Scientific Research & Studies Center-Faculty of Science- Zagazig University- Egypt

Biochemistry Letters

Potential Association of Poly(ADP-ribose) Polymerase-1 (PARP-1) with CD133 and G2/M as Independent Predictors in Colorectal Cancer Development.

Hoda M. El-Emshaty[1], Amina A. Abdelfatah[2], Hisham Ismail [3], Fathy M El-Taweel[2], Mohamed M. El-Hemaly[1]

[1]Gastroenterology Surgical Center, Faculty of Medicine, Mansoura University, Egypt
[2]Biochemistry Department, Faculty of Science, Damietta University, New Damietta, Egypt

[3]Biochemistry Division, Faculty of Science, Minia University, Minia, Egypt

ARTICLE INFO

Keywords:
CD133; PARP-1; DNA cell cycle; Colorectal cancer (CRC); Ulcerative colitis (UC); Flow cytometry.

ABSTRACT

Objective: PARP inhibitor therapy was intensively investigated in colorectal cancer (CRC). Therefore, our aim was to investigate the potential association of DNA repair protein PARP-1 with cancer stem cell CD133 and DNA cell cycle abnormalities in colorectal cancer patients and ulcerative colitis (UC) as a diagnostic tool for differential diagnosis, evaluation of tumor progression and prediction of patient's disease outcome to benefit in therapeutic response.

Materials and Methods: Thirty seven (20 colorectal cancer and 17 Ulcerative colitis) patients and ten tissue specimens of normal colon mucosa used as non-disease control group. Tissue specimens from all individuals were collected at surgery and examined for PARP-1, CD133 and DNA Cell cycle by flow cytometry.

Results: CD133 and PARP-1 were gradually increased from UC to CRC ($p<0.0001$) in CD133 and PARP-1 ($p=0.02$). DNA cell cycle abnormalities showed significant difference between CRC and UC groups only in G2/M ($p<0.0001$). CRC showed higher expression of CD133 in Stage III compared to stage I ($p=0.01$) but the difference in tumor site was recorded between transverse colon and rectum in S phase ($p=0.04$) and G0/1 ($p=0.016$) and between transverse and Lt Colon in G0/1 ($p=0.016$). Multiple regressions for PARP-1, CD133 and G2/M showed higher prediction for CRC progression.

Conclusion: PARP-1, CD133 and G2/M could be considered as additional biomarkers to increase the diagnostic potential in CRC patients, in predicting tumor development and monitoring therapeutic response.

INTRODUCTION:

Ulcerative colitis (UC) is a chronic inflammatory bowel disease with an increased risk of developing colorectal cancer (CRC) [1]. Chronic inflammation in UC is considered to cause colonic epithelial injury, repair and regeneration, or UC-CRC development [2]. CRC is one of the most common diagnosed malignancies in the world [3,4]. Although

Corresponding Author
Dr. Hoda Mohamed El-Emshaty
Gastroenterology Surgical Center - Mansoura University, Mansoura, Egypt
Tel.:002(050)2275524
e-mail: elemshaty_h@yahoo.com,

patients with early stage CRC generally have a good prognosis, patients with metastatic disease to lymph nodes (stage III) or distant organs (stage IV) have marked increased recurrence and mortality rates. Therefore, approximately 50% of patients present with advanced disease requiring multimodal therapy including surgery and chemotherapy [5].

Several studies have identified subpopulations of colorectal cancer cells that are more resistance to cancer treatment such as chemotherapeutic and radiation [4,6]. These cells are often referred to as cancer stem cells (CSCs) or tumor-initiating cells, and successful treatment is dependent on the elimination of these highly resistant subpopulations [7]. CSCs were first identified by John Dick in acute myeloid leukemia in the late 1990s. CSCs are cancer cells with features associated with normal stem cells, specifically the ability to give rise to all cell types found in a particular cancer sample. CSCs are therefore tumorigenic (tumor-forming), may be in contrast to other nontumorigenic cancer cells [8].

CSCs were detected in many tumor types using specific markers, such as c-kit, CD133, CD90, CD44, CD326 and OV6, and it has been proposed that they are the origin of circulating tumor cells [9,10]. CD133+ population is enriched in many tumor tissues including CRC [11]. CD133 (also called Prominin-1) is believed to be associated with tumorigenicity and progression of the disease. The up-regulation of CD133 in colorectal cancer correlates strongly with poor prognosis and synchronous liver metastasis [12], although the precise role and function of CD133 is unknown.

Colorectal cancer is one of the most common tumor entities which are causally linked to DNA repair defects and inflammatory bowel disease (IBD). The role of DNA repair protein poly(ADP-ribose) polymerase-1 (PARP-1) in CRC and tissue microarray analysis revealed PARP-1 over expression in human CRC, correlating with disease progression [13]. PARP-1 is a nuclear enzyme belonging to the DNA damage surveillance network and a founding member of the PARP superfamily [14]. Following DNA strand breaks generated by genotoxic stress such as chemotherapy, PARP catalyze transfer of ADP-ribose polymers to downstream substrates that include numerous DNA repair enzymes, facilitating DNA repair [15]. Consequently combining the standard chemotherapy agents with drugs that inhibit the DNA repair mechanism such as PARP inhibitor will theoretically create synthetic lethality [16]. Therefore, this study was conducted to correlate DNA repair protein PARP-1 and CD133 cancer stem cell with DNA cell cycle abnormalities in colorectal cancer patents and ulcerative colitis.

SUBJECTS AND METHODS

The present study involved thirty seven (17 men and 20 women, mean age 45.73±15.74 years) patients admitted at Gastroenterology surgical center, Mansoura university, Egypt. Tissue specimens of all cases were collected at surgery; one part fixed in neutral buffered formalin, embedded in paraffin and processed for histopathological diagnosis and the other part was stored at -70ºC until used. All cases were grouped according to the pathological diagnosis into 17 (mean age 38.47±13.7 yrs) cases with UC and 20 (mean age 51.9±14.96 yrs) cases with CRC. Also, ten tissue specimens of normal colon mucosa (4 men and 6 women; mean age 42.6±15.4 yrs) were used as non disease control group. A written informed consent to use the samples and clinical data for research purposes was obtained from all patients before starting the study protocol according to the ethical guidelines of Helsinki Declaration (World Medical Association, 2014). The study protocol was approved by the institutional review board of the Gastroenterology Surgical Center, Mansoura University, Egypt.

Blood samples were collected from colorectal patients and examined for tumor markers CEA (BIOTECH, Cairo, Egypt) and CA19-9 (Enzyme immunoassay test kit, Catalogue No. TM E-4500, LDN Labor Diagnostika Nord GmbH Co. KG, Germany). Fresh tissue specimens were processed for flow cytometric analysis of CD133, PARP-1 and DNA cell cycle. DNA cell cycle abnormalities was detected using propidium iodide (Sigma Aldrich Co., USA) and flow cytometric analysis of cancer stem cell marker CD133 (TMP4, eBioscience Inc., Alfymetrix Company, www.eBioscience) and biomarker for DNA repair protein PARP-1 [BD Pharmingen™ PARP antibodies, Clone 4C10-5 (Cat No. 556494), Clone 7D3-6 (Cat No. 55493), and Clone C2-10 (Cat No. 556362)] was also performed according to the manufacturer's protocols using fluorescent labeled antibodies.

Statistical analysis

Statistical analysis was performed using SPSS 17.0 (version 17, Sydney, NSW, Australia). Data were expressed in continuous variables as mean±SD and categorical variables as frequencies and percentages. The significance in continuous variables was calculated in Kruskal Wallis and Mann-Whitney U test. The association between variables was analyzed using Spearman correlation's coefficient and linear regression analysis was used for predictions. Differences between variables were considered significant at $p<0.05$ [17].

RESULTS

Demographic and clinicopathological characteristics of all patients were listed in **Table** (**1**). Thirty seven (17 men and 20 women; mean age 45.73±15.74 years) patients admitted at Gastroenterology Surgical center were histopathologically categorized into: 17(45.9%) patients diagnosed as UC and 20 (54.1%) patients diagnosed as CRC. Also, ten tissue specimens of normal colon mucosa (4 men and 6 women; mean age of 42.6±15.4 years) were included as non disease control group.

Clinicopathological characteristics and Flow Cytometric analysis of CD133, PARP-1 and DNA cell cycle in all study groups were listed in (**Table 2**). There is no significant difference as regard to gender or HCV viral infection but in age significant difference was recorded between CRC and UC (51.9±14.96 vs 38.47±13.7, p=0.012). Serum level of CEA and CA19-9 was recorded in all CRC patients and in only 9 UC showing significant difference in CA19-9 between UC and CRC (p=0.027). DNA cell cycle in CRC was differed compared to control group as regard to G0/1 (56.7±11.5 vs 81.3±0.8, p<0.0001), S phase (22.03±17.4 vs 0.88±0.19, p=0.001) and G2/M (11.87±5.7 vs 0.23±0.08, p<0.0001). Also, highly significant difference (p<0.0001) was detected between UC and control groups as regard to G0/1 (59.2±16.3 vs 81.3±0.8), S phase (21.26±10.93 vs 0.88±0.19) and G2/M (4.79±4.17 vs 0.23±0.08). Cell cycle abnormalities between CRC and UC was detected only in G2/M (11.87±5.7 vs 4.79±4.17, p<0.0001).

Flow cytometric analysis of CD133 and PARP-1 (**table 2**) showed significant difference (p<0.0001) between CRC and control group in CD133 (54.4±7.28 vs 18.96±2.1) and PARP-1 (45.9±15.9 vs 21.53±5.52). UC and ND groups showed significant difference in CD133 only (38.5±10.9 vs 18.96±2.1, p<0.0001). CRC compared to UC showed CD133 (54.4±7.28 vs 38.5±10.9, p<0.0001) and PARP-1 (45.9±15.9 vs 31.4±22.9, p=0.02). Incidence of CD133, PARP-1 and DNA cell cycle in UC was recorded according to the severity of the disease **(table (3)**. Severe and moderate activity of UC differed significantly in CD133 (p=0.006), PARP-1 (p=0.044) and G2/M (p=0.017). Also, severe and mild active UC showed significant difference in PARP-1 and G2/M (p=0.01). However, no significant difference was detected between moderate

and mild active UC patients. In CRC patients, PARP-1, CD133 and DNA cell cycle abnormalities was recorded with different stages and in different location of CRC **(table 3**). Significant difference was recorded between stage I and III only in CD133 (p=0.021) but as regard to tumor site, transverse colon showed significant difference with rectum in S phase (p=0.04) and G0/1 (p=0.016). Also significant difference was recorded between transverse colon and Lt colon in G0/1 (p=0.016).

Significant correlation between CD133 with PARP-1 and G2/M in disease group patients was illustrated in **figure (1)**. In UC patients, PARP-1 and CD133 showed positive correlation with G2/M (rho=0.489, p=0.046 and rho=0.553, p=0.021 respectively). Also, PARP-1 was correlated significantly with CD133 (rho=0.525, p=0.03) and S phase (rho=0.601, p=0.011) but negative correlation was recorded with Sub G1 (rho=-0.656, p=0.004). In CRC patients, significant positive correlation was recorded between PARP-1 and G2/M (rho=0.486, p=0.03) but negative correlation was recorded between S phase and Sub G1 (rho=-0.598, p=0.005) and G0/1(rho=-0.642, p=0.002). Multiple regression analysis showed significant prediction for PARP1, CD133 and G2/M in CRC development as independent predictors (**Table 4**).

DISCUSSION

Colorectal cancer (CRC) is a major cause of cancer related mortality in the world [18]. The most important currently available markers in CRC that provide prognostic or predictive information are serum markers such as CEA and CA19-9 [19]**.** Our results showed elevated serum level of CEA in UC and CRC patients compared to control group but no significant difference between UC and CRC patients as recorded by **Mohamed et al.** [20]. **Basbug et al.** [21] found that mean values of CA19-9 were elevated in CRC than controls concluding that CA19-9 should be used for the study of this kind of malignancy, and this is in agreement with our results showed higher expression of CA19-9 in CRC compared to UC patients (p=0.027).

Recently, a number of studies have demonstrated that cancer stem cells (CSCs) present in CRC tissues, are responsible for resistant to conventional therapies [18] and multiple cell surface and enzymatic markers have been characterized to identify CSCs within a heterogeneous tumor [22]. CD133 is currently considered the most robust surface marker for colorectal CSCs [23]. However, the clinical and prognostic significance of CD133 expression in CRC remains unclear, it is therefore of great interest to study the role of CD133 in CRC and UC patients and its correlation with the clinicopathological characteristics and DNA cell cycle analysis.

CD133expression was associated with the degree of tumor differentiation and tumor size suggesting that CSCs might play a critical role in the progression of colorectal cancer [23]. Currently, CD133 showed significant elevation in CRC patients compared to UC and control group (p<0.0001) and the difference in CD133 expression was recorded with tumor stage (p=0.021) but no significant difference as regard to tumor site. Also, UC patients showed comparable elevation of CD133 expression (p<0.0001) to control group and significant difference was noted with higher activity of UC (p=0.006). Based on above reports and our data, CD133may play a significant role in CRC development and disease progression.

CRC is one of the most common tumor entities which are causally linked to DNA repair defects and inflammatory bowel disease (IBD). Poly(ADP-ribose) polymerase-1 (PARP1) is a DNA repair protein and part of the genome maintenance network [13]. Here, we found that PARP-1is abundantly expressed in human CRC (p<0.0001) compared with non disease control and UC patients (p=0.02), PARP-1 expression was

progressively increased with tumor stage correlating with disease progression consistent with **Dörsam et al.** [13]. **Sulzyc-Bielicka et al.** [24] reported a significant association of PARP-1 expression with the site of CRC and Astler-Cooller Stage. PARP-1 expression in UC patients showed also significant difference compared to control group *(P<0.0001)* and CRC (*P=0.02)* and higher expression of PARP-1 was differed between severe and moderate (p=0.04) and between severe and mild active patients (p=0.01)**;** therefore, PARP-1 may play an important role in carcinogenesis of CRC [25, 26].

It was well established that the loss of key cell cycle check points is a hall mark of cancer cells, which lead to abnormal proliferation and facilitates oncogenic transformation [27]. Analysis of DNA cell cycle in current study showed significant difference between CRC and control group as regard to G0/1 (p<0.0001), S phase (p=0.001) and G2/M (p<0.0001). Also, highly significant difference (p<0.0001) was detected between UC and control group as regard to G0/1, S phase and G2/M. CRC and UC groups showed significant difference only in G2/M (p<0.0001).

CD133+ CSCs have shown resistance to chemotherapy and radiotherapy due to their slower cell cycle, lower proliferation, and higher expression of DNA repair and anti-apoptotic genes [28, 29]. Currently, CD133 was associated with higher expression of PARP-1 and G2/M in CRC suggesting the clinical significance of PARP inhibitor for CRC patients [30] and regression analysis of PARP-1, CD133 and G2/M showed significant prediction in CRC development. Thus, PARP inhibition with chemotherapy was suggested to impair the ability of cancer initiating cells to drive CRC maintenance and recurrence and the assessment of tumor samples may improve selection of CRC patients for PARP inhibitor therapy [24].

CONCLUSION

Our results suggest that PARP-1 could be used simultaneously with CD133 and G2/M as independent predictors for monitoring tumor progression and therapeutic response in colorectal cancer patients and also to improve selection of CRC patients to PARP inhibitor therapy.

ACKNOWLEDGEMENT

We would like to express our gratitude to members from the Gastroenterology Center in Mansoura for their collaboration during the study.

REFERENCES:

1. Yasuda H., Tanaka K., Okita Y., Araki T., Saigusa S., Toiyama Y., Yokoe T., Yoshiyama S., Kawamoto A., Inoue Y., Miki C., Kusunoki M., (2011); "CD133,OCT4, and NANOG in ulcerative colitis-associated colorectal cancer". ONCOLOGY LETTERS 2: 1065-1071.
2. Bartsch H., and Nair J., (2006); " Chronic inflammation and oxidative stress in the genesis and perpetuation of cancer: role of lipid peroxidation, DNA damage and repair". Langenbecks Arch Surg., 391: 499-510.
3. Sahlbberg SH., Spiegelberg D., Glimelius B., Stenerlöw B., Nestor M., (2014); "Evaluation of cancer stem cell markers CD133, CD44, CD24: Association with AKT isoforms and radiation resistance in colon cancer cells". PLOS ONE, 9(4)/e94621 (www.plosone.org).
4. Abdullah LN., Chow EK., (2013); "Mechanisms of chemoresistance in cancer stem cells". Clin Transl Med., 2:3.
5. SEER Cancer Statistics Review, 1975-2014. Available at https://Seer. Cancer. gov/archive/csr/1975-2014.

6. Rich JN., (2007); "Cancer stem cells in radiation resistance". Cancer Res., 67:8980-8984.

7. Baumann M., Krause M., Thames H., Trott K., Zips D., (2009); "Cancer stem cells and radiotherapy". Int J Radiat Biol., 85:391-402.

8. Moharil RB., Dive A., Khandekar S., Bodhade A.**,** (2017); "Cancer stem cells: An insight". J Oral Maxillofac Pathol., 21:463.

9. Rountree CB., Ding W., He L., Stiles B., (2009); "Expression of CD133-expression liver cancer stem cells in liver-specific phosphates and tensin homology deleted on chromosome 10-deleted mice". Stem cells, 27:290-299. [PMID:190008348 DOI: 10.1634/stem cells. 2008-0332].

10. Yoon SK., (2012); "The biology of cancer stem cells and its clinical implication in hepatocellular carcinoma". Gut Liver, 6:29-40 [PMID:22375168 DOI: 10.5009/gn.2012.6.1.29].

11. O'Brien CA., Pollett A., Gallinger S., Dick JE., (2009) "A human colon cancer cell capable of initiating tumor growth in immunodeficient mice". Nature, 445:106-110.

12. Horst D., Scheel SK., Liebmann S., Neumann J., Maatz S., (2009); "The cancer stem cell marker CD133 has high prognostic impact but unknown functional relevance for the metastasis of human colon cancer". J. Pathol., 219:427-434.

13. Dörsam B., Seiwert N., Foersch S., Stroh S., Nagel G., Begaliew D., Diehl E., Kraus A., Mckeague M., Minneker V., Roukes V., ReiBig S., Waisman A., Moehler M., Stier A., Mangerich A., Dantzer F., Kaina B., Fahrer J., (2018); "PARP-1 protects against colorectal tumor induction, but promotes inflammation-driven colorectal tumor progression". PNAS., 115(17)ıE4061-E4070.

14. Gupte R., Liu Z., Kraus WL., (2017); " PARPs and ADP-ribosylation: Recent advances linking molecular functions to biological outcomes". Genes Dev., 31:10-126.

15. Farres J., Martin-Caballero J., Martinez C., (2013); "Parp-2 is required to maintain hematopoiesis following sublethal-irradiation in mice". Blood, 122:44–54.

16. Venere M., Hamerlik P., Wu Q., (2013); "Therapeutic targeting of constitutive PARP activation compromises stem cell phenotype and survival of glioblastoma-initiating cells". Cell Death Diff., 21:258–269.

17. Lvesque R., (2007) "programming and Data management: a guide for SPSS and SAS users, 4th edn, SPSS Inc., Chicago III.

18. Wang K., Xu J., Zhang J., Huang J., (2012); "Prognostic role of CD133 expression in colorectal cancer: a meta-analysis". BMC Cancer, 12:573.

19. Vukobrat-Bijedic Z., Husic-Selimovic A., Bijedic N., Mujkic A., Sofic A., Gogov B., Mehmedovic A., Bjelogrlic I., Glavas S., Djuran A., (2014); "Sensitivity of SymptomatologyVersus Diagnostic Procedures and Concentration of CEA and CA19–9 in the Early Detection of Colorectal Cancer". Acta Informatica Medica, 22(2): 89–93.

20. Mohamed FS., Elkady MM., Mohamed EF., Hussein BE., Elsaied WSH., (2015); "Study of Glutathione-S-Transferase, Cancer Embryonic Antigen and Carbohydrate Antigen 19-9 in Ulcerative Colitis and Colorectal Cancer". Basic Science of Medicine, 4(4): 58-66.

21. Basbug M., Arikanoglu Z., Bulbuller N., Cetinkaya Z., Aygen E., Akbulut S., Satici O., (2011); " Prognostic

value ofpreoperative CEA and CA 19-9 levels in patients with colorectal cancer". Hepatogastroenterology, 58(106):400-405.

22. Desai A., YAN Y., Gerson SL., (2019); "Concise Reviews: Cancer Stem Cell targeted Therapies: Toward Clinical Success". STEM CELLS TRANSLATIONAL MEDICINE, 8:75–81. www.StemCellsTM.com.
23. Kazama S., Kishikawa J., Kiyomatsu T., Kawai K., Nozawa H., Ishihara S., Watanab T., (2017); "Expression of the stem cell marker CD133 is related to tumor development in colorectal carcinogenesis". Asian Journal of Surgery, http://dx.doi.org/10.1016/j.asjsur.2016.12.002.
24. Sulzyc-Bielicka V., Domagala P., Hybiak J., Majewicz-Broda A., Safranow K., Domgala W., (2012); "Colorectal cancers differ in respect 0f PARP-1 protein expression". POL J PATHOL., 2: 87-92
25. Nosho K., Yamamoto H., Mikami M., (2006); "Overexpression of poly(ADP-ribose) polymerase-1 (PARP-1) in the early stage of colorectal carcinogenesis". Eur J Cancer, 42: 2374-2381.
26. Stern MC., Conti DV., Siegmund KD., (2007); "DNA repair singlenucleotide polymorphisms in colorectal cancer and their role as modifiers of the effect of cigarette smoking and alcohol in the Singapore Chinese Health Study". Cancer Epidemiol Biomarkers Prev., 16: 2363-2372.
27. Lin W., Zheng L., Zhuang Q., Shen A., Liu L., Chen Y., Sferra T.J., Peng J.(2013); “Spica prunellae extract inhibits the proliferation of human colon carcinoma cells via the regulation of the cell cycle”, Oncology Letters, 6(4): 1123-1127.
28. Zinzi L., Contino M., Cantore M., Capparelli E., Leopoldo M., Colabufo NA., (2014); "ABC transporters in CSCs membrames as a novel target for treating tumor relapse". Front pharmacol., 5:163.
29. Morrison R., Schleicher SM., Sun Y., Niermann KJ., Kim S., Spratt DE., Chung CH., Lu B. (2011); "Targeting the mechanisms of resistance to chemotherapy and radiotherapy with the cancer stem cell hypothesis". J Oncol., 2011:941876.
30. JARRAR A., Lotti F., Devecchio J., Ferrandon S., Gantt G., Mace A., Karagkounis G., Oirloff M., Venere M., Hitomi M., Lathia J., Rich JN., KaladyMF., (2019); "Poly(ADP-Ribose) Polymerase Inhibition Sensitizes Colorectal Cancer-Initiating Cells to Chemotherapy". STEM CELLS, 37:42–53 www.StemCells.com.

Table (1) Clinicopathological characteristics of the study group patients

Variable	
• No	37
• Gender (M/F)	17/20
• Age (Yrs)	45.73±15.74
• Positive HCV infection	10/37 (27%)
• Pathological Diagnosis	
Ulcerative colitis	17/37(45.9%)
Colorectal cancer	20/37(54.1%)
• Severity of ulcerative colitis	
Mild	6/17(35.3%)
Moderate	5/17(29.4%)
Severe	6/17(35.3)
• Site of colorectal cancer	
Ascending colon	2/20(10.0%)
Descending colon	1/20(5.0%)
Transverse colon	2/20(10.0%)
Sigmoid colon	5/20(25.0%)
Rectosigmoid	4/20(20%)
Caecum	2/20(10.0%)
Ileocolic	1/20(5.0%)
Rectum	3/20(15.0%)
• Grouping according to site of CRC	
Rt colon	5/20(25.0%)
Lt colon	10/20(50.0%)
Transverse colon	2/20(10.0%)
Rectum	3/20(15.0%)
• Tumor staging of CRC	
Stage I	7/20(35.0%)
Stage II	6/20(30.0%)
Stage III	7/20(35.0%)
• Tumor grade	
Grade I	3/20(15.0%)
Grade II	17/20(85.0%)
Grade III	0/20(0.0%)

Data are expressed as n(%) or mean±SD

Biochemistry letters, 14(12) 2019, Pages 156- 166

Table (2) Clinicopathological characteristics and Flow Cytometric analysis of CD133, PARP-1 and DNA cell cycle in study group patients and non-disease control group

Variable	Non-disease control	Ulcerative Colitis	Colorectal cancer	P value
Age (yrs) Gender (M/F) +ve HCV infection	42.6±15.39 (43) 4/6 -	38.47±13.7 (35.0)* 9/8 4/17(23.5%)	51.9±14.96 (55.0)* 8/12 6/20(30%)	0.039 0.117 0.16
CA19-19 CEA	- -	7.5 ± 2.75* 2.59±0.2	52.9 ± 10.55* 7.69±8.89	0.027 0.52
PARP-1 CD133 SubG1 G0/1 S phase G2/M	21.53±5.52(23.0) º 18.96±2.1(18.7)• º 10.18±0.53(10.3) 81.3±0.8(81.4) •º 0.88±0.19(0.8) •º 0.23±0.08(0.25) •º	31.4±22.9(17.8)* 38.5±10.9(34.7)*• 13.56±5.19(12.4) 59.2±16.3(63.2) • 21.26±10.93(18.1)• 4.79±4.17(3.4)*•	45.9±15.9(47.2)* º 54.4±7.28(53.6)* º 17.65±12.5(14.5) 56.7±11.5(55.9) º 22.03±17.4(21.85) º 11.87±5.7(11.85)*°	0.002 <0.0001 0.166 <0.0001 0.0001 <0.0001

*Significant difference was detected between UC and CRC in mean age (p=0.012), CA19-9 (p=0.027), PARP-1 (p=0.02), CD133 and G2/M (p<0.0001)

•Significant difference (p<0.0001) was detected between non disease control and UC in CD133, G0/1, S, G2/M

°Significant difference was detected between non disease control and CRC in PARP-1, CD133, G0/1, G2/M (p<0.0001) and S phase (p=0.001)

Mean difference was calculated using Kruskal-wallis H and Mann-whitney-U

Table (3) PARP-1, CD133 and DNA cell cycle abnormalities with the clinicopathological characteristics of UC and CRC

	No.	PARP-1	CD133	Sub G1	G0/1	S	G2/M
Tumor Stage	**Stage I (n=7)**	42.08±16.82	48.5±5.44*	23.32±15.3	53.42±5.64	19.11±15.4	11.7±7.72
	Stage II (n=6)	44.7±10.3	51.8±6.07	15.22±12.2	59.9±16.9	25.58±20.1	14.3±6.89
	Stage III (n=7)	49.45±20.7	60.16±8.5*	16.5±10.7	58.75±9.6	23.98±18.77	9.8±3.3
	P value	7.6	**0.021**	0.507	0.59	0.815	0.414
Tumor Sites	**Rt colon (n=5)**	47.0±17.76	58.18±9.01	13.26±5.4	57.98±9.89	27.34±14.28	9.66±2.05
	Lt Colon (n=10)	49.73±15.77	52.54±9.98	21.86±13.7	54.51±6.7*	17.26±15.5	13.9±6.45
	Transverse colon (n=2)	47.75±9.12	53.4±1.69	21.05±24.67	77.7±21.35*#	2.8±2.96 #	15.8±5.37
	Rectum (n=3)	30.3±13.08	55.13±4.24	8.7±1.08	47.996±5.7#	41.86±15.7#	6.2±2.8
	P value	0.399	0.712	0.349	**0.016**	**0.04**	0.11
Activity of UC	**Severe**	57.42±20.67*#	47.6±4.56*	10.81±2.6	63.46±8.1	24.4±11.2	9.23±4.024*#
	Moderate	17.6±1.75*	30.5±3.84*	14.28±3.61	52.04±27.65	16.8±6.1	2.6±1.45*
	Mild	16.93±1.53#	36.18±13.34	15.72±7.33	60.93±9.7	21.85±13.97	2.18±1.15#
	P value	**0.021**	**0.036**	0.273	0.919	0.578	**0.013**
	Severe vs Mod	**0.044**	**0.006**	0.144	0.715	0.273	**0.017**
	Severe vs Mild	**0.010**	0.20	0.2	0.749	0.522	**0.01**
	Mod vs Mild	0.522	0.361	1.0	0.927	0.855	0.584

Table (4) Regression analysis of CD133, PARP-1 and DNA cell cycle abnormalities as independent predictors for colorectal cancer development

Variable	R	R2	*P value*	B
PARP-1	0.357	0.128	**0.03**	0.009
CD133	0.644	0.415	**<0.0001**	0.026
Sub G1	0.208	0.043	0.216	0.011
G0/1	0.092	0.008	0.589	-.003
S	0.026	0.001	0.877	0.001
G2/M	0.58	0.337	**<0.0001**	0.048

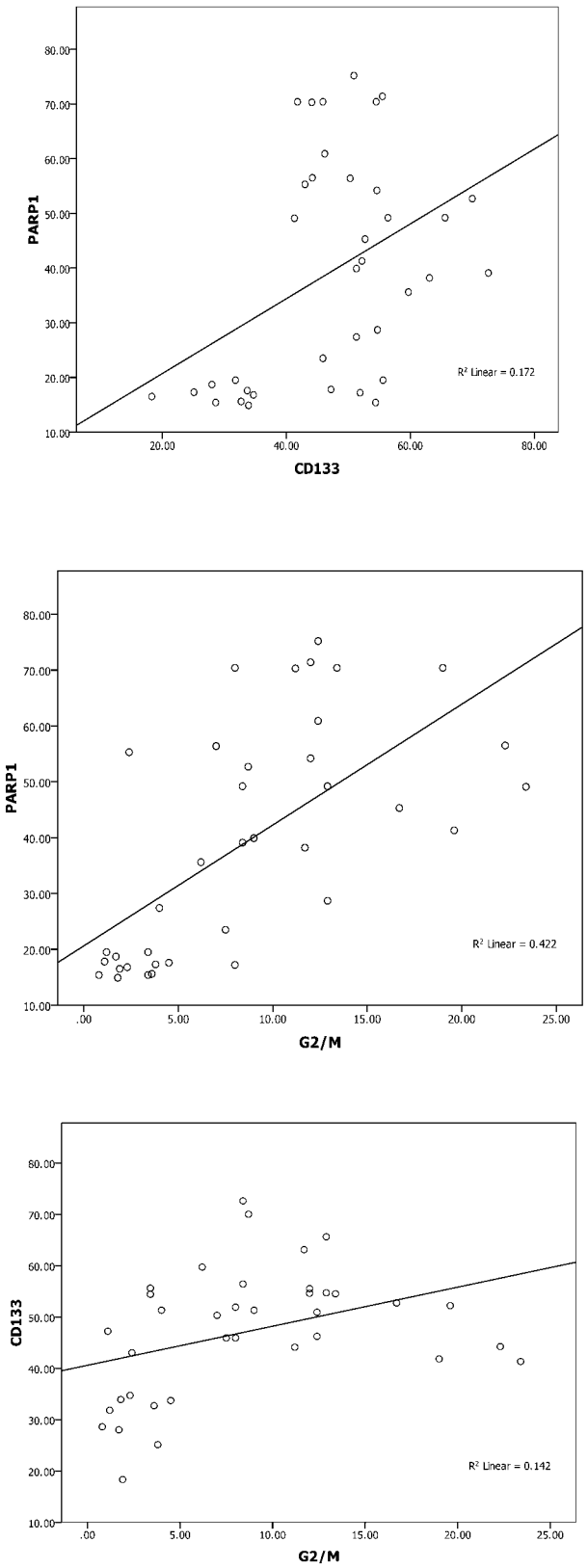

Figure 1: Correlation between CD133, PARP-1 and G2/M in colorectal cancer development