Abstract
Oesophageal cancer is the eighth commonest form of cancer world-wide with variable incidence rates across different geographical regions. Squamous cell type is the commonest histological type in developing countries. This study aimed to determine the pathological subtypes of oesophageal cancer and the probable roles of some markers in the metastatic potential of uncommon subtypes. The study Proposal was approved by the institutional review board. Clinical data and histopathology results of 55 resection specimens from oesophageal cancer cases were reviewed. Two uncommon tumours, a basaloid squamous carcinoma and a squamous cell carcinoma that invaded the stomach were analyzed by immuno-histochemistry for stemness and pathogenesis. All specimens received were stage T3N2 or T3N3 and had carbon pigment in the draining lymph nodes. The most common histological type was squamous cell carcinoma of different degrees of differentiation (74%). Adenocarcinoma was less common and was reported in 14.6% of the cases. The basaloid squamous carcinoma consisted of well differentiated squamous cells and basaloid spindle cells. Both the squamous and basaloid components were positive for P63. Basaloid cells were positive for CD44, weakly positive for OCT4 and negative for E-Cadherin. This part metastasised to regional lymph nodes. The oesophageal carcinoma that invaded the stomach was positive for CD44 but negative for E-Cadherin. The CD44 and E-Cadherin changes described in the basaloid squamous carcinoma were in the gastric component which metastasised to abdominal lymph nodes.

Conclusion: Squamous cell carcinoma of different degrees of differentiation is the commonest type of oesophageal cancer in Sudan with variations in male: female ratios. Over expression of CD44 and down regulation E-Cadherin are probably associated with aggressiveness and metastasis.

Keywords: Oesophageal cancer, squamous cell, pathogenesis, Sudan

Introduction
Cancer of the oesophagus is the eighth most common cancer worldwide with extreme geographical differences in incidence that is more extreme than any other cancer [1,2]. A high-risk area known as the “oesophageal cancer belt” extends from northern Iran all the way to north central China [2]. Squamous cell carcinoma with different degrees of differentiation is the most common in developing countries. On the other hand, the incidence of oesophageal adenocarcinoma has increased in the last 30 years particularly among white males in the U.S.A. and several European countries [3-7]. The true incidence of the disease in Sudan is not known with certainty because most of the published work was before endoscopy was introduced in the country and consequently the figures published are an underestimate of the true incidence [8].

The most comprehensive publication on cancer and its incidence rate was published from two centres in Sudan: Radiation and Isotope Center in Khartoum (RICK) located in the national capital Khartoum, Khartoum State, and the National Cancer Institute of the University of Gezira (NCI-UG) in Wadmadani,
capital of the Central State [9]. Of the all 26,652 new cancer cases registered between 2000 and 2006 in both sexes, the top five cancers were cancer of the breast 4652 (17.5%) followed by leukemia 2282 (8.6%), cancer of the oesophagus 1426 (5.4%), non-Hodgkin lymphoma 1336 (5%), and cancer of the cervix 1139 (4.3%) [9].

The aim of this article is to report on the frequency and histological types of oesophageal cancer in a single centre in Khartoum Sudan. The pathogenesis of some rare variants of oesophageal tumours is presented in the context of stem cells and adhesion molecules.

Materials and methods
The study Proposal was approved by the institutional review board. The histopathology and available clinical data of 55 cases of oesophageal cancer referred to El Hassan Centre for Histopathology in Khartoum were reviewed. Selected rare variants were analyzed by immuno-histochemistry to determine their pathogenesis. Pathogenesis studies aimed at determining the probable role of stem cells and adhesion molecules in the pathogenesis of these tumours. The factors studies included OCT-4, P63, CD44 (Thermo Scientific, UK), E-Cadherin, CD99, Ki67, and EMA (Dako, Copenhagen, Denmark). The instructions of the manufacturers were followed.

Results
The total number of oesophageal cancer cases reviewed was 55. All specimens received as a complete resection of the oesophagus were stage T3N2 or T3N3. One feature of almost all resected specimens was the presence of carbon pigment in the draining lymph nodes (Figure 1). The mean age was 48 years with a range from 14 to 80 years. The male to female ratio was 1.8:1. The most common histological type was squamous cell carcinoma of different degrees of differentiation (Table 1). Adenocarcinoma was less common and was reported in 14.6% of the cases.

The uncommon tumours seen recently are Basaloid squamous carcinoma and the so called blue cell tumours. The latter include Ewing family of tumours, carcinoids and lymphomas. Two uncommon oesophageal tumours and their pathogenesis are described in this article. A rare basaloid squamous cell carcinoma was seen in a 70 years old female who presented with dysphagia and loss of weight. The tumour was in the lower part of the oesophagus. As the name implies it consisted of squamous cells and basaloid spindle cells as shown in Figure 2. The squamous cell component was well differentiated, showed intercellular bridges and contained keratin. The basaloid cells were spindle shaped with dark nuclei and scanty cytoplasm. They showed a much higher proliferation index (ki67) than the squamous cells.

The basaloid cells were different from basal cells in normal epidermis and oesophagus. For one thing they were EMA

Table 1. Histological types of 55 oesophageal carcinomas. The squamous cell carcinomas were graded according to WHO grading system (Seen in 2012 and 2013).

| Histological type      | No. of cases | Frequency (%) |
|------------------------|--------------|---------------|
| Well differentiated SCC | 11           | 20.0          |
| Moderately differentiated SCC | 11        | 20.0          |
| Poorly differentiated SCC | 19         | 34.6          |
| Adenocarcinoma         | 08           | 14.6          |
| Others                 | 06           | 10.8          |

Figure 1. Squamous cell carcinoma of the lower oesophagus infiltrating the whole thickness of the oesophagus. Note the carbon pigment in the large lymph nodes.

Figure 2. (a) Well differentiated squamous cell component with an epithelial pearl. (b) Basaloid component composed of spindle shaped cell with dark nuclei.
negative while basal cells were positive (Figures 2 and 3).

Both the squamous and basaloid components were positive for P63 but the basaloid cells showed a stronger reaction (Figure 4). Basaloid cells were positive for CD44, weekly positive for OCT4 and negative for E-Cadherin (Figure 5). The opposite was true for the squamous cells. The tumour was negative for chromogranin and CD56 thus distinguishing it from small cell carcinoma of the oesophagus.

The second case was a poorly differentiated squamous cell carcinoma (SCC) of the gastro-oesophageal junction that invaded the proximal stomach. In a small area the cells showed intercellular bridges and no glands. The tumor in the oesophagus.
Phagus was spreading directly into the stomach without any interruption or a gap in the tumor. Thus it was not a metastasis from the oesophagus to the stomach but a direct invasion. The main part of the tumour was in the stomach not in the oesophagus which is unusual. It metastasized to the abdominal and thoracic lymph nodes. The nodes contained carbon pigment. The appearance of the tumour in the H&E section and the expression of the tumour markers are shown in Figure 6. The tumour expressed P63 which is essential for its proliferation. This started in the hyperplastic epithelium proximal to the tumour and involved all tumour cells. Even at an early stage the tumour expressed P63 which is essential for its proliferation and stemness. It expressed CD44 which is important for adhesion of tumour cells to each other and with the stroma. CD44 also presents growth factors and is anti-apoptotic. We and others showed that when the tumour metastasized there was focal or total loss of expression of E-Cadherin and up-regulation of CD44. It seems that E-Cadherin is important in localizing the tumour on its own or through optimum expression of CD44 [10].

Figure 6. Poorly differentiated esophageal SCC. (A) Hyperplastic stratified squamous epithelium away from tumor. (B) Hyperplastic epithelium as it approaches the tumor. (C) CD63 in stratified epithelium near tumor. Invasive tumor beneath is CD 63 positive. (D) Poorly differentiated SCC. (E) E-Cadherin was negative in the invasive tumor. (F) CD44 is strongly positive.

Discussion
Carcinoma of the oesophagus is among the top ten malignant tumours worldwide including developing countries. The most common histological type is a squamous cell carcinoma with different degrees of differentiation. While squamous cell carcinoma is still a major form in developing countries adenocarcinoma is on the increase. The dominant form in USA and some European countries is adenocarcinoma. In Sudan all recent publications confirm that squamous cell carcinoma is dominant but adenocarcinoma is on the increase [1-6,9,11].

The sex ratio of oesophageal cancer in Sudan varies in the different parts of the country. In some areas the disease was reported more frequently among males while in other areas females were more frequently affected. Reasons for these differences remain to be elucidated.

In a total of ninety patients reported in a major research centre in Khartoum, 78 were squamous cell and 12 were adenocarcinoma [11]. Males were more affected than females [M:F ratio of 1.8:1]. There may be a bias in this sex ratio since the material was from a research centre engaged in studying the role of snuff dipping and smoking on cancer. Snuff dipping and smoking are more common in males. The authors used matched controls and snuff dipping and smoking were found to be significantly associated with oesophageal cancer. We agree that this may be the case in some patients. We have shown here that about 80% of surgically removed oesophageal tumours, the local lymph nodes contain carbon deposits in both men and women. Men who smoke are by far more than females. Females are subjected to smoke inhalation in a local practice in Sudan in which married females use what is known as Dukhan. They expose their skin to smoke from Acacia seyal (Figure 7). Dukhan is not practiced by men.

Figure 7. Shows a young lady using Dukhan. She is intimately surrounded by smoke from Acacia seyal wood burning in a hole in the floor.

In this article we reported on the pathology and the pathogenesis of two unusual carcinomas of the oesophagus: a Bas-aloid squamous cell carcinoma and a poorly differentiated squamous cell carcinoma. We used the stem cell markers P63, OCT4 and CD44 in both cases. We also used the marker of adhesion molecule E-Cadherin because it is responsible for adhesion of cancer cells to each other and to the connective tissue matrix. Down regulation of E-Cadherin upregulates CD44 leading to metastasis [10].

We studied stem cells in the unusual oesophageal cancers by using the important stem cell markers. Cancer cells arise from a single progenitor stem cell that accumulates multiple genetic and epigenetic mutations over a long period of time. Because of their long life span the stem cells are more likely to accumulate these mutations than the normal short-lived cells. Further more they resist chemotherapy and are responsible for recurrences.
CD44 is a ubiquitous multi-structural and multi-functional cell surface adhesion molecule involved in cell-cell and cell-matrix interactions. The CD44 is involved in cell motility and migration. It has several isoforms but it was shown that CD44std over expression is associated with aggressiveness and metastasis of tumours [10]. Various isoforms of CD44 were studied in basal cell carcinoma, a tumour that almost never metastasizes. It was shown that CD44v6 or 10 are not linked to the metastatic potential of tumours originating from keratinocytes [12]. It was suggested that the very low expression of CD44std may be one of the factors which block the formation of metastases in basal cell carcinoma of the skin [12]. CD44std is also a marker of oesophageal squamous cell carcinoma and its expression is associated with metastasis and a poor prognosis. We therefore used CD44std (standard) in the present study. Metastasis happens in cases where another molecule E-Cadherin that maintains adhesion of tumour cells as well, is lost. Its loss up-regulates CD44 and the tumour becomes invasive and capable of metastasizing.

Basaloid squamous carcinoma of the oesophagus is extremely rare. As the name implies it consists of squamous cells and basaloid spindle cells [13]. They are different from basal cells in normal epidermis and oesophagus. For one thing they are EMA negative while basal cells are positive. The tumor has also been described in the hypo-pharynx, base of the tongue and larynx.

In the present case the basaloid cells unlike the squamous component were negative for EMA. They were strongly positive for P63 and weakly for OCT4. They were also strongly positive for P63 and weakly for OCT4. The tumour was aggressive and the patient died before therapy was initiated. Basaloid squamous cell carcinoma should be distinguished from small cell carcinoma of the oesophagus which, unlike basaloid squamous carcinoma is positive for chromogranin and CD56. These markers were negative in our case.

We made a comparison between basal cell carcinoma of the skin and basaloid SCC of the oesophagus. The reason we made this comparison is because there is a belief that both tumours arise from similar basal cells. As far as we are aware we are the first that addressed this issue. We showed that the two tumours are different and arise from different “basal cells”. Basal cell carcinoma is CD44 negative. CD44 is important for adhesion of tumour cells to each other and to the matrix. The question was how do cells in basal cell carcinoma adhere if they are CD44 negative? In 1971 Riedbord et al., showed that Desmosomes, the ultra-structural analogues of intercellular bridges, were seen under the electron microscope in basal cell carcinoma and were responsible the adhesions between the tumour cells [14].

The poorly differentiated squamous cell carcinoma of the oesophagus that extended into the stomach with the main tumour in the gastric part is unusual. One case of a synchronous oesophageal and gastric squamous cell carcinoma was described in a Korean patient in 2012 [15].

Our case was positive for the stem cell markers tested. The major part of the tumour was positive for E-Cadherin except in one area. This was associated with up-regulation of CD44 leading to invasion and metastasis starting at the site of the up-regulated CD44.

Conclusion
Squamous cell carcinoma of different degrees of differentiation is the commonest type of oesophageal cancer in Sudan with variations in male: female ratios. Stem cell markers, particularly over expression of CD44 and down regulation E-Cadherin are associated with the more aggressive and metastatic tumours.

Competing interests
The authors declare that they have no competing interests.

Authors’ contribution

| Authors’ contributions | AME | WME | LAME | GDAE | AMM | EAA | MEI | EAGK |
|------------------------|-----|-----|------|------|-----|-----|-----|------|
| Research concept and design | ✓ |   |     |     |     |     |     |      |
| Collection and/or assembly of data |     | ✓ | ✓ |     |     |     |     |      |
| Data analysis and interpretation | ✓ | ✓ |     |     |     |     |     |      |
| Writing the article | ✓ | ✓ |     |     |     |     |     |      |
| Critical revision of the article | ✓ | ✓ |     |     |     |     |     |      |
| Final approval of article |     | ✓ | ✓ |     |     |     |     |      |
| Statistical analysis | ✓ | ✓ |     |     |     |     |     |      |

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References
1. Parkin DM, Pisani P and Ferlay J. Estimates of the worldwide incidence of 25 major cancers in 1990. Int J Cancer. 1999; 80:827-41. | Article | PubMed
2. Stewart BW and Kleihues P. World cancer report. Lyon (France):International Agency for Research on Cancer. 2003. | Website
3. Wabinga HR, Parkin DM, Wabwire-Mangen F and Mugerwa JW. Cancer in Kampala, Uganda, in 1989-91: changes in incidence in the era of AIDS. Int J Cancer. 1993; 54:26-36. | Article | PubMed
4. Vizcaino AP, Parkin DM and Skinner ME. Risk factors associated with oesophageal cancer in Bulawayo, Zimbabwe. Br J Cancer. 1995; 72:769-73. | Article | PubMed Abstract | PubMed Full Text
5. Sitas F, Madhoo J and Wessie J. Incidence of histologically diagnosed cancer in South Africa, 1993–1995. National Cancer Registry of South Africa, South African Institute for Medical Research. 1998.
6. Segal I. The gastro-oesophageal reflux disease complex in sub-Saharan Africa. Eur J Cancer Prev. 2001; 10:209-12. | Article | PubMed
7. Daoud EH, El Hassan AM, Zak F and Zakova N. Aspects of malignant
disease in the Sudan in Proceedings of a Conference on Cancer in Africa. Nairobi Kenya published. Eds. Peter Clifford, C. Allen Linsell and Geoffrey L. Timms. East African Publishing House, Nairobi. 1968; 7.

8. Mohamed ME, Hassan Ammar, Abdel hadi Hala, Elsadig MG, Adam Dalal, ElTamouni K, Hamid Rania, Elias Hiba, Abdullah M, Abdelkarim Z, Elwali N and Mohamed Sulma. The burden and pattern of cancer in the Sudan. British Journal of Medicine & Medical Research. 2014; 4:1231-1243. | Article

9. Bollschweiler E, Wolfgarten E, Gutschow C and Holscher AH. Demographic variations in the rising incidence of esophageal adenocarcinoma in white males. Cancer. 2001; 92:549-55. | Article | PubMed

10. Le Bras GF, Allison GL, Richards NF, Ansari SS, Washington MK and Andl CD. CD44 upregulation in E-cadherin-negative esophageal cancers results in cell invasion. PLoS One. 2011; 6:e27063. | Article | PubMed Abstract | PubMed Full Text

11. Ahmed ME. Tobacco consumption among oesophageal cancer patients in the Sudan. UICC World Cancer Congress. Washington DC, USA. 2006. | Website

12. Baum HP, Schmid T, Schock G and Reichrath J. Expression of CD44 isoforms in basal cell carcinomas. Br J Dermatol. 1996; 134:465-8. | Article | PubMed

13. Zhang XH, Sun GQ, Zhou XJ, Guo HF and Zhang TH. Basaloid squamous carcinoma of esophagus: a clinicopathological, immunohistochemical and electron microscopic study of sixteen cases. World J Gastroenterol. 1998; 4:497-403. | Article | PubMed

14. Reidbord HE, Wechsler HL and Fisher ER. Ultrastructural study of basal cell carcinoma and its variants with comments on histogenesis. Arch Dermatol. 1971; 104:132-40. | Article | PubMed

15. Lim SM, Jung ES, Shin SK, Chung HS, Kim HI, Kim do W and Cho BC. A case of synchronous squamous cell carcinoma in the esophagus and stomach. Gut Liver. 2012; 6:118-21. | Article | PubMed Abstract | PubMed Full Text

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ElHassan AM, Ealamin WM, Elhassan LAM, Awad Elkareem GDa, Musa AM, Abd Alla EA, Ibrahim ME and Khalil EAG. Oesophageal cancer: Pathological subtypes and markers of metastasis. J Histol Histopathol. 2015; 2:11. http://dx.doi.org/10.7243/2055-091X-2-11