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Highlights

- Giant Cell Carcinoma
- Fluke Infection in Ruminants
- Biochemical Study of Liver
- Aberrant Cytoplasmic Localization

Discovering Thoughts, Inventing Future

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Giant Cell Carcinoma of Lung with Aberrant Cytoplasmic Localization of P63 Protein

By Cyrus Parsa, Robert Orlando, Parham Naghdechi & Navneet K Singh

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Abstract- Giant Cell Carcinoma is a rare, aggressive type of lung malignancy and very few cases have been extensively studied and reported to date. Because of its rare occurrence, this neoplasm is often not included in the classifications of usual lung cancers. It is considered by some as a variant of undifferentiated large cell carcinoma. Giant cells have, however, been found to occur in different types of lung cancers and their presence in any histologic type is associated with a significantly worse outcome. We present a non-smoking female who was diagnosed with lung adenocarcinoma composed of many giant cells showing cytoplasmic expression of p63 protein. Admitting imaging studies revealed metastases to liver, lymph nodes and the pelvic region. Due to the rarity of this cancer, the diagnosis as well as therapeutic and prognostic features of giant cell carcinoma is often overlooked.

Keywords: giant cell carcinoma, aberrant p63, lung carcinoma.

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Giant Cell Carcinoma of Lung with Aberrant Cytoplasmic Localization of P63 Protein

Cyrus Parsa [α], Robert Orlando [σ], Parham Naghdechi [ρ] & Navneet K Singh [ω]

Abstract- Giant Cell Carcinoma is a rare, aggressive type of lung malignancy and very few cases have been extensively studied and reported to date. Because of its rare occurrence, this neoplasm is often not included in the classifications of usual lung cancers. It is considered by some as a variant of undifferentiated large cell carcinoma. Giant cells have, however, been found to occur in different types of lung cancers and their presence in any histologic type is associated with a significantly worse outcome. We present a non-smoking female who was diagnosed with lung adenocarcinoma composed of many giant cells showing cytoplasmic expression of p63 protein. Admitting imaging studies revealed metastases to liver, lymph nodes and the pelvic region. Due to the rarity of this cancer, the diagnosis as well as therapeutic and prognostic features of giant cell carcinoma is often overlooked. This neoplasm has been shown to have dismal response to chemotherapy when compared to other non-small cell lung carcinomas. Cytoplasmic localization of p63, only rarely described, is also associated with poor patient survival.

Keywords: giant cell carcinoma, aberrant p63, lung carcinoma.

I. Case Presentation

A 59 year-old non-smoking female presented to the Emergency Department with worsening fever of one day duration reaching up°F to 102 associated with hemoptysis, mild shortness of breath and hematochezia. She complained of upper abdominal and epigastric pain, which was worse with coughing, dull, burning and radiating to the back. She also complained of nausea but denied vomiting, diarrhea, and melena.

Physical examination revealed a well-developed, well-nourished female in no acute distress. The patient's vital signs were as follows: temperature, 99°Fahrenheit, pulse of 103/minute, blood pressure of 110/79 mmHg, respiratory rate of 18/minute and O_2 saturation of 97% on room air. On auscultation of the chest, there were diminished breath sounds throughout the left lung field. Right lung was clear to auscultation. There were no other significant abnormal findings on further physical examination.

Significant laboratory findings included leukocytosis of 15.0 x $10^3/\mu L$ with band neutrophils of 31%, hemoglobin of 9.3 g/dL and albumin of 1.5 g/dL. The latter finding was later attributed to a colonos-copyically diagnosed inflammatory bowel disease (IBD). Chest X-ray showed a consolidation at the left lung base and an infiltrate in the left mid-lung.

The clinical impression on admission was, "post-obstructive pneumonia likely secondary to a lung mass and anemia of chronic disease."

Upon admission, the patient received 2 units of packed red blood cells and was started on antibiotics for suspected pneumonia and on metronidazole for her colitis, which started to improve her hematochezia.

High resolution CT of the chest with contrast showed a large left pneumothorax and a left lower lobe cavitary lesion with fluid level and surrounding infiltration (figure 1) "of uncertain etiology, possibly carcinoma or infectious process such as tuberculosis". A subsequent abdominal CT revealed multiple lesions in the liver and a complex lesion in the left pelvis measuring 3.4 x 3.6 cm, which was considered to represent a necrotic lymph node or left adnexal mass. There was diffuse thickening of the wall of the distal descending and sigmoid colon, suggestive of IBD.

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Figure 1 : CT of the chest (with contrast) showed prominent cavitary lesion in the left lower lobe of lung, possibly a neoplasm or granulomatous disease

Core needle biopsies of the lung lesion showed irregular cords and sheets of loosely cohesive malignant neoplastic cells with markedly pleomorphic bizarre nuclei, prominent nucleoli, and abundant faintly eosinophilic cytoplasm (figures 2 and 3). The scant supporting stroma was sprinkled with moderate numbers of mononuclear inflammatory cells accompanied by occasional eosinophils. There were extensive areas of tumor necrosis.

The neoplastic cells showed an immunohistochemical staining pattern characteristic of primary lung adenocarcinomas, i.e. positive for CEA(p), CK7 (figure 4), TTF-1 (figure 5), and focally for napsin-A (figure 6). There was nonspecific weakly positive staining for Hepar-1 and focally weak positive staining for S-100. The neoplastic cells stained negative for CK-5/6, CK 20, GCDFP - 15, GPC - 3, ALK - 1, B - HCG, chromogranin, PAX-8, AFP, and p63 (nuclear). The latter, essential in the differentiation of stratified squamous epithelia, is a common nuclear marker used in the diagnosis of squamous cell carcinomas. There was however aberrant strong p63 cytoplasmic expression by the tumor cells (figure 7) of our patient.

The immunehistochemical pattern and the light microscopic features described supported the diagnosis of poorly differentiated adenocarcinoma, large cell (pleomorphic giant cell) type. Molecular analysis of EGFR mutation was negative and FISH analysis of ALK gene showed no evidence of gene re-arrangement. However, 20% of the cells showed 1 or more additional fusion signals for ALK DNA sequence located at the 2p; likely representing an aneuploidy population with extra copies of chromosome 2/2p ALK region.

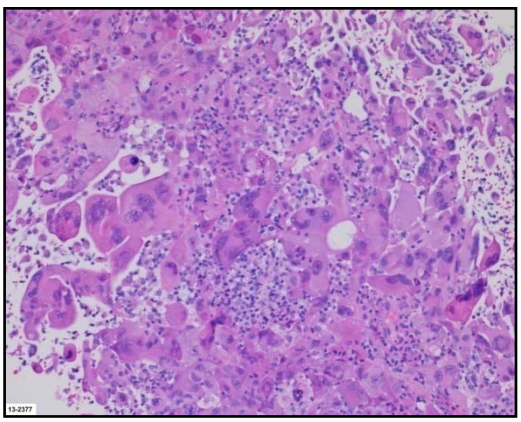

Figure 3 : Higher power of the lesion shows many malignant neoplastic giant cells

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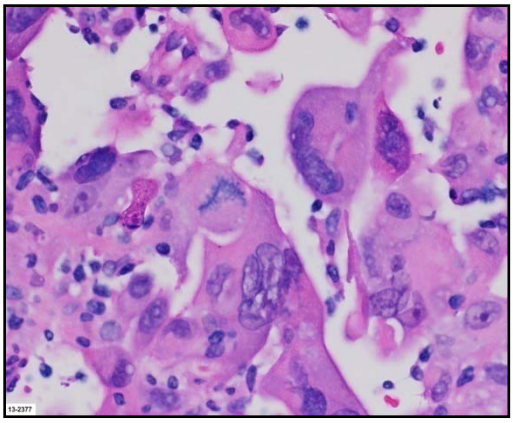

Figure 4 : Immunohistochemical stain for cytokeratin-7 (CK-7) shows characteristic cytoplasmic staining

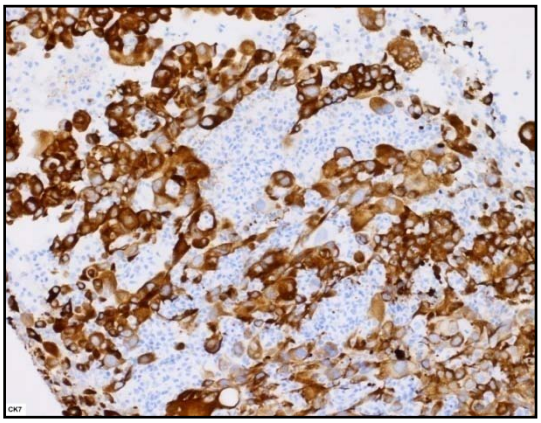

Figure 5 : Thyroid transcription factor-1 (TTF-1): positive nuclear staining, a sensitive marker for primary adenocarcinomas of lung

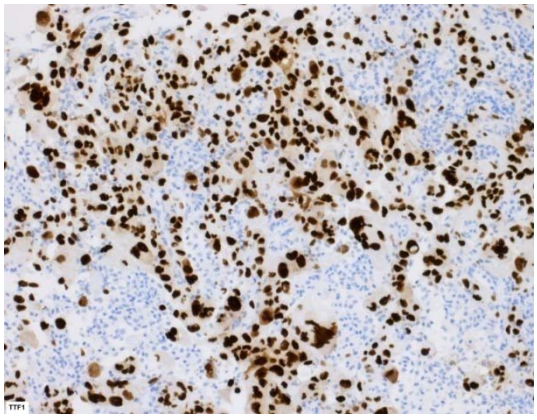

Figure 6 : Napsin A: focal positive cytoplasmic staining, a specific marker for primary adenocarcinomas of lung

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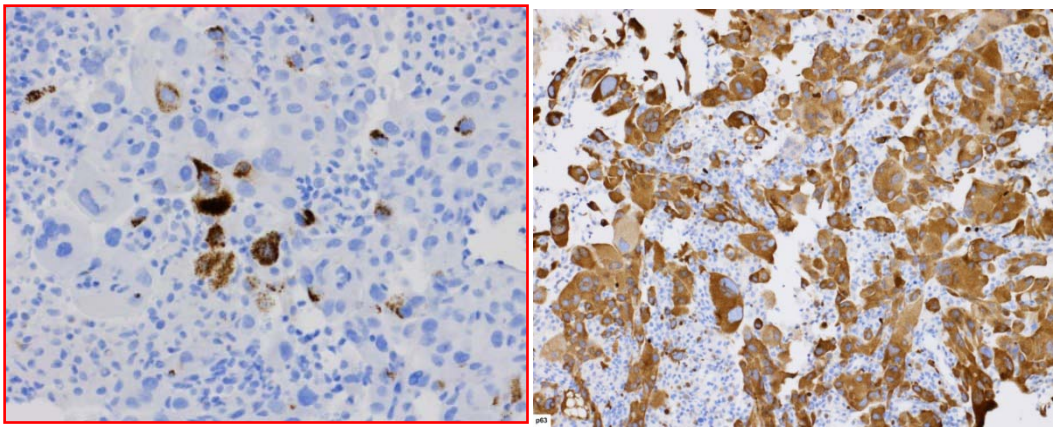

Figure 7 : Aberrant cytoplasmic staining of the neoplastic cells by p63

Due to the advanced stage of her metastatic lung cancer involving the liver, lymph nodes and pelvic area, oncology recommendation offered only supportive and palliative follow-up chemotherapy on an outpatient basis. However, before discharge, the patient expressed interest in hospice, although she would still follow up with oncology regarding the prognosis of her metastatic carcinoma. The patient was treated with IV Albumin during the course of her hospitalization for hypoalbuminemia and was started on mesalamine for her colitis before being discharged on prednisone.

II. Discussion

Large cell carcinomas are peripherally located tumors, which are defined as poorly differentiated carcinomas of the lung composed of larger malignant neoplastic cells without histomorphologic evidence of squamous, glandular, or neuroendocrine differentiation[1]. These tumors usually consist of sheets of large malignant cells, often with associated necrosis. Four variants of undifferentiated large cell carcinoma have been described: giant cell carcinoma, lymphoepithelioma-like carcinoma, large cell neuroendocrine carcinoma and non-small cell carcinoma with neuroendocrine features[9].

The term giant cell carcinoma is restricted to tumors in which multinucleated giant cells (greater than 40 microns) make up at least 10 percent of the neoplastic population[1]. The tumor cells alternate with mononuclear forms in a solid fashion, usually with a heavy neutrophilic infiltration and peripheral leukocytosis. This tumor comprises 1-5% of all lung cancers[4]. Most tumors are quite extensive at diagnosis. The presence of tumor giant cells in any histologic type is significantly associated with a worse outcome.

Giant tumor cells have been found to occur in different types of primary lung cancers as well as primary sarcomas, pleural mesotheliomas, pulmonary choriocarcinomas and metastatic tumors. Giant cells can also be found in many lung tumors after irradiation. Immunohistochemical staining profile and clinical history are helpful in these differential diagnostic considerations. Other helpful features, cited by some authors, include the presence of neutrophils and large pleomorphic phagocytic cells engulfing the neutrophils[4].

There has been mixed opinions in the sliterature as to whether giant cell carcinoma is an entity unto itself or a dedifferentiated adenocarcinoma. Addis et al., suggested that giant cell carcinoma arises by a process of dedifferentiation with eventual loss of epithelial markers showing unique ultrastructural features that support a diagnosis of giant cell carcinoma as a separate entity [5].

Wang et al. described several features specific to giant cell carcinoma in their study. Giant cell carcinomas were characterized by concentric whorls of fine fibrils. There was an abundance of mitochondria recorded, which may represent the high degree of activity of giant tumor cells. The tumor cells in giant cell carcinoma lack the well developed Golgi apparatus as seen in usual types of adenocarcinomas. Also, they lack dense bodies, usually seen in brochioloalveolar carcinomas. The cytoplasmic fibrils, frequently associated with desmosomes in squamous cell carcinomas, are found in lesser quantity in giant cell carcinomas[6]. On the other hand, Attanoos et al.[2] suggested that giant cell carcinoma of the lung does not appear to be a distinct entity, instead, a morphological phenotype expressed by a heterogenous set of tumors. They suggested that the term *Giant Cell Carcinoma* should not be used for any carcinoma with an abundance of giant tumor cells, instead, terms such as "pleomorphic" or "anaplastic" describe tumors that have no specific differentiation pattern but display a variety of morphological features such as giant cell formation, clear cells or spindle cells.

Nevertheless, carcinomas of the lung with giant cells are characterized as having a more aggressive course compared to the common types of non-smoker associated lung carcinomas. Multiple ribosomes, poorly developed Golgi apparatus, and the poor aggregation

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of the endoplasmic reticulum are characteristics of the rapidly advancing nature of these tumors. These tumors characteristically present with early distant metastases, short survival times, and worse overall outcomes[4].

There have been several studies on the efficacy of surgical resection and chemotherapy for advanced pleomorphic carcinomas. Yuki et al.[8] followed patients with pulmonary pleomorphic carcinoma who had undergone early surgical resection. Patients without lymph node metastases, pN0, frequently manifested vascular invasion supporting the aggressive nature of the tumor. The five year overall survival rate and the disease free survival was 39.2% and 47.1% respectively. Patients with pN1/N2 disease had a significantly worse outcome than patients with pN0 disease. The recurrence within 6 months after resection was found in 50% of patient and the median survival time after initial relapse was 2.6 months. Hong et al.[7] reported on the clinical course of patients undergoing palliative chemotherapy with advanced pulmonary pleomorphic carcinoma. The median overall survival time from the initiation of the chemotherapy was eight months with the median follow-up of 26 months.

Lung adenocarcinomas with giant cell features are aggressive tumors which have not been found to be responsive to surgical resection or chemotherapy due to the early advance stage of these tumors at presentation. Our patient presented in an advanced stage with early metastasis to the liver, lymph nodes and the pelvic area and was thus not a suitable candidate for surgical resection and chemotherapy. Furthermore, aberrant cytoplasmic localization of p63, evident in our patient, was reported in one study to represent an unfavorable prognostic feature associated with poor patient survival in lung adenocarcinomas[10]. Additionally, absence of EGFR mutation and ALK gene re-arrangement precluded any attempt at targeted therapeutic regimens. There has been limited research on the pathogenesis of adenocarcinomas with giant cell features due to its rarity. Future studies should further investigate and evaluate the molecular pathogenesis of these tumors in order to gain insight regarding an approach to development of efficacious targeted treatment modalities.

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Pathological and Serum Biochemical Study of Liver Fluke Infection in Ruminants Slaughtered at ELFORA Export Abattoir, Bishoftu, Ethiopia

By Dinaol Belina Kitila & Yoseph Cherinet Megersa
Haramaya University, Ethiopia

Abstract- A cross sectional study was conducted from September 2013 to July 2014 on a total of 284 ruminants (77 cattle, 99 sheep and 108 goats) to assess pathological and biochemical changes on liver infected with fluke at ELFORA export abattoir in Bishoftu, Ethiopia. Liver and blood samples of the same animals were collected using systematic random sampling. Gross and histopathological lesions and serum biochemical alterations were assessed. On the basis of gross pathological examination study animals were grouped into three: group-A (78.87%) showed no fasciola *spp*. and no visible gross lesion (taken as control groups), group-B (12.32%) confirmed with fasciola and fasciola indicative lesion and, group-C (8.80%) were co-infected (fasciola presence, fasciola indicative lesion and other lesions).

Keywords: ELFORA-abattoir, serum biochemicals, liver, liver fluke, pathological changes, ruminant, bishoftu, ethiopia.

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Pathological and Serum Biochemical Study of Liver Fluke Infection in Ruminants Slaughtered at ELFORA Export Abattoir, Bishoftu, Ethiopia

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Abstract- A cross sectional study was conducted from September 2013 to July 2014 on a total of 284 ruminants (77 cattle, 99 sheep and 108 goats) to assess pathological and biochemical changes on liver infected with fluke at ELFORA export abattoir in Bishoftu, Ethiopia. Liver and blood samples of the same animals were collected using systematic random sampling. Gross and histopathological lesions and serum biochemical alterations were assessed. On the basis of gross pathological examination study animals were grouped into three: group-A (78.87%) showed no fasciola *spp.* and no visible gross lesion (taken as control groups), group-B (12.32%) confirmed with fasciola and fasciola indicative lesion and, group-C (8.80%) were co-infected (fasciola presence, fasciola indicative lesion and other lesions). The gross lesions in fasciola positive livers include firm, enlarged livers with tense capsule, haemorrhagic spots, multi focal nodules and enlarged hepatic lymph nodes. The bile ducts were thickened and distended with adult fluke especially in chronic cases. Frequently observed histologic lesions were, hepatic portal fibrosis with large amount of fibrin in the portal area, hepatocytes degeneration, fatty changes and periportal necrosis. Fibrous connective tissue of various amount with, fibroblasts and infiltration of mononuclear cells, in particular lymphocytes were common lesions in tracts migrated by parasites; biliary cirrhosis with fibrous connective tissue and with epithelial hyperplasia were also observed. Eosinophilic hepatitis, talengechtasis and hepatocytes degeneration and necrosis were lesions in the parenchyma. Activity of serum ALT and AST were higher in cases of acute parenchymal lesions like eosinophilic hepatitis and necrosis, while ALP was significantly elevated with fibrosis and cirrhosis. The findings of the present study indicated that serum biochemical changes were consistent with pathological lesions; hence serum biochemical analysis could be used with other tests in diagnosis of ruminant fasciolosis. However, additional studies would be needed to establish association between serum biochemical and pathological changes with ruminants' liver fluke infection.

Keywords: ELFORA-abattoir, serum biochemicals, liver, liver fluke, pathological changes, ruminant, bishoftu, ethiopia.

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I. Introduction

Liver fluke infection caused *Fasciola hepatica* and *F.gigantica* remains economically significant parasite of livestock and is emerging zoonotic infection. It causes morbidity and mortality in most mammalian species and by far important in sheep and cattle (Hodzic *et al.*, 2013). A study conducted by Keyyu *et al.* (2006), reported up to 100% liver condemnation rates in slaughter slabs in Iringa region in Tanzania in cattle. In Ethiopia the prevalence of fasciolosis is as high as 83.08% (Mulualem, 1998) in cattle, 62.7% (Zeleke *et al.,* 2013) in sheep and 17.2 % (Sirajudin *et al.,* 2012) in goats. The variation in climato-ecological conditions such as altitude, rainfall and temperature, and livestock management system influences the prevalence of fasciolosis together with survival and distribution of the parasites as well as their intermediate host (snails).

Clinical examination of the Fasciola infected animal showed pale visible mucous membrane or anemia (Radostits *et al.,* 2000). On post mortem fasciola infected liver is an irregular outline, and pale and firm. According to Talukder *et al.* (2010), gross pathology of chronic fasciolosis is characterized by reduced in size of the organ and thickened bile ducts. Several types of fibrosis such as post necrotic scarring, ischemic fibrosis and peribiliary fibrosis may also present (Steyl, 2009). In cattle calcification of bile ducts, enlargement of the gallbladder and aberrant migration of flukes are more common. Fluke eggs may sometimes stimulate a granuloma-like reaction with obliteration of the affected bile ducts. Histopathologically, infiltration of fibroblasts admixed with lymphocytes and few mononuclear cells in the area previously migrated by young flukes are appreciated. Mature flukes cause necrosis and ulceration of the epithelium and severe hyperplasia of the epithelial layer.

Acute fasciolosis is associated with immature flukes migrating through the liver parenchyma and create migratory tracts. In this, grossly the liver is enlarged and haemorrhagic with fibrinous to fibrous exudates on the capsular surface. Numerous haemorrhagic spots and focal necrosis are found on the cut surface of liver parenchyma. The migratory tracts from direct trauma of this parasite is grossly seen as dark acute haemorrhagic streaks of typical post necrotic

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scarring and granulation(Affroze *et al.*, 2013). Histopathologically migratory tracts created by immature flukes migrating through the liver parenchyma are seen as necrotic tracts (Steyl, 2009).

The lesions in the liver are only partially a result of mechanical action of liver fluke, because the injury of the liver can be induced by parasites excretory products, decomposed products of parasites, bile and hepatic tissue. Hence serum biochemical tests including serum liver enzymes are also helpful to assess the severity of hepatocellular injury and to monitor the progress of the disease in ruminants (Lee *et al.*, 2005). Serum biochemical analysis also used to evaluate the degree of cholestasis and synthesizing capacity of the liver (Hodzic *et al.*, 2013). Therefore, the objectives of this study were to assess pathological changes of the liver, gall bladder and hepatic lymph nodes; to evaluate serum biochemical changes associated with liver fluke infection, and to establish association between serum biochemical parameters and pathological changes of the ruminants liver.

II. Materials and Methods

a) *Study Area*

The study was conducted from September 2013 to July 2014 at ELFORA export abattoir. Currently the abattoir is one of the most facilitated modern export abattoirs in Ethiopia and is exporting meat of small ruminants though cattle are slaughtered for local market. During the study on average 400 and 500 sheep and goats respectively, were slaughtered at this abattoir per day. On the other hand, on average 70-85 cattle were slaughtered per week based on local market needs. The ruminant animals slaughtered at the abattoir were purchased from different zones of the country particularly Borana, Arsi, Bale, Gondar, Jimma and some parts of SNNP region. Therefore, animals were encountered different ecological areas and management conditions at their origin.

b) *Study Design and Study animals*

A cross-sectional study was conducted on systematically selected local breeds of apparently healthy cattle, sheep and goats destined for slaughter. The study animals were only males of different body condition and age groups, and they were transported to the abattoir by vehicles.

i. *Sample size determination*

The sample size was determined by the formula described by Thrusfield (2005), at 95% confidence level and 5% precision, and considering the previous combined prevalence of 12.37% ruminant fasciolosis at ELFORA export abattoir (Meskerem, 2006); accordingly, the total sample size would be 167. However, to maximize the precision the sample size was increased by 1.7 folds and a total of 284 ruminants (77 cattle, 99 sheep and 108 goats) were included.

ii. *Sampling Method and procedures*

Animals were included in the study using systematic random sampling method where only the first animal was chosen randomly. After such selection animals were grouped in to young and adult according to Gatenby (1991), Steele (1996) and Johnson (1998) and moderate and excellent body condition scores (Belina *et al.*, 2012). Then blood sample was taken during ante mortem and livers of the same animals were appropriately inspected for presence of fasciola *spp*. and gross liver pathology after slaughter. After slaughter animals were clustered in to three groups based on presence of liver fluke and gross liver lesions: group-A/control (no liver fluke and no visible gross lesion), group-B/only affected by fasciola (fasciola presence and fasciola indicative lesion) and, group-C/ co-infection (fasciola presence and fasciola indicative lesions together with other lesions). Livers showing absence of fasciola *spp*. and fasciola indicative lesion, but with evidence of non fasciola lesions were totally excluded from the study.

Liver with irregular outline, pale, firm and with bile duct distention and thickening, and calcified bile ducts were grouped as chronically infected. When migrating flukes were observed in biliary tract and gallbladder, and adhesions of the gallbladder to adjacent organs were present the lesion also taken as chronic fasciolosis (Meskerem, 2006). When the liver is enlarged with blunt edge and the capsule is tense it was grouped as acute fasciolosis. Presence of haemorrhagic spots with fibrinous exudates on the capsular surface and the dark acute haemorrhagic streaks and juvenile flukes in the migratory tunnels were also considered as acute fasciolosis (Steyl, 2009; Affroze *et al.*, 2013).

c) *Study Methodology*

i. *Blood samples and serum biochemical analysis*

8ml of blood were collected from jugular vein using sterile plain vacutainer tubes, labeled according to the neck tag of animals and taken to laboratory. At the laboratory, blood samples were rendered to stand at room temperature for 3 hours to allow serum separation (Hodzic *et al.*, 2013). Then sera were transferred in to 2ml eppendrof tubes and stored at -70c° until the time of analysis. Analysis of samples were then took place after bringing the samples to room temperature. The serum sample was analyzed with ALT, AST and ALP commercially available respective enzyme working reagent of the test kits, using humastar 80 chemistry analyzer. The instrument humastar 80 chemistry analyzer was calibrated using calibrator (Autocal), and quality control samples normal (Humatrtol N) and pathological (Humatrol P) each day before running the samples.

ii. *Pathological examination and tissue sampling*

The livers and gallbladders were appropriately examined for the presence of fasciola and its gross

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pathology, during which the fasciola *spp*. and all gross pathological changes were noted and recorded. At first liver and bile duct were systematically inspected for the presence of fasciola *spp*. by applying the routine internal organ inspection procedures, if evidence of fascioliasis is found, they were classified as mature or immature and gross lesions were characterized (Sohair and Eman, 2009). Accordingly the primary examination involves visualization and palpation of the organs; secondary examination involves more incision of liver; opening of bile duct and hepatic lymph nodes. For generalized liver fluke infection (fascioliosis) incision were made in different parts of the liver to check the presence of fluke in the parenchyma. The cut liver was pressed to squeeze out flukes from the tissue and smaller bile ducts. The gross pathological changes of hepatic lymph nodes as well as the distribution of the lesion to hepatic lobes were also thoroughly examined. Then parts of the affected organ were sampled into 10% neutral buffered formalin.

For histological lesion characterization, the fixed tissue samples were trimmed to 5mm and processed (dehydrated through a series of ascending grades of alcohols, cleared in three changes of xylene and impregnated with paraffin wax) and finally, embedded in melted paraffin (60c°). The tissues were then sectioned at 5μm and stained routinely with haematoxylin and eosin (Okaiyeto *et al.,* 2012), and examined.

d) Data Management and Analysis

Data was entered into Microsoft excel 2010 and analyzed by SPSS statistical software version 20. Prevalence differences of study variables were analyzed by chi- square and descriptive statistics. P≤ 0.05 was considered as statistically significant at 95% CI. The serum biochemical parameters infected groups were compared with those of the controls. The data was expressed as mean ± standard error and range; one way ANOVA was used for multiple comparisons and to see their correlation.

III. Results

a) Prevalences

The results of the gross pathology showed that 21.13% (n=284) of ruminants were found to be infected with fasciolosis. The result also depicted the infection prevalence was significantly higher (p=0.00) in cattle (50.65%) than sheep (16.16%) and goats` liver (4.63%) (Table: 1).

Table 1 : Prevalence of liver fluke infection by age and body condition score among cattle, sheep and goats

Variables	Levels	No. examined	No. positive (%)	P value
Body condition score	Moderate	201	51(25.37)	0.00
	excellent	83	9(10.84)	
Age	Young	94	16(17.02)	0.20
	Adult	190	44(23.16)	
Species	Cattle	77	39(50.65)	0.00
	Sheep	99	16(16.16)	
	Goats	108	5(4.63)	
	Total	284	60 (21.13)	

The pathological examination revealed that 47(78.33%) acute and 13(21.67%) chronic liver lesions proportion with 8(61.54%) cattle, 3(23.08%) sheep and 2(15.38%) goats, were suffering with chronic liver fluke infection. On the other hand 35(58.33%) livers were only positive for fasciola and fasciola indicative lesions, whereas 25(41.67%) of livers showed lesions that indicate co-infection (Table: 2).

Table 2 : Proportion of liver fluke lesions (acute and chronic) among cattle, sheep and goats

Lesion type	Animal spp.	Status of fasciola infection (%)			P value
		Only fasciola	Co-infection	Subtotal	
Acute	Cattle	20(64.52)	11(35.48)	31(66.00)	0.00
	Sheep	7(53.85)	6(46.15)	13 (27.67)	
	Goats	1(33.33)	2(66.67)	3(6.38)	
	Subtotal	28 (59.57)	19 (40.42)	47 (78.33)	
Chronic	Cattle	5(62.50)	3(37.50)	8(61.54)	
	Sheep	1(33.33)	2(66. 67)	3(23.08)	
	Goats	1(50.00)	1(50.00)	2(15.38)	
	Subtotal	7(53.85)	6(46.15)	13(21.67)	
Total	-	35 (58.33)	25(41.67)	60 (21.13)	

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b) Pathological Lesions

In this study, the gross pathological changes observed in cattle and sheep livers were almost similar except repeated severe calcification in chronically infected cattle livers. The commonly observed gross lesions include firm, pale and irregularly outlined liver with tough consistency. In chronic cases variably sized livers, focal and multifocal nodules, Pin-point hemorrhages on the parietal surface of the liver were also examined. When a section of the bile duct was cut through there was aberrant migration of flukes and in some cases evidence of calcification was noted. Thickened and distended bile ducts containing adult flukes, decomposed materials and cholangitis were also observed at post mortem (figure.1). The ventral lobe was mostly affected and reduced in size. In goats` liver however, no multifocal nodules and less extensive ductular thickness was found in the chronic form of the infection.

In acute fluke infection, on the other hand the liver was highly enlarged (swollen) with rounded edges and the color was paler than normal with numerous small and large hemorrhagic patches scattered over the parietal surface. The capsule was tense with fibrous exudates on the capsular surface of the liver. Hepatic lymph nodes were enlarged and an abnormally cloudy thick fluid oozed up on cutting, and flukes were also observed in the migratory tunnels of the parenchyma. In cattle hard, dark and brown color liver with multiple soft abscesses surrounded by hyperemic zone on the surface were noted and up on cutting section, a viscous yellow material oozed from the cut ends. Grossly in co-infected livers abscesses of different sizes and consistency, and cysts were observed in both acute and chronically infected livers in addition to fasciola and fasciola indicative lesions examined.

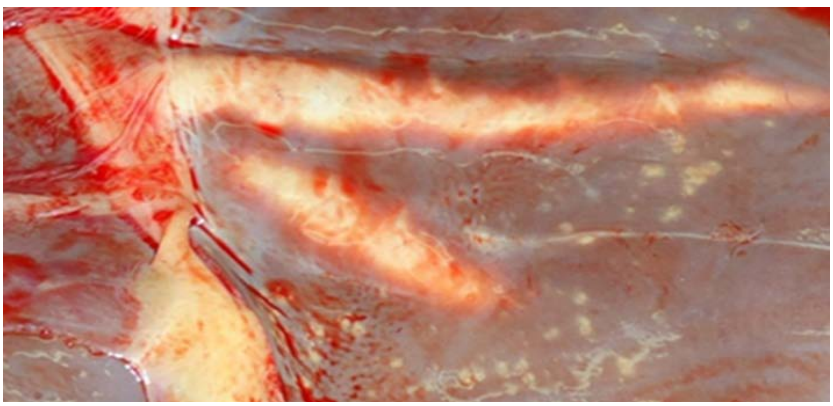

Figure 1 : Chronic liver fluke infection in cattle, with bile duct distension (Gross)

The histopathological changes observed in this study showed both parenchymal and biliary tract lesions. Fibroblasts admixed with lymphocytes, plasma cells and few macrophages in the area previously migrated by the young fluke in cattle and sheep liver parenchyma with mild portal fibrosis (figure. 2B) and biliary hyperplasia were lesions repeatedly examined in chronic cases. Histologically, in chronic infection there was no differences between lesions of co-infected and, fasciola and fasciola indicative lesion positive livers. Atrophy, fatty necrosis, focal and multi focal hepato-necrosis, peribiliary epithelial inflammation and biliary tract fibrosis with severe hyperplasia of the epithelial layer and mild liver parenchymal necrosis were also examined in some cases of chronic fasciolosis. Peri-acinar necrosis, cholangitis dominated by mononuclear cell infiltration, hepatocyte pyogranuloma, and portal fibrosis and cirrhosis with fibrous connective tissue were also encountered in some cattle and sheep livers though fibrosis was less severe in sheep. One liver taken from cattle showed multilobular parenchymal fibrosis with large amount of fibrin in the portal area and complete biliary cirrhosis. Granuloma lesion with aggregation of epitheloid cells and mononuclear cells, intrahepatic ductular fibrosis surrounded by normal area, portal fibrosis with mononuclear cell infiltration and lymphocytic multifocal hepatitis were also examined. On the other hand, focal coagulative hepatocyte necrosis, fatty degeneration, portal fibrosis with mild biliary hyperplasia and peri-acinar necrosis were histopathologic changes observed in chronically affected goats liver. In goats however, cirrhosis was not encountered, even in chronic fasciolosis.

Microscopic lesion patterns were more or less similar in acutely affected cattle and sheep but less pronounced in goats liver. The biliary tract was less affected than liver parenchyma in acute form of the fluke infection. Migratory tracts traveled by fasciola *spp.* were infiltrated with macrophages and eosinophils (figure.2A). Multifocal hepatitis and the necrotic lesions with deep eosinophilic cytoplasm, karyorhexis and karyolysis were examined.

Focal hepatitis with neutrophils and eosinophils infiltration in parenchyma was another lesion types frequently observed. Numerous eosinophils admixed with few lymphocytes surrounded by hemorrhagic and

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edematous area in which hepatic blood vessels were dilated, engorged with blood, occluded by thrombi and foci with extensive hemorrhagic streaks were significantly observed. There were also mild fibrosis and bile duct proliferation and distortion of the hepatic cords in some areas. Moreover, congestion with focally extensive parenchymal necrosis and the degenerative changes manifested by vacuolation of the hepatocytes particularly around central vein were noted. In acute infection edema and neutrophilia were more severe in co-infected livers than in livers only fasciola and fasciola indicative lesion positives.

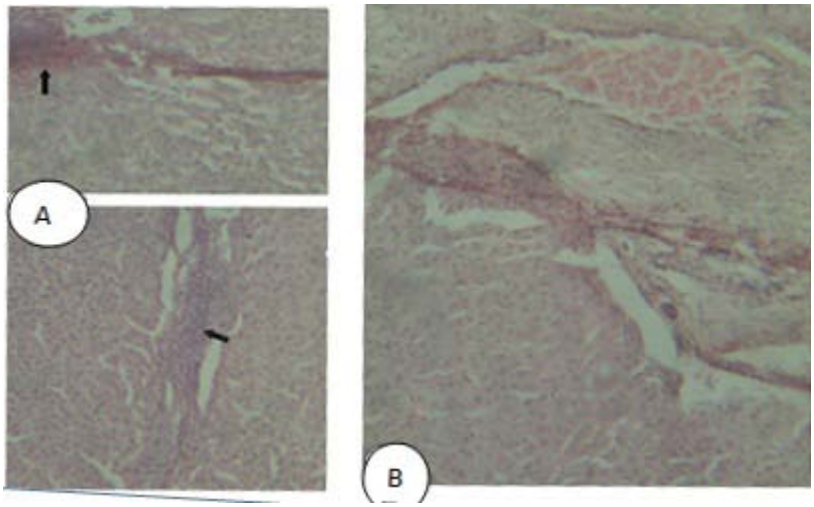

Figure 2 : A. Fluke's migratory tract infiltrated with macrophages and eosinophils, and few neutrophils (arrows), B. Chronic liver fasciolosis with portal fibrosis (H and E, 40x)

c) Serum Biochemical Analysis

Serum biochemical parameters were compared among animals with no visible gross lesion and no liver fluke (group A), fasciola indicative lesions and fasciola presence (group B), and liver fluke, liver fluke indicative lesions together with other non fasciola like lesions/ co-infected (group C) (*Tables* 4, 5 and 6). When comparing mean values with that of group A, animals with acute fasciolosis and hepato-necrosis have significantly elevated activity of serum ALT and AST (F*calcul.*>F*tabul.*; P<0.05). However, cases with chronic biliary cirrhosis showed decreased ALT and AST activity. In cattle AST and ALT were statistically higher in animals with hepatocyte degeneration, and eosinophilic hepatitis but ALP was significantly increased (F*calcul.*>F*tabul.*; P<0.05), in animals with cholangitis and biliary fibrosis. Similar serum biochemical changes were observed in sheep and goats. The serum liver AST, ALT and ALP activities were also positively correlated (0<r<1) with pathologic lesions (Tables 3, 4 and 5).

Table 3 : Comparisons of some serum liver enzymes among group-A/controls, group-B/ affected with liver fluke only, and group-C/ co-infected cattle (Mean±S.E)

Groups	Lesion condition	AST(U/L)	ALT(U/L)	ALP(U/L)
Group A	No Visible Lesion	56.76±1.08^{x}	14.68±0.51^{y}	147.96±4.09^{z}
	Hyperplastic cholangitis	61.00±2.40^{l}	17.60±2.47	396.45±21.06^{e}
	Hepatocyte necrosis	87.00±4.78^{k}	24.99±0.88^{b}	161.88±7.41
	Hepatocyte fibrosis	59.95±0.04	21.30±1.74^{c}	179.45±2.00^{f}
	Biliary tract fibrosis	52.17±2.90	11.86±0.45	373.07±47.34^{g}
Group B	Biliary cirrhosis	53.55±2.34	11.80±0.74	384.20±15.96^{h}
	Fatty necrosis	60.90±1.31^{j}	19.35±0.86^{c}	274.70±36.74^{i}
	Eosinophilic hepatitis	111.16±6.79^{m}	28.90±2.45^{d}	160.14±4.57
	Hepatocyte degeneration with telangiectasis	105.44±6.75^{n}	25.60±1.13^{b}	149.28±4.14
		F=26.44, p=0.00, r=0.739	F=22. 98, p=0.00, r=0.662	F= 49.84, p=0.00 r=0.106
Group C	Hyperplastic cholangitis	63.00±2.44^{j}	16.86±0.65	393.49±20.66^{e}
	Fatty necrosis	62.65±7.81^{j}	20.95±0.46^{c}	272.28±26.34^{i}
	Hepatocyte necrosis	84.00±2.68^{k}	26.65±0.87^{b}	159.80±2.81
	Biliary tract fibrosis	54. 67±1.22	12.84±0.61	374.07±46.12^{g}
	Hepatocyte degeneration with telangiectasis	108.24±6.62^{n}	26.60±4.43^{b}	146.42±2.14

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		F= 18.41, p=0.01 r=0.421	F=12, p=0.02 r=0.367	F= 21.62, p=0.02 r=0.089

Note : Rows with superscripts ^{x}, ^{y} and ^{z} are significantly different (Fcalcul.>Ftabul.; p≤0.05; 0<r<1) from(^{j},^{k},^{m} and ^{n}), (^{b},^{c} and ^{d}) and (^{e},^{f},^{g},^{h} and ^{i}), respectively; and similar superscripts in different rows indicate equal significance from respective healthy mean values

Table 4 : Comparisons of some serum liver enzymes among group- A/controls, group- B/ affected with liver fluke only and group- C/ co-infected sheep (Mean ± S.E)

Groups	Lesion condition	AST(U/L)	ALT(U/L)	ALP(U/L)
Group A	No Visible Lesion	86.89±2. 53^{x}	19.58± 1.04^{y}	140.60±6.12^{z}
Group B	Hepatocyte necrosis	213.13±6.80^{j}	40.10±1.94^{a}	166.10±16.68
	Biliary cirrhosis	82.33±1.15	18.9± 0.27	297.70±20.69^{f}
	Eosinophilic hepatitis	311.38±16.67^{k}	44.28 ±3.39^{c}	131.74±2.40
	Hepatocyte degeneration with Telangiectasis	204.68±9.10^{m}	39.32±2.79^{d}	117.38±2.89
		F= 75.20, p=0.00, r=0.276	F= 15.96, p=0.00, r=0.339	F= 12.53, p=0.00, r=0.475
Group C	Hyperplastic cholangitis	88.10±1.24	15.95±3.52	247.05±2.39^{h}
	Hepatocyte fibrosis	241.8 ±3.47^{n}	28.70±1.93^{b}	202.20±12.06^{i}
	Hepatocyte necrosis	218.13±4.64^{j}	38.10±1.4^{a}	171.21±14.57
	Fatty necrosis	83.95±2.31	27.70±6.31	252.25±1.89^{p}
	Biliary tract fibrosis	73.55±4.88	22.65±4.22	291.40±11.06^{q}
	Hepatocyte degeneration with Telangiectasis	207.48±7.89^{m}	38.36±2.86^{d}	101.18±4.6
		F=48.21, p=0.00, r=0.083	F= 8.43, p=0.00, r=0.173	F= 28.62, p=0.00, r=0.436

Note : Rows with superscripts ^{x}, ^{y} and ^{z} are significantly different (Fcalcul.>Ftabul.; p≤0.05; 0<r<1) from(^{j},^{k},^{m} and ^{n}), (^{b},^{c} and ^{d}) and (^{e},^{f},^{g},^{h} and ^{i}), respectively; and similar superscripts in different rows indicate equal significance from respective healthy mean values

Table 5 : Comparisons of some serum liver enzymes among group- A/controls, group- B/ affected with liver fluke only and group- C/ co-infected goats (Mean ± S.E)

Groups	Lesion condition	AST(U/L)	ALT(U/L)	ALP(U/L)
Group A	No Visible Lesion	62.65±3.32^{x}	20.54±1.02^{y}	121.88±9. 56^{z}
Group B	Hyperplastic cholangitis	74.50±3.46	24.60±1.46	802.20±51.48^{e}
	Biliary tract fibrosis	68.00±1.09	29.00±2.45	701.50±106.70^{f}
	Eosinophilic hepatitis and hepatocyte necrosis	208.50±44.24^{j}	80.85±8.33^{c}	125.75±17.75
	Hepatocyte degeneration with Telangiectasis	215.50±46.58^{k}	54.85±5.92^{d}	118.55±1.02
		F=4.17, p=0.02, r=0.813	F=7.45, p=0.01, r=0.821	F=4.63, p=0.01, r=0.230
Group C	Hyperplastic cholangitis	74.28±2. 81	23. 34±4.14	792.12±14.32^{e}
	Fatty necrosis	82.41±6.45^{m}	26.85±2.5	462. 64±10.71^{h}
	Hepatocyte degeneration with Telangiectasis	212.50±42.64^{k}	57.15±2.76^{d}	114.52±4.26
		F=1.67, p=0.04, r=0.103	F=4.25, p=0.02, r=0.216	F=1.46, p=0.04, r=0.108

Note : Rows with superscripts ^{x}, ^{y} and ^{z} are significantly different (Fcalcul.>Ftabul.; p≤0.05; 0< r<1) from(^{j},^{k},^{m} and ^{n}), (^{b},^{c} and ^{d}) and (^{e},^{f},^{g},^{h} and ^{i}), respectively; and similar superscripts in different rows indicate equal significance from respective healthy mean values

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Our result showed variation in mean values of the enzymes between group-A (those taken as controls) and infected animals (group B and C) in age wise comparisons. 778.40 U/L of ALP and 12.00 U/L of ALT were recorded in adult goats as the maximum and minimum ranges of the enzyme values in infected and control groups, respectively (Table. 6).

Table 6 : Age wise comparisons of serum liver enzymes in fasciola infected and non infected groups

Enzymes	Levels	Ruminants	Mean ± S.E	Range
AST	Young control	cattle	57.07±1.35	30.40
		sheep	85.41±6.22	56.00
		goat	73. 5±4.99	42.00
	Young infected	cattle	88.11±10.29	98.70
		sheep	153.05±32.27	327.40
		goat	127.40±38.89	220.00
	Adult control	cattle	56.03±1.86	35.40
		sheep	88.00±3.93	58.90
		goat	54.54±3.45	37.00
	Adult infected	cattle	92.42±4.07	139.80
		sheep	214.63±18.79	270.80
		goat	140.25±46.20	203.00
ALT	Young control	cattle	15.06±0.65	16.60
		sheep	20.95±1.94	22.60
		goat	22.61±1.57	15.23
	Young infected	cattle	22.76±3.01	33.60
		sheep	28.87±2.96	29.30
		goat	38.08±7.47	39.90
	Adult control	cattle	13.9045±0.87	18.00
		sheep	18.55±1.04	15.40
		goat	18.56±1.20	12.00
	Adult infected	cattle	24.04±1.84	25.20
		sheep	38.99±2.42	37.66
		goat	53.50±16.42	66.20
ALP	Young control	cattle	167.43±3.80	102.10
		sheep	162.70±8.36	96.00
		goat	144.33±10.73	90.06
	Young infected	cattle	207.39±34.33	318.00
		sheep	221.34 ± 23.55	221.80
		goat	474.92±148.40	748.80
	Adult control	cattle	119.00±3.65	57.50
		sheep	124.03±6.63	83.69
		goat	108.32±13.67	165.70
	Adult infected	cattle	183.49±11.39	319.70
		sheep	156.88±16.59	190.00
		goat	303.23±186.46	778.40

In current study age and status of fluke infection wise multiple comparisons were also made and the results showed statistically significant differences ($p<0.05$) of serum ALT, AST and ALP values between asciola positives (group-B and C), and control (group-A) animals. In age wise comparisons between infected young and adult cattle, sheep and goats, transaminases showed statistically no differences except significantly higher serum activity of ALT found in adult infected sheep (Tables: 7, 8 and 9).

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Table 7 : Multiple comparisons of serum AST, ALT and ALP in cattle

Enzymes	Levels(i)	Levels(j)	Mean Diff.(i-j)	Sig. (p value)	95% Confidence Interval	
					Lower Bound	Upper Bound
AST	Young control	Young- infected	-31.039	0.001	-51.665	-10.413
		Adult control	1.043	1.000	-14.644	16.731
		Adult infected	-35.348	0.000	-48.257	-22.440
	Young infected	Adult control	32.082	0.001	10.215	53.949
		Adult infected	-4.309	1.000	-24.276	15.657
	Adult control	Adult infected	-36.391	0.000	-51.202	-21.580
ALT	Young control	Young- infected	-7.695	0.002	-13.194	-2.195
		Adult control	1.160	1.000	-3.022	5.343
		Adult infected	-8.973	0.000	-12.415	-5.531
	Young infected	Adult control	8.855	0.001	3.025	14.685
		Adult infected	-1.278	1.000	-6.602	4.045
	Adult control	Adult infected	-10.133	0.000	-14.082	-6.184
ALP	Young control	Young- infected	-39.963	0.003	0.276	18.349
		Adult control	48.421	0.024	4.068	92.775
		Adult infected	-16.067	1.000	-52.562	20.427
	Young infected	Adult control	88.385	0.001	26.563	150.207
		Adult infected	23.896	0.002	25.554	80.347
	Adult control	Adult infected	-64.489	0.000	-106.363	-22.615

Table 8 : Multiple comparisons of serum AST, ALT and ALP in sheep

Enzymes	Levels(i)	Levels(j)	Mean Diff.(i-j)	Sig. (p value)	95% Confidence Interval	
					Lower Bound	Upper Bound
AST	Young Control	Young- infected	-67.635	0.037	-39.598	4.328
		Adult control	-2.591	1.000	-66.774	61.591
		Adult infected	-129.220	0.000	-193.403	-65.037
	Young Infected	Adult control	65.043	0.067	-2.707	132.794
		Adult infected	-61.585	0.095	-129.336	6.166
	Adult control	Adult infected	-126.628	0.000	-186.050	-67.206
ALT	Young Control	Young- infected	-7.914	0.019	-6.953	1.124
		Adult control	2.403	1.000	-5.657	10.464
		Adult infected	-18.034	0.000	-26.096	-9.973
	Young Infected	Adult control	10.317	0.010	1.8083	18.827
		Adult infected	-10.120	0.012	-18.629	-1.610
	Adult control	Adult infected	-20.438	0.000	-27.901	-12.974
ALP	Young Control	Young- infected	-58.640	0.008	-18.555	1.275
		Adult control	38.673	0.314	-14.764	92.111
		Adult infected	5.824	1.000	-47.613	59.262
	Young Infected	Adult control	97.313	.000	40.904	153.722
		Adult infected	64.464	0.017	8.055	120.873
	Adult control	Adult infected	-32.849	0.045	-22.323	16.624

Table 9 : Multiple comparisons of serum AST, ALT and ALP in goats

Enzymes	Levels(i)	Levels(j)	Mean Diff.(i-j)	Sig. (P Value)	95% Confidence Interval	
					Lower Bound	Upper Bound
	Young control	Young- infected	-53.900	0.014	-37.352	29.5529
		Adult control	18.954	1.000	-49.065	86.9743
		Adult infected	-66.750	0.050	-56.392	22.8928

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AST	Young infected	Adult control	72.854	0.084	-6.100	151.8093
		Adult infected	-12.850	1.000	-111.048	85.3488
	Adult control	Adult infected	-85.704	0.039	-171.175	-.2335
ALT	Young control	Young-infected	-15.471	0.007	-8.266	7.3242
		Adult control	4.053	1.000	-14.526	22.6331
		Adult infected	-30.891	0.008	-15.377	-6.4051
	Young infected	Adult control	19.524	0.094	-2.042	41.0913
		Adult infected	-15.420	0.668	-42.243	11.4033
	Adult control	Adult-infected	-34.944	0.001	-58.291	-11.5979
ALP	Young control	Young- infected	-330.590	0.011	-251.773	-9.4064
		Adult control	36.010	1.000	-225.775	297.7973
		Adult infected	-158.895	1.000	-503.901	186.1115
	Young infected	Adult control	366.600	0.012	62.729	670.4726
		Adult infected	171.695	0.006	206.240	549.6307
	Adult control	Adult infected	-194.905	0.008	-23.856	134.0449

IV. Discussions

The overall combined prevalence of fasciolosis in cattle, sheep and goats was 21.13% and this is higher when compared to reports of Meskerem (2006), who reported 12.37% from the same abattoir. However, the present prevalence was by far lower than (31.70%) Ozung *et al.* (2011), reported from Ikom abattoir of Nigeria. The variation in the prevalence may be due to geographical origin of studied animals. According to Yohannes and Abebaw (2012), the difference in incidence and severity of the fluke infection are evident in various geographical regions depending on the local climatic condition, availability of permanent water and system of management.

Statistically, in agreement with the report of Meskerem (2006), in cattle, sheep and goats and Ayana, *et al.* (2009), only from sheep and goat; our current study showed significant differences in the prevalence of liver fluke infection among cattle, sheep and goats. Accordingly, cattle were found highly infected and goats were the least infected ones. These prevalence differences among species might have some connection with their feeding habits as goats are commonly browsers. Similar to the findings of Talukder *et al.* (2010) and El-Hallawany and Abdel-Aziz (2012), both chronic and acute forms of liver fluke infection were detected in the present study. Adult flukes mainly localized in the bile ducts and gallbladder and they cause chronic fasciolosis while the immature flukes wander in the parenchyma and are responsible for the acute fluke infection.

The gross pathological changes observed in the chronically infected livers were reduction in size and pin-point hemorrhages on the parietal surface which is partly due to the inflammatory changes and later fibrosis that took place in the parenchyma. These findings are partially in agreement with the report of Okaiyeto *et al.* (2012), as they reported hemorrhages on the surface of the liver in chronic fasciolosis from dairy farm. The pin-point hemorrhagic foci on the surface represented the points of entrance of the immature parasites into the liver structure (Borai *et al.*, 2013). In agreement with report of Jones *et al.* (1997), Molina *et al.* (2005), and Sayed *et al.* (2008), chronic cases of the current study also revealed that the livers were firm, tough in consistency and the cut section showed evidence of calcification while the affected ducts were enlarged/distended, thickened and cholangitis with adult fluke in the ducts. These might be because of the immunological reaction of macrophages and lymphocytes infiltration that merges with fibrotic healing of the necrotic areas during the later stage of fasciolosis. Progressive irritation by the adult flukes wandering in the biliary tract cause biliary inflammation like hyperplastic cholangitis, ductular wall thickening, and intra- and extrahepatic biliary dilatation (Catalano *et al.*, 2009). Evidence of calcification showed the brilliant cell in the adenomatous proliferation of the biliary epithelium particularly in cattle liver. However, bile ducts in goats (Talukder *et al.,* 2010) and sheep (Okaiyeto *et al.,* 2012) under chronic liver fluke infection never calcified. The differences in calcification might be due to species variation showing the possibility of direct relationship to host resistance. It is also probable that the calcification seen in cattle interferes with feeding habits of fluke and these effects are noted in its size.

In this study no multifocal nodules were noted and even less extensive ductular thickness was recorded in chronic form of goats' fasciolosis. However, El-Hallawany and Abdel-Aziz (2012), reported focal and or multi focal liver nodules, from small ruminants which might be due to abnormal host immune response that

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resulted against fasciola eggs, dead larvae or the fluke byproducts trapped into the liver parenchyma. Attributing to our finding Talukder *et al. (*2010), reported extensive enlargement and huge ductular fibrosis resulting the thickening of the bile ducts which also confirms the findings of Sohair and Eman (2009). Because the inflammatory changes in the parenchyma leads enlargement of the liver in size, and presence of adult flukes in the bile duct cause continuous irritation resulting hyperplastic proliferations and extensive ductular fibrosis.

Enlarged hepatic lymph nodes and multiple soft abscesses on the liver surfaces surrounded by hyperemic zone was also seen during the study, such findings were also reported by Molina *et al.* (2005) and Sohair and Eman (2009). According to Adrien *et al.* (2013), the liver is enlarged in response to acute inflammation as the wandering juvenile fluke mechanically damage the parenchyma. Molina *et al.* (2005), also stated at the cut surface, the liver is irregular, firm and edematous with hemorrhagic channels and adhered fibrosis in the hepatic parenchyma and also fibrinous tags on the capsule and exudates between organs. These authors justified fibrinous tags on the capsule are due to capsular reaction to the flukes penetrating it. Supporting the current study Adrien *et al.* (2013), reported the hepatic and mesenteric lymph nodes are reactive and enlarged and they ooze edematous material up on opening. Hepatic abscesses are usually resulted from bacterial infections and subsequent lysis of neutrophils and are later surrounded by a fibrous capsule. Therefore, multiple soft hepatic abscesses in our study might be resulted from secondary bacterial complication. On the other hand, this may be due to host response against parasitic infestation and continuous mechanical irritation along with parenchymal destruction accompanied by intensive haemorrhagic lesions and immunological reactions (El-Hallawany and Abdel-Aziz, 2012).

Microscopically fibroblasts admixed with lymphocytes, plasma cells and few macrophages in the area previously migrated by the young fluke was observed in chronic fluke infection of this study. This might be indicative of granulomatous hepatitis which is resulted from aggregations of activated macrophages with an epitheloid appearance, mostly accompanied by lymphocytes and plasma cells (Sohair and Eman, 2009). Fatty degeneration which is manifested by lipid accumulation inside hepatocytes may be due to tissue hypoxia resulted from anemia and vascular damage (Abd El-Baky and Salem, 2011), as more than 0.5ml blood per fluke can be lost per day when adults become established in the bile ducts (Molina *et al.*, 2005) and consequently the tissue is atrophied as in this study. The ductular epithelium inflammation and focal and multi focal hepato-necrosis of the current study were in agreement with the reports of Mahmoud *et al.* (1989) and Sayed *et al.* (2008), which are due to the effect of toxic products liberated by fasciola worms. Hyperplastic bile duct is produced as an attempt to regenerate hepatic tissue when the liver cells have lost their capacity to regenerate themselves (Kelly, 1985). Also, Sohair and Eman (2009), mentioned that the hyperplasia of the ductular epithelium occurs as a result of fluke toxic products which cause changes in the structural integrity of the ductular cells in non specific and potentially destructive manner. The presence of mature worms within the lumen of intrahepatic bile ducts also brings about continuous irritations that lead to hyperplastic proliferations.

Portal and multilobular biliary cirrhosis in cattle and sheep, and coagulative hepatocyte necrosis in goat were the microscopic findings of the chronic fasciolosis in present study. Sayed *et al.* (2008) and Darwish (1996), also found similar pathological changes from infected livers of buffalo and camel, respectively. A progressive mechanical irritation of hepatocyte by the liver fluke in chronic fasciolosis enhances hepatocyte destruction with persistent healing which results fibrous connective tissue proliferation leading to hepatic cirrhosis as well as mononuclear cell infiltrations, and if the cirrhosis is developed in the portal triad, it is called portal cirrhosis (Heidelbaugh and Bruderly, 2006). On the other hand, the parasite itself chemically digest and destruct the liver parenchyma and biliary epithelium by enzymes like proteases and toxins that produced by the fluke, and such condition later come up with hepatic fibrosis and cirrhosis (Sohair and Eman 2009). In addition to this, the obstruction to intrahepatic bile flow leads to upstream bile ductular proliferation, inflammation and necrosis of adjacent periportal hepatic parenchyma, generalized choleostasis, portal tract scarring and bridging fibrosis. Moreover, chronic extrahepatic and intrahepatic biliary obstruction, and choleostasis together with chronic hepatitis develop to cirrhosis (Salem and Hassan, 2011). Mild irritation to the hepatocyte by fasciola also causes a decrease in blood flow to the irritated area followed by protein denaturation which forms coagulative hepatocyte necrosis (Molina *et al.*, 2005). Granulomatous lesion of the current study indicates circumscribed focal aggregation of epitheloid cells and mononuclear cells, and encapsulated by delicate fibrous connective tissue capsule. Such lesion is created due to host immune response that resulted against fasciola eggs or dead larvae trapped in the liver parenchyma that causes granulomatous reaction (El-Hallawany and Abdel-Aziz, 2012). Zongping (2003), stated the hepatic necrosis may be related to the enteric infection which can invade the liver by way of septicemia or ascending cholangio-billary infection, and adult flukes moving in the biliary tree also cause biliary colic or cholangitis. Furthermore, invasion of the liver by migrating immature liver fluke damages the tissue and results in reduction of the oxygen tension (anaerobic

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condition), that allowed the germination and proliferation of closteridial spores with concomitant release of its toxins and induce hepatocellular necrosis (Jones *et al.*, 1997; Sayed *et al.*, 2008).

In our current study, the histopathological examination also revealed the presence of numerous eosinophils admixed with few lymphocytes and accompanied by hemorrhage and edema in acute fascioliosis. These lesions partially correlated with the findings of Talukder *et al.* (2010), from goats` liver fluke infection, and completely agreed with report of Borai *et al.* (2013). Eosinophilia occurs due to sensitivity to the foreign protein of the parasite, which may be a part of immune phenomena. Thus, eosinophilia is likely to be seen when parasites are migrating through the tissue in large animals (Kerr, 2002; Borai *et al.*, 2013).

Edema/excessive fluid in hepatocyte interstitial space may be due to decrease in plasma colloidal osmotic pressure as a result of hypoalbuminemia. The acute cases in this study also revealed dilated blood vessels with disorganized and distortion of the hepatic cords while the hepatic cells showed variable degrees of necrosis and degeneration, which are in agreement with Mac Gavin *et al.* (2001), Sayed *et al.* (2008) and Sohair and Eman (2009). These authors support the result as it might be due to mechanical and toxic effects of fasciola *spp.* plus immunological reactions affecting the complex vascular and biliary systems in the liver accompanied by vascular obstruction and distension. Proliferation and mild fibrosis in some areas of biliary tract in acute fluke infection of our study might indicate the previous infection as asymptomatic infection can occur, especially in cattle. According to Adrien *et al.* (2013), extensive liver damage with hemorrhagic dark red tracts of necrotic parenchyma, presence of immature flukes and thrombosis of the hepatic vessels during the migratory phase are consistent features of the liver fluke infection in all species, and thrombosis occurs over areas of localized phlebitis produced by fluke migration.

a) Serum Biochemical Analysis

Fasciola *spp.* causes the release of reactive oxygen species that produce damage to cell wall and hepatic tissue necrosis. These changes have an influence on biochemical parameters in serum including liver specific enzymes (Hodzic *et al.*, 2013). Differences in activities of liver enzymes like AST, ALT, GGT, ALP, LDH and GLDH in serum are generally indicate pathological changes of tissue and organ (Tanritanir *et al.*, 2009). In agreement to these statements, the results of the current study showed a variation in ALT, AST and ALP values among cattle, sheep and goats affected by different stages of fasciolosis in comparisons with animals with no fasciolosis (Tables:8, 9 and 10). Whenever the liver is injured or damaged, the liver enzymes spill into the blood, causing elevations of serum liver enzyme (Mert *et al.*, 2006). Therefore, in combinations with the physical examination and history, the evaluation of serum liver enzymes should aid in differentiating the source of increased enzymes like AST, ALT and ALP levels (Yasuda, 1988). Different mean values of AST, ALT and ALP were also measured among animal *spp.*, which may arise from physiological adaptations, maturation of metabolic pathways, growth effects, body composition, and or nutrition (Sharon, 2013). The variation among animal species might also due to variability in parasite load that indicate innate differences in the host immune systems (Semrad and Gay, 2013). Furthermore, in "Overview of hepatic disease in large animals" these authors stated the serum levels of hepatic enzymes vary even with ages, breeds and sexes.

In present study, the mean value of serum liver AST and ALT were sufficiently higher ($P<0.05$) with acute lesions like hepatocyte degeneration, telangiectasis and eosinophilic hepatitis unlike with chronic lesion like biliary cirrhosis and fibrosis, which are in agreement with statements of (Yasuda, 1988), in large animals, serum concentrations of liver specific enzymes like transaminases are generally higher in acute liver disease than in chronic liver disease. They may be within normal limits in the later stages of chronic hepatic disease. Consequently AST markedly increased with intrahepatic cholestasis and mildly increased with extrahepatic cholestasis. This increment of serum transaminases at the early stage of the infection could be related to the hepatocellular necrosis and degenerative changes produced by migrating juvenile flukes through the liver parenchyma (Dias, 1996). In previous findings of experimental study on *F. hepatica* in goats, activity of AST highly elevated first and returns to normal values 11 weeks post infection (Hodzic *et al.*, 2013). The elevation of serum AST and ALT activity in affection with liver parenchyma than in biliary tract damages in our study also partially agreed with the findings of Al-Quraishy and Al-Moussawi (2001) in cows, (Adama *et al.*, 2011) in goats, that the elevation of AST indicates injury to liver parenchyma and or acute liver fluke infection. The cytosolic location of transaminases allows their immediate release with even minor changes in hepatocellular membranes as a result raise their concentration in serum (Sharon, 2013). AST is present in both the cytoplasm and mitochondria of hepatocytes and will elevate together with ALT in states of altered membrane permeability (Yasuda 1988), though the mitochondrial AST isoenzyme is less likely released with most of the conditions which result in increased membrane permeability (Kerr and Steiner, 2012).

ALT is present in high concentration in the cytoplasm of hepatocytes and is considered to be liver specific in small animals and ruminants like camel. Its plasma concentration increases with hepatocellular damage/necrosis or degeneration and hepatocyte proliferation (Hodzic *et al.*, 2013). The previous findings of Mbuh and Mbwaye (2005), also detected the raise of

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ALT in fasciolosis may be due to hepatocyte destruction since ALT is primarily found in the liver parenchyma. On the other hand, the raise in mean value of ALT in this study may be due to hepatocyte death from liver fluke infection causing complete or partial bile ducts obstruction and then returning of bilirubin to hepatocyte (Dias, 1996; Kilad *et al.*, 2000). Accoding to Adama *et al.* (2011), serum ALT remain raised for some days after acute liver fluke infection indicating epithelial damage in the bile ducts which agrees with elevated serum ALT in hyperplastic cholangitis of cattle and goats but not in sheep, in our study. Thus, in hyperplastic cholangitis AST and ALT elevation may be due to parenchymal damage as a secondary effect of cholestasis (Salem and Hassan, 2011).In current study, though it was statistically insignificant the serum activity of AST in cases of biliary fibrosis and cirrhosis of sheep and cattle was declined compared to its activity in control groups; ALT activity was also lowered with biliary cirrhosis of sheep and cattle, plus in hyperplastic cholangitis of sheep. Supporting our findings Okaiyeto *et al.* (2012), dictated that atrophy or fibrosis of an organ can lead to low plasma activities of AST. However, Mert *et al.*, (2006) and Igado *et al.* (2011), reported significant elevation of AST in chronic fasciolosis.

Apart from serum transaminases, ALP serum activity showed insignificant decrease with parenchymal lesions like hepatocyte degeneration in sheep and goats, with eosinophilic hepatitis in sheep, and significantly elevated with chronic liver lesions in all animals. Mbuh and Mbwaye (2005), also reported significantly elevated ALP in chronic goats fasciolosis. Serum ALP is known to be excreted via the bile duct and its elevation is usually significant as fascioliasis progresses which may be synchronized with the arrival of flukes to the bile ducts constraining biliary clearance (Adama *et al.,* 2011). Attributing to our current study, Hacariz *et al.* (2009), also justified that ALP is significantly higher in chronic cases such as cirrhosis and fibrosis in sheep, because ALP is derived from the liver or biliary tract as the adult flukes reside in the bile ducts. In line with this, ALP was evidently raised in cases of hyperplastic cholangitis of the present study. The elevation of ALP is associated with irritation or destruction of biliary epithelium and biliary obstruction (Semrad and Gay, 2013). Attributing to the report of Kocatepe (2012), elevated serum ALP was however, also recorded with hepatocytes necrosis in this study; thus, hepatic ALP can rise following hepatocytes necrosis due to secondary intrahepatic biliary obstructions and as part of the nodular regeneration process.

Age was another variable used for comparisons of mean values of serum liver enzymes of cattle, sheep and goats in this study. Accordingly transaminases were statistically revealed no differences between infected youngs and adults, except significantly higher serum activity of ALT found in infected adult sheep (*Table*.9). These findings were partially in agreement with reports of Rumosa *et al.* (2012), who found insignificant differences in serum activity of both AST and ALT between young and mature goats suffering with liver infection. However, Kocatepe (2012), evaluated significantly elevated AST in adult goats with liver infection than in infected youngs. Semrad and Gay (2013), also reported that AST levels in foals is elevated compared to its values in adults for many months.

In this study, highly significant differences were detected in ALP mean values between infected young and adult groups of each animal *spps.*; which is in agreement with the study of Kocatepe (2012). Accordingly higher values of ALP were found in young cattle, sheep and goats than in their adults, which could be due to leakage of the enzyme from the growing bones and intestines into the blood, beside ALP released from the affected liver in high concentration. On the other hand, Rumosa *et al.* (2012), reported lower values of ALP from infected mature goats, which correspond with the lower values of phosphorus in adult animals serum compared to their equivalent youngs as ALP activity is associated with the process of calcification that accompanies growth.

V. Conclusion and Recommendations

This study attempted to assess liver lesions and serum biochemical alteration that accompany liver fluke infection in ruminants. Grossly livers were: irregular in outline, firm, pale and tough in consistency. Multifocal nodules, thick and distended bile ducts were also examined in chronic cases whereas enlarged liver with tense capsule, numerous hepatocyte haemorrhagic spots, enlarged hepatic lymph nodes and multiple soft abscesses were found in acute fasciolosis. Parenchymal and biliary fibrosis, atrophy, fatty changes, hyperplasia, cirrhosis and necrosis were histopathologic changes observed in chronic forms of the fluke infection though goats' liver was not registered as cirrhotic. On the other hand, multifocal eosinophilic hepatitis, talengechtasis and different hepatocyte necrosis and degeneration were histopathologic changes observed in acute cases. Concentration of serum liver enzyme analysis was also used to evaluate liver fluke infection and indicated significant elevation of serum AST and ALT with acute parenchymal lesions and ALP activity with chronic lesions. Based on this study the following recommendations were forwarded:

In these study results of serum biochemical changes were also consistent with pathological findings and therefore, serum biochemical analysis could be used as complementary in diagnosis of ruminant liver fluke infection. In the study we used ruminants with no liver fluke and no gross liver lesions at all, as control groups for serum biochemical comparisons but further experimental studies with case-control should be done

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to establish better association between liver pathological changes and serum biochemical alteration.

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Comparison of Clinicopathological Characteristics of BRCA1 Associated Breast Cancer Cases with BRCA1 Negative Breast Cancer Cases: A Prospective Study of 100 Women in a Tertiary Care Cancer Hospital

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Abstract- Aims and Objectives: Mutations in BRCA1 gene are associated with greater risk of developing breast cancer. We determined whether clinicopathological characteristics of the tumour differ in patients with and without BRCA 1 expression.

Materials and Methods: The study was conducted in 100 cases of breast carcinomas received as lumpectomy or mastectomy specimens in the Department of Pathology, Sri Guru Ram Das Institute of Medical Sciences, Amritsar over a period of one year(between November 2013 and October 2014).

Keywords: breast carcinoma, immunohistochemistry, ER, PR, BRCA 1.

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a) Haematoxylin and Eosin for histopathology typing and grading
b) All cases were subjected to immunohistochemistry for ER, PR and BRCA1 expression.

Results: The most common age group was 41-60 years (60%).The grade II tumours were the commonest (67%). An immunohistochemical evaluation revealed 35 out of 100 cases as ER, PR+ and 36 cases as positive for BRCA1 expression. BRCA1 positive cases showed a significant lower hormone receptor expression as compared to BRCA 1 negative cases with P=0.008. Similarly BRCA 1 positivity was associated with other poor prognostic factors significantly as with higher grade of tumour (p=0.015), lymph node metastasis (p=0.001) and with greater tumour size (p<0.001).

Conclusion: In this study, BRCA1 expression seems to be associated with adverse prognostic factors. Further studies should seek to determine whether patients with BRCA1 expression respond to treatment differently than BRCA 1 negative patients with similar tumour pathology.

Keywords: breast carcinoma, immunohistochemistry, ER, PR, BRCA 1.

I. Introduction

Breast cancer is the most common malignancy in women. It is the commonest cause of death in developed countries in middle aged women and is becoming frequent in developing countries as well.[1] It has been estimated that by 2020, 14% of the world's cancer cases will be in India. An analysis of breast cases among women in Delhi, Mumbai, Chennai and Bangalore between 1982 and 2005 performed by the ICMR revealed that the number of breast cancer cases have more than doubled in the last 10 years.[2] Mutations in the tumour suppressor gene BRCA 1 are believed to be responsible for majority of hereditary breast cancers. It is estimated that women with BRCA 1 mutations have a lifetime risk of developing breast cancer as high as 87% and 50% of increased chances of its expression in blood relatives of BRCA1 positive patients.[3]

Breast cancer susceptibility gene 1 (BRCA 1) associated breast cancers often occur in younger women and such tumours are of high grade and lack hormone receptors according to several studies.[4,5]

Many studies have shown that BRCA 1 associated breast cancers are associated with adverse histopathological features suggestive of aggressive cancer phenotype[6,7] but other studies failed to demonstrate such association.[8]

This study on 100 breast cancer patients has been done in an attempt to better define the relationship between BRCA1 mutation and tumour grade, tumour stage and Estrogen receptor (ER), Progesterone Receptor (PR) expression.

II. Materials and Methods

a) Histopathology

Haematoxylin-Eosin stained sections from 100 formalin fixed breast cancer specimens received as mastectomy and lumpectomy specimens over the period of one year (Nov 2013-Oct 2014), were diagnosed in the Department of Pathology, Sri Guru Ram Das Institute of Medical Sciences and research, Amritsar. The tumours were graded from grade I to grade III according to Nottingham Modification of Bloom- Richardson method taking into account the parameters- tubule formation, nuclear grading and number of mitosis /HPF. The tumours were evaluated for the histological types, lymphocytic stromal response, nuclear chromatin pattern and nucleoli. Lymph nodes

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recovered were evaluated for the presence of metastatic deposits.

b) Immunohistochemistry

IHC was performed by using the antibodies against the estrogen receptors (ER), the progesterone receptors (PR) (Diagnostic Biosystem) and BRCA1(Biocare Medical).The antigen retrieval was done by using pressure cooker method with 10 mmol citrate buffer at pH 6.0. Tris buffer was used as the wash buffer and Diaminobenzene tetrahydrochloride (DAB) was used as the chromogen. The endogenous activity was blocked by using hydrogen peroxide. After protein blocking, the slides were incubated overnight with the available ER, PR and BRCA-1primary antibodies and they were conjugated with streptavidin Horse Radish Peroxidase (HRP). The slides were counterstained with hematoxylin and were examined by light microscopy. ≥ 10% nuclei stained brown were taken positive for ER and even 1% stained were taken positive for PR. For BRCA-1 this value for positive stained nuclei was ≥ 30%.

III. Results

The age of presentation ranged from 26-70 years with a mean of 52.3 years. The maximum number of patients were in the age group of 41-60 years (60% of the patients). Left sided breast cancer cases (55%) outnumbered the right sided cases (45%), and the upper outer quadrant being the most commonly involved site (61%).

The size varied from 1.5-5.0 cms with maximum number of cases being > 2cms (65%). All the tumours were infiltrating ductal carcinoma NOS (not otherwise specified). 4/100 cases were graded grade I, 67/100 as grade II and 29/100 as grade III. Lymph nodes were recovered in 86 cases out of which 50 cases showed lymph node involvement. 30 cases were ER + and PR + and 5 cases were ER + and PR-. They were taken together as positive for ERPR (35 cases). BRCA 1 positivity was seen in 36/100 cases. Out of these 36 BRCA1 positive cases, 06 cases also showed positivity for ERPR. Rest 29 positive ERPR cases were BRCA 1 negative. While correlating ER, PR and BRCA1 expression it was concluded that BRCA1 positive cases showed a significantly lower ER PR expression as compared to BRCA 1 negative cases with p=0.008 (Table 1).BRCA1 positivity was found to be associated with higher grade of tumour (p=0.015) (Table 2). Similarly lymph node involvement was significantly higher in BRCA 1 positive cases as compared to BRCA 1 negative cases.(p=0.001) (Table 3).BRCA 1 positivity was higher in cases where tumour size was >2 cms (p<0.001)(Table 4).

In contrast, ER PR expression was associated with favourable prognostic markers- lower tumour grade, smaller tumour size and less number of lymph nodes involved.

Table 1 : Correlation of ER, PR, and BRCA 1

ER PR status	BRCA 1 Positive	BRCA1 Negative	Total
ER + PR-	02	03	05
ER- PR+	00	00	00
ER + PR +	04	26	30
ER- PR-	30	35	65

Table 2 : Correlation of ER, PR and BRCA 1 with Tumor Grade

Grade	ERPR+	BRCA 1+	ER-PR-	BRCA 1-
1	02	-	02	04
2	28	14	39	53
3	05	22	24	07

Table 3 : Correlation of ER, PR, and BRCA 1 with Lymph Node

Lymph Nodes	ER+ PR+	ER-PR-	BRCA 1 +	BRCA 1-
N0	13	23	05	31
N1 (1-3)	10	04	09	05
N2 (4-9)	07	19	16	10
N3 (≥10)	03	07	06	04

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Table 4 : Correlation of ER, PR and BRCA 1 with Tumor Size

Size	ER+PR+	ER-PR-	BRCA 1 +	BRCA 1-
<2cm	22	13	09	26
>2cm	13	52	27	38

Figures

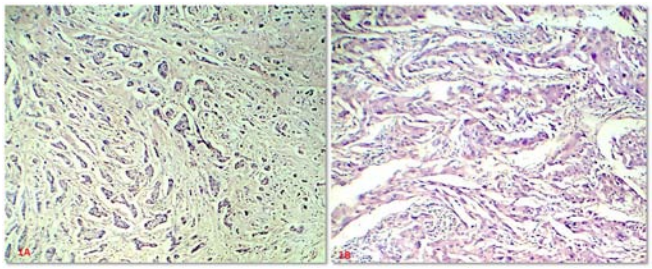

Figure 1 (A, B)

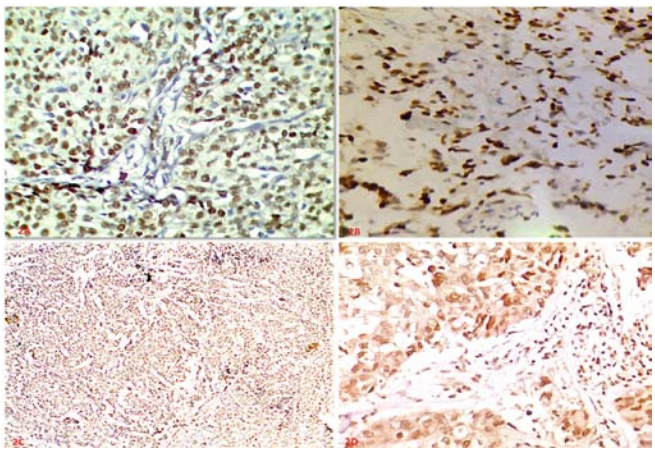

Figure 2 (A, B, C, D)

Figure Legends

Figure 1 (A): Infiltrating Ductal Carcinoma- grade II (H&E, 100X)
Figure 1 (B): Infiltrating Ductal Carcinoma- grade III (H&E, 100X)
Figure 2 (A): ER positivity (Nuclear) – IHC (400X)
Figure 2 (B): PR positivity (Nuclear) – IHC (400X)
Figure 2 (C): BRCA 1 positivity (Nuclear) – IHC (100X)
Figure 2 (D): BRCA 1 positivity (Nuclear) – IHC (400X)

IV. Discussion

The incidence of breast cancer in India is on the rise and is rapidly becoming the number one cancer in females pushing cervical cancer to second spot.[2] It is widely acknowledged that breast cancers show several characteristics which play an important role in their diagnosis and treatment. So all the variables including age of the patient, grade of the tumor, immunohistochemistry and genetic profiles are important associated factors.

Age is an important factor as with the advancing age risk of development of breast cancer increases. Age of the patients varied from 26-70yrs with maximum number of cases in the age group of 41-60 yrs (60%) with mean age of 52.3 years. In different parts of the country similar results regarding maximum number of cases in this age group have been reported. In these studies mean age of breast cancer patient was found to be lower than the western countries counterpart by an average difference of one decade.[9,10]

In the immunohistochemical profile –ER PR positivity was calculated to be 35 % which is in concordance with various studies conducted in this part of continent.[11,12] ER PR positivity was found to correlate significantly with favourable prognostic factors as lower grade of tumour (p=0.012), less number of lymph nodes involvement (p=0.009) and smaller size of the

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tumour at the time of presentation (p<0.001) .This result is consistent with previous reports.[13,14]

BRCA1 positivity was seen in 36 out of 100 cases which is similar to other various studies where positivity varied from 25% to 35%.[15,16] In this study we compared the clinical and pathologic characteristics of BRCA 1 positive cases with BRCA 1 negative cases. Significantly more of the BRCA1 positive were ERPR negative (30/36-83%) than BRCA1 negative cases (35/64-54%). Several studies have evaluated decreased hormone expression in BRCA 1 mutation carriers. In these studies 60%-85% of BRCA 1 carriers were diagnosed ERPR negative as compared to 20%-40% of BRCA 1 non carriers.[16,17,18]

Similarly more of the BRCA1 related cancers had higher tumour grade i.e. grade III (22/36-61%) as compared to BRCA1 negative cases (7/64-11%). This result is in concordance with previous studies which have shown that BRCA 1 related cancers were of higher histological grade.[16,17] In a study conducted in Jewish women, 76.5% of BRCA 1positive tumours had a higher nuclear grade as compared to only 27.3% of BRCA 1 negative tumours.[17]

The individuals with BRCA1 mutation were significantly less likely to present with stage1 disease. BRCA1positive cases had higher number of lymph nodes involvement (p= 0.001) and tumour size > 2 cms at the time of presentation (p<0.001). These results are similar to those reported previously in the literature.[18]

It was proposed that there is some intriguing mechanism of interaction between BRCA 1 and other molecular markers. BRCA 1mutation is followed by p53 dysfunction and cancer cells become ERPR negative, hence favouring the unique profile of BRCA 1mutant tumours.[19]

Despite the presence of association of BRCA 1with poor prognostic markers, several studies have failed to demonstrate a worse clinical outcome in such patients as compared to their counterparts. No significant difference was seen between BRCA 1 associated and non BRCA 1associated cases in five year relapse free survival, five year event free survival and five year overall survival. However women with germline BRCA 1mutation significantly belong to younger age group and more likely to develop contralateral breast cancer and ovarian cancers.[5]

In the above study, several statistically significant correlations were found between BRCA 1positivity, clinical variables and molecular markers. Women with BRCA 1 mutations have a life time risk of developing breast cancer as high as 87% and 50% of increased chances of its expression in blood relatives of positive BRCA 1.[3] So all the blood relatives of patients with BRCA 1positivity on IHC should be screened for BRCA 1 gene mutation.

Our findings suggest that BRCA 1 positive tumours have a unique molecular profile and a different mechanism of tumorigenesis. These tumous are of high grade, higher stage at presentation and ERPR negative. Whether the more aggressive biological phenotype of BRCA1 tumours justifies more aggressive treatment is still a matter of debate. Further studies should determine whether treatment outcome differs for BRCA 1 mutation carriers as compared to BRCA 1negative counterparts.

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Note :

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- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone. "

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1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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- Submitting a manuscript with pages out of sequence

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· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
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- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
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- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

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Approach:

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- Materials may be reported in a part section or else they may be recognized along with your measures.

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- Report the method (not particulars of each process that engaged the same methodology)
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- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
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- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
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What to keep away from

- Resources and methods are not a set of information.
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The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

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Approach

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- Give details all of your remarks as much as possible, focus on mechanisms.
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- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

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References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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