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EXPRESSION OF *APC*TUMOR SUPPRESSOR GENE IN COLORECTAL CANCER: RETROSPECTIVE STUDY

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ABSTRACT
Background: Despite the advances in diagnosis of colorectal cancer it is still the third malignancy worldwide. Adenomatous polyposis coli *(APC)* gene responsible for familial adenomatous polyposis (FAP), an autosomal dominant hereditary disease, characterized withhundreds to thousands of adenomatous polyps in colon and rectum. The majority of both sporadic and familial forms of adenomatous polyposis (FAP) in colorectal cancer (CRC)originates from inactivation of *APC* (Adenomatous Polyposis Coli) tumor suppressor gene. Authors aimed to investigate the clinical significance of *APC* in colorectal cancer progression. **Materials and methods:** Expression of *APC* was detected in 50 formalin fixed paraffin using quantitative PCR (QPCR) and their levels were analyzed versus clinicopathological factors and the overall survival (OS) of CRC patients. **Results:** Significant relation was reported young female and CRC as compared to their counterparts from male individuals. *APC* Expression was increased significantly with young CRC and reported significant correlation with advanced stage and high grade tumors. Worse OS was reported among patients with decreased APC expression. **Conclusion:** *APC* expression was significantly related to differential grading and patient's outcome thus pointing out their potential role as predictive markers for CRC prognosis.

KEYWORDS: Colorectal cancer; tumor suppressor genes; *APC*; progression, prediction.

INTRODUCTION
Colorectal cancer (CRC) is a malignant neoplasm of thecolon, rectum, and appendix and is a major health load, causing one-third of cancer-related deaths.[1] Time of diagnosis seriously influences on the whole survival rate of patients with CRC. Five-year survival rates are estimated to be between 85 and 90% for patients with localized cancer tocolon or rectum. Survival decreases considerably for patientswith distant metastasis, with 5-year survival of only 12.5%. Appendix cancers exhibit higher survival rates in all stages.[1] The numeral of cases of CRC has decreased due toadvances in screening and diagnostic methods.[2] CRC develops as a result of genetic and epigeneticalterations, as well as environmental factors. CRC occurs as familial, inherited, and sporadic disease, where less than10% reported cases are inherited CRCs. Lynch syndrome, familial adenomatous polyposis (FAP), and Peutz-Jeghers syndrome are inherited diseases which have a tendency to progress to CRC. Familial form of CRC records for 25% of all cases. The largest group is the sporadic form of CRC, which records for 70% of all CRCs. With this form of CRC etiological, dietary and environmental factors are associated.[3] CRC is a heterogeneous disease, arising through different molecular mechanisms. Currently, three chief molecular mechanisms throughout which CRC carcinogenesis can progress are chromosomal instability (CIN), microsatellite instability(MSI), and the CpG island methylator phenotype (CIMP). Imbalances in chromosome number and a loss of heterozygosity(LOH) are characteristic for CIN molecular subtype.[3] Mutations in mismatch repair genes, such as MLH1, MSH2, and MSH6, are characteristic for MSI. MSI molecular subtype is interrelated to inherit CRC. CIMP is found in sporadiccases of CRC and is characterized by aberrant methylation of tumor suppressor genes silencing them. The survival of CRC affected patients depends highlyon early recognition.[4] The main screening approaches include direct structural examinations and fecal tests. Fecal tests are used to detect blood in the stool, which can be detected with hemoglobin test or immunohistochemistry. Fecal test is not definite for CRC and direct structural exam, such as flexible

sigmoidoscopy and colonoscopy, must succeed fecal test. The structural examinations are persistent procedures and reduce the compliance of a patient to participate in CRC screening. Improving the patients' prognosis, treatment reaction prediction, and return risk would be enabled with dependable biomarkers for early detection of CRC.

Adenomatous polyposis coli (*APC*) gene mutations are the initial events in the multi-step colorectal cancer development Mutations in *APC* gene on chromosome 5q21 locus are well thought-out as one of the earliest events in the initiation and progression of colorectal cancer. In FAP patients, allelic mutation of *APC* gene followed by a loss of heterozygosity (LOH) is a frequent feature. Notably, mutations in *APC* gene also are found in 60 to 80% of sporadic colorectal cancers and adenomas. FAP patients with *APC* mutations are prone to hundreds to thousands of colorectal adenomas and early onset carcinoma. FAP patients also are prone to small intestinal adenomas (and carcinomas), intra-abdominal desmoids and osteomas tumors (Gardner's syndrome), congenital hypertrophy of retinal pigment epithelium (CHRPE), fundic gland polyps in the stomach, pancreas and thyroid, dental abnormalities, and epidermal cysts.[5] Mutations in *APC* are also associated with malignant brain tumors known as Turcot's syndrome.

The study was conducted to investigate the expression of *APC* tumor suppressor gene among colorectal cancer patients to detect their role in colorectal cancer progression and their predictive significance for CRC patients. Also their correlation with other clinicopathological factors will be determined.

PATIENTS AND METHODS

Sample selection

Fifty formalin fixed paraffin embedded (FFPE) tumors from Egyptian patients diagnosed with primarycolorectal cancer were enrolled in the study, all samples were tissue blocks obtained from patients after surgical resection. Exclusion criteria will be patients with pre-surgically treated cancer, recurrent colorectal cancer, or other known malignancies. Before RNA extraction, representative sections were stained with H&E and analyzed and samples with tumor percentage more than 80% were only included in the study. Tumor staging were performed according to TNM classification using classification of the International Union Against Cancer[6], and the analyzed pathological feature as defined by the Collage of American Pathologists consensus declaration[7] were lymphatic invasion, tumor pattern, histological grading.

Survival data for enrolled individuals were obtained, and the endpoint for the current study was selected as the overall survival (OS) which resembles the time (months) from diagnosis to the end of the study with a follow up duration of nearly 3 years (36 months).

Purification of RNA

Total RNA was isolated from FFPE samples following the manufacture instruction protocol (Cat. no. 73504, Qiagen, USA). Breifly, deparaffinization treatment for the FFPE tissue samples was carried out using deparaffinization solution (Cat. no. 19039, Qiagen, USA). Then samples were incubated at 56°C with lysis buffer containing protinase K to release RNA from the paraffin sections. Then DNase treatment was carried out to eliminate of genomic DNA, and ethanol was added to provide binding conditions for RNA. After words the samples were applied to RNeasy Min Elute spin columns to wash away any contaminants and total RNA was eluted using RNase-free water. Total RNA concentration was detected using Q-5000 spectrophotometer nanodrop (Quawell Technology, Inc., San Jose, USA) at A260/A280. The ratio of purified RNA wasranged between 1.8-2.0, then they were divided into aliquots and stored at –80°C for complementary DNA (cDNA) synthesis.

Reverse transcription to synthesize cDNA

Reverse transcription process was carried out using QuantiTect reverse transcription kit (Cat no. 205311, Qiagen, USA) and cDNA was synthesized according the manufacturer instruction by adding 1µg of RNA template to reverse transcription master mix (reverse transcriptase, RT primer mix and RT buffer)forming a total volume of 20µl and PCR thermal cycler (SureCycler 8800, Agilent Technologies, Germany) was adjusted as following: samples were incubated for 30 minutes at 45°C, then 3 minutes at 95°C. Synthesized cDNA was divided into aliquots and stored at –80°C for gene expression analysis.

Gene expression analysis

APC expression was carried out using quantitative real-time PCR (QPCR) (Stratagen 3005MxP, Agilent Technologies, Germany) and theirprimers, as listed in Table (1) with SYBR Green chemistry according to the manufacture's recommended protocol of Quanti Tect SYBR Green PCR (Cat. no. 204143, Qiagen, USA). In brief, 500ng/reaction from cDNA was add to tubes each containing SYBR green master mix, primers for *APC* gene (forward 3′- TCRC AGT CTC TTC GAG CRCT T -5′and reverse 3′- TCRC CCT AAC ATA CAG GGT GA -5′)[8] and RNase free water to form a total volume 50µl, the thermal conditions were: initial activation for 15 minutes at 95°C followed by 40 cycles of: denaturation for 15 seconds at 94°C, annealing for 30 seconds at 54°C, then extension for 30 seconds at 72°C. The internal control used to normalize the expression of the investigated genes was *GAPDH*primer (forward 3′-ATGGGGAAGGTGAAGGTCG-5′, and reverse 3′-GGGTCATTGATGGCAACAATATC-5′[9] and calculations of the gene expression analysis were conducted using comparative C_T ($2^{-\Delta CT}$ as ΔCt = Target gene − Reference gene).[10]

Statistical analysis

The results were analyzed using Statistical Program for Social Science version 16 (SPSS). Non-parametric analysis using Wilcoxon and Kruskal Wallis tests for two and three groups, respectively. Also chi-square analysis was used to compare between qualitative parameters. Overall survival analyses were investigated versus gene expression using Kaplan Meier curves. *P*-value was two-tailed test and it was considered significant if less than or equal 0.05.

RESULTS

Fifty CRC FFPE samples were enrolled in the current study, according to their gender they were divided into 27 males and 23 females with median (range) age 44years (18-57 years) and 48 years (18-67.7 years), respectively (F=0.694, *P*= 0.409). The clinical data for studied CRC was presented in Table (1). Among the studied group, age of CRC cancer patients showed significant difference with grading as those with poor grading reported higher mean age (50 ± 6.6) as compared to those with wild grading (36.2 ± 16) at (F=8, *P*=0.007), while the correlation between age and clinical stage or tumor location did not reach significant difference.

As shown in Table (2), the level of *APC* gene was reported regarding investigated clinicopathological factors. Significance difference was reached between *APC* level and age of enrolled individuals as the level was higher in younger CRC patients (≤ 39 years) as compared to those higher than 39 years at (F11, *P*<0.001). Similarly, its level was significantly correlated with histological grading as its median level was increased with wild grading compared to those with poor histological grading (F=16.3, *P*<0.001). No significant level was reached between *APC* expression level and staging although its median was increased among those with early stage, in the same current its expression was increased among those with rectum cancer as compared to colon cancer.

Among this retrospective study the follow-up period was ranged between 20-36 moths and median 28 months, accordingly the mean expression level was assessed as 5.7 fold change and considered as a cutoff point for survival analysis hence the expression level of *APC* reveled significant difference (Log Rank= 3.9, *P*=0.04) with OS analysis by considering grading status as those with low expression of *APC* and poor grading reported worse OS as compared to those with high *APC* expression and wild grading status as presented in Figure (1).

Table (1): Clinical data for enrolled 50 CRC patients.

Parameter	Number (n)	Percentage %
Age		
≤ 39 years	21	42%
>39 years	29	58%
Gender		
Male	27	54%
Female	23	46%
Clinical Stage		
early	33	66%
late	17	34%
Histological Grade		
wild	38	76%
poor	12	24%
Tumor location		
Rectum	22	44%
Colon	28	56%

Table (2): Level of *APC* gene among other clinicopathological data.

Clinical pathological of date	Median	Range	Statistics
Age			
≤39 year (n=21)	12.3	10-13.4	F= 211, *P*<0.001
>39 year (n=29)	5.9	5.7 - 9.8	
Gender			
Male (n=27)	6.3	5.7-13.4	F=0.044, *P*=0.836
Female (n=23)	6.3	5.7- 13.4	
Clinical stage			
Early(n=33)	9.8	5.7 - 13.4	F=0.536, *P*=0.468
Late(n=17)	6.3	5.7 - 13.4	
Histological grading			
Wild (n=38)	10	5.7 - 13.4	F=16.3, *P*<0.001
Poor (n=12)	5.9	5.7 - 6.3	
Tumor location			
Rectum (n=22)	9.9	5.7 - 13.4	F=0.856, *P*=0.359
Colon (n=28)	6.3	5.7 - 13.4	

- Statistical analysis using ANOVA test.

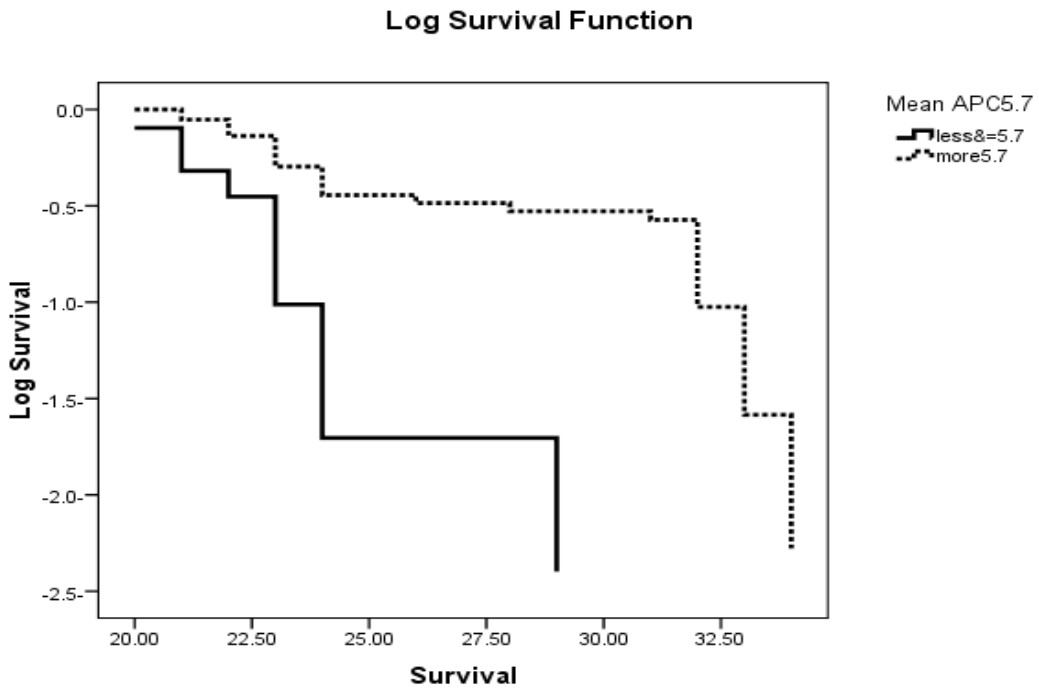

Figure (1): Kaplan Meier curves for comparing between *APC* and histological differentiation. The poor differentiation is represented by straight line while the well-moderate differentiation is represented by dashed line.

DISCUSSION

Colorectal cancer is the third most frequently type of cancer in oncologic pathology.[11] Presently, it is the most common malignant cancer in the gastrointestinal tract, instead of 13% of all malignant tumors, and it is considered the second the majority frequent cause of death correlated to cancer affecting men as women in the same manner globally for both developed and un-developedcountries, and it is predictable to conquer the mortality rate of heart diseases in the pending years.[12] Adenomatous *polyposis coli (APC)* gene mutations are the initial events in the multi-step for development of colorectal cancer; mutations in the *APC*gene on chromosome 5q21 locus are well thought-out as one of the earliest events in the initiation and progression of colorectal cancer. In FAP patients, allelic mutation of the *APC*gene followed by a loss of heterozygosity (LOH) is a common feature. In the current study include 50 CRC patients were enrolled (54)% of the cases were males and (46)% of them were females, accordingly gender status revealed increment of CRC percentage among males than females, these results were consistent with Purim and his colleagues[13] reported that men have a higher incidence of CRC than women. Moreover by considering the relation between staging and gender our results reported that 64.7% of late stage were male while the remaining were females these observations indicate males and females show many epidemiological, clinical and pathological differences With respect to cancer localization, left-sided CRC tends to be more frequent in men while right-sided CRC more frequent in women.[14]

Additionally, women with CRC seem to have an age-dependent survival advantage over men.[15] In this study 58% of cases were >39 years these results consistent with previous study reported that CRC is typically a disease of the elderly, with over 90% of cases in old patients.[16,17] In this study young patients manifested a wild grade prognosis these results consistent with Marble and his collage.[17,18] CRC patients with age ≤ 39 years represented 71.4% of enrolled cases were at early stage, this manifestation has been considered as a predictor for poor survival as previously stated.[19] The relation between age and clinical staging was also investigated and revealed that early stage was detected in 45.5% of patients were ≤ 39 years and detected in 54.5% for patients > 39 years these results were consistent with Fazeli and his colleagues as they observed that diagnosis in young patients is always difficult, because both patient and the doctor usually don't give credit to the presenting symptoms, leading to a frequent unfavorable outcome of the disease.[20]

In the current study 55.3% of patients were young (≤ 39 years) were represented with wild grade these results were consistent with Sinicrope and his collages they observed that hereditary non polyposis CRC tumors are associated with a better prognosis in young patients.[21]

By considering tumor location, among the enrolled cases the incidence of rectum cancer was more than in colon among young age (≤ 39 years), these results were agreed with previous report showing an increment of rectal

cancer for patients with age range 35- 49 years.[22] In this study 50% of patients were ≤ 39 years with rectal carcinoma and the remaining were > 39 years, for rectal adenocarcinoma the male survival was about 55% compared to over 60% for women. The reasons for the improvementsin survival have been suggested to be earlier diagnosis and better health status and care.[23] Tumor localization was a strong predictor of high T, N and stage but a weaker predictor of M. These results were consistent with Baxter and his collages they observed that Rectal tumors had the most favorable characteristics compared to any colonic sub-site[24], while numerous researches are investigating markers to forecast the prognosis of colorectal cancer, still there is a need to discover markers that may improve this subject amongst these markers is the recognition of gene expression.

*APC*gene expression was investigated in a FPEE samples using real-time PCR as sensitive and applicable technique for accurate quantitation for gene expression[25] and their levels was normalized against GAPDH as house-keeping gene. The relation between investigated gene and clinicopathological factors were assessed. Age is amongst the main risk factors that lead to colorectal cancer[26] in this study the age range for the enrolled samples were 18-67years and those with age less than 39 years represented (42%) indicating that it iscolorectal cancer is a major risk in younger ages as in older ones, but its detection among those with young ages represent a percentage higher than reported in Westcountries which may concern the epidemiological trends among Egyptians these results agreed with previous reports.[27] In this study the median level of *APC*was similar in both gender which disagree with other studies reported increase of CRC among males as compared to women.[28] This discrepancies may be attributed to the different selected population in both studies. The enrolled patients were categorized according to their clinical stage into early stage (stage I-II) and late stage (stage III), the median level of *APC* of at early stage 9.8 and at late stage 6.3 that indicates that the level of *APC* decrease with CRC progression. As reported by Ting and his colleagues nearly 90% of early diagnosed colorectal cancer patients have reached the 5- year survival on the contrary less than 10% reached this survival rate when diagnosed in metastatic stage.[29]

Standard grading classifiedcolorectal cancer into grade I, grade II and gradeIII and it is dependent on individual estimation of the histopathologist. Diversity of differentiation inthe similar cancer samples frequently leads tosignificant inter-and intra-observer difference in grading.[30] In the currentstudy expression level of *APC* was correlated significantly with tumor variation as their expression was decreased in grade III. Mutations in APC gene result in transcriptional activation through β-catenin; principal cell-cell adhesion molecule, to transcriptional activate a family of transcription factors which further modulate tumorgenesis.[31] In the current study About 66% of colorectal cancer patients relapsed although early diagnosed. It has been reported that although colorectal cancer patients are being classified by clinicopathological features, still the treatment reaction is diverse which may stress for the understanding the molecular events initiating colorectal cancer.[32] Thus, in this study the expression of *APC* gene was different from rectum to colon, as its median level was 9.9 in rectum and was 6.3 in colon although no significant different was reached, theis may clarify that the expression of *APC*gene is affected by the site of tumor, these results were consistent with a previous report by Birgisson and his colleagues as they observed thatthe relatively favorable tumor characteristics of rectal cancer would have predicted a survival better than that for colon cancer.[33]

The expression for *APC* gene was investigated with the OS of the enrolled individuals and hence significant correlation was reported between the increased expression of APC amongst on the whole survival which points out its efficacy as a predictor for the prognosis of colorectal cancer patients. In this study colorectal cancer patients with grade III and exhibiting decreased tumor suppressor gene expression exposed worse survival as compared to those with grade I-II and high gene expression, which emphasize the importance for analyzing *APC* gene among CRC patients to predict the patients outcome hence direct for a suitable treatment strategy.

In conclusion, detection of APC expression using quantitative PCR on FFPE samples may provide a better insight into CRC progression or identifying patinets outcome and their better treatment strategies.

Conflicts of interest

The authors have nothing to disclose.

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REFERENCES

1. R. Siegel, C. Desantis, and A. Jemal, "Colorectal cancer statistics, 2014," CA: A Cancer Journal for Clinicians, 2014; 64(2): 104–117. 2011, 103: 863–875. 69: 866–872.
2. R. A. Smith, D. Manassaram-Baptiste, D. Brooks et al., "Cancer screening in the United States, 2015: a review of current American cancer society guidelines and current issues in cancer screening," CA: A Cancer Journal for Clinicians, 2015; 65(1): 30–54.
3. M. S. Pino and D. C. Chung, "The chromosomal instability pathway in colon cancer," Gastroenterology, 2010; 138(6): 2059–2072.

4. J. B. Rawson and B. Bapat, "Epigenetic biomarkers in colorectal cancer diagnostics," Expert Review of Molecular Diagnostics, 2012; 12(5): 499–509.
5. Fearnhead NS, Britton MP, Bodmer WF: The ABC of APC. *Hum Mol Genet*, 2001; 10: 721-733.
6. Sobin LH. TNM classification of malignant tumors. New York: Wiley-Liss, 1997; 5: 66-69.
7. Compton CC, Fielding LP, Burgart LJ, Conley B, Cooper HS, Hamilton SR, Hammond ME, Henson DE, Hutter RV, Nagle RB, et al: Prognostic factors incolorectal cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med, 2000; 124: 979-994.
8. Fang Z, Xiong Y, Li J, Liu L, Li M, Zhang W, Shi L, Wan J. Detection of APC gene deletions in colorectal malignancies using quantitative PCR in a Chinese population. Pathol. Oncol. Res., 2011; 17: 657–661.
9. Kontos CK, Papadopoulos IN, Fragoulis EG and Scorilas A. Quantitative expression analysis and prognostic significance of L-DOPA decarboxylase in colorectal adenocarcinoma. British Journal of Cancer, 2010; 10: 1384–1390.
10. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using realtime quantitative PCR and the 2(-Delta Delta C(T)) Method, 2001; 25: 402–408.
11. Smith RET, Renaud RC, Hoffman E. Colorectal cancer market. Nat Rev Drug Discov., 2004; 3: 471-2.
12. Dobre M, Dinu DE, Panaitescu E, Bîrlă RD, Iosif CL, Boeriu M, et al. KRAS gene mutations prognostic factor in colorectal cancer? Rom J Morphol Embryol, 2015; 56: 671-8.
13. Purim, O.; Gordon, N.; Brenner, B. Cancer of the colon and rectum: Potential effects of sex–age interactions on incidence and outcome. *Med. Sci. Monit.*, 2013; *19:* 203–209.
14. Takada, H.; Ohsawa, T.; Iwamoto, S.; Yoshida, R.; Nakano, M.; Imada, S.; Yoshioka, K.; Okuno, M.;Masuya, Y.; Hasegawa, K.; *et al.* Changing site distribution of colorectal cancer in Japan. *Dis. Colon Rectum*, 2002; *45:* 1249–1254.
15. Micheli, A.; Ciampichini, R.; Oberaigner, W.; Ciccolallo, L.; de Vries, E.; Izarzugaza, I.; Zambon, P.; Gatta, G.; de Angelis, R. EUROCARE Working Group. The advantage of women in cancer survival: An analysis of EUROCARE-4 data. *Eur. J. Cancer*, 2009; *45:* 1017–1027.
16. Atkin WS, Cuzick J, Northover JM, Whynes DK: Prevention of colorectal cancer by once-only sigmoidoscopy. Lancet, 1993; 341: 736–740.
17. Andrew McKay, Jeniva Donaleshen, Ramzi M Helewa, Jason Park, Debrah Wirtzfeld, David Hochman, Harminder Singh and Donna Turner World Journal of Surgical Oncology, 2014; 12: 370. http://www.wjso.com/content/12/1/370 .
18. Marble K, Banerjee S, Greenwald L: Colorectal carcinoma in young patients. J Surg Oncol, 1992; 51: 179–182.
19. Fancher TT, Palesty JA, Rashidi L, Dudrick SJ. Is gender related to the stage of colorectal cancer at initial presentation in young patients? J Surg Res., 2011; 165(1): 15-8.
20. Fazeli MS, Adel MG, Lebaschi AH. Colorectal carcinoma: a retrospective, descriptive study of age, gender, subsite, stage, and differentiation in Iran from 1995 to 2001 as observed in Tehran University. Dis Colon Rectum, 2007; 50(7): 990-5.
21. Sinicrope FA, Foster NR, Thibodeau SN, Marsoni S, Monges G, Labianca R, Kim GP, Yothers G, Allegra C, Moore MJ, Gallinger S, Sargent DJ: DNA mismatch repair status and colon cancer recurrence and survival in clinical trials of 5-fluorouracil-based adjuvant therapy. J Natl Cancer Inst., 2011; 103: 863–875.
22. Bailey CE, Hu CY, You YN, Bednarski BK, Rodriguez-Bigas MA, Skibber JM, et al. Increasing disparities in the age-related incidences of colon and rectal cancers in the United States, 1975-2010. JAMA Surg., 2015; 150(1): 17-22.
23. Lango H, Palmer CN, Morris AD, Zeggini E, Hattersley AT, McCarthy MI, et al: Assessing the combined impact of 18 common genetic variants of modest effect sizes on type 2 diabetes risk. Diabetes, 2008; 57: 3129-35.
24. Baxter NN, Goldwasser MA, Paszat LF, Saskin R, Urbach DR, Rabeneck L: Association of colonoscopy and death from colorectal cancer. Ann Intern Med., 2009; 150: 1-8.
25. Sanders BD, et al. Structural basis for sirtuin function: What we know and what we don't. Biochim Biophys Acta., 2010; 1804(8): 1604-16.
26. Hao YP, Jemal A, Zhang XY, Ward EM. Trends in colorectal cancer incidence rates by age, race/ethnicity, and indices of access to medical care, 1995–2004 (United States). Cancer Cause Control, 2009; 20(10): 1855–1863.
27. Johnson CM, Wei C, Ensor JE, Smolenski DJ, Amos CI, Levin B, et al. Meta-analyses of colorectal cancer risk factors. Cancer Causes Control, 2013; 24: 1207–22.
28. Matanoski G, Tao XG, Almon L, Adade AA and Davies-Cole JO: Demographics and tumor characteristics of colorectal cancers in the United States, 1998-2001. Cancer, 2006; *107*: 1112-1120.
29. Ting WC, Chen LM, Pao JB, et al. Common genetic variants in Wnt signaling pathway genes as potential prognostic biomarkers for colorectal cancer. PLoS One., 2013; 8: e5619.
30. Fernando M, Amarsekara LR. The pathologist and colorectal cancer. Ceylon Med J., 1998; 43: 167.
31. Schneikert J, Behrens J. The canonical Wnt signalling pathway and its APC partner in colon cancer development. Gut., 2007; 56(3): 417-25.
32. Souglakos J, Philips J, Wang R, et al. Prognostic and predictive value ofcommon mutations for treatment response and survival in patients with metastatic colorectal cancer. Br. J. Cancer., 2009; 101: 465–472.
33. Birgisson H, Ghanipour A, Smedh K, Pahlman L, Glimelius B: The correlation between a family history of colorectal cancer and survival of patients with colorectal cancer. Fam Cancer, 2009.