Colorectal cancer in patients from Uganda: A histopathological study.

DN Dijxhoorn[1,4], A Boutall[1], CJ Mulder[4], R Ssebuufu[3], A Mall[1], S Kalungi[3], C Baigrie[2], PA Goldberg[1]

[1]Colorectal Surgery Unit and the Surgical Laboratory, Department of Surgery, University of Cape Town and Groote Schuur Hospital, Cape Town, South Africa

[2]Path Care Laboratory Cape Town, South Africa

[3]Department of Pathology, Makerere University College of Health Sciences, Kampala, Uganda.

[4]Gastroenterology and Hepatology, VU University Medical Center, Amsterdam, The Netherlands

Correspondence to: Prof. P.A. Goldberg**,** Email: Paul.Goldberg@uct.ac.za

Background: ***Approximately one million people are diagnosed with colorectal cancer (CRC) globally each year. Uganda is a developing country with an estimated total population of 32.9 million. The incidence of colorectal cancer is reported to be low. The clinical impression is that it occurs predominantly in young people who live in rural areas This study was aimed at determining the demographic and morphological features of a cohort of patients withc olorectal cancer from in Mulago and Mbarara hospitals in Uganda.***

Methods: ***Histopathologicalspecimens of 81 Ugandan patientswith CRC (2006-2010) wereretrospectivelyreviewed. Demographic data was retrievedfrom the medical records. Tumourswereexaminedtodeterminehistological features suggestive of mismatchrepair gene mutations (accordingto the revisedBethesdaguidelinesformicrosatelliteinstabilitytesting).***

Results: ***The medianage of thosewith CRC was 55 years (range 20-89) at diagnosis. 30.6% and 6.9% of the patientswere< 50 and< 30 yearsoldrespectively. 94,9% of the tumourswerelocated in the left side of the colon. Mucinous adenocarcinoma was significantly more common in patientsunder the age of 50. Based on the histologicalanddemographic features, 22/81 (27.3%) patients met at leastone of the criteria of the revisedBethesdaGuidelinesfor MSI testing.***

Conclusions: ***Histologicalanddemographic features suggestive of the geneticpathwaytypicallyseen in HNPCC wereidentified in 27.3% of patients. These features appeartobequite different frompublished data from first worldcountries. The limited availability of flexiblecolonoscopy, maycontributeto the greatpreponderance of left-sidedneoplasms (especiallylocated in the rectum) andunderdiagnoses of right sidedtumours.***

Keywords: Colorectal cancer, HNPCC, Endoscopy, Uganda, Histopathology, Lynch syndrome.

Introduction

Approximately one million people are diagnosed with colorectal cancer (CRC) worldwide each year. In 2008, CRC was the third most prevalent cancer in men and the second in women[1]. The incidence of CRC varies considerably throughout the world with Japan, Australia/New Zealand and Western Europe having the highest rate and Asia and Africa the lowest[1,2,3]. South Africa is reported to have a relatively low incidence of CRC, which is now the fourth commonest cancer in men, and the third most common in women in the country[4].

HNPCC, also referred to as Lynch Syndrome, is the most common cause of hereditary CRC. It is an autosomal dominant condition caused by a germ line mutation of the mismatch repair genes (MMR-genes). It is associated with early onset CRC and the underlying mutations have been identified in the MLH1, MSH2, MSH6, PMS1 and PMS2 genes[7].MLH1 and MLH2 account for almost 90% of all disease-causing mutations[8]. As a result of defects in MMR-genes, mutations occur frequently in microsatellites[9] and can ultimately lead to the occurrence of colonic and extra colonic tumours. Extracolonic sites include the endometrium, stomach, small intestine, hepatobiliary tract, ovary, renal pelvis and the ureter[10,11]. Microsatellite instability (MSI) occurs in cancers with MMR gene deficiency.MSI is detected in about 15% of all colorectal cancers. About 3% are associated with HNPCC syndrome and 12% are caused by sporadic, acquired hypermethylation of the promoter of the

MLH1 gene[12]. Cancers that are MMR gene deficient have certain morphological features which are suggestive of MMR gene deficiency.

Previous studies have reported that a disproportionately large number of young (<50 years) black South Africans are presenting with CRC[13]. They also presented more often with features suggestive for HNPCC[8,13]. The Northern Cape Province of South Africa is known to have a very large family with an *h*MLH1 gene mutation. Long term research done at Groote Schuur Hospital on CRC in this Province, reported that the inherited form may contribute a greater proportion of the overall CRC burden than it would in a high incidence area[14]. The Northern Province is a sparsely populated region with a very low incidence of carcinoma. Wentink et al[18] established the age standardize rate (ASR) to be 4.2 per 100.000 world standard population per year. This is 10% of the rate reported in the first world countries.10.5% of these colorectal cancers are MMR-gene product deficient and this is approximately three times the reported rate in high incidence areas. Patients seem younger and the disease is more advanced at diagnosis[14].

Uganda is a developing country with an estimated total population of 32.9 million[15]. The incidence of colorectal cancer is reported to be low[1]. The clinical impression is that it occurs predominantly in young people who live in rural areas. Access to facilities for early diagnosis and treatment contribute to poor prognosis[16].The purpose of this study was to identify the demographic and morphologic features in patients with colorectal cancer in Uganda.

Patients and Methods

All patients diagnosed with colorectal cancer between 2006 and 2010, were identified from the records of the Departments of Pathology, Makerere University College of Health Sciences and Mbarara University Teaching Hospital. Archived paraffin blocks of pathological tissue, where available, were retrieved. Tissue section were cut and reviewed to confirm the presence of colorectal adenocarcinoma. 3-5 micrometer sections from 120 patients identified were placed on slides and heat fixed on at 60 ˚C in an incubator for 60 minutes. Clinical features of the 120 patients were retrieved from the medical records of the relevant hospitals. The microscope slides were sent to the University of Cape Town, General Surgery Research Laboratory, in Cape Town, South Africa.

Haematoxylin and Eosin staining was performed on one section of each case in Cape Town using standard methods. In brief, the sections were dewaxed 3 times in xylol for 3 minutes then rehydrated in graded alcohol. Sections were stained with Mayers Haematoxylin for 9 minutes, destained in Acid Alcohol for 10 seconds and then blued in Scott's tap water substitute for 2 minutes. They were counterstained in eosin for 2 minutes and dehydrated through graded alcohols to xylol. All slides were mounted in Entellan (Merck KGaA, Darmstadt, Germany). A single pathologist (CB) assessed all sections and documented the presence of adenocarcinoma. If a carcinoma was present, the following morphological features were documented: differentiation (well, moderate or poor), presence of mucinous and signet ring cells, nature of the edge as pushing or infiltrative and lymphocytic infiltration present or absent. Patients were analysed for sex (male vs. female), age (<50 years vs. ≥50 years) and site of tumour (left vs. right).

Demographic and pathological features were retrieved from the medical records of the relevant hospitals and histological features suggestive for HNPCC were reported, to determine which patients should be tested for microsatellite instability (MSI), based on the revised Bethesda Guidelines.

Statistical analysis was performed using*Statistica 10* software. Categorical data were compared using the two-sided Fisher's exact tests as appropriate. P-values < 0.05 were considered significant.

Ethical consideration

Anonymity of the subjects was assured as each subject was assigned a unique number in Uganda. Personal information was not available to the research group in South Africa. Ethics approval for the

study was obtained by the Health Sciences Faculty Research Ethics Committee of the University of Cape Town, project reference No 128/2010 and by the Research Ethics Committee, Mulago Hospital, Uganda.

Results

AQ total of 120 cases of adenocarcinoma of the colon or rectum were identified in Uganda and forwarded to the University of Cape Town. In 39 (32%) patients, no adenocarcinoma could be identified on the Haematoxylin and Eosin staining. These were excluded from further analysis, 81 cases were thus available for further analysis.

Patient demographics

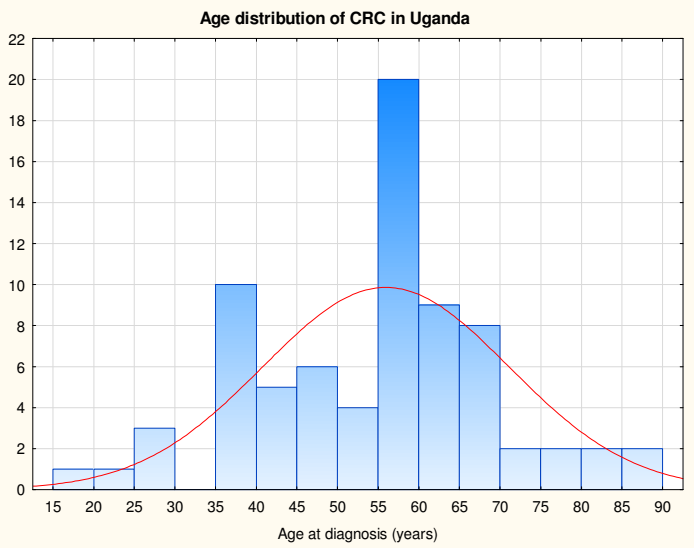

Figure 1. Age distribution CRC in Uganda

The ages were not available in 9 out of 81 patients diagnosed with adenocarcinoma. The median age at which colorectal cancer was diagnosed in the other 72 patients was 55 years (range 20-89). The age distribution is illustrated in Figure 1. Patients aged under 50 years were 22 (30.6%) while 5 (6.9%) were < 30 years. Fifty (69.4%) of the patients were aged 50 years and above.

The sex was not available in 8 out of 81 patients diagnosed with adenocarcinoma. Of the other 73 patients, 34 were male (46.6%) and 39 women (53.4%). There was no significant difference in age of diagnosis between female and male (p=0.4510).

Site

The site of the colorectal cancer could be identified by the pathology report in 39 cases. 2 tumours (5.1%) were located in the right side of the colon and 37 (94.9%) tumours were located in the left side of the colon. 32 of the 37 left-sided tumours were located in the rectum (86.5%). There was no significant difference in tumour site between males and females (p=1.0000), or younger and older patients (<50 vs. ≥50 years. p=0.5238).

Histological features

Histological features were reported by the pathologist in all 81 cases. Table 1 summarises the mucinous and signet ring cell carcinoma by age (<50 vs. ≥50). The age was not given in 1 patient with mucinous carcinoma. Mucinous adenocarcinoma was more common in younger compared to older patients (9.7% vs. 5.6%. p= 0.0276), though the presence of signet ring cells was similar (p=1.0000).

Grade

A total of 72 (88.9%) of cases were moderately differentiated and 9 (11.1%) were poorly differentiated. Younger patients were more likely to have a poorly differentiated adenocarcinoma than those diagnosed at an older age (8.3% vs. 4.2%. p=0.0198).

Table 1. Histological Type CRC by Age

Histological type	<50 years *n*(%)	≥ 50 years *n*(%)	Total *n*(%)	P value
Mucinous/signet ring cell carcinoma	7(58.3)	5(41.7)	12 (100)	< 0.05
Mucinous adenocarcinoma	7(63.6)	4(36.4)	11 (100)	> 0.05
Signet ring cell carcinoma	0 (0)	1 (100)	1 (100)	> 0.05

Table 2. Features suggestive of microsatellite instability (MSI)

Feature*	Count	N < 50 years	N ≥ 50 years	% of total CRC
Age < 50 years	22			**27.3**
MSI-H histology**		7 (58.3%)	5 (41.7%)	16
Mucinous/signet ring cell differentiation	13	8 (29.6%)		38.3
Presence of tumour infiltrating lymphocytes	31	0 (0%)	23 (70.4%)	1.2
Crohn's like lymphocytic reaction	1		1 (100%)	

*Features comprise the Revised Bethesda Guidelines, with the exception of criteria 2, 4 (concerning the presence of synchronous/metachronous or other HNPCC-related tumours), and 5 (concerning family history)

**Medullary growth patterns were not mentioned specifically in any of the pathology reports.

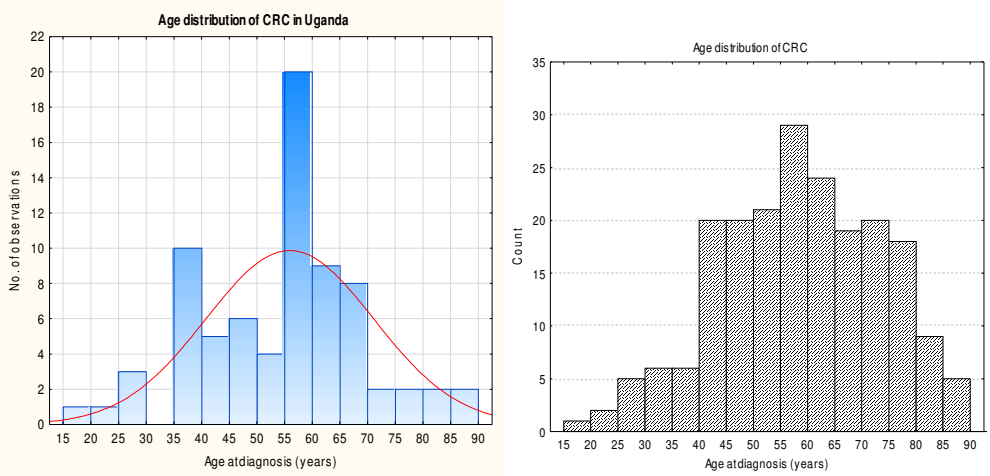

Figure 2. Age distribution CRC in Uganda (left) and The Northern Cape Province (right)

Edge

Regarding the edges, 79 (97.5%) of the tumours had an infiltrating edge and 2 (2.5%) tumours had a pushing edge. The patients with tumours with a pushing edge were 25 and 39 years old.

Revised Bethesda guidelines

Table 2 shows the frequency of histological and demographic features suggestive of microsatellite instability.22 patients were under the age of 50 years and 1 patient presented with MSI-H histology, but was not less than 60 years old. This means that 22/81 (27.3%) patients met at least one of the criteria of the revised Bethesda Guidelines for MSI testing (Appendix 1).

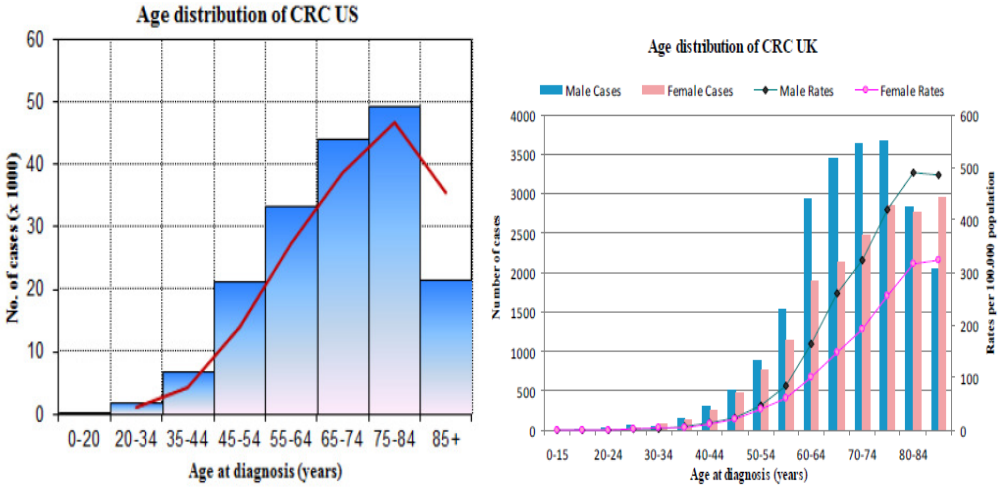

Figure 3. Age distribution CRC in the Western world: US (left) and UK (right)

Discussion

The aim of this study was to identify patients with demographic and histological features suggestive for inherited colorectal cancer. Comparing these data with data reported in the Northern Cape of South Africa, is extremely relevant to establish if the Northern Cape is unique or a model. The median age at which colorectal cancer was diagnosed in Uganda was 55 years and 22/72 (30,6%) were under the age of 50 years. This is almost equal with the median age of 59 years (range 14-100 and 16-90) reported in studies from both South Africa and The Northern Cape of South Africa[17,18]. Moreover, 27% (472/1731) and 26% (54/205) were under the age of 50 reported in respectively South Africa and the Northern Cape. This is different from statistics reported in the SEER programme (1975-2007) of the US (70 years) and the UK (73 years)[19.20]. The US reported that 17.4% of the patients were diagnosed under the age of 54 and the UK reported that 16% of the patients were diagnosed under the age of 60.The age distribution curve of Uganda, the Northern Cape, the US and the UK are illustrated in figure 2 and 3. Africa reported a lower median age and a higher percentage of patients under the age of 50, compared to developed countries. This could be due to other demographics (a younger population), a shorter life expectancy or may reflect to different tumour biology. Comparing these data with the Northern Cape Province will establish if the Northern Cape has an unique morphological pattern of CRC.

In our study, only 2 (5.1%) tumours were located in the right side of the colon. Of the 37 left sited tumours, 32 were located in the rectum. No difference in location of the tumour between older and younger patients was recognized in our study (p=0.5238). In the Northern Cape, 58 (31%) tumours were located in the right side and 132 (69%) tumours in the left side of the colon. Subjects who were diagnosed with CRC before the age of 50 years were more likely to have right- sided cancers than those who were diagnosed when they were older than 50 (48% vs. 25% p=0.007). Moreover, in both our study and the study done in the Northern Cape (p=0.55) no significant difference in location of the tumour was seen in men and women.

An important finding of our study is the fact that younger patients were more likely to have a poorly differentiated and mucinous adenocarcinoma. This is recently described in a case report, which presented a 27 years old man from Kampala with a mucinous adenocarcinoma.[21]Other studies in South Africa[17], Taiwan[6] and the US[22] also reported that younger patients with colorectal cancer had more poorly differentiated tumours and mucin production. This suggests that MSI-H type tumours are more prevalent in younger patients.

Based on the age and the presence of MSI-H histology in patients less than 60 years of age, 27.3% (22/81) of the patients met at least one of the criteria of the Revised Bethesda Guidelines. Unfortunately, no data is available about the family history and the presence of synchronous/metachronous colorectal, or other HNPCC-associated tumours. It is often problematic to obtain family history from affected individuals in developing countries due to factors such as language barriers, fears of isolation and being prejudiced against and overall patient's reluctance to follow-up after initial diagnosis. They neither have Colon Cancer Family Registries (C-CFR-S) nor standardized instruments and protocols for collecting data of colorectal cancers in Uganda. Moreover, the tumours are not screened for HNPCC. More patients could probably have been selected to test for MSI in this study (based on the Revised Bethesda guidelines), if more data were available. 36% of the patients from The Northern

Appendix 1

The Revised Bethesda Guidelines for testing colorectal tumours for microsatellite instability (MSI)
Tumors from individuals should be tested for MSI in the following situations:
1. Colorectal cancer diagnosed in a patient who is less than 50 years of age.
2. Presence of synchronous, metachronous colorectal, or other HNPCC associated tumours,* regardless of age.
3. Colorectal cancer with the MSI-H† histology‡ diagnosed in a patient who is less than 60 years of age. §
4. Colorectal cancer diagnosed in one or more first-degree relatives with an HNPCC-related tumour, with one of the cancers being diagnosed under age 50 years.
5. Colorectal cancer diagnosed in two or more first- or second-degree relatives with HNPCC-related tumours, regardless of age.
*Hereditary nonpolyposis colorectal cancer (HNPCC)-related tumours include colorectal, endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, and brain (usually glioblastoma as seen in Turcot syndrome) tumours, sebaceous gland adenomas and keratoacanthomas in Muir–Torre syndrome, and carcinoma of the small bowel *(48)*.
†MSI-H _ microsatellite instability–high in tumours refers to changes in two or more of the five National Cancer Institute-recommended panels of microsatellite markers.
‡Presence of tumour infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation or medullary growth pattern.
§There was no consensus among the Workshop participants on whether to include the age criteria in guideline 3 above; participants voted to keep less than 60 years of age in the guidelines.

Cape met at least one criterion of the Bethesda Guidelines.[18] This higher percentage could also be due to the fact that more data about the features of MSI was available.

Sigmoidoscopies are more common and available in these rural areas. Colonoscopies are only available in Kampala, the Capital city, with 3 units serving a population of about 33 million. In the county of Kampala, Kyadondo County, the age standardized rate was 7.5 and 9.0 per 100.000 in between 2002 and 2006, for respectively males and females. This is an increase compared to 1996 [23]. Moreover, there are few trained endoscopists. As a result, many right-sided tumours may go undiagnosed and most diagnosed cancers are rectal cancers. However, because of this limitation and the lack of received data of the tumour site, we cannot conclude that colon cancers generally arise in the rectum.

Interestingly, 33% of the patients were excluded, because there was no histological evidence of CRC on their slides. Instead, some cases were lymphomas, squamous cell carcinomas etc. This could be due to inappropriate biopsy submitting, wrong diagnosis at histology or error in filing the histology blocks in Uganda. In this first report about HNPCC in Uganda, 27.3% of the patients were identified with histological and demographic features similar to those in MSI tumours (based on the Revised Bethesda Guidelines). The limited availability of flexible endoscopy may contribute to the preponderance of left-sided neoplasms, especially located in the rectum. Patients under the age of 50 were more likely to be diagnosed with mucinous and poorly differentiated adenocarcinoma, which is suggestive for MSI-H tumours being more prevalent in younger patients.

The age distribution and morphological pattern of CRC in Uganda, is comparable with published data reported in the Northern Cape Province and differ with published data reported in the US and UK. Further immunohistochemical staining and MSI testing in the Northern Cape Province and Uganda is recommended to establish the prevalence of HNPCC. Additionally, it is recommended to ask patients, with suspected or diagnosed CRC, about any relatives who might have developed cancer.

Acknowledgements

We wish to thank Marilyn Tyler from Surgery laboratory and the Surgery laboratory and Pathcare Laboratory as organisations.

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. GLOBOCAN 2008, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10. 2010; Available at: http://globocan.iarc.fr/.
2. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005 Mar-Apr; 55(2):74-108.
3. Center MM, Jemal A, Ward E. International Trends in Colorectal Cancer Incidence Rates. Cancer Epidemiology Biomarkers & Prevention 2009 June 01;18(6):1688-1694.
4. Mqoqi N, Kellett P, Sitas F, Jula M (2004). Incidence of histologically diagnosed cancer in South Africa, 1998-1999. National Cancer Registry of South Africa, National Health Laboratory Service, Johannesburg.
5. Fearon, ER, Vogelstein, B. A genetic model for colorectal carcinogenesis. Cell 1990 Jun 1; 61(5):759–67.
6. Liang JT, Huang KC, Cheng AL, Jeng YM, Wu MS, Wang SM. Clinicopathological and molecular biological features of colorectal cancer in patients less than 40 years of age. British Journal of Surgery 2003;90(2):205-214.
7. Calvert PM, Frucht H. The Genetics of Colorectal Cancer. Annals of Internal Medicine 2002 October 01; 137(7):603-612.
8. Guttmacher AE, Collins FS, Lynch HT, de la Chapelle A. Hereditary Colorectal Cancer. N Engl J Med 2003 03/06;348(10):919-932.

9. Hsieh P, Yamane K. DNA mismatch repair: Molecular mechanism, cancer, and ageing. Mech Ageing Dev 2008 8;129(7-8):391-407.
10. Watson P, Lynch H. Extracolonic cancer in hereditary nonpolyposis colorectal cancer. Cancer 1993;71(3):677-685.
11. Watson P, Riley B. The tumor spectrum in the Lynch syndrome. Fam cancer 2005;4(3):245-248.
12. Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology. 2010 Jun;138(6):2073-2087
13. Cronjé L, Becker PJ, Paterson AC, Ramsay M. Hereditary non-polyposis colorectal cancer is predicted to contribute towards colorectal cancer in young South African blacks. S Afr J Sci 2009;105(1-2):68-72.
14. Vergouwe F, Boutall A, Goldberg PA, Stupard D, Algar U, Govender AD, et al. Mismatch repair gene mutations in colorectal cancer patients in a low incidence area. S Afr J Surg (in press) 2013.
15. TP3: Mid-year population estimates and projections for Uganda, 1992 – 2011. 2011; Available at: http://www.ubos.org/onlinefiles/uploads/ubos/pdf%20documents/TP32010.pdf.
16. Gondos A, Brenner H, Wabinga H, Parkin DM. Cancer survival in Kampala, Uganda. Br J Cancer 2005 05/09;92(9):1808-1812.
17. Cronje L, Paterson A, Becker P. Colorectal cancer in South Africa: A heritable cause suspected in many young black patients. S Afr Med J 2009;99(2):103-106.
18. Wentink MQ, Rakers M, Stupard DA, Algar U, Bradshaw D, Ramesar R, et al. Incidence and histological features of colorectal cancer in the Northern Cape Province of South Africa. SAJS 2010; 48(4):109-113.
19. Altekruse SF, Kosary CL, Krapcho M, Neyman N, Aminou R, Waldron W, et al. *SEER Cancer Statistics Review, 1975-2007*, National Cancer Institute. Bethesda, MD, 2010; Available at: http://seer.cancer.gov/csr/1975_2007/.
20. Barrett J, Jiwa M, Rose P, Hamilton W. Pathways to the diagnosis of colorectal cancer: an observational study in three UK cities. Family Practice February 2006 February 2006;23(1):15-19.
21. Tumwine LK, Kagimu M, Ocama P, Segamwenge I, Masiira-Mukasa N, Wamala D, et al. Atypical presentation of colon adenocarcinoma: a case report. J Med Case Rep. 2012; 6:58-1947-6-58.
22. O'Connell JB, Maggard MA, Liu JH, Etzioni DA, Livingston EH, Ko CY. Rates of Colon and Rectal Cancers Are Increasing in Young Adults. Am Surg 2003 10;69(10):866-872.
23. Parkin DM, Nambooze S, Wabwire-Mangen F, Wabinga HR. Changing cancer incidence in Kampala, Uganda, 1991-2006. International Journal of Cancer 2010; 126(5):1187-1195.