INTRODUCTION

• Octamer 4 (OCT4) is a transcriptional factor important in maintaining stem cells pluripotency and self-renewal (Brumbaugh et al., 2012; Kim & Nam, 2011).
• The expression of OCT4 found to be associated with many somatic cell cancers.
• OCT4 can be posttranslationally controlled by phosphorylation (Brumbaugh et al., 2012).
• Phosphorylation of OCT4 serves an important role in its stability, nuclear localization and transcription (Lin et al., 2012).
• Immunofluorescence revealed the presence of phosphorylated OCT4 at threonine 235 (OCT4-pT235) in glioblastoma and liver cancer cells (Zhao et al., 2015).
• Breast Cancer is a heterogeneous group of diseases and classified generally into luminal-like, HER2 positive and triple negative breast cancer.
• Herein, we test the association between OCT4-pT235 and different breast cancer subtypes and disease characteristics.

METHODS:

Cell culture:
The Breast cancer cell lines MDA-MB-231 and MCF-7 were maintained in RPMI-1640 medium, all supplemented with fetal bovine serum (FBS), antibiotics and antimycotics (ABM). HMLE cell line was maintained in serum free WIT-P medium supplemented with ABM.

Immunofluorescence:
Immunofluorescence was performed on cytospined cells after 48 hours of culture cell confluency (60-70%). Oct4-pT235 was detected using rabbit anti-Oct4 antibody at 1:700 dilution, which was incubated overnight at a dilution of 1:700. Alexa 594 goat anti-rabbit was used as the conjugated secondary antibody at 1:400 dilution in addition to DAPI.

Expression analysis of OCT4-pT235:
Quantification of fluorescence intensity was done using BD pathway 855 system, which uses a program called a macro to quantify Oct4-pT235 level in each cell. The data was then analyzed by BD Image Data Explorer.

RESULTS:

DISCUSSION:
Out of the seven breast cell lines tested: HMLE, which is a normal human mammary epithelial cell line, had the lowest OCT4-pT235 level. Among breast cancer cell lines triple negative breast cancers showed the highest level of OCT4-pT235. MDA-MB-468, MDA-MB-231, BT-20 cell lines have mutation in their tumor suppressor gene PTEN, which encodes for PTEN a phosphatase that inhibits the PI3K/AKT signaling pathway and its deletion results in hyper-activation of the pathway which is responsible for OCT4 phosphorylation (P. Liu, Cheng, Roberts, & Zhao, 2009). Moreover, all of the triple negative cell lines show mutation in TP53 gene. Importantly, the deletion of both tumor suppressors results in hyper-activation of AKT pathway in triple negative breast cancer cells (J. C. Liu et al., 2014), which phosphorylates OCT4 (Zhao et al., 2015). Both luminal like MCF-7 and triple negative BT-20 cells show mutation in PIK3CA gene, which encodes for the catalytic subunit p110 of PI3K. Mutations in this gene found to increase AKT activity.

Phosphorylation of OCT4 at T235 plays a major role in it stabilization (Zhao et al., 2015), thus maintaining cancer stem cells. Expression of OCT4 is associated with cancer stem cells (Kim & Nam, 2011), in which a subpopulation of cancer cells plays a role in maintaining the tumor.

CONCLUSION
• OCT4-pT235 level is higher in triple negative breast cancer when compared with normal, luminal like and Her2-positive cell lines.
• OCT4 expression is associated with cancer stem cells and its phosphorylation at threonine 235 stabilizes it.