Evaluation of Telomerase Activity in Ascitic Fluid of Benign & Malignant Origins

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List of Abbreviations

AASLD	American association of the study of liver disease
Ab	Antibody
AFP	Alpha-fetoprotein
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
Anti-id	Anti-idiotype
AST	Aspartate aminotransferase
BsAb	Bispecific antibody
CA	Catumaxomab
Ca 125	Cancer antigen 125
CA 19-9	Carbhydrate antigen 19-9
CGRP	Calcition gene related peptide
CLD	Chronic liver disease
CT	Computerized tomography
DNA	Deoxyribonucleic Acid
EDTA	Ethylene diamine tetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
EPCAM	Epithelial cell adhesion molecule
ER	Estrogen receptor
ET-1	Endothelin-1
FDA	Food and drug adminstration
Hb	Hemoglobin
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus

HIPEC	Intraperitoneal hypothemic chemotherapy
HTERT	Human telomerase trasncriptase
HTR	Human telomerase
INF	Interferon
INR	International normalization ratio
IP	Intraperitoneal
M	Mean
M1	Mortality stage 1
M2:	Mortality stage 2
MMP	Matrix metalloproteinase
MRI	Magnetic resonance imaging
No	Nitric oxide
P	P-value
PC	Peritoneal carcinomatosis
PCR	Polymerase chain reaction
PgR	Progesterone receptor
PIVKA-II	Prothrombin induced by Vit-K antagonist II
PLT	Platelets
RAAS	Renin angiotension aldosterone system
RNA	Ribonucleic acid
SAAG	Serum ascites albumin gradient
SBP	Spontaneous bacterial peritonitis
SD	Standard deviation
SNS	Sympathetic nervous system
TIPS	Transjugular intrahepatic porto-systemic stent.
TLC	Total leucocytic count
TRAP	Telomeric repeat amplification protocol

US	Ultrasound
VEGF	Vascular endothelial growth factor
VPF	Vascular permeability factor

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Introduction

Telomerase is one of the DNA modifying enzymes whose history dates back to 1938 when Hermann Müller exposed the flies of the species Drosophila Melanogaster to X rays and observed that the ends of the irradiated chromosomes, different from the other genome, did not present alterations such as deletions or inversions, thanks to the presence of a protective cap that he himself called “terminal gene” and afterwards “telomere”, from the Greek terms “telos” = end and “meros” = part **(Müller, 1938)**. The role of telomerase was discovered in 1985 by Elizabeth Blackburn, Carol Greider and Jack Szostak **(Blackburn et al, 1985)**. They received a Nobel Prize for this discovery in 2009 **(Yucheng et al, 2011)**.

Telomerase is a ribonucleoprotein that is an enzyme which adds DNA sequence repeats ("TTAGGG" in all vertebrates) to the 3' end of DNA strands in the telomere regions, which are found at the ends of eukaryotic chromosomes. This region of repeated nucleotide called telomeres contains non-coding DNA and hinders the loss of important DNA from chromosome ends. As a result, every time the chromosome is copied only 100–200 nucleotides are lost, which causes no damage to the organism's DNA. Telomerase is a reverse transcriptase that carries its own RNA molecule, which is used as a template when it elongates telomeres, which are shortened after each replication cycle. Telomerase consists of a protein component (TRT) and an RNA component (TR) containing the template for synthesis of the repeat unit added onto the ends of chromosomes **(Blackburn, 1992)**.

It is known that telomerase is active in 85% of cancer tumors, while in the other 15% of cases different mechanisms of telomere length maintenance based on recombination are active **(Bollman, 2007)**. It should be noted that telomerase activity is not found in usual somatic tissues. As a result, already beginning from the first years of investigation of telomerase the enzyme was considered as a universal target that could be used in the development of anticancer therapy.

Ascites is the pathologic accumulation of fluid in the peritoneal cavity. It is an important clinical finding and its appropriate treatment depends on correct diagnosis **(Habeeb et al, 1997)**. Different diseases can cause different types of ascites. Some of the known low gradient ascites include tuberculous peritonitis-induced ascites, malignancy-induced ascites (for instance, peritoneal carcinomatosis, gastric and ovarian cancer metastases), pancreatic ascites, renal ascites, biliary ascites, bacterial peritonitis and serositis-induced ascites **(Fariborz et al, 2005)**. On the other hand, high gradient ascites are uncomplicated cirrhosis-induced ascites, heart-failure-induced ascites, those induced by extensive liver metastases and other circumstances such as fulminant hepatic failure.

The differential diagnosis between malignancy related ascites and non- malignancy related ascites has remained a clinical challenge. Although cytological examination of ascitic fluid is considered the gold standard in terms of diagnostic specificity, its sensitivity in detecting malignancy related ascites is low, ranging between 40% and 60%. Various laboratory parameters such as total protein, lactate dehydrogenase, fibronectin, and cholesterol from ascitic fluid, as well as the serum-ascites albumin gradient (SAAG), have been evaluated, but none of them are satisfactory

as a single diagnostic test. Likewise, several tumor markers such as carcinoembryonic antigen, cancer antigen 125 (CA 125), and carbohydrate antigen 19-9 (CA 19-9) have also been evaluated, but discrepancies among these tests still persist. Therefore, a more reliable test with higher sensitivity and specificity to discriminate between malignancy related ascites and non-malignancy related ascites is required **(Tangkijvanich et al, 1999)**.

Aim of work

This thesis aims at determining the diagnostic value of ascitic fluid telomerase activity in discriminating between malignancy related and non-malignancy related ascites.

Review

Ascites is a Greek term “ASKOS” which refers to a bag or sac **(Doddamani et al, 2010)**. It is the pathologic accumulation of fluid in the peritoneal cavity **(Habeeb et al, 1997)**. A normal peritoneal cavity contains no fluid, although in women a small amount 20ml or less can occasionally be found depending on the menstrual cycle **(Aslam et al, 2012)**.

Pathologically, ascites results from:

1. **Increased hydrostatic pressure associated with portal hypertension**, as in cirrhosis, alcoholic hepatitis, fulminant hepatic failure, fatty liver of pregnancy, hepatic fibrosis, Budd-Chiari syndrome, constrictive pericarditis, congestive heart failure, veno-occlusive disease
2. **Decreased colloid osmotic pressure secondary to hypoalbuminemia**, as in end stage liver disease with poor protein synthesis, nephrotic syndrome with protein loss, malnutrition, protein-losing enteropathy
3. **Increased permeability of peritoneal capillaries**, as in tuberculous peritonitis, bacterial peritonitis, fungal peritonitis, HIV associated peritonitis
4. **Leakage of fluid into the peritoneal cavity**, as in bile ascites, pancreatic ascites, chylous ascites, urine ascites
5. **Malignant conditions**, as in peritoneal carcinomatosis, hepatocellular carcinoma, hepatic metastasis, pseudomyxoma peritonei, mesothelioma, and cancers associated with breast, large bowel, bronchus, stomach, pancreas, ovary and endometrium