stage of appendicitis, as postulated by Aschoff, and supported by Stambolis and Wagner,[8] since our result shows expression of different CAM in the HNA patient group. The positive expression of ICAM-3 and the higher expression of VCAM-1 and ICAM-1 when compared with the control group may support our demonstration. Thus, immunohistochemistry detection of CAM is more sensitive than routine histopathological analysis. These findings may help in reducing the discrepancies between the surgical and pathological opinions. Moreover, ICAM-3 expression can be used to differentiate between 2 overlapped groups (HNA and normal appendix) in addition to the level of expression of 1CAM-l and VCAM-1, which were increased in HNA. The level of IL-10 mRNA expression was significantly higher in the patients group and it was different among the patients groups. Interleuken-10 expression was mainly in mucosal (epithelial) and submucosal layer while in advanced stages it was expressed in subserosal layer also beside mucosal and submucosal expression. These data were comparable with previous studies carried out by Autsbach et al[10] that stated "IL-10 is constitutively expressed in normal human gut tissue and its major source was the intestinal epithelial cells in non-inflamed gut. In the inflamed gut tissue, at mRNA level, increased IL-10 production was mainly apparent in the submucosal compartment of gut wall." Our data (the negative correlation of IL-10 with duration and with histopathological stage, and the association of IL-10 decreased with shifting of cases from early acute appendicitis to gangrenous) may provide an excellent evidence of the IL-10 function, as a protective factor against the inflammation of the appendix, which could keep it free from inflammation. These data differs from previous studies carried out by Yoon et al[11] and Rivera-Chavez et al.[12] The discrepancy could be expected as they detect the serum or plasma level (namely systemic detection of IL-10 in the circulation), whereas in the present study, we detected the local expression of IL-10 mRNA. It is noteworthy that IL-10 most likely exerts its anti-inflammatory effect when produced locally in the vascular wall. The level of INF-gamma mRNA expression was significantly higher in the patient groups and the expressing cells were mainly in mucosal (epithelial layer) and submucosal layer while in advanced stages it was expressed in mucosal, submucosal and subserosal layer as well, the expression was in scattered fashion that may indicate its source (the inflammatory cells in this case). All patients groups except HNA and control, the cytokine was higher than IL-10 also it was associated with increasing levels of ICAM-1, ICAM-3 and VCAM-1. This goes with previous study carried out by Carol et al.[13]

We conclude that the kinetics of CAM expression was tightly correlated to the balance between IL-10 and INF-gamma especially after 12.5 hours from the first symptom experienced by the patient. The INF-gamma was the main player and was the most significant factor that leads to shifting of cases towards gangrenous appendicitis, may be by direct effect on CAM-expression with up regulation of CAM, mainly the ICAM-1. Intracellular adhesion molecule-3, with modest down regulation of VCAM-l, this will lead to infiltration of large number of leukocyte, especially the neutrophils, and this will result an increased tissue damage. Thus, whenever INF-gamma is increased and IL-10 decreased, the patient will have more aggressive form of appendicitis. We can postulate that when the ratio of IL-10/IFN-gamma was more than one, the patient had a histological normal appendix but when the ratio is <1, the patient has an appendicitis with definite histopathological findings.

References

1. Bernard AB, Stephanie RW. Appendicitis at the millennium. ***Radiology*** 2000; 2: 337-348.
2. Addiss DG, Shaffer N, Fowler BS, Tauxe RV. The epidemiology of appendicitis and appendectomy in the United States. ***Am J Epidemiol*** 1990; 132: 910-925.
3. Condon R, Telford G. Appendicitis. In: Sabistan I, editor. Textbook of surgery. 14 ed. Philadelphia (USA): WB Saunders Company; 1991. p. 884-898.
4. Zahid A. The vermiform appendix: not a useless organ. ***J Coll Physicians Surg Pak*** 2004; 14: 256-258.
5. Janeway CA, Travers P, Walport M, Shlomchik M. Immunobiology, 5th ed. New York, London: Garland Publishing; 2001.
6. Fiocchi C. Intestinal inflammation: a complex interplay of immune and non-immune cell interaction. ***Am J Physiol Gastrointest Liver Physiol*** 1997; 273: 769-775.
7. Bittinger F, Brochhausen C, Kohler H, Lehr HA, Otto M, Skarke C, et al. Differential expression of cell adhesion molecules in inflamed appendix: correlation with clinical stage. ***J Pathol*** 1998; 186: 422-428.
8. Panes J, Perry M, Granger DN. Leukocyte-endothelial cell adhesion: avenues for therapeutic intervention. ***Br J Pharmacol*** 1999; 126: 537-550.
9. Pieper R, Kager L. The incidence of acute appendicitis and appendectomy: an epidemiological study of 971 cases. ***Acta Chir Scand*** 1982; 148: 45-49.
10. Autschbach F, Braunstein J, Helmke B, Zuna I, Schurmann G, Niemir ZI, et al. In situ expression of interleukin-10 in noninflamed human gut and in inflammatory bowel disease. ***Am J Pathol*** 1998; 153: 121-130.
11. Yoon DY, Chu J, Chandler C, Hiyama S, Thompson JE, Hines OJ. Human cytokine levels in nonperforated versus perforated appendicitis: molecular serum markers for extent of disease? ***Am Surg*** 2002; 68: 1033-1037.
12. Rivera-Chavez FA, Wheeler H, Lindberg G, Munford RS, O'Keefe GE. Regional and systemic cytokine responses to acute inflammation of the vermiform appendix. ***Ann Surg*** 2003; 237: 408-416.
13. Carol M, Lambrechts A, Van Gossum A, Libin M, Goldman M, Mascart-Lemone F. Spontaneous secretion of interferon gamma and interleukin 4 by human intraepithelial and lamina propria gut lymphocytes. ***Gut*** 1998; 42: 643-649.